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Biodegradation potential of MTBE and BTEX under aerobic, nitrate reducing, and methanogenic conditions at a gasoline-contaminated site

Ku-Fan Chen*, Yu-Chen Chang, Sheng-Chih Huang

Department of Civil Engineering, National Chi Nan University, 1 University Rd, Puli, Nantou 54561, Taiwan
Tel. +886 49 2910960x4983; Fax: +886 49 2918679; email: kfchen@ncnu.edu.tw

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ABSTRACT

In this study, a gasoline-contaminated site was selected to evaluate the biodegrading potential of methyl *tert*-butyl ether (MTBE) and benzene (B), toluene (T), ethylbenzene (E) and xylenes (X), collectively known as BTEX under aerobic, nitrate reducing, and methanogenic conditions at the site. Results show that in situ microorganisms had capabilities of degrading MTBE and BTEX using MTBE and BTEX as major carbon sources under aerobic condition. Under conditions of nitrate reduction and methanogenesis, BETX could be decomposed by in situ microorganism; but MTBE was not decomposed. The decomposition of BTEX under anaerobic condition was much slower than aerobic condition. Benzene had the slowest degrading rate. In addition, BTEX was decomposed faster and more completely under methanogenic condition than nitrate reducing condition. The on-site activated sludge system is effective in decomposing BTEX but not effective in decomposing MTBE. Results of this study reveal that the enhanced aerobic bioremediation method is more feasible for renovating this site. Injecting air, pure oxygen or oxygen-releasing chemicals will raise the removal rate of pollutants. Results of this study will be helpful in designing a practical system for bioremediation of this and other similar sites.

Keywords: MTBE; BTEX; Biodegradation; Enhanced aerobic bioremediation

1. Introduction

The extensive use of petroleum hydrocarbons leads to common but serious environmental pollution problems. Fuel constitutes about 40% of various petrochemical products; gasoline is one of the commonly used fuels. It consists of hydrocarbons of 5–12 carbons with 40% aliphatic and branched alkanes, 20% cycloalkanes and 25% aromatics; the rest components are additives for increasing the fuel octane value [1]. Among the many components of gasoline, aromatics and additives cause the most concern because of their polluting potentials. The aromatics mainly include benzene (B), toluene (T), ethylbenzene (E) and xylenes (X), collectively known as BTEX, whereas methyl *tert*-butyl ether (MTBE) is an extensively used fuel additive nowadays. Benzene, toluene and ethylbenzene are included in the list of priority pollutants compiled by the US EPA [2]. In Taiwan, MTBE, benzene and xylene are regulated as controlled toxic substances with restrictive standards of BTEX in soil and groundwater [3]. BTEX may cause nerve damages, and benzene may cause leukemia [4]; MTBE has been proved to be carcinogens to animals. Hence, the US EPA has classified MTBE as a possible cancer-causing substance in human body [5]. MTBE and BTEX are the pollutants commonly found in groundwater often caused by accidental leaking of petroleum fuel from ground or underground storage tank and pipeline [6–8].

*Corresponding author.

There are several technologies for remediating polluted soil and groundwater. Bioremediation is economically feasible and environmental friendly so that it is becoming an attractive alternative soil and groundwater remediation method. Results of numerous studies show that MTBE and BTEX can be degraded by aerobic and anaerobic microorganisms [9–13]. Under aerobic condition, MTBE and BTEX are effectively decomposed by microorganisms using direct metabolism or cometabolism [14,15], whereas anaerobic microorganisms may degrade MTBE and BTEX under conditions of nitrate reduction, sulfate reduction, iron reduction and methanogenesis [16–21]. Generally, aerobic biodegradation of MTBE and BTEX is faster and more effective than anaerobic biodegradation [10,17,22–24]. However, the polluted groundwater is often anaerobic so that the aerobic biodegradation method may not be feasible for all polluted site especially those with low permeability. Therefore, the biodegradation and removal of BTEX and MTBE under anaerobic conditions needs to be evaluated in order to understand how anaerobic biodegradation can contribute to intrinsic biodegradation at the polluted site [18,25,26].

