Premio a la Mejor Tesis de Licenciatura de la UANL 2004

Publicaciones Nacionales e Internacional derivadas de la tesis de licenciatura de:

LQI Miriam Verónica Gracia Lozano

Que lleva por titulo:

"Evaluación de las constantes cinéticas de biodegradación de hidrocarburos de la gasolina sin plomo por un consorcio microbiano mixto aclimatado a estos en matrices acuosas y de suelo en presencia y ausencia de un surfactante no iónico"

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):

$$\text{Model I } S_t = S_0 \exp(-Kt) \quad (1)$$

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})

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):

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

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:

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

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-

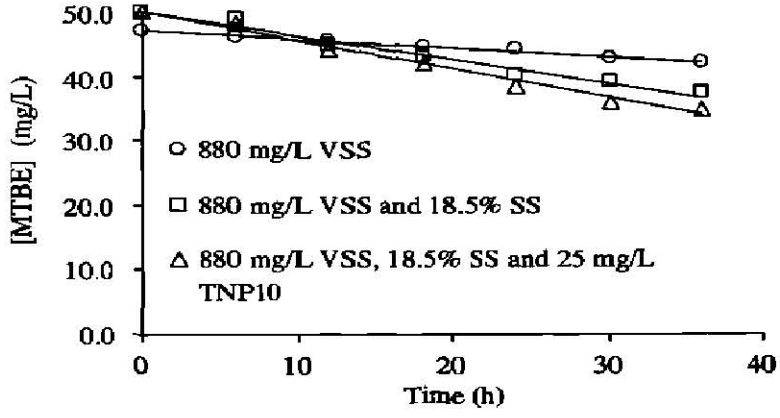

Figure 1 MTBE biodegradation kinetics with 200 mg/L total BTEoX in the presence of 880 mg/L VSS

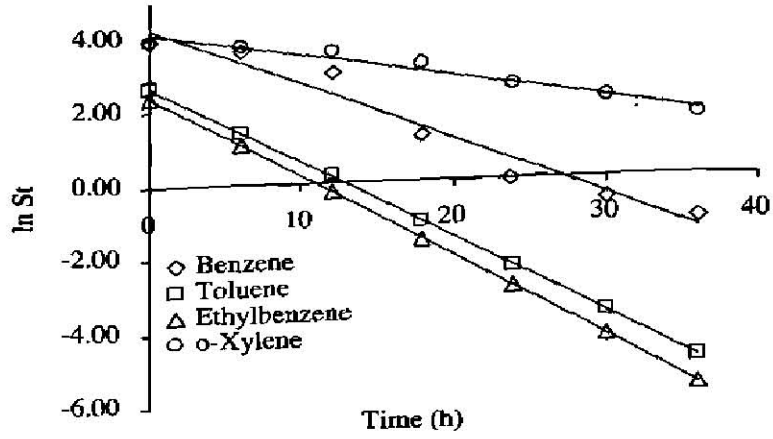

Figure 2 BTEoX biodegradation kinetics with 50 mg/L MTBE in the presence of 880 mg/L VSS

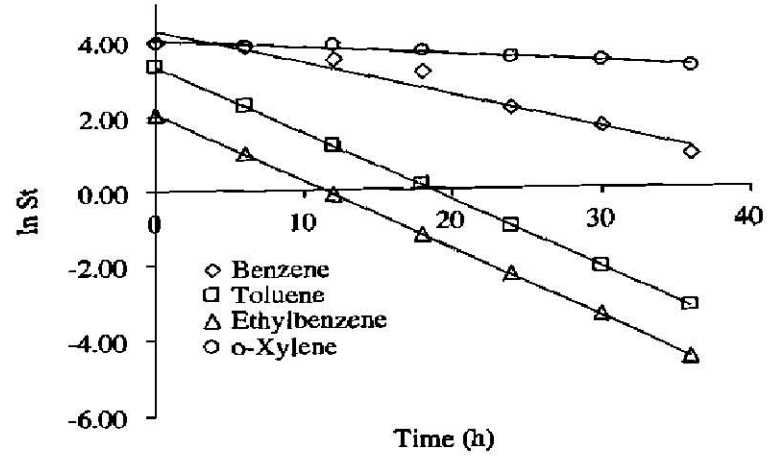

Figure 3 BTEoX biodegradation kinetics with 50 mg/L MTBE, 880 mg/L VSS and 18.5% SS

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.

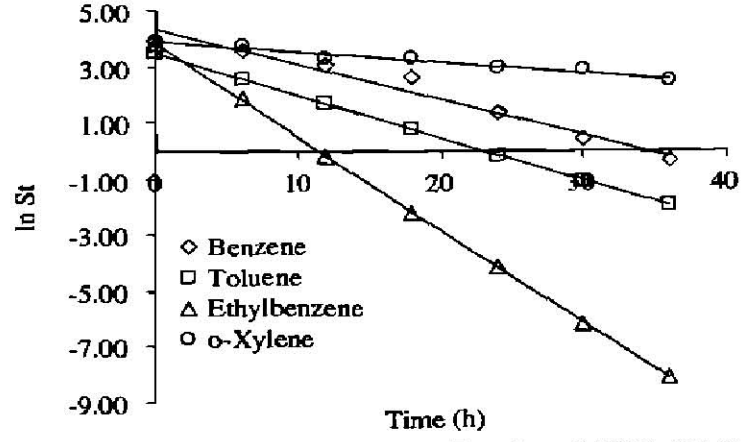

Figure 4 BTEoX biodegradation kinetics with 50 mg/L MTBE, 880 mg/L VSS, 18.5% SS and 25 mg/L of TNP10

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

The authors are grateful to Juan M. Oviedo-Tristan for helping in laboratory equipment calibration. This research was supported under the auspices of the Instituto Mexicano del Petroleo research project FIES96-F-48VI; CONACYT research project I32968-B and fellowship 990345. UANL PAICYT projects SA591-01 and SA672-02 are also acknowledged.

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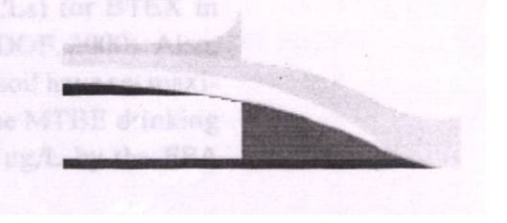

BTE-OX biodegradation kinetics with MTBE through bioaugmentation

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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):

$$\text{Model I } S_t = S_0 \exp(-Kt) \quad (1)$$

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})

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):

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

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:

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

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-

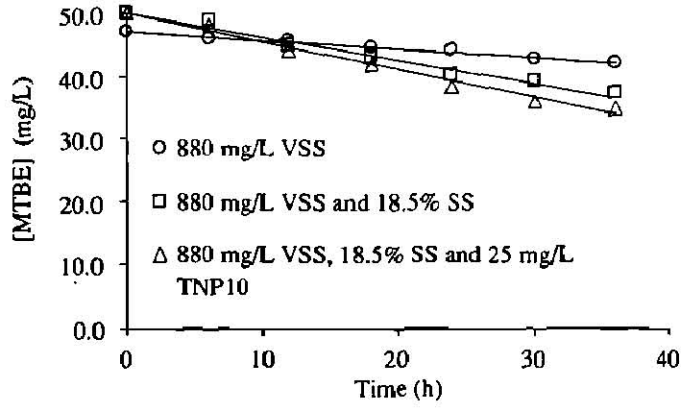

Figure 1 MTBE biodegradation kinetics with 200 mg/L total BTEoX in the presence of 880 mg/L VSS

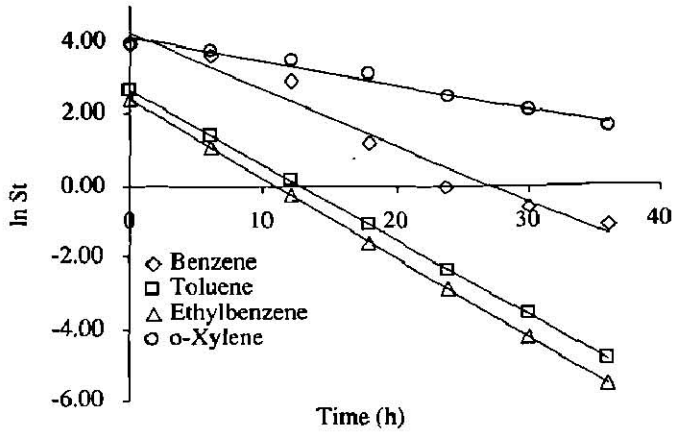

Figure 2 BTEoX biodegradation kinetics with 50 mg/L MTBE in the presence of 880 mg/L VSS

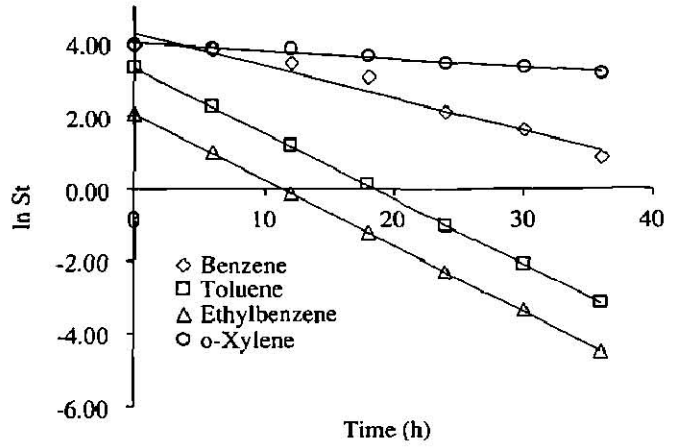

Figure 3 BTEoX biodegradation kinetics with 50 mg/L MTBE, 880 mg/L VSS and 18.5% SS

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.

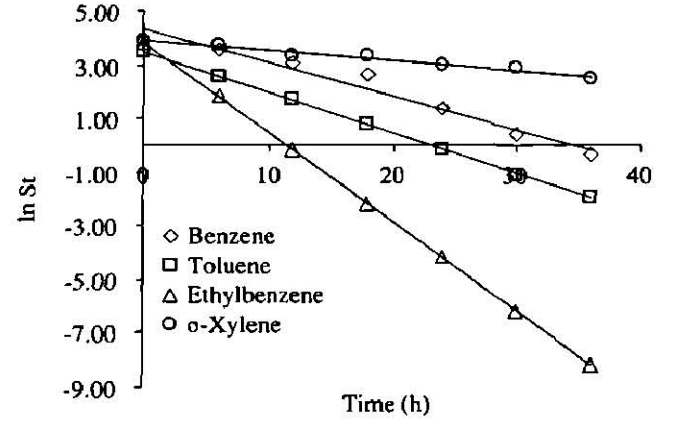

Figure 4 BTEoX biodegradation kinetics with 50 mg/L MTBE, 880 mg/L VSS, 18.5% SS and 25 mg/L of TNP10

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

The authors are grateful to Juan M. Oviedo-Tristan for helping in laboratory equipment calibration. This research was supported under the auspices of the Instituto Mexicano del Petroleo research project FIES96-F-48VI; CONACYT research project I32968-B and fellowship 990345. UANL PAICYT projects SA591-01 and SA672-02 are also acknowledged.

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Ecohazard 2003

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PROCEEDINGS OF THE 4TH IWA SPECIALIZED CONFERENCE ON ASSESSMENT AND CONTROL OF HAZARDOUS SUBSTANCES IN WATER - ECOHAZARD 2003 -

Edited by Horst Fr. Schröder

Institute of Water and Waste Management, Aachen University

Aachen, Germany
14-17 September, 2003

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Ecohazard2003
14 - 17 September 2003

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Date: July 15th, 2003

INVITATION
for
Dr. Karim Acuna-Askar, Facultad de Medicina UANL, Monterrey, N.L.

Dear Dr. Acuna-Askar,

I am pleased to inform you that your abstract reference numbers 007 and 008 have been accepted for oral and poster presentation, respectively, at the 4th Specialised IWA Conference on Assessment and Control of Hazardous Substances in Water, (ECOHAZARD 2003) to be held in Aachen, Germany, 14-17 September 2003.

Following is information pertaining to the above abstracts:

Abstract 007, title: *BTE-oX biodegradation kinetics with MTBE through bioaugmentation.*
Paper 22 B Presentation group VI B Chemical analysis and fate studies.
Date and time: Tuesday 9/16/03, 11:20 a.m.

Abstract 008, title: *Effect of soil on BTE-oX biodegradation patterns in the liquid phase in batch.*
Poster 25 Topic IV Analysis and fate of hazardous substances.
Date and time: Posters will be displayed throughout the Conference schedule.

We look forward to your participation at ECOHAZARD 2003 in Aachen.

Sincerely,

Prof. Dr. H. Fr. Schroeder (Chairman)
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Aachen University
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The 4th IWA Specialized Conference on Assessment and Control of Hazardous Substances in Water

- ECOHAZARD 2003 -

14 - 17 September 2003

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Foreword

It is my great pleasure to welcome you to the 4th International Water Association Specialized Conference on Assessment and Control of Hazardous Substances in Water (ECOHAZARD 2003). Our event, from the 14th to the 17th of September 2003, in Aachen, Germany, follows the conferences organised in Otsu, Shiga, Japan and Copenhagen, Denmark. The Specialist Group on Assessment and Control of Hazardous Substances in Water (ACHSW), the International Water Association, the Institute for Water- and Waste Management (ISA) and Aachen University (RWTH) are the organisers of the ECOHAZARD 2003 Conference.

With this book the organisers will provide an overview of the conference themes covering the main issues of hazardous substances in water: endocrine-disrupting compounds, pharmaceuticals, persistent polar pollutants, industrial wastewater, re-use of wastewater, chemical analytical and toxicity testing methods and applications, analysis and fate of hazardous substances in treatment systems and the environment, natural attenuation in soil, contaminated sediments, risk assessment, and river basin monitoring programmes. The present publication testifies to the essential role of interdisciplinary communication in solving today's environmental tasks.

In the name of the organisers I would like to thank the authors of oral and poster presentations for preparing their papers so timely that we can make these proceedings available to the participants before the start of ECOHAZARD 2003. In addition, I would like to thank all the co-workers involved in the organisation of ECOHAZARD 2003 and the edition of this conference publication.

Aachen, September 2003

Prof. Dr. Horst Fr. Schröder
Chairman of the Organising Committee

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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 95.4-99.7% for benzene, toluene and ethylbenzene. O-xylene and MTBE percent removals ranged 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 (USEPA, 2002). Underground storage tanks (UST's), 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. The MSCC are divided in three categories: the residential soil cleanup levels; the industrial/commercial soil cleanup levels and the soil-to-water maximum contaminant concentration (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). Emerging environmental regulations for BTX contaminated soil in Mexico, have set the maximum contaminant levels (MCLs) as 20.00, 40.00 and 40.00 mg/Kg, respectively, for agricultural, residential and commercial settings, and 50.00, 100.00 and 100.00 mg/Kg (as total xylenes), respectively, for industrial use (DOF, 2002). The MTBE drinking water health advisory level for taste and odor has been set at 20-40 ug/L by the EPA (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). The physical-chemical properties of BTEX allow them to partially adsorb on to the soil particles before they reach the groundwater, therefore, 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.