In general, each polluted site has its own unique characteristics. Hence, understanding the potential of in situ microorganisms to degrade pollutants and the mechanism of degradation prior to conducting bioremediation is important for providing a feasible and effective remediation strategy to be referenced for future implementation. In this study, a gasoline-contaminated site was selected to evaluate the biodegrading potential of MTBE and BTEX under aerobic, nitrate reducing, and methanogenic conditions at the site. Additionally, since the process wastewater that contains petroleum hydrocarbons is currently treated in an activated sludge plant at the pollution site, the degradation of hydrocarbon pollutants in the activated sludge process was also evaluated. Major objectives of this study were:

1. to investigate the potential of in situ microorganisms to degrade MTBE and BTEX under aerobic condition and anaerobic conditions of nitrate reduction and methanogenesis,
2. to investigate the potential of activated sludge process to degrade MTBE and BTEX,
3. to evaluate the mechanism of natural biodegradation of MTBE and BETEX, and
4. to evaluate the feasibility of applying bioremediation for renovating the polluted site.

2. Description of the polluted site

The site selected for conducting this research is an active oil-refinery facility, which has been polluted by spills of gasoline. The soil consists of silty sand, silt, and clay with silty sand being the major component. There is a weak clay layer of 5–10 m thickness located 40 m below the surface. The groundwater is 3–7 m during dry season and 2–5 m during wet season below the ground; its velocity is estimated to be between 0.2 and 1.4 m/day [7].

Results of previous studies show that the groundwater at this site contained the highest MTBE and total BTEX concentrations of 0.2 and 200 mg/L, respectively, with dissolved oxygen of less than 1 mg/L. Additionally, *tert*-butyl alcohol (TBA), the biodegrading byproduct of MTBE, is also detected to exist in the groundwater with the highest concentration of about 1 mg/L. The aquifer soil at the polluted site contains about 10^6–10^7 CFU/g soil of total aerobic microorganisms and 10^5–10^6 CFU/g soil of total anaerobic microorganisms. Additionally, nitrate reducing microorganisms, iron reducing microorganisms, sulfate reducing microorganisms and methanogens have been proved to exist at the site; their total population numbers 10^1–10^3 CFU/g soil [27].

3. Materials and methods

3.1. Source of inocula and growth nutrient

The inocula used in this research were obtained from three sources: (1) the aquifer sediments 4 m below ground, (2) extracted supernatant of the aquifer sediments, and (3) the wastewater treatment plant activated sludge. For extracting the microorganisms contained in the aquifer soil, 10 g of the collected soil was mixed with 200 mL of growth nutrient, and the mixture was incubated for 7 days in 160 rpm shaker bath with temperature controlled at 30 °C. The incubated microbial suspension was centrifuged to remove the clear liquid. The remaining solids were rinsed three times with inorganic nutrient solution to remove any trace amount of organic matter that may interfere with subsequent analyses. Inorganic nutrient solution was used as the nutrient to carry out batch studies in this research. Compositions and preparation of the nutrient solution, e.g. buffer solution, calcium and magnesium solutions, and trace elements contained in the nutrient solution, are the same as those shown in the previous study [28].

3.2. Batch biodegradation experiments

Serum bottles of 60 mL volume were used as the batch reactors to carry out aerobic, nitrate reduction and methanogenesis biodegradation studies. After preselected amounts of microbial inoculums, MTBE or BTEX, and nutrient solution or ground water were added, the reactor was immediately sealed with Teflon-covered aluminum cap. The prepared reactors were then placed in a dark container with temperature

maintained constant at 25 °C to incubate with the content periodically removed for analyses. For the anaerobic biological degradation process, mixture of N_2/CO_2 (80/20) (for methanogenesis group) or N_2 (for nitrate reduction group) was blown into the reactor through the bottle neck before the reactor was inoculated. In addition, 0.05% of sodium sulfide ($Na_2S{\cdot}9H_2O$) was added to the methanogenic group to reach strict anaerobic conditions. The reactor was placed in an anaerobic box for adding microbial inoculum and other ingredients. The initial pH values in all microcosms were adjusted to around 7, and the initial dissolved oxygen (DO) concentrations in the aerobic biodegradation experiments were approximately 8 mg/L. DO concentrations were monitored during the aerobic experiments. When DO was less than 2 mg/L, pure oxygen was injected into the headspace to supply enough DO for aerobic biodegradation. Each group of batch studies consists of a sterilization control. Table 1 lists the experimental parameters of the batch experiments.