Methods

Chemicals. Benzene, toluene, ethylbenzene, mixed xylenes, o-, m-, p-xylene, methyl tertiary butyl ether (MTBE) and Tergitol NP-10 (nonionic surfactant) were purchased from Sigma-Aldrich (Mexico) and were above 98% purity. Unleaded gasoline (UG) Premium was purchased from a gas station. Nutrient agar (Difco Laboratories, Detroit, MI) and bacteriological agar (BIOXON, Becton-Dickinson, Mexico) were purchased from Casa Rocas Fisher Scientific (Mexico).

Culture conditions. 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.*, 2002): 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 concentration range of nonionic surfactant (Tergitol NP-10) where a sudden variation in the relation between both culture medium density and culture surface tension occurred was chosen as the critical micelle concentration (CMC). The amount of Tergitol NP-10 added to experimental bioassays was slightly below the CMC based on prior studies (Acuna-Askar *et al.*, 2002).

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. 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. 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 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 the grown in the 20-L biomass acclimation batch reactor. This procedure was made for each of the three replicates.

Experimental bioassays. 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 Tergitol NP-10 (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.

Sample and control sterilization. 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.

Isolation of acclimated bacteria. UG-acclimated bacteria were grown in UG agar plates and incubated in a gravity flow Isotemp incubator (Fisher Scientific, USA), model 537D at 28-30°C for 72 hours. Acid production in UG agar plates was identified by change of color from blue to green, and in some cases from blue to yellow using bromothymol blue as indicator. For identification purposes, bacteria isolates were grown in nutrient agar plates.

Mechanical shakers and sonication. 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

Gas chromatography and sample concentrator. 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) 100m x 0.25mm ID x 0.5µm 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, point at which temperature was held for 20 minutes. A second temperature rate followed and varied 30°C/min from 90°C up to 150°C, point at which 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

First-order one-phase model. For the three sample sets, the overall benzene and o-xylene removal rate constants K were obtained by the following equation (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})

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

First-order two-phase model. For the three sample sets, the overall toluene and ethylbenzene removal rate constants K were obtained by the following equation (Hu *et al.*, 2002):

Model II $S_t = S_1 \exp(-K_1 t) + S_2 \exp(-K_2 t)$ (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})

The overall removal rate constants K were obtained from the method of residuals (Hu *et al.*, 2002).

Zero-order model. For the three sample sets, the overall MTBE removal rate constants K were obtained by the following equation:

Model III $S_t = - Kt + S_0$ (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 (Fig. 1). All BTE-oX chemicals biodegraded in the presence of biougmented 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.

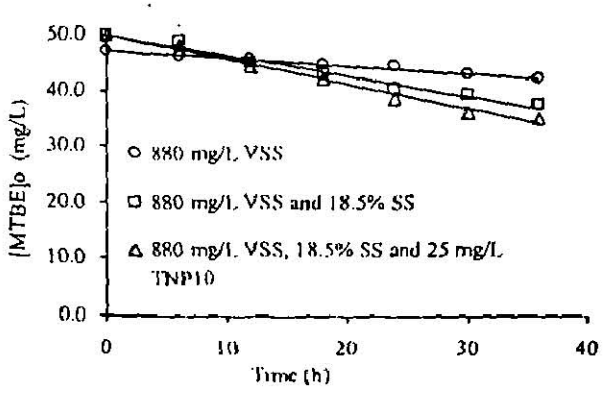

Figure 1 MTBE biodegradation kinetics with 200 mg/L total BTEoX in the presence of 880 mg/L VSS.

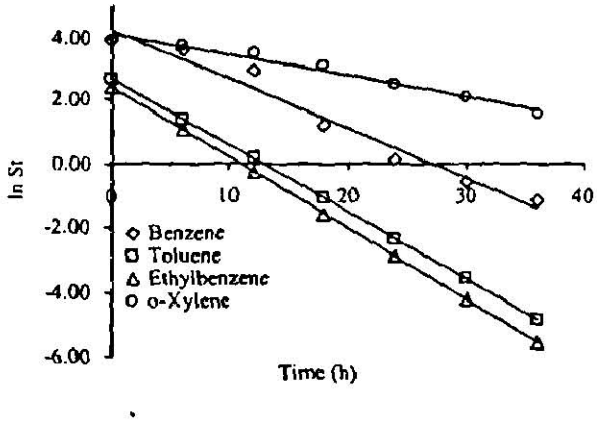

Figure 2 BTEoX biodegradation kinetics with 50 mg/L MTBE in the presence of 880 mg/L VSS.

As indicated in Figure 3, soil had a negative impact on the biodegradation rates of all BTE-oX chemicals, but 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 substrate 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 Tergitol NP-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 Tergitol NP-10 to the slurry samples. Toluene removal rate, however, had a significant negative impact by the addition of Tergitol NP-10.

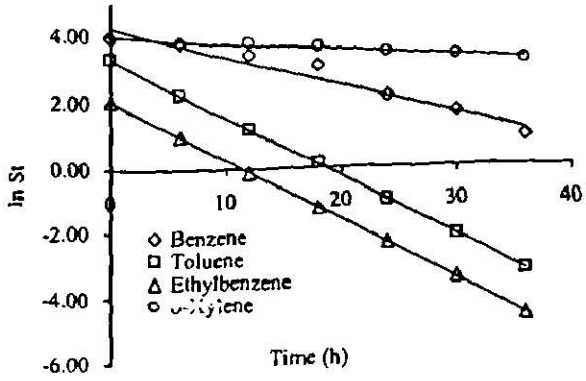

Figure 3 BTEoX biodegradation kinetics with 50 mg/L MTBE, 880 mg/L VSS and 18.5% SS

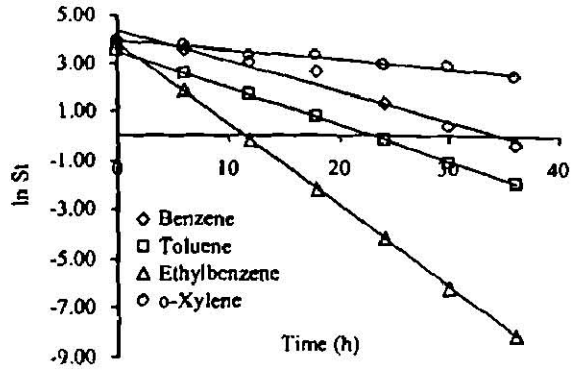

Figure 4 BTEoX biodegradation kinetics with 50 mg/L MTBE, 880 mg/L VSS, 18.5% SS and 25 mg/L of TNP10.

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 were consistently higher in all experimental bioassays than o-xylene removal rate constants.

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 + 880 mg/L VSS
** SMM + 18.5% SS + 880 mg/L VSS
*** SMM + 18.5% SS + 880 mg/L VSS + 25 mg/L TNP-10

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 the MTBE biodegradation. The presence of soil, however, had a positive effect on MTBE removal rate by three-fold. Tergitol NP-10 showed a slight increase on MTBE removal rate.

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 + 880 mg/L VSS
** SMM + 18.5% SS + 880 mg/L VSS
*** SMM + 18.5% SS + 880 mg/L VSS + 25 mg/L TNP-10

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 the mixture. The low biodegradation of MTBE was not unexpected because previous work (Acuna-Askar *et al.*, 2000; Hu *et al.*, 2002; Stringfellow and Oh, 2002; Pruden *et al.*, 2003) 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 Tergitol NP-10, however, helped increase o-xylene percent 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). Benzene, toluene and ethylbenzene percent removals ranged 95.4-99.7%. Although, the overall removal rate constants of these three substrates were negatively impacted by soil, the overall percent removals did not change significantly at the end of 36 hours.

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

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 Tergitol NP-10 to aqueous samples containing soil, showed a increase on 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 ethylbenzene biodegradation rate. MTBE showed the lowest biodegradation rate among the substrates evaluated. Substrate percent removals ranged 95.4-99.7% for benzene, toluene and ethylbenzene. O-xylene and MTBE percent removals ranged 55.9-90.1% and 15.6-30.1%, respectively.

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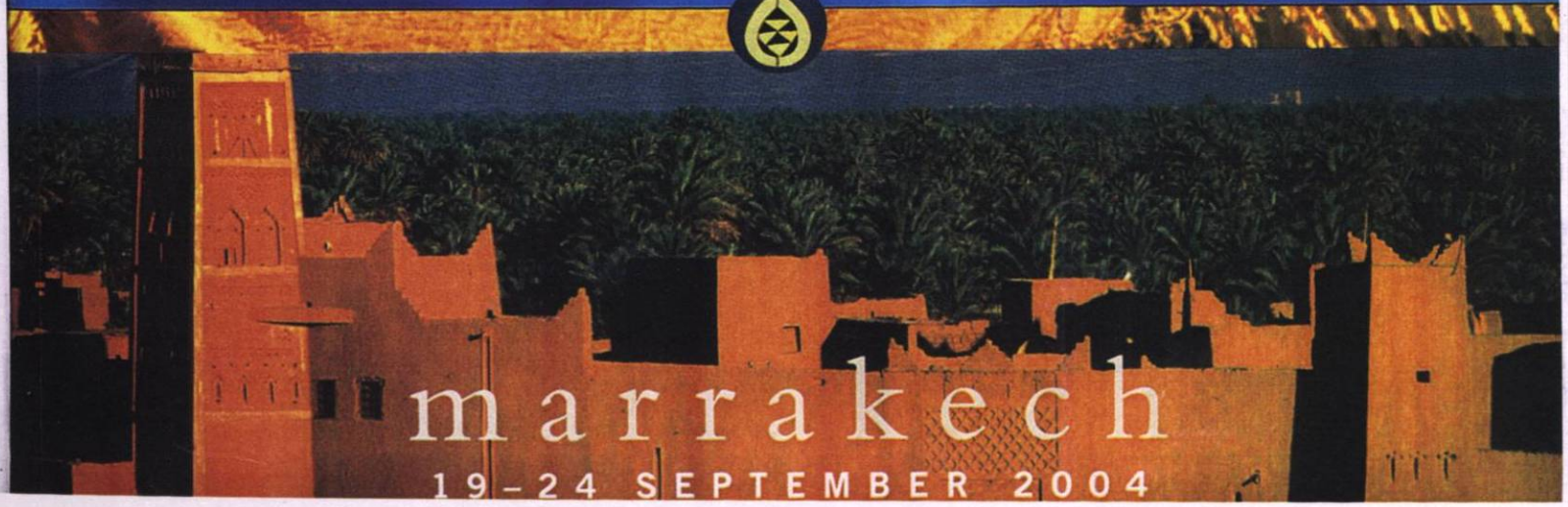

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Biodegradation of Hazardous Compounds

PaperID 116318

Effect of soil and a nonionic surfactant on BTE-oX and MTBE biodegradation kinetics

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The biodegradation kinetics of BTE-oX and MTBE, mixed all together, in the presence of 905 mg/L VSS of BTEX-acclimated biomass was evaluated. Effects of soil and Tergitol NP-10 in aqueous samples on substrate biodegradation rates were also evaluated. Biodegradation kinetics was evaluated for 36 hours, every 6 hours. MTBE biodegradation followed a first-order one-phase kinetic model in all samples, whereas benzene, toluene and ethylbenzene biodegradation followed a first-order two-phase kinetic model in all samples. O-xylene biodegradation followed a first-order two-phase kinetic model in the presence of biomass only. Interestingly, o-xylene biodegradation was able to switch to a first-order one-phase kinetic model when either soil or soil and Tergitol NP-10 were added. The presence of soil in aqueous samples retarded benzene, toluene and ethylbenzene removal rates. O-xylene and MTBE removal rates were enhanced by soil. 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 77-99.8% for benzene, toluene and ethylbenzene. O-xylene and MTBE percent removals ranged 50.1-65.3% and 9.9-43.0%, respectively.

PaperID 116148

Role of hydrophilic organic matter on developing toxicity in decay process of activated sludge

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It is known that the toxicity of effluent is more intensive than that of influent in the activated sludge process. In this study, we applied bioassay using cultured human cell lines to decay process of activated sludge in order to evaluate the toxicity of organic matter generated and/or released from activated sludge bacteria. We also applied this bioassay to hydrophilic fraction of samples, The bioassay results showed that (1) the variation in the dose-response relationship obtained from assay with original samples was observed during decay; (2) on the other hand, the response curves of only hydrophilic fraction at each time show the same relationship between TOC and viability of MCF7 cells; (3) this trend was confirmed by plotting the time course of EC50. These results implied that (1) the hydrophilic organic matter controlled for developing toxicity during decay process of activated sludge; and (2) the character of hydrophilic organic matter is not changed during the experimental period.

PaperID 116378

Removal of PhACs in Nitrifying-Denitrifying Plants

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The behaviour of 9 pharmaceutically active compounds (PhACs) of different diagnostic groups is studied during a nitrifying-denitrifying process in an activated sludge system. The compounds selected cover a wide range of frequently used substances such as anti-epileptics (carbamazepine), tranquillisers (diazepam), anti-depressants (fluoxetine and citalopram), anti-inflammatories (ibuprofen, naproxen and diclofenac) and estrogens (estradiol and ethinylestradiol). The main objective of this research is to investigate the effect of acclimation of biomass on the removal rates of these compounds, either by maintaining a high sludge retention time or at long-term operation. The removal rates achieved for nitrogen and carbon in the experimental unit exceed 90% and were not affected by the addition of PhACs. Carbamazepine, diazepam and diclofenac were only removed to a small extent. On the other hand, higher removal rates have been observed for naproxen and ibuprofen (68% and 82%), respectively.

PaperID 116150

Cometabolic Transformation of cis-1,2-Dichlororethylene andcis-1,2-Dichloroethylene Epoxides by a Butane-Grown Mixed Culture

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Aerobic cometabolism of cis-1,2-dichloroethylene (c-DCE) by a butane-grown mixed culture was evaluated in batch kinetic tests. The transformation of c-DCE resulted in the coincident generation of c-DCE epoxide. Chloride release studies showed ~75% oxidative dechlorination of c-DCE. Mass spectrometry confirmed the presence of a compound with mass-to-charge-fragment ratios of 112, 83, 48, and 35. These values are in agreement with the spectra of chemically synthesized c-DCE epoxide.

Effect of Soil and a Nonionic Surfactant on BTE-oX and MTBE Biodegradation Kinetics

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Abstract The biodegradation kinetics of BTE-oX and MTBE, mixed all together, in the presence of 905 mg/L VSS of BTEX-acclimated biomass was evaluated. Effects of soil and Tergitol NP-10 in aqueous samples on substrate biodegradation rates were also evaluated. Biodegradation kinetics was evaluated for 36 hours, every 6 hours. MTBE biodegradation followed a first-order one-phase kinetic model in all samples, whereas benzene, toluene and ethylbenzene biodegradation followed a first-order two-phase kinetic model in all samples. O-xylene biodegradation followed a first-order two-phase kinetic model in the presence of biomass only. Interestingly, o-xylene biodegradation was able to switch to a first-order one-phase kinetic model when either soil or soil and Tergitol NP-10 were added. The presence of soil in aqueous samples retarded benzene, toluene and ethylbenzene removal rates. O-xylene and MTBE removal rates were enhanced by soil. 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 77-99.8% for benzene, toluene and ethylbenzene. O-xylene and MTBE percent removals ranged 50.1-65.3% and 9.9-43.0%, respectively.

Keywords biodegradation; BTEX; MTBE

Introduction

Benzene, toluene, ethylbenzene and mixed xylenes (BTEX) along with methyl tertiary-butyl ether (MTBE) are among the unleaded gasoline compounds of major environmental concern usually found in petroleum-contaminated sites. Discharges from chemical factories and petroleum refineries, leaching from gasoline storage tanks, improper waste management practices and accidental spills are common sources of soil and groundwater contamination by BTEX and MTBE (USEPA, 2000). Global concern on soil and groundwater cleanup has led to the establishment of enforceable or recommended maximum contaminant levels of BTEX and MTBE. In the United States, BTEX are included in the National Primary Drinking Water Regulations (USEPA, 2001), and some states have set maximum soil contaminant concentrations for a number of hydrocarbons including BTEX (NCDENR, 2002). The European Union has included benzene in the List of Priority Substances in the Field of Water Policy and Amending Directive (OJEC, 2001). In Japan, benzene is included in the Environmental Quality Standards lists for groundwater and soil pollution (JME, 1997). In Mexico, BTEX are included in drinking water regulations (DOF, 2000) and benzene, toluene and xylenes are also regulated in soil (DOF, 2002).

Recent research has shown that among xylene isomers, o-xylene appears to be the most recalcitrant (Stewart and Kamarthi, 1997). Additionally, *meta* and *para* isomers can be metabolized by revertant strains grown on o-xylene (Di Lecce *et al.*, 1997). The migration of the BTEX contaminant plume through the soil is a function of the structural properties of BTEX which allow the molecules to partially adsorb on to the soil particles before they reach the groundwater, therefore, the use of nonionic surfactants offer a potential alternative to enhance hydrocarbon bioavailability (Volkering *et al.*, 1995) and dissolution rate (Grimberg *et al.*, 1996). Hydrocarbon polluted site cleanup efforts require the development of new and more efficient technologies to treat contaminated sites.

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 BTEX. The effects of soil and the addition of nonionic surfactant Tergitol NP-10 on BTE-oX and MTBE biodegradation kinetics were also evaluated.

Methods

Experimental design

Chemicals and culture conditions. Benzene, toluene, ethylbenzene, mixed xylenes, o-xylene, methyl tertiary butyl ether (MTBE) and Tergitol NP-10 (TNP-10, a nonionic surfactant) were purchased from Sigma-Aldrich (Mexico) and were above 98% purity. Nutrient agar (Difco Laboratories, Detroit, MI) and bacteriological agar (BIOXON, Becton-Dickinson, Mexico) were purchased from Fisher Scientific (Mexico). 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., 2003a*): 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.

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 each BTEX chemical 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 850-950 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 905 mg/L VSS, which was a concentration similar to the 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 BTEX-acclimated biomass. Controls and three sets of samples were evaluated. Controls had only SMM. Set 1 contained SMM and 905 mg/L VSS of microbial inoculum. Set 2 contained SMM, 18.5% sterilized soil (SS) and 905 mg/L VSS of microbial inoculum. Set 3 contained SMM, 18.5% SS, 905 mg/L VSS of microbial inoculum and 25 mg/L TNP-10. The amount of TNP-10 added to experimental bioassays was slightly below the critical micelle concentration (CMC) based on prior studies (Acuna-Askar *et al.*, 2003a). 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 volume available for respiration. Three replicates were run to evaluate substrate biodegradation kinetics.

Sample and control sterilization. 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. Soil-free 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.

Sample shaking, sonication and analysis. Samples and controls used for biotransformation studies were shaken using a New Brunswick oscillating incubator shaker (Fisher-Scientific, Pittsburg, PA) model R76. 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) 100m x 0.25mm ID x 0.5μm 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, point at which temperature was held for 20 minutes. A second temperature rate followed and varied 30°C/min from 90°C up to 150°C, point at which 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

First-order one-phase model. For the three sample sets, the overall o-xylene and MTBE removal rate constants K were obtained by the following equation (Acuna-Askar *et al.*, 2000):

Model I $S_t = S_0 \exp(-Kt)$ Where: S_t = Substrate concentration at time t, (mg/L)
S_0 = Substrate concentration at time zero, (mg/L);
K = overall first order constant, $K = k\,Xv$; Xv = VSS, (mg/L)
k = specific rate constant, $h^{-1}(mg/L)^{-1}vss$; t = time, (h)
The overall removal rate constants K were obtained from the slope by plotting ln S_t versus t.

First-order two-phase model. For the three sample sets, the overall benzene, toluene and ethylbenzene removal rate constants K were obtained by the following equation (Hu *et al.*, 2004): Model II $S_t = S_1 \exp(-K_1 t) + S_2 \exp(-K_2 t)$

Where: S_t = Substrate concentration at time t, (mg/L); S_1 = First phase concentration at time zero, (mg/L); S_2 = Second phase concentration at time zero, (mg/L); K_1 = First phase kinetic rate constant, (h^{-1}); K_2 = Second phase kinetic rate constant, (h^{-1})
The overall removal rate constants K were obtained from the method of residuals.

Results and Discussion

Effect of bioaugmentation, sterile soil (SS) and surfactant on BTE-oX and MTBE.

All BTE-oX and MTBE chemicals followed a first-order removal rate in the presence of 905 mg/L VSS of BTEX-acclimated biomass during the time frame evaluation of 36 hours. As shown in Figure 1, toluene biodegraded slightly faster than ethylbenzene and these two substrates were removed faster than benzene, o-xylene and MTBE. Recent studies have shown that unleaded-gasoline acclimated biomass under experimental conditions similar to the present study, removed toluene and ethylbenzene faster than benzene, o-xylene and MTBE (Acuna-Askar *et al.*, 2003b). Prior research has shown that the biotransformation of BTEX mixtures, in the absence of MTBE, followed the order of ethylbenzene, toluene, benzene and xylenes (Deeb and Alvarez-Cohen, 1999). In addition, inhibitory effects of ethylbenzene and xylenes on MTBE biodegradation have been reported (Deeb *et al.*, 2001).

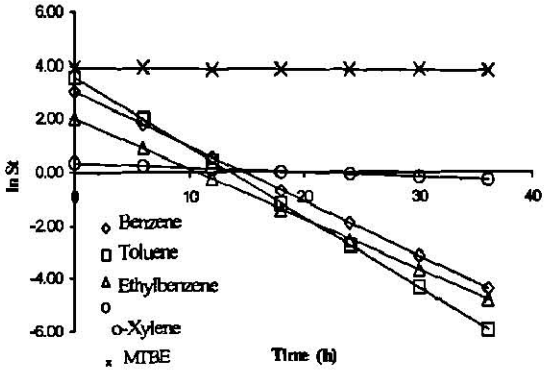

Figure 1 BTEoX biodegradation kinetics with 50 mg/L MTBE in the presence of 905 mg/L VSS.

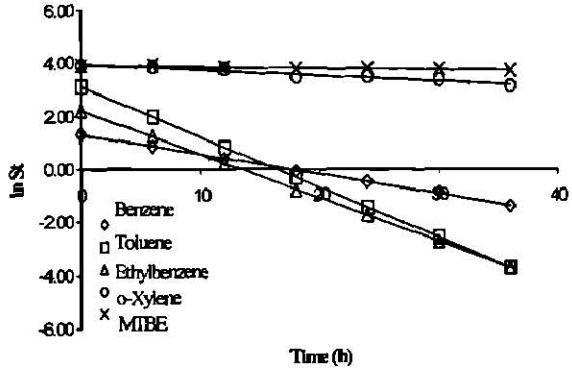

Figure 2 BTEoX biodegradation kinetics with 50 mg/L MTBE, 905 mg/L VSS and 18.5% SS.

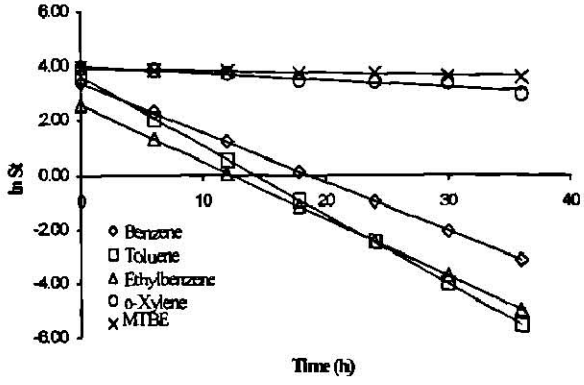

Figure 3 BTEoX biodegradation kinetics with 50 mg/L MTBE, 905 mg/L VSS, 18.5% SS and 25 mg/L of TNP-10.

As shown in Figure 2, soil had a negative impact on the biodegradation rates of benzene, toluene and ethylbenzene. As can be seen from comparing Figures 1 and 2, the addition of soil to the substrate mixture slightly increased o-xylene and MTBE removal rates.

Differences of the effect of soil on all these substrates may be due to the major role that sorption-desorption kinetics plays in the bioavailability properties of chemicals (Braida *et al.*, 2002) as well as the effect of electron-accepting conditions on substrate response variability (Ruiz-Aguilar *et al.*, 2002). As can be seen from comparing Figures 2 and 3, the addition of TNP-10 enhanced overall BTE-oX and MTBE removal rates.

Table 1 Two-phase kinetic model reaction rate constants vs. experimental bioassay samples

	Benzene		Toluene		Ethylbenzene	
Set 1 Samples						
Overall K rate	K_1	K_2	K_1	K_2	K_1	K_2
[h^{-1}]	0.0109	0.2059	0.0060	0.2647	0.0135	0.1898
(r)	(0.999)		(0.999)		(0.999)	
Specific k rate	k_1	k_2	k_1	k_2	k_1	k_2
[$h^{-1}(mg/L)^{-1}$]	$1.20x10^{-5}$	$2.28x10^{-4}$	$6.63x10^{-6}$	$2.92x10^{-4}$	$1.49x10^{-5}$	$2.10x10^{-4}$
(r)	(0.999)		(0.999)		(0.999)	
Set 2 Samples**						
Overall K rate	K_1	K_2	K_1	K_2	K_1	K_2
[h^{-1}]	0.0080	0.0739	0.0145	0.1888	0.0138	0.1657
(r)	(0.999)		(0.999)		(0.999)	
Specific k rate	k_1	k_2	k_1	k_2	k_1	k_2
[$h^{-1}(mg/L)^{-1}$]	$8.84x10^{-6}$	$8.17x10^{-5}$	$1.60x10^{-5}$	$2.09x10^{-4}$	$1.52x10^{-5}$	$1.83x10^{-4}$
(r)	(0.999)		(0.999)		(0.999)	
Set 3 Samples***						
Overall K rate	K_1	K_2	K_1	K_2	K_1	K_2
[h^{-1}]	0.0072	0.1816	0.0104	0.2526	0.0348	0.209
(r)	(0.999)		(0.999)		(0.999)	
Specific k rate	k_1	k_2	k_1	k_2	k_1	k_2
[$h^{-1}(mg/L)^{-1}$]	$7.96x10^{-6}$	$2.01x10^{-4}$	$1.15x10^{-5}$	$2.79x10^{-4}$	$3.85x10^{-5}$	$2.31x10^{-4}$
(r)	(0.999)		(0.999)		(0.999)	

r = correlation coefficient
*SMM + 905 mg/L VSS
** SMM + 18.5% SS + 905 mg/L VSS
*** SMM + 18.5% SS + 905 mg/L VSS + 25 mg/L TNP-10

Benzene, toluene and ethylbenzene followed a first-order two-phase removal rate kinetic model in the three sample sets evaluated (Table 1). Kinetic models for mixtures of BTEX and MTBE, all together, are limited in the literature. Reliable fit of data consistently showed that benzene, toluene and ethylbenzene had a biphasic removal rate with a strong slope change at 12 hours. The first phase kinetic rate constants of benzene, toluene and ethylbenzene were significantly lower than the corresponding second phase kinetic rate constants, suggesting that benzene, toluene and ethylbenzene removal rates may have been influenced by some type of substrate interaction (Chang *et al.*, 2001; Deeb *et al.*, 2001). The second phase kinetic rate constants of benzene, toluene and ethylbenzene were reduced by 64%, 28% and 13% respectively, when soil was added to the BTEX-acclimated biomass. The addition of TNP-10 to Set-2 samples enhanced benzene, toluene and ethylbenzene second phase kinetic rate constants by 145%, 34% and 26%, respectively. Overall o-xylene and MTBE kinetic rate constants were enhanced by the addition of soil by 22% and 75%, respectively. Also, the addition of TNP-10 to Set-2 samples enhanced o-xylene and MTBE

kinetic rate constants by 17% and 80%, respectively (Table 2). A comparison of the overall kinetic rate constants of Set-2 samples reported in this study with those aerobic k rates of BTEX-MTBE mixtures in aquifer materials reported earlier (Ruiz-Aguilar *et al.*, 2002), shows that benzene and o-xylene k rates were 1.7 and 2.7 times higher, respectively, in the prior study. The overall toluene kinetic rate constant in the present study, however, was nearly 6.5 times higher than that reported by these authors in those mixtures. Surprisingly, the overall ethylbenzene kinetic rate constants were similar in the two studies.

Table 2 Kinetic model reaction rate constants vs. experimental bioassay samples

	o-Xylene		MTBE
Set 1 Samples *			
Overall K rate	K_1	K_2	K
[h^{-1}]	0.0095	0.0178	0.0028
(r)	(0.999)		(0.978)
Specific k rate	k_1	k_2	k
[$h^{-1}(mg/L)^{-1}$]	$1.05x10^{-5}$	$1.97x10^{-5}$	$3.09x10^{-6}$
(r)	(0.999)		(0.978)
Set 2 Samples**			
Overall K rate	K		K
[h^{-1}]	0.0218		0.0049
(r)	(0.977)		(0.972)
Specific k rate	k		k
[$h^{-1}(mg/L)^{-1}$]	$2.41x10^{-5}$		$5.41x10^{-6}$
(r)	(0.977)		(0.978)
Set 3 Samples***			
Overall K rate	K		K
[h^{-1}]	*0.0256*		*0.0068*
(r)	(0.966)		(0.981)
Specific k rate	k		k
[$h^{-1}(mg/L)^{-1}$]	$2.83x10^{-5}$		$9.72x10^{-6}$
(r)	(0.966)		(0.981)

r = correlation coefficient
*SMM + 905 mg/L VSS
** SMM + 18.5% SS + 905 mg/L VSS
*** SMM + 18.5% SS + 905 mg/L VSS + 25 mg/L TNP-10

Table 3 Biodegradation percentage vs. experimental bioassay samples

	Benzene (%)	Toluene (%)	Ethylbenzene (%)	o-Xylene (%)	MTBE (%)
SMM + 905 mg/L VSS	99.0	99.8	99.2	50.1	9.9
SMM + 905 mg/L VSS + 18.5% SS	*77.0*	*97.9*	*98.3*	*54.8*	16.1
SMM + 905 mg/L VSS + 18.5% SS + 25 mg/L TNP10	96.3	99.7	99.7	65.3	43.0

As indicated in Table 3, benzene, toluene and ethylbenzene had percent removals above 99% in the presence of biomass only. When soil was present, however, benzene percent removal was reduced by 28%. Soil did not significantly affect the percent removal of either toluene or ethylbenzene. The addition of TNP-10 helped increased benzene percent removal by 25%. TNP-10 did not have any significant effect on either toluene or ethylbenzene percent removals. O-xylene percent removal was 50.1% and had a slight increase to 54.8% with the addition of soil and a significant further increase to 65.3% when TNP-10 was added to the mixture. MTBE showed the lowest percent removal among all substrates evaluated,

however, the addition of soil enhanced MTBE removal by 62%. Interestingly, the addition of TNP-10 to soil-containing samples enhanced MTBE percent removal by nearly three-fold. The low biodegradation performance of the biomass on MTBE was presumably due to the presence of other easily assimilated carbon sources such as toluene, ethylbenzene and benzene. Also, prior studies have shown that different conditions are needed to significantly achieve MTBE biodegradation (Acuna-Askar *et al.*, 2000; Stringfellow and Oh, 2002; Pruden *et al.*, 2003; Hu *et al.*, 2004). The significant increase of benzene, o-xylene and MTBE percent removals by the addition of TNP-10 might suggest that the addition of nonionic surfactant at a concentration lower than the CMC was able to enhance substrate interaction with the microbial population. This finding is relevant because earlier research has indicated that hydrocarbon availability can be constrained by micellization (Grimberg *et al.*, 1996).

Conclusions

Benzene, toluene and ethylbenzene biodegradation followed a first-order two-phase kinetic model in all samples, whereas MTBE biodegradation was well described by a first-order one-phase kinetic model. O-xylene biodegradation followed a first-order two-phase kinetic model in the presence of biomass only. The addition of soil or soil and TNP-10 to the biomass allowed o-xylene biodegradation to switch to a first-order one-phase kinetic model. The addition of soil reduced benzene, toluene and ethylbenzene removal rates and enhanced o-xylene and MTBE removal rates. The addition of TNP-10 to aqueous samples containing soil allowed BTE-oX and MTBE removal rates to increase in all samples. Benzene biodegradation rates were slower than the corresponding toluene and ethylbenzene biodegradation rates in nearly all samples. O-xylene biodegraded slower than benzene and MTBE showed lower biodegradation rates than the corresponding BTE-oX removal rates. Substrate percent removals ranged 77-99.8% for benzene, toluene and ethylbenzene. O-xylene and MTBE percent removals ranged 50.1-65.3% and 9.9-43.0%, respectively.

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ABSTRACT BOO

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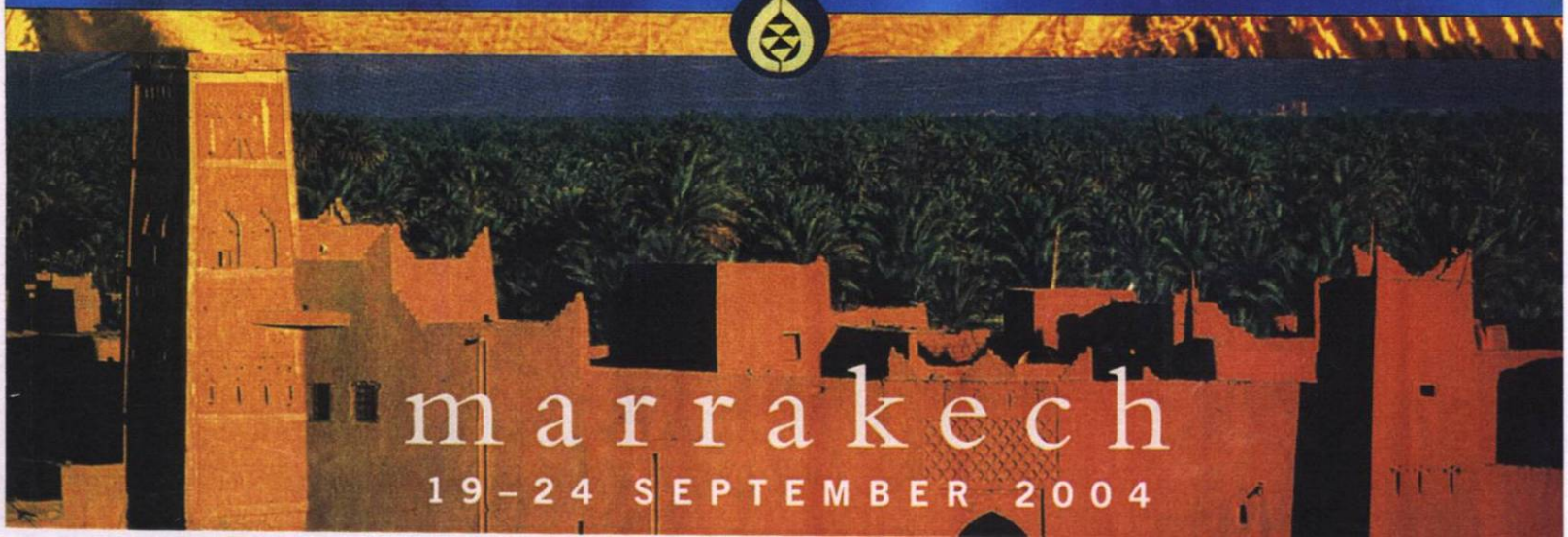

World Water
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IWA 4TH WORLD WATER CONGRESS

19 – 24 September 2004. Marrakech, Morocco

Abstract Book

INTRODUCTION

The IWA World Water Congress has received unprecedented interest from authors wishing to share their knowledge and experiences on an international platform.

A total of 1,900 paper outlines were submitted via the Associations new web-based paper submission platform. Unfortunately, this volume of papers could not be accommodated in the Congress programme and, following a review process, approximately 900 authors were requested to submit manuscripts. A further review will be undertaken on a selection of papers following the Congress, and these papers will be published in one of IWA Publishing's journals.

The abstract book for Congress is an integral part of the programme and allows delegates to locate summaries of all platform and poster presentations as submitted by the author. Used in conjunction with the programme booklet, this book should enable you to benefit fully from the technical programme and also be a useful resource beyond the 2004 Congress.

M. Ali Fassi Fihri

President, 4th IWA World Water Congress

Jerry Gilbert

Chair, IWA International Programme Committee

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Table of Contents

Innovation in Drinking Water Treatment

survey, destruction pilot studies at OCWD, and formation/reformation pathways and control options.

Because of its soluble characteristics, NDMA cannot be absorbed by activated carbon and is not removed from water using air stripping. However, UV photolysis and advanced oxidation (UV/peroxide) of NDMA has been successful. UV destruction pilot tests at OCWD, conducted using flow through low- and medium-pressure UV systems as well as natural sunlight indicated that UV photolysis is effective in destruction of NDMA. Hydrogen peroxide addition improved NDMA removal efficiencies and reduced reformation potential after chlorination with free chlorine.

Sunlight exposure was very effective and reduced NDMA levels from 500 ppt to <1ppt in three hours. As a result, OCWD converted some existing structures to plug-flow shallow basins for sunlight exposure/NDMA destruction. The NDMA destruction efficiency of these basins is discussed in this paper. Additionally, OCWD added advanced oxidation processes for NDMA destruction at Water Factory 21, at water wells, and at the proposed Ground Water Replenishment System. Testing was also conducted to study reformation potential of UV exposed samples after chlorination (free chlorine and chloramine) at various holding times. The results of this testing for various water quality matrices is also discussed in this paper.

NDMA removal/formation potential by conventional treatment processes was also evaluated. This evaluation indicates that cellules acetate and thin film composite RO membranes remove <10% and >50% NDMA, respectively, but hydrogen peroxide or pure oxygen treatment alone does not remove NDMA appreciably. The information presented here benefits many other agencies facing similar water quality concerns.

PaperID 26992

About the Examination of Ion Exchanger Beads Through Optical and Electronic Microscopy

Author *Dr. Federico Mijangos, Spain*
Presenting Author *Dr. Federico Mijangos, Spain*
Corresponding Author ,

Kinetic of simultaneous load of cobalt and copper onto an iminodiacetic-type resin is here investigated. Analysing a semireacted bead under the microscope, two different coloured layers surrounding the central core have been observed. Metal concentration profiles inside the particles at different reaction times were measured by the Scanning Electron Microscopy-Energy Dispersive X-Ray (SEM- EDX) technique, which allows one to obtain a linescan along diametrical positions.

Copper diffuses following a monotonous increasing path to the resin, and when the reaction is finished, metal uptake achieves the equilibrium value which is the maximum load. Using experimental result one can conclude by comparing the load rates for both metals, that at the beginning of the reaction they diffuse in parallel but finally copper is displacing cobalt from the central core, so the process is merely and copper/cobalt ion exchange reaction.

The swelling/shrinking behaviour of a commercial chelating resin as a consequence of the ion exchange reaction is reported in this paper. Measurements of the volume variation were carried out for every step of an operational cycle, metal load, elution and regeneration of the ion exchanger, using an optical cell and an image treatment.

On the other hand, using the X-ray microprobe it has been concluded that the coloured layers observed under the optical microscope, do not show constant metal concentration in a radial direction, as it could be assumed. On the contrary, irregular concentration profiles are appreciated, largely affected by species in solution and even for trace metal introduced in the reaction vessel with the reactives.

PaperID 27205

The Role of a Nonionic Surfactant on Biodegradation Efficiency Kinetic Models of BTE-oX and MTBE

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The biodegradation efficiency kinetic models of BTE-oX and MTBE mixtures in the presence of soil and Tergitol NP-10 (TNP-10) by unleaded gasoline acclimated biomass were evaluated. MTBE and BTE-oX concentrations were monitored for 36 hours every 6 hours. MTBE biodegradation efficiency showed a zero-order rate in all samples. Benzene biodegradation efficiency showed a first-order two-phase reaction rate in the absence of surfactant. Interestingly, however, with the addition of TNP-10, benzene biodegradation efficiency switched to a first-order one-phase kinetic rate model. Toluene and ethylbenzene biodegradation efficiencies showed a first-order two-phase kinetic rate model in all samples. O-xylene biodegradation efficiency followed a first-order one-phase kinetic rate model in all samples. The addition of TNP-10 significantly increased the biodegradation efficiency percent removals of those recalcitrant substrates such as o-xylene and MTBE by 40% and 35.5%, respectively.

The role of a nonionic surfactant on biodegradation efficiency kinetic models of BTE-oX and MTBE

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Keywords: BTEX; MTBE; Tergitol NP-10

ABSTRACT

The biodegradation efficiency of BTE-oX and MTBE, all together, in the presence of 18.5% soil with and without 25 mg/L Tergitol NP-10 (TNP-10) by 880 mg/L VSS unleaded gasoline acclimated biomass was evaluated. MTBE and BTE-oX concentrations were evaluated for 36 hours every 6 hours. MTBE biodegradation efficiency showed a zero-order rate in all samples. Although, the addition of surfactant enhanced MTBE biodegradation efficiency rate by 22.6%, MTBE efficiency percent removal was not above 28.4%. Benzene biodegradation efficiency showed a first-order two-phase reaction rate in the absence of surfactant. Interestingly, however, with the addition of TNP-10, benzene biodegradation efficiency switched to a first-order one-phase kinetic rate model. Toluene and ethylbenzene biodegradation efficiencies showed a first-order two-phase kinetic rate model in all samples. The addition of TNP-10 had a major selective impact with a significant increase on toluene and ethylbenzene biodegradation efficiency second phase kinetic rate constants. This selective enhancement showed a trend for toluene to reduce differences between the distribution and elimination phase kinetic rate constants. O-xylene biodegradation efficiency followed a first-order one-phase kinetic rate model in all samples. The addition of TNP-10 significantly increased the biodegradation efficiency percent removals of those recalcitrant substrates such as o-xylene and MTBE by 40% and 35.5%, respectively. The addition of TNP-10, however, did not have a significant effect on the biodegradation efficiency percent removals of those easily assimilated substrates such as benzene, toluene and ethylbenzene.

Keywords
Bioaugmentation; biodegradation; bioremediation; BTEX; MTBE; Tergitol NP-10

INTRODUCTION

The major chemicals of enviromental concern from unleaded gasoline are methyl tertiary-butyl ether (MTBE) and benzene, toluene, ethylbenzene and total xylenes (BTEX) due to their carcinogenic potential and other toxicity and their impact on property value (Hartley et al.,1999; Acuna-Askar, et al., 2000; Chang, et al.,2001; Wilson, et al., 2001). Underground storage tanks (UST's), 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). 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. The MSCC are divided in three categories: the residential soil cleanup levels; the industrial/commercial soil cleanup levels and the soil-to-water maximum contaminant concentration (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). Emerging environmental regulations for benzene, toluene and total xylene contaminated soil in Mexico, have set the maximum contaminant levels (MCLs) as 20.0, 40.0 and

40.0 mg/Kg, respectively, for agricultural, residential and commercial settings, and 50.0, 100.0 and 100.0 mg/Kg, respectively, for industrial use (DOF, 2002). The MTBE drinking water health advisory level for taste and odor has been set at 20-40 ug/L by the EPA (USEPA, 1997).

METHODS

Chemicals. Benzene, toluene, ethylbenzene, mixed xylenes, o-, m-, p-xylene, methyl tertiary butyl ether (MTBE) and Tergitol NP-10 (nonionic surfactant) were purchased from Sigma-Aldrich (Mexico) and were above 98% purity. Unleaded gasoline (UG) Premium was purchased from a gas station. Nutrient agar (Difco Laboratories, Detroit, MI) and bacteriological agar (BIOXON, Becton-Dickinson, Mexico) were purchased from Casa Rocas Fisher Scientific (Mexico).

Culture conditions. 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.*, 2002): 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, 50 mg/L MTBE and 18.5% sterilized soil (SS). The pH of MMII and SMM was 7.0-7.5.

Critical micelle concentration. The concentration range of nonionic surfactant (Tergitol NP-10) where a sudden variation in the relation between both culture medium density and culture surface tension occurred was chosen as the critical micelle concentration (CMC). The amount of Tergitol NP-10 added to experimental bioassays was slightly below the CMC based on prior studies (Acuna-Askar *et al.*, 2002).

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. 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. 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 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 the grown in the 20-L biomass acclimation batch reactor. This procedure was made for each of the three replicates.

Experimental bioassays. 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. Two controls and two sets of samples were evaluated. First controls had only SMM. Second controls had SMM and 25 mg/L Tergitol NP10 (TNP-10). Set 1 contained SMM and 880 mg/L VSS of microbial inoculum. Set 2 contained SMM, 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.

Sample and control sterilization. 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.

Isolation of acclimated bacteria. UG-acclimated bacteria were grown in UG agar plates and incubated in a gravity flow Isotemp incubator (Fisher Scientific, USA), model 537D at 28-30°C for 72 hours. Acid production in UG agar plates was identified by change of color from blue to green, and in some cases from blue to yellow using bromothymol blue as indicator. For identification purposes, bacteria isolates were grown in nutrient agar plates.

Mechanical shakers and sonication. 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

Gas chromatography and sample concentrator. 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) 100m x 0.25mm ID x 0.5µm 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, point at which temperature was held for 20 minutes. A second temperature rate followed and varied 30°C/min from 90°C up to 150°C, point at which 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

First-order one-phase model. Model I (Acuna-Askar *et al.*, 2000) was used to evaluate the overall removal rate constants K for benzene in set 2 samples and for o-xylene in sets 1 and 2 samples.

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)

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

First-order two-phase model. Model II (Hu *et al.*, 2002) was used to evaluate the overall removal rate constants K for benzene in set 1 samples and for toluene and ethylbenzene in sets 1 and 2 samples.

Model II $S_t = S_1 \exp(-K_1 t) + S_2 \exp(-K_2 t)$ (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})

The overall removal rate constants K were obtained from the method of residuals.

Zero-order model. Model III was used to evaluate the overall removal rate constants K for MTBE in sets 1 and 2 samples.

Model III $S_t = - Kt + S_0$ (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

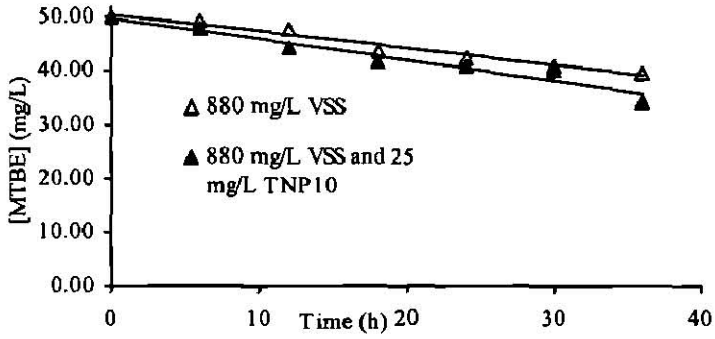

Figure 1 *MTBE biodegradation efficency kinetics with 200 mg/L* total BTEoX in the presence of 18.5% SS

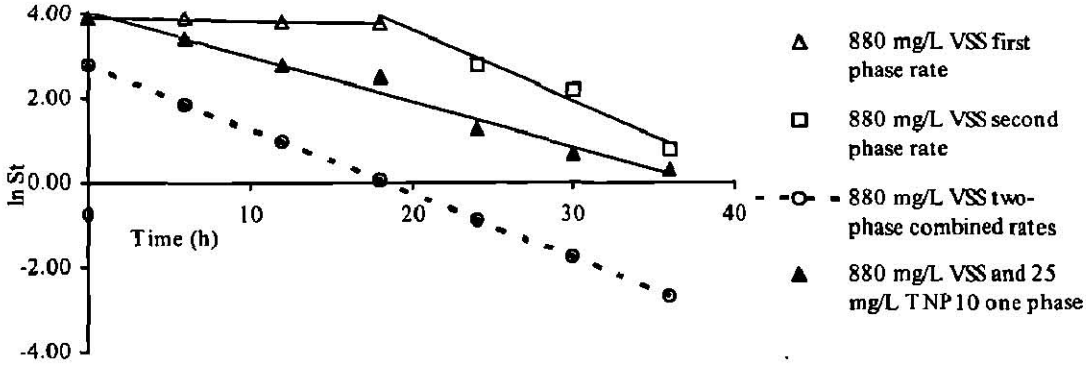

Figure 2 Benzene biodegradation efficiency kinetics with 200 mg/L total BTEoX and 50 mg/L MTBE in the presence of 18.5% SS

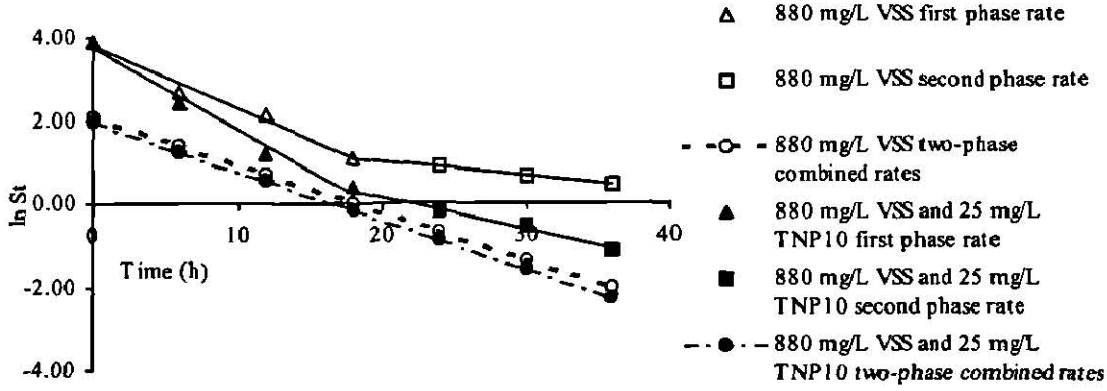

Figure 3 Toluene biodegradation efficiency kinetics with 200 mg/L total BTEoX and 50 mg/L MTBE in the presence of 18.5% SS

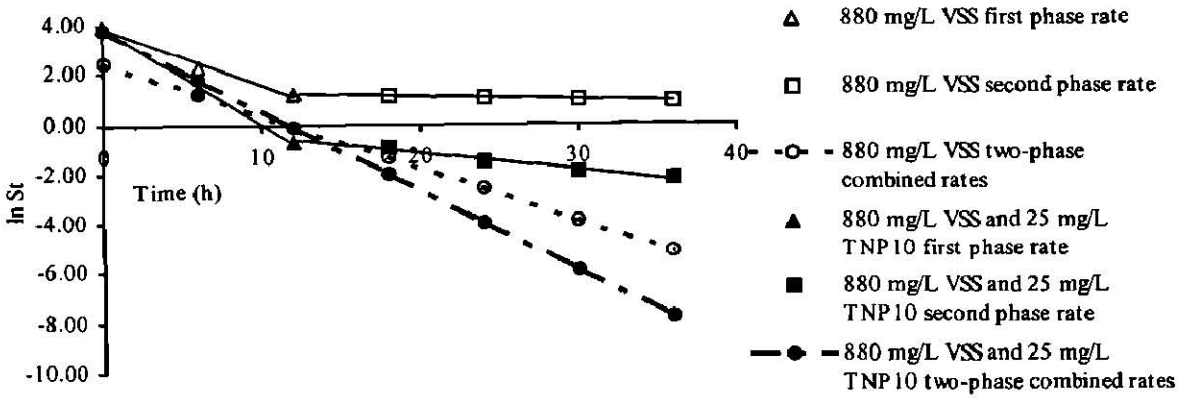

Figure 4 Ethylbenzene biodegradation efficiency kinetics with 200 mg/L total BTEoX and 50 mg/L MTBE in the presence of 18.5% SS

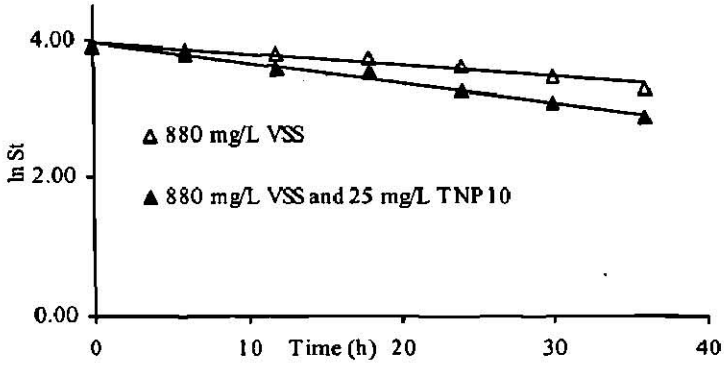

Figure 5 O-Xylene biodegradation efficiency kinetics with 200 mg/L total BTEoX and 50 mg/L MTBE in the presence of 18.5% SS

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

	Benzene		Toluene		Ethylbenzene		o-Xylene
Set 1 Samples*							
Overall K rate	K_1	K_2	K_1	K_2	K_1	K_2	K
$[hr^{-1}]$	0.0085	0.1514	0.1143	0.0362	0.2105	0.0123	0.0498
(r)	(0.985)		(0.999)		(0.989)		(0.983)
Specific k rate	k_1	k_2	k_1	k_2	k_1	k_2	k
$[hr^{-1}(mg/L)^{-1}vss]$	9.66×10^{-6}	1.72×10^{-4}	1.13×10^{-4}	0.411×10^{-4}	2.39×10^{-4}	0.14×10^{-4}	0.566×10^{-4}
(r)	(0.999)		(0.999)		(0.999)		(0.985)
Set 2 Samples**							
Overall K rate	K		K_1	K_2	K_1	K_2	K
$[hr^{-1}]$	0.1063		0.1181	0.0798	0.3206	0.0639	0.0291
(r)	(0.989)		(0.993)		(0.993)		(0.990)
Specific k rate	k		k_1	k_2	k_1	k_2	k
$[hr^{-1}(mg/L)^{-1}vss]$	1.20×10^{-4}		1.34×10^{-4}	0.907×10^{-4}	3.64×10^{-4}	0.726×10^{-4}	0.331×10^{-4}
(r)	(0.989)		(0.999)		(0.999)		(0.990)

r = correlation coefficient
*SMM + 880 mg/L VSS (control 1 as SMM)
** SMM + 880 mg/L VSS + 25 mg/L TNP-10 (control 2 as SMM + 25 mg/L TNP-10)

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

	Set 1 Samples*	Set 2 Samples**
Overall K rate	K	K
$[mgL^{-1}hr^{-1}]$	0.3175	0.3895
(r)	(0.983)	(0.975)
Specific k rate	k	k
$[mgL^{-1}hr^{-1}(mg/L)^{-1}vss]$	3.61×10^{-4}	4.53×10^{-4}
(r)	(0.983)	(0.975)

r = correlation coefficient
* SMM + 880 mg/L VSS (control 1 as SMM)
** SMM + 880 mg/L VSS + 25 mg/L TNP-10 (control 2 as SMM + 25 mg/L TNP-10)

Table 3. Biodegradation efficiency percentage vs. experimental bioassay samples

	Benzene (%)	Toluene (%)	Ethylbenzene (%)	o-Xylene (%)	MTBE (%)
SMM + 880 mg/L VSS*	95.6	96.9	95.0	46.1	20.9
SMM + 880 mg/L VSS + 25 mg/L TNP10**	97.3	99.3	99.7	64.6	28.4

* Control 1 as SMM
** Control 2 as SMM + 25 mg/L TNP10

CONCLUSION

ACKNOWLEDGEMENTS

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BIODEGRADACIÓN AERÓBICA DE BTEX EN PRESENCIA DE EMTB EN MUESTRAS DE SUELO

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ABSTRACT

El proposito de este trabajo fue el de investigar el poder de biodegradación de los BTEX y el EMTB por un consorcio microbiano mixto aclimatado. Se encontró que el orden de biodegradación va de la siguiente manera: Etilbenceno > Tolueno > o-Xileno > Benceno, mientras que el EMTB no fue afectado por el consorcio.

INTRODUCCIÓN

México es unos de los mayores productores de petróleo en el mundo, ocupando el 5° lugar con una producción de más de 3 millones de barriles diarios.

Los derrames de hidrocarburos, por las sustancias que involucran, se consideran como emergencias ambientales, debido a que pueden poner en peligro la integridad de los ecosistemas, así como la preservación de los recursos naturales.[2] (Fig. 1)

Figura 1. Vía usual de contaminación de los BTEX y EMTB

La biorremediación consiste en la utilización de métodos biológicos para transformar o inmovilizar contaminantes que se encuentren en el suelo y/o en el agua[1]

Diversos grupos de investigación en todo el mundo, se han dedicado a estudiar la biodegradación de estos contaminantes, utilizando para ello, una gran variedad de parámetros. Sin embargo, no se han realizado estudios donde se evalúe la biodegradabilidad de BTEX y EMTB por biomasa aclimatada a BTEX.

METODOLOGÍA

RESULTADOS Y CONCLUSIONES

El consorcio microbiano aclimatado a BTEX no puede degradar el EMTB (Fig. 2), sin embargo, logra degradar completamente 50 ppm de cada BTEoX en un máximo de 48 Horas. (Fig. 3 – 6)

Los resultados obtenidos de las gráficas indican que el compuesto de más fácil degradación por el consorcio es el Etilbenceno iniciando su biodegradación a partir de las 8 horas y siendo totalmente biodegradado a las 40 horas. (Fig. 5)

El benceno es el compuesto más recalcitrante de los BTE-oX iniciando su biodegradación a las 32 horas y finalizando esta a las 48 horas. (Fig. 3)

Se encontró que el orden de biodegradabilidad es el siguiente:

Etilbenceno > Tolueno > o-Xileno > Benceno

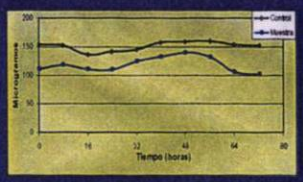

Figura 2. Biodegradación de EMTB

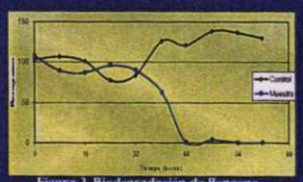

Figura 3. Biodegradación de Benceno

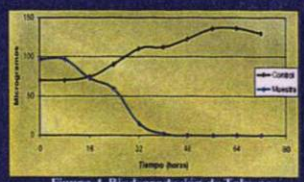

Figura 4. Biodegradación de Tolueno

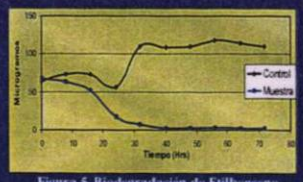

Figura 5. Biodegradación de Etilbenceno

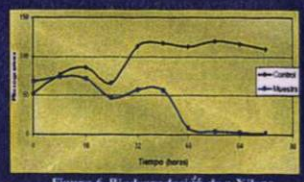

Figura 6. Biodegradación de o-Xileno

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Biodegradación aeróbica de BTEX en presencia de EMTB en muestras de suelo

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ANTECEDENTES

Una preocupación importante en el campo de la contaminación ambiental, la constituye la presencia en el ambiente de compuestos químicos que presentan efectos adversos a la salud, entre los cuales se encuentran algunos de los componentes de la gasolina, particularmente los BTEX (benceno, tolueno, etilbenceno y los isómeros del xileno) (Dean, 1978) y el EMTB (éter metil terbutílico) (Hartley *et al.*, 1999).

Los BTEX están entre los compuestos de la gasolina más solubles en agua, encontrándose que llegan a constituir cerca del 90% de la fracción soluble de la gasolina en agua y son superados solamente por el EMTB que es añadido intencionalmente a la gasolina para utilizarlo como aditivo y mejorar su combustión y reemplazar otras sustancias tóxicas como el plomo (Chang *et.al*, 2001). En conjunto los BTEX pueden llegar a representar hasta el 15% en peso de la gasolina sin plomo (ATSDR, 2002).

El EMTB es un compuesto sintético muy soluble en agua y no es adsorbido por el suelo, por lo que llega directamente hasta los mantos freáticos, que son utilizados como fuentes de suministro de agua potable (Squillace *et al.*, 1996).

Se ha demostrado que la vía usual de contaminación de los suelos por estas sustancias la constituyen las fugas en los tanques de producción y almacenamiento subterráneos, derrames accidentales y/o prácticas de disposición inadecuadas (Eweis, 1999).

Todos estos contaminantes han sido catalogados como altamente peligrosos debido a los graves efectos que pueden provocar en la salud humana, incluso, el benceno ha sido catalogado como cancerígeno por la Agencia de Protección Ambiental de los Estados Unidos de América (USEPA) (ASTDR, 2002).

La biorremediación consiste en la utilización de métodos biológicos para transformar o inmovilizar contaminantes que se encuentren en el suelo y/o en el agua (Kilroy y Gray, 1995). Las tecnologías de biorremediación generalmente utilizan consorcios microbianos, debido a que aportan interacciones positivas, donde una o varias especies salen beneficiadas de los procesos metabólicos de otras. Dentro de las ventajas que se han encontrado, han sido la capacidad de mineralizar los compuestos contaminantes y el de mejorar el tiempo de degradación, entre otros (Eweis, 1999).

Se ha reportado la biodegradación de EMTB en reactores de lote en un intervalo de 67-73% en muestras acuosas (Acuna-Askar, 1998) y con un incremento en la eficiencia de biodegradación del 70% al 99% en condiciones de flujo continuo vertical (Acuna-Askar *et al.*, 2000; Hu *et al.*, 2002). Por otra parte, se ha demostrado que los BTEX se biodegradan en reactores de lote al 99%, en muestras acuosas en condiciones de bioestimulación y bioaumentación (Acuna-Askar *et al.*, 2002), pero que también se biodegradan en suspensiones de suelo (Alfaro, 2002), en los cuales es imprescindible realizar el balance de masas en la fase líquido-vapor, involucrando, para ello, la ley de Henry (Acuna-Askar *et al.*, 2003a). Recientes hallazgos muestran que durante la biodegradación de los BTEX pudieran existir mecanismos concertados, en los que destacan patrones de inducción-inhibición, probablemente estimulados por cometabolismo (Acuna-Askar *et al.*, 2003b).

Los BTEX y el EMTB van acompañados en la pluma contaminante tanto en los mantos freáticos como en los suelos expuestos a derrames, por lo que es importante realizar el estudio detallado para generar la tecnologia que permita la biodegradación de estos compuestos en conjunto para la biorremediación de sitios contaminados (Acuna-Askar *et al.*, 2003c).

OBJETIVO

El objetivo de esta investigación es el de determinar la biodegradabilidad de los BTE-oX (se utilizó solamente orto-xileno debido a su alta recalcitrancia comparada con los otros isómeros de xilenos) en presencia de EMTB, por un consorcio microbiano mixto aclimatado solamente a BTEX.

METODOLOGÍA

Preparación de biomasa.

Se concentró la biomasa proveniente del reactor de aclimatización en tubos Falcon™ a una velocidad de centrifugación de 6000 rpm por 3 minutos y se lavó para eliminar los residuos y sustratos presentes, hasta obtener una concentración de 8,000 mg/L

Preparación de muestra de suelo.

Se tamizó el suelo a través de un tamiz de malla número 8 y se pesó un gramo del mismo y se esterilizó en 3 ciclos a 15 psi por 15 minutos cada uno.

Bioensayos experimentales.

Se llevaron a cabo 2 bioensayos simultáneamente, uno para control y el otro para la determinación de la biodegradación de los BTE-oX, todos ellos en 10 viales cada uno de 40 mL, avalados por la USEPA para la determinación de compuestos orgánicos volátiles (COV), en los cuales se colocaron 20 mL de medio de cultivo con 50 mg/L cada uno de los BTE-oX y 50 mg/L de EMTB, con la singular diferencia que en el bioensayo para la determinación de la biodegradación, se le añadió un gramo de suelo estéril y 2 mL de biomasa concentrada. Los viales se colocaron en una incubadora de movimiento oscilatorio a 200 revoluciones por minuto (rpm) y a 30°C. Las muestras se retiraron en intervalos de 8 horas y se analizaron en un cromatógrafo de gases.

RESULTADOS Y DISCUSIONES

Los resultados obtenidos indican que los cuatro BTEo-X se degradan al 99% por el consorcio microbiano, sin embargo el EMTB no se consume por el consorcio, confirmando así su alta recalcitrancia en reactores de lote (Figs. 1-5).

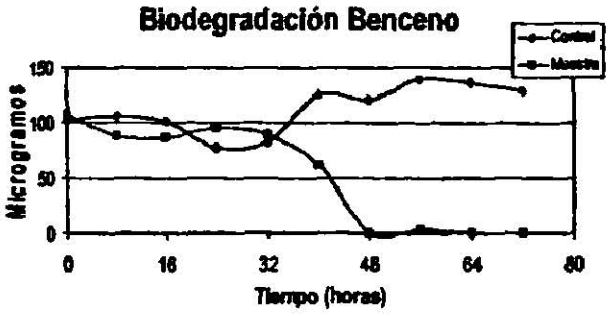

Figura 1.

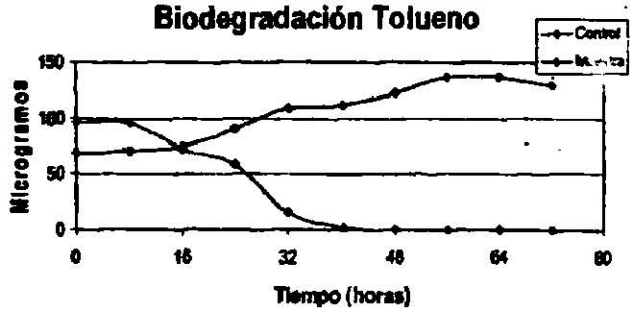

Figura 2.

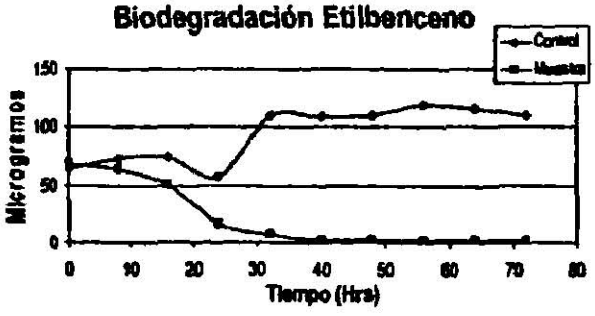

Figura 3.

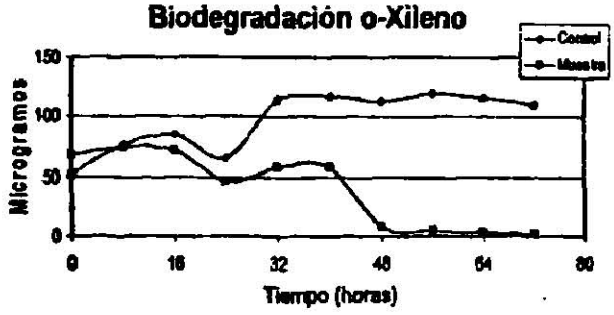

Figura 4.

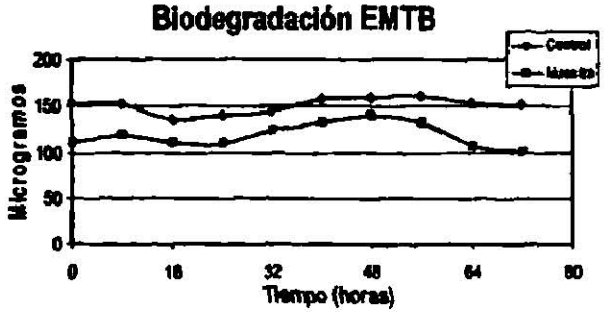

Figura 5.

Las gráficas obtenidas nos indican que el primer compuesto en ser biodegradado es el etilbenceno a las 32 horas, confirmando lo reportado por Acuna-Askar *et al.*, (2002); en cambio, el benceno y el orto-xileno presentaron mayor recalcitrancia entre los BTE-oX al ser degradados 16 horas después. Sin embargo, la biodegradación del benceno presentó inhibición por la mayor asimilación de los aromáticos sustituidos (Acuna-Askar *et al.*, 2003b).

La Figura 2 muestra que el tolueno inicia primero la biodegradación, la cual se desacelera por la presencia del etilbenceno (Figura 3), lo cual sugiere, que en las primeras horas de la biodegradación, el etilbenceno funciona como un inhibidor de la biodegradación del orto-xileno y el benceno, así como del tolueno mismo.

Al ser consumidos los compuestos de mayor asimilación, como son el etilbenceno y tolueno, el consorcio tiende a consumir rápidamente los de menor asimilación, como son el benceno y orto-xileno. Esto se comprueba al observar la meseta de la biodegradación del orto-xileno entre cero y 40 horas (Figura 4); tiempo durante el cual la concentración del benceno se mantiene en estado estacionario (Figura 1). Es relevante mencionar la mayor prolongación que presenta la meseta del ortoxileno en relación a la del benceno; destacando, sin embargo, que esta meseta muestra una ligera biodegradación al observarse una ligera inclinación en la curva de biodegradación, lo cual no sucede con el benceno. La presencia del EMTB en los bioensayos realizados en la presente investigación sugiere que su alta recalcitrancia podría haber beneficiado la biodegradación de los BTE-oX en un menor tiempo, considerando previos estudios comparativos sin la presencia de EMTB (Acuna-Askar *et al.*, 2003c).

CONCLUSIONES

Con los resultados arrojados de los experimentos se concluye que el orden de biodegradabilidad de los BTEo-X, en presencia de EMTB, por el consorcio, es el siguiente: Etilbenceno > Tolueno > o-Xileno > Benceno. Esto demuestra la alta estabilidad química del anillo aromático del benceno en el metabolismo de los microorganismos y de cómo se ve influenciada esta estabilidad por la presencia de grupos sustituyentes.

Podemos concluir que el consorcio microbiano mixto aclimatado a BTEX es capaz de biodegradar 50 ppm de estos contaminantes, sin embargo, no logra su adaptación para metabolizar el EMTB en condiciones de lote.

RECOMENDACIONES

Con el fin de lograr la biodegradación del EMTB en presencia de los BTEX se deben iniciar bioensayos que permitan inducir la producción de enzimas requeridas para estimular la ruptura de enlaces carbono-carbono o carbono-oxígeno presentes en el EMTB.

RESUMEN

Se presentan los resultados obtenidos de una cinética de biodegradación de una mezcla BTE-oX y EMTB por un consorcio microbiano aclimatado a 300 ppm de Benceno, 300 ppm de Tolueno, 300 ppm de Etilbenceno y 300 ppm de Xilenos. Se encontró que el consorcio no es capaz de biodegradar el EMTB, sin embargo logra degradar completamente 50 ppm de cada BTE-oX en un máximo de 48 horas. El orden de biodegradabilidad que se encontró fue el siguiente: Etilbenceno > Tolueno > o-Xileno > Benceno.

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From Sm9uZy1BIEtpbQ== <always@gist.ac.kr>
Date Monday, May 9, 2005 1:01 am
To kaskar@fm.uanl.mx
Subject QWNjZXB0YW5jZSBMZXR0ZXIgZm9yIFdSUlMyMDA1

Date: 2005.5.9
Paper #: 6-10
Paper title: The effect of a nonionic surfactant on the biodegradation efficiency of BTE-oX and MTBE
Corresponding author: K. Acuna-Askar

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The effect of a nonionic surfactant on the biodegradation efficiency of BTE-oX and MTBE

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ABSTRACT

The biodegradation efficiency of BTE-oX and MTBE, all together, in the presence of 18.5% soil with and without 25 mg/L Tergitol NP-10 (TNP-10) by 880 mg/L VSS unleaded gasoline acclimated biomass was evaluated. Substrate biodegradation efficiency was defined as the pure effect of the biomass versus two types of controls. Type 1 controls consisted of dissolved substrates and sterilized soil. Type 2 controls consisted of dissolved substrates, sterilized soil and TNP-10. MTBE and BTE-oX concentrations were evaluated for 36 hours every 6 hours. MTBE biodegradation efficiency showed a zero-order rate in all samples. Although, the addition of surfactant enhanced MTBE biodegradation efficiency rate by 22.6%, MTBE efficiency percent removal was not above 28.4%. Benzene biodegradation efficiency showed a first-order two-phase reaction rate in the absence of surfactant. Interestingly, however, with the addition of TNP-10, benzene biodegradation efficiency switched to a first-order one-phase kinetic rate model. Toluene and ethylbenzene biodegradation efficiencies showed a first-order two-phase kinetic rate model in all samples. The addition of TNP-10 had a major selective impact with a significant increase on toluene and ethylbenzene biodegradation efficiency second phase kinetic rate constants. This selective enhancement showed a trend for toluene to reduce differences between the distribution and elimination phase kinetic rate constants. O-xylene biodegradation efficiency followed a first-order one-phase kinetic rate model in all samples. The addition of TNP-10 significantly increased the biodegradation efficiency percent removals of those recalcitrant substrates such as o-xylene and MTBE by 40% and 35.5%, respectively.

Keywords

Biodegradation;BTEX; MTBE; Tergitol NP-10

INTRODUCTION

The major chemicals of enviromental concern from unleaded gasoline are methyl tertiary-butyl ether (MTBE) and benzene, toluene, ethylbenzene and total xylenes (BTEX) due to their carcinogenic potential and other toxicity and their impact on property value (Hartley *et al.*,1999; Acuna-Askar, *et al.*, 2000; Chang, *et al.*,2001; Wilson, *et al.*, 2001). Underground storage tanks (UST's), 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). 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. The MSCC are divided in three categories: the residential soil cleanup levels; the industrial/commercial soil cleanup levels and the soil-to-water maximum contaminant concentration (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). Environmental regulations for benzene, toluene, ethylbenzene and total xylene contaminated soil in Mexico, have set the maximum contaminant levels (MCLs) as 6, 40, 10 and 40.0 mg/Kg, respectively, for agricultural and residential settings, and 15, 100, 25 and 100.0 mg/Kg, respectively, for industrial use (DOF, 2005). The MTBE drinking water health advisory level for taste and odor has been set at 20-40 ug/L by the EPA (USEPA, 1997).

METHODS

Chemicals.

Benzene, toluene, ethylbenzene, mixed xylenes, o-, m-, p-xylene, methyl tertiary butyl ether (MTBE) and Tergitol NP-10 (nonionic surfactant) were purchased from Sigma-Aldrich (Mexico) and were above 98% purity. Unleaded gasoline (UG) Premium was purchased from a gas station. Nutrient agar (Difco Laboratories, Detroit, MI) and bacteriological agar (BIOXON, Becton-Dickinson, Mexico) were purchased from Casa Rocas Fisher Scientific (Mexico).

Culture conditions.

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, 50 mg/L MTBE and 18.5% sterilized soil (SS). The pH of MMII and SMM was 7.0-7.5.

Critical micelle concentration.

The concentration range of nonionic surfactant (Tergitol NP-10) where a sudden variation in the relation between both culture medium density and culture surface tension occurred was chosen as the critical micelle concentration (CMC). The amount of Tergitol NP-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. 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.

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 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 the grown in the 20-L biomass acclimation batch reactor. This procedure was made for each of the three replicates.

Experimental bioassays.

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. Two controls and two sets of samples were evaluated. First controls had only SMM. Second controls had SMM and 25 mg/L Tergitol NP10 (TNP-10). Set 1 contained SMM and 880 mg/L VSS of microbial inoculum. Set 2 contained SMM, 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.

Sample and control sterilization.

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.

Isolation of acclimated bacteria.

UG-acclimated bacteria were grown in UG agar plates and incubated in a gravity flow Isotemp incubator (Fisher Scientific, USA), model 537D at 28-30°C for 72 hours. Acid production in UG agar plates was identified by change of color from blue to green, and in some cases from blue to yellow using bromothymol blue as indicator. For identification purposes, bacteria isolates were grown in nutrient agar plates.

Mechanical shakers and sonication.

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

Gas chromatography and sample concentrator.

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 Petrocholl™ (Supelco, Bellefonte, PA) 100m x 0.25mm ID x 0.5µm 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, point at which temperature was held for 20 minutes. A second temperature rate followed and varied 30°C/min from 90°C up to 150°C, point at which 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

First-order one-phase model. Model I (Acuna-Askar *et al.*, 2000) was used to evaluate the overall removal rate constants K for benzene in set 2 samples and for o-xylene in sets 1 and 2 samples.

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)

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

First-order two-phase model. Model II (Hu *et al.*, 2004) was used to evaluate the overall removal rate constants K for benzene in set 1 samples and for toluene and ethylbenzene in sets 1 and 2 samples.

Model II $S_t = S_1 \exp(-K_1 t) + S_2 \exp(-K_2 t)$ (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})

The overall removal rate constants K were obtained from the method of residuals.

Zero-order model. Model III was used to evaluate the overall removal rate constants K for MTBE in sets 1 and 2 samples.

Model III $S_t = - Kt + S_0$ (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

MTBE biodegradation efficiency showed a zero-order rate in all samples (Figure 1), whereas benzene biodegradation efficiency showed a first-order two-phase reaction rate in the absence of surfactant. Interestingly, however, with the addition of TNP-10, benzene biodegradation efficiency switched to a first-order one-phase kinetic rate model (Figure 2). Research reports on the switch of substrate kinetics models under the effect of nonionic surfactants are limited in the literature. Substrate bioavailability may not only be handled as a function of the CMC, but it may also be related to changes in substrate kinetic rates. Precise determinations of changes in chemical kinetic rates under the effect of nonionic surfactants may improve prediction on biomass capability to efficiently biodegrade the substrates of interest.

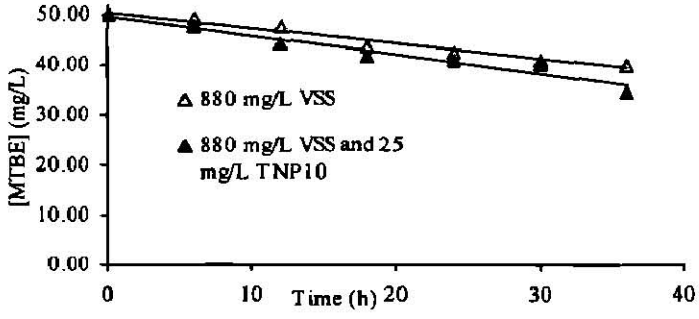

Figure 1 MTBE biodegradation efficency kinetics with 200 mg/L total BTEoX in the presence of 18.5% SS

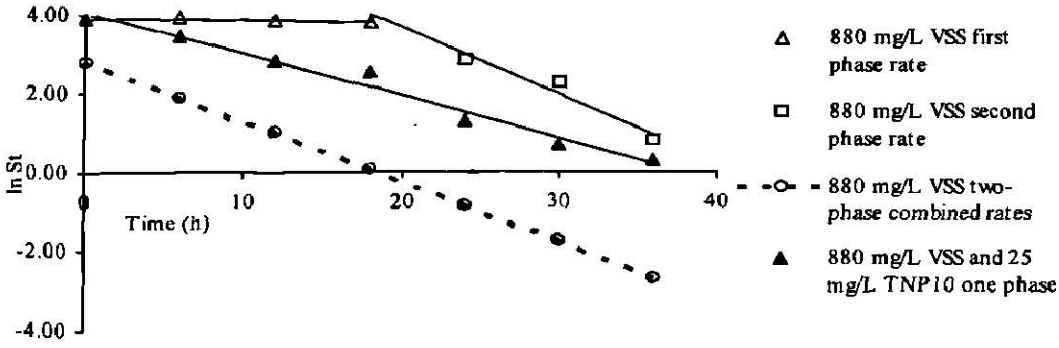

Figure 2 Benzene biodegradation efficiency kinetics with 200 mg/L total BTEoX and 50 mg/L MTBE in the presence of 18.5% SS

Toluene and ethylbenzene biodegradation efficiencies showed first-order two-phase kinetic rate models in all samples (Figures 3 and 4), which could have been due to the higher assimilative capacity of the biomass for theses substrates as compared to the assimilative capacity to biodegrade MTBE (Acuna-Askar *et al.*, 2004). The addition of TNP-10 had a major selective impact with a significant increase on toluene and ethylbenzene biodegradation efficiencies, particularly on the second phase kinetic rate constants (Table 1).

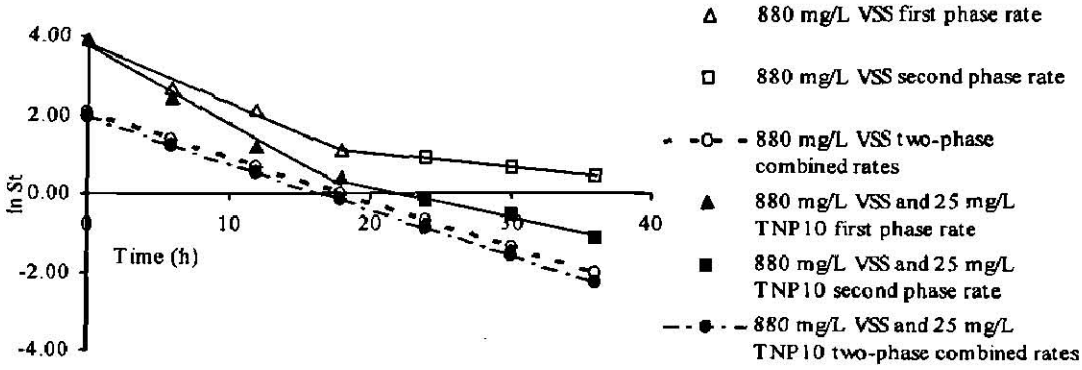

Figure 3 Toluene biodegradation efficiency kinetics with 200 mg/L total BTEoX and 50 mg/L MTBE in the presence of 18.5% SS

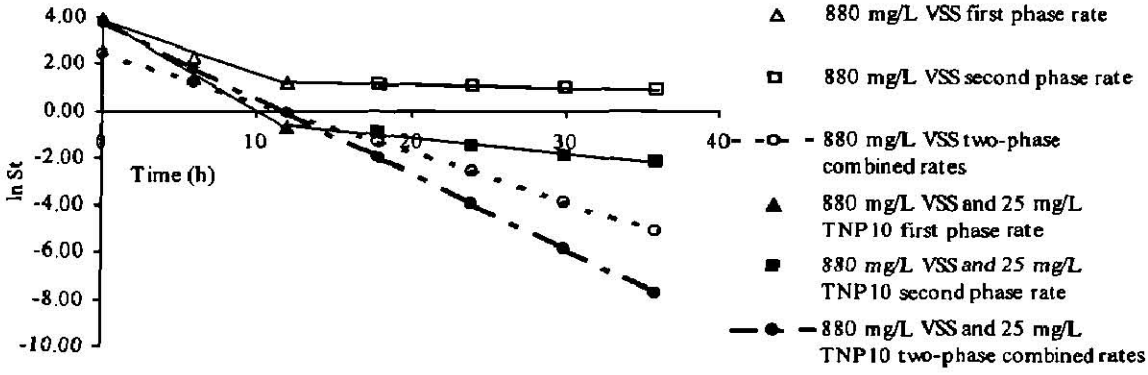

Figure 4 Ethylbenzene biodegradation efficiency kinetics with 200 mg/L total BTEoX and 50 mg/L MTBE in the presence of 18.5% SS

O-xylene biodegradation efficiency followed a first-order one-phase kinetic rate model in all samples (Figure 5). The addition of TNP-10 significantly increased the biodegradation efficiency percent removals of those recalcitrant substrates such as o-xylene and MTBE by 40% and 35.5%, respectively (Table 3).

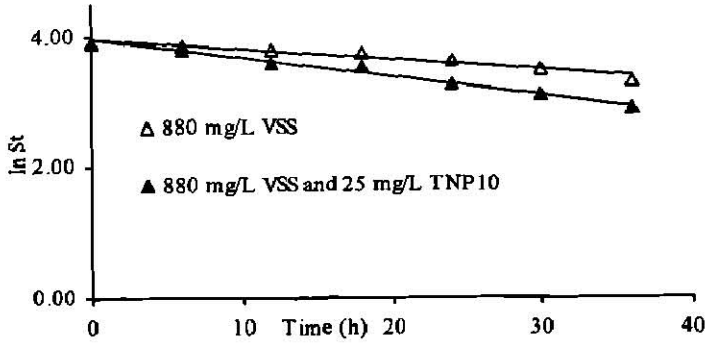

Figure 5 O-Xylene biodegradation efficiency kinetics with 200 mg/L total BTEoX and 50 mg/L MTBE in the presence of 18.5% SS

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

	Benzene		**Toluene**		**Ethylbenzene**		**o-Xylene**
Set 1 Samples*							
Overall K rate	K_1	K_2	K_1	K_2	K_1	K_2	K
$[hr^{-1}]$	0.0085	0.1514	0.1143	0.0362	0.2105	0.0123	0.0498
(r)	(0.985)		(0.999)		(0.989)		(0.983)
Specific k rate	k_1	k_2	k_1	k_2	k_1	k_2	k
$[hr^{-1}(mg/L)^{-1}vss]$	9.66×10^{-6}	1.72×10^{-4}	1.13×10^{-4}	0.411×10^{-4}	2.39×10^{-4}	0.14×10^{-4}	0.566×10^{-4}
(r)	(0.999)		(0.999)		(0.999)		(0.985)
Set 2 Samples**							
Overall K rate	K		K_1	K_2	K_1	K_2	K
$[hr^{-1}]$	0.1063		0.1181	0.0798	0.3206	0.0639	0.0291
(r)	(0.989)		(0.993)		(0.993)		(0.990)
Specific k rate	k		k_1	k_2	k_1	k_2	k
$[hr^{-1}(mg/L)^{-1}vss]$	1.20×10^{-4}		1.34×10^{-4}	0.907×10^{-4}	3.64×10^{-4}	0.726×10^{-4}	0.331×10^{-4}
(r)	(0.989)		(0.999)		(0.999)		(0.990)

r = correlation coefficient

*SMM + 880 mg/L VSS (control 1 as SMM)

** SMM + 880 mg/L VSS + 25 mg/L TNP-10 (control 2 as SMM + 25 mg/L TNP-10)

MTBE biodegradation efficiency kinetic rate was higher in those samples containing TNP-10 as compared to those in the absence of TNP-10, suggesting that MTBE bioavailability was enhanced by TNP-10 (Table 2).

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

	Set 1 Samples*	Set 2 Samples**
Overall K rate	K	K
$[mgL^{-1}hr^{-1}]$	0.3175	0.3895
(r)	(0.983)	(0.975)
Specific k rate	k	k
$[mgL^{-1}hr^{-1}(mg/L)^{-1}vss]$	3.61×10^{-4}	4.53×10^{-4}
(r)	(0.983)	(0.975)

r = correlation coefficient

* SMM + 880 mg/L VSS (control 1 as SMM)

** SMM + 880 mg/L VSS + 25 mg/L TNP-10 (control 2 as SMM + 25 mg/L TNP-10)

A point where the addition of surfactant may no longer have a significant effect on substrate bioavailability would be expected, since breakdown mechanisms depend on chemical structure, among other factors. MTBE recalcitrance to biodegradation is a clear example of the molecular hindrance even though its solubility in water is higher than that of BTEX. Overall, the addition of TNP-10 increased the biodegradation efficiency percent of all chemicals and demonstrated that limitations other than those related to substrate-micelle interactions may influence chemical breakdown (Table 3). In addition, the results of this study suggest that changes in substrate biodegradation kinetic rates under the effects of surfactants should be monitored to improve multi-substrate biodegradation efficiency prediction, especially for scale-up applications.

Table 3. Biodegradation efficiency percentage vs. experimental bioassay samples

	Benzene (%)	Toluene (%)	Ethylbenzene (%)	o-Xylene (%)	MTBE (%)
SMM + 880 mg/L VSS*	95.6	96.9	95.0	46.1	20.9
SMM + 880 mg/L VSS + 25 mg/L TNP10**	97.3	99.3	99.7	64.6	28.4

* Control 1 as SMM

** Control 2 as SMM + 25 mg/L TNP10

Comparison and discussion of the results presented in this research with others previously published either in batch or continuous cultures by an ample variety of authors is ongoing and will complete the present work.

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From Ogarit Uhlmann <ogarit@fu-confirm.de>
Date Thursday, March 10, 2005 4:05 am
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Subject ConSoil 2005
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About 600 abstracts were received and all have now been reviewed by the Program Committee.

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Your abstract for an oral presentation has been ranked by the Program Committee. The ranking position of your abstract did not fit in the limited number of oral presentations in your theme.

The Program Committee has acceptedyour contribution entitled:

The effect of a nonionic surfactant on BTEOX and MTBE solubility in soil slurries

for **poster presentation** in *Theme C:* **SITE CHARACTERISATION & RISK ASSESSMENT**

Please note that the working language of the Conference is English.

Poster display and contact poster author – delegates

The posters will be displayed during the four conference days. Posters should be mounted on Monday 3 October 2005 from 8 until *10 a.m.*

The posters will be clustered by themes and as far as possible within a theme by sub-themes.

The ConSoil organization will provide notes that can be stuck to the poster panels by:

<!--[if !supportLists]-->• <!--[endif]-->a delegate to inform the poster presenter at what time (s)he would like to meet the poster author near the poster

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Poster discussions

We **intend** to organize poster discussions on Tuesday and Thursday with a limited number of poster authors in a limited number of sub-themes (**so not for all poster authors**). We will select topics for discussion. The decision of having poster discussions will depend on the poster authors registered for the conference.

During these poster discussions, the selected poster authors will be near their posters. There will be a discussion with the poster authors and delegates facilitated by a discussion leader.

At the conference, delegates and poster authors can submit issues they would like to discuss during the poster discussions.

As already mentioned, **if there will be** poster discussions they will be on Tuesday and Thursday. We will divide the conference themes (A-G) over these two days. In the Final Announcement (see below), you will find over which days the themes have been divided.

In the Poster program (handed out at the conference) we will inform you which posters have been selected for the poster discussions.

Poster awards

Three best PhD-posters will be awarded. The delegates will vote for the best PhD-posters. The awards will be presented during the Closing Session.

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Please note the enclosed Guidelines for poster presentation.

Paper in the Conference Proceedings

Please find enclosed the instructions for a PAPER in the Proceedings.

The strict deadline for submission of your PAPER to the Proceedings Secretariat (see below) is: **18 May 2005**.

It will be much appreciated if you could send the paper amply before this date. If your paper is received after the deadline, it will **not** be included in the Proceedings.

The Proceedings will be available at the Conference.

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We will ensure that you will receive the Final Announcement and Registration/Hotel Accommodation Form by regular mail. The distribution is planned for April/May 2005.

The Final Announcement can also be downloaded from www.consoil.de by the time of distribution.

Please note that you, as a poster presenter (only one poster presenter per poster), have to return the Registration Form.

If we do not receive your registration 3 September 2005 ultimately there will be no panel for your poster.

Conference fee:

The organizing Committee offers you as a poster presenter a reduced fee. Please tick in this special fee on the Registration Form in the Final Announcement.

This reduced fee is only for you (and not for your co-authors).

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ConSoil 2005 - Abstract form

Title of abstract	**The effect of a nonionic surfactant on BTEOX and MTBE solubility in soil slurries**
Author(s) + organisation, company, university	K. Acuna-Askar*, M.V. Gracia-Lozano*, J.F. Villarreal-Chiu*, M.T.Garza-Gonzalez**, I.P. Rodriguez-Sanchez***,H.A. Barrera-Saldana*** and B. Chavez-Gomez**** *Laboratorio de Biorremediacion Ambiental, Facultad de Medicina, UANL, Av. Madero Pte y Dr. Aguirre-Pequeno, 64460 Monterrey, N.L., Mexico (Corresponding author: kaskar@fm.uanl.mx). **Facultad de Ciencias Quimicas, UANL, Pedro de Alba s/n, Cd. Universitaria, 66400 San Nicolas de los Garza, N.L., Mexico (tgarza@fcq.uanl.mx). ***ULIEG, Depto. de Bioquimica, Facultad de Medicina, UANL, Av. Madero Pte y Dr. Aguirre-Pequeno, 64460 Monterrey, N.L., Mexico (irodriguez@fm.uanl.mx;hbarrera@fm.uanl.mx) ****Instituto Mexicano del Petroleo, Eje Central Lazaro Cardenas 152, Delegación Gustavo A. Madero, 07730 Mexico, D.F (bchavez@imp.mx).

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Abstract

Introduction

The major chemicals of environmental concern from unleaded gasoline are methyl tertiary-butyl ether (MTBE) and benzene, toluene, ethylbenzene and total xylenes (BTEX) due to their carcinogenic potential and other toxicity and their impact on property value (Hartley *et al.*,1999; Acuna-Askar *et al.*, 2000; Chang *et al.*, 2001; Wilson *et al.*, 2001). Underground storage tanks (UST's), 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 and MTBE are included in the current United States Environmental Protection Agency (USEPA) drinking water standards and health advisories under the National Primary Drinking Water Regulations (NPDWRs) (USEPA, 2004). The maximum drinking water levels for BTEX are 0.005, 1.0, 0.7, and 10 mg/L, respectively (USEPA, 2004). 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). The MTBE drinking water health advisory levels for taste and odor have been set at 40 and 20 ug/L by the EPA (USEPA, 2004). To date, no MTBE drinking water health advisory level has been set in Mexico.

Although BTEX can be adsorbed on to soil due to hydrophobic properties, MTBE is more hydrophilic (Sangster, 1989). The soluble fraction of BTEX, however, can infiltrate through the soil and reach groundwater bodies, where further liquid and vapor phase equilibrium occurs in the subsoil-groundwater compartment. It is important to understand the fate of BTEX and MTBE as the contaminant plume approaches the subsoil-groundwater compartment so that the risk of environmental impact can be assessed and appropriate bioremediation actions can be undertaken. Physical chemical parameters including BTEX octanol-water partition coefficients applicable to chemical solubility and substrate bioavailability need to be considered for site bioremediation strategies. It has been reported that 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 soil-groundwater compartments. This study was aimed to evaluate the solubility of BTEOX and MTBE in soil slurries in the presence of a nonionic surfactant, Tergitol NP-10, which could be used as a substrate bioavailability enhancer.

Materials and methods

Chemicals

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. Mineral medium I (MMI) was prepared for controls and for soil slurry samples and had the following composition (in g/L) (Acuna-Askar *et al.*, 2003): 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 assays to evaluate the effect of TNP-10 on BTEX and MTBE solubility and consisted of MMI, 50 mg/L of each BTE-oX component and 50 mg/L MTBE. The pH of MMI and SMM was acidified with H_3PO_4 down to 2.5-3.0.

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 assays was 25 mg/L based on prior studies (Acuna-Askar *et al.*, 2003).

Experimental assays

Controls and two sets of samples were evaluated. Controls had only SMM. Set 1 contained SMM and 18.5% sterilized soil (SS). Set 2 contained SMM, 18.5% SS and 25 mg/L TNP-10. MTBE and BTE-oX were monitored for 36 hours every 6 hours. Experimental assays 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. Experiments were run three times separately.

Sample sterilization

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 (*Standard Methods*, 1998). Controls were autoclaved following one sterilization cycle.

Sample shaking and gas chromatography

Samples and controls 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. 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) 100m x 0.25mm ID x 0.5 m 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, point at which temperature was held for 20 minutes. A second temperature rate followed and varied 30°C/min from 90°C up to 150°C, point at which 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.

Results and discussions

The addition of soil decreased the solubility of all chemicals, and this effect is more noticeable after 18 hours, with approximately 50% of BTE-oX concentration being partitioned onto soil. Among all chemicals, MTBE showed highest solubility, as it would be according to its physical chemical properties (Figure1).

In Figure 2, it can be seen that BTE-oX showed higher concentration in the aqueous phase when TNP-10 was added to the slurries, suggesting that chemical soil-to-water ratio was reduced by the addition of the nonionic surfactant. When surfactant-free slurries were compared against slurries containing surfactant to obtain the corresponding chemical concentration ratio, it was clearly seen that the solubility of BTE-oX and MTBE significantly increased (Figure 3).

Discussions on results will be extended as more results are obtained during the course of this research, before final submittal of the full manuscript.

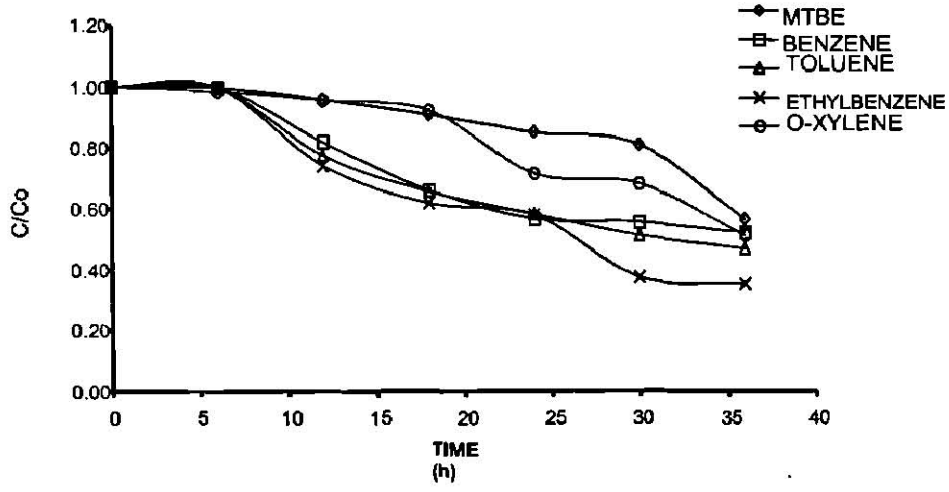

Figure 1 Chemical concentrations in soil slurries without TNP-10

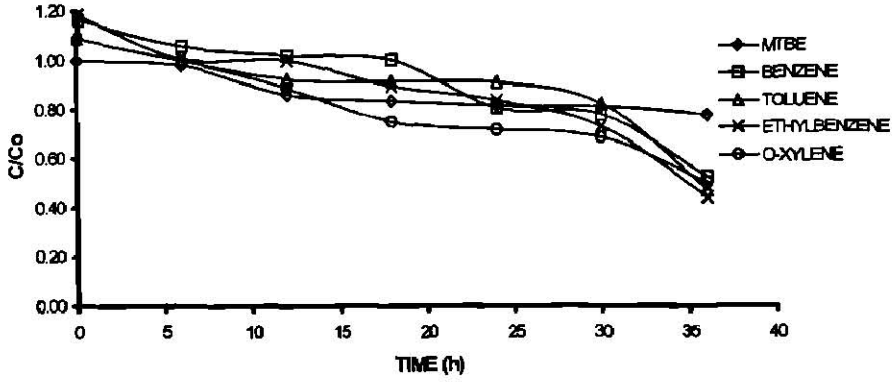

Figure 2 Chemical concentrations in soil slurries with TNP-10

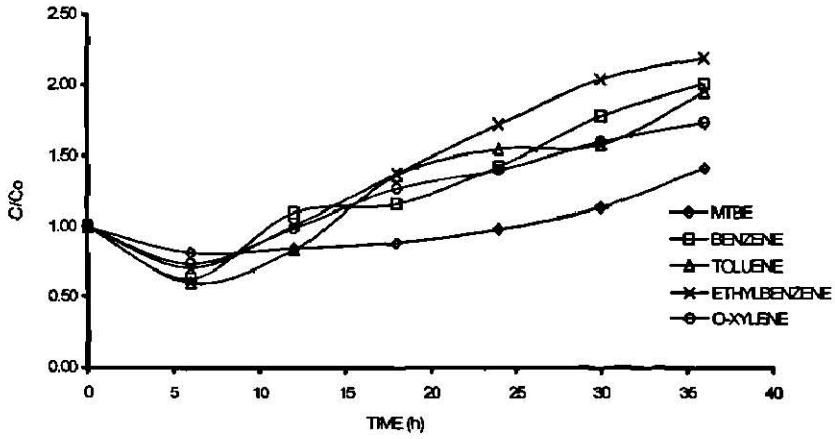

Figure 3 Resultant chemical solubility increase by the addition of TNP-10 to soil slurries.

Conclusions

The addition of the nonionic surfactant Tergitol NP-10 significantly increased the solubility of all chemicals, with BTEoX being the more favored as compared to MTBE.

More conclusions from this work will be drawn as more results will be obtained and further discussed during the course of this research before final submittal of the full manuscript.

Acknowledgements

This research was supported under the auspices of the Instituto Mexicano del Petroleo, CONACYT, and UANL PAICYT research projects.

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We hope you will consider submitting a abstract for the 106th General Meeting in Orlando, FL. This meeting will take place May 21- 25, 2006. Information relating to this meeting will be posted to the General Meeting Pages on the ASM website after the completion of this year's meeting.

March 7, 2005

RE: Abstract 05-GM-A-3000-ASM

Juan F Villarreal-Chiu
ULIEG Depto. Bioquimica
Facultad de Medicina UANL
Madero Pte. y Dr. Aguirre-Pequeno
Col. Mitras Centro
Monterrey NL 64460
Mexico

Dear Dr. Villarreal-Chiu

I am pleased to inform you that your abstract has been accepted for a poster presentation at the 105th General Meeting, which will be held at the Georgia World Congress Center, from June 5 through June 9, 2005 in Atlanta, GA.

The following information refers to the above abstract:

Abstract Title: Biodegradation of BTEOX and MTBE in Soil Slurries by Biomass Grown in a BTEX-fed Batch Reactor
Authors: **J. F. Villarreal-Chiu**[1], M. V. Gracia-Lozano[1], K. Acuna-Askar[1], M. T. Garza-Gonzalez[2], I. P. Rodriguez-Sanchez[1], H. A. Barrera-Saldana[1];
[1]UANL Facultad de Medicina, Monterrey, MEXICO, [2]UANL Facultad de Ciencias Quimicas, San Nicolas de los Garza, MEXICO.
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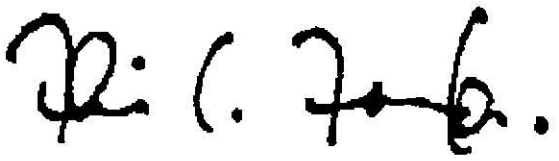
Please recall that you agreed to present your poster as scheduled. If you fail to do so, you will be prohibited from submitting abstracts to ASM-sponsored meetings for 3 years. If unable to present your poster, notify ASM before March 21, 2005, so that your abstract will not be published.

Please check our website at http://www.asm.org/Meetings/index.asp?bid=745 for information regarding presentation hints. Select "Poster Guidelines" from the sidebar. While you are at the website, do not forget to register for the General Meeting since you must be a registered attendee to present your abstract. The link to the on-line registration can be found under "Registration and Housing" at http://www.asm.org/Meetings/index.asp?bid=23083.

As an additional note, for those who submitted a request for a Student Travel Grant, confirmation notices will be sent under separate cover March 25.

Sincerely,



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Selected Proceedings of the 4th IWA Specialised Conference on Assessment and Control of Hazardous Substances in Water (ECOHAZARD2003), held in Aachen, Germany, 14-17 September 2003

Editor(s): H. F. Schröder

The risks of ecohazards have never been more prominent than today; better risk management to minimise the adverse impacts of chemical compounds or their degradation products has become a key objective. The diverse but interlinked array of environmental issues discussed at Ecohazard 2003 encompassed the condition of water in the ecosystem, its fate in wastewater treatment plants, chemical analysis and toxicity testing, natural attenuation in soil, contaminated sediments, monitoring programmes and risk assessment.

These topics are being tackled by collaborative efforts by an equally broad range of specialists – academic and non-academic, in the life sciences, engineering, industry as well as politics. Work in life sciences and chemistry highlights the often crucial importance of practical tasks. It exemplifies the interlocking of science with the practical, economic and political realisation of innovation that must underpin the realm of directives and legislation.

After a full peer review 51 papers (from over a hundred on the programme) were selected for these proceedings. Topics covered include: wastewater treatment and reuse; special treatment and fate aspects; industrial wastewaters; chemical and biochemical analytical methods and applications; endocrine disruptors; persistent polar pollutants; pharmaceuticals; fate of chemicals in the environment; toxicity; and natural attenuation.

The general management of chemical contaminants in the environment continues to broaden its scope and to enforce interdisciplinary co-operation to which the engineers and natural scientists are lending a systemic perspective.

These proceedings record the latest findings and summarise the insights of some the world's leading authorities in this field. They will prove invaluable to anyone seeking to understand both the technological limits and the possibilities facing current and future generations of decision-makers and to become acquainted with recently developed tools for environmental risk assessment and monitoring.

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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