3.3. Methods of analyses

Samples to be analyzed for volatile organics including MTBE, TBA, and BTEX were pre-treated using a purge and trap equipment prior to gas chromatograph/flame ionization detector (GC/FID) analyses. The GC/FID used a capillary column (HP-1, 30 m × 0.53 mm ×0.5 μm); nitrogen gas (99.9995% purity) flowing at 10 mL/min was used as the carrying gas. The operating temperatures were maintained at 180 °C for the injector, and 250 °C for the detector. The oven temperature was maintained at 35 °C for 5 min, then elevated at the ramp temperature of 11 °C/min to 115 °C, and held at 115 °C for 6 min. Afterward, the temperature was raised at the ramp temperature of 7 °C/min to 130 °C, and then held at 130 °C for 3 min. Concentrations of BTEX presented in this study were total BTEX concentrations.

4. Results and discussion

4.1. The aerobic biodegradation group

4.1.1. Aquifer sediments

The MTBE molecule has ether bond and tertiary butyl structure, it is thus difficult to be degraded biologically [14]. Hence, the biodegradability of MTBE at the polluted site is first investigated using MTBE solution with an initial concentration of 50 mg/L. Fig. 1 shows the degradation of MTBE in aquifer sediments under aerobic condition. The degradation of MTBE was noted to begin after 20-day reaction time. During 200 days of incubation, in situ microorganisms decomposed more than 99.9% of MTBE. The byproduct of MTBE degradation, i.e. TBA, started to form after 20 days and the highest accumulated TBA concentration was reached after 50 days. Afterward, the accumulated TBA concentration continually decreases until day 85 when no TBA is detected. These observations indicate that in situ microorganisms have the capability of degrading MTBE and TBA.

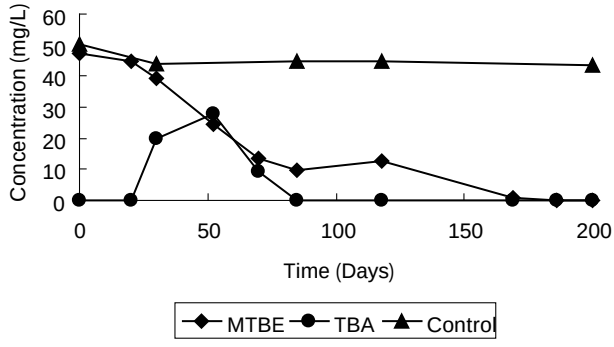

Fig. 1. Degradation of MTBE by aquifer sediments under aerobic condition.

Table 1
Experimental parameters of the batch experiments

Trial	Redox condition	Inocula	Constituent
1	Aerobic	Aquifer sediments	Aquifer sediments 20 g + in situ groundwater 35 mL + MTBE 50 mg/L
2	Aerobic	Extracted supernatant of the aquifer sediments	Extracted supernatant of the aquifer sediments 1 mL + in situ groundwater 35 mL + MTBE 50 mg/L + BTEX 50 mg/L
3	Aerobic	Activated sludge	Activated sludge 5 mL + nutrient 35 mL + MTBE 50 mg/L + BTEX 50 mg/L
4	Nitrate reduction	Aquifer sediments	Aquifer sediments 20 g + in situ groundwater 35 mL + MTBE 50 mg/L + NO_3-N 100 mg/L + BTEX 20 mg/L
5	Methanogenesis	Aquifer sediments	Aquifer sediments 20 g + in situ groundwater 35 mL + MTBE 50 mg/L + BTEX 20 mg/L + reductant

4.1.2. Extracted supernatant of aquifer sediments

In order to exclude the influence of organic substances in aquifer sediments on MTBE and BTEX biodegradation, the extracted supernatant of aquifer sediments and inorganic nutrient were used to confirm that if in situ microorganisms could utilize MTBE and BTEX as the major source of carbon. Fig. 2 displays the degradation of MTBE and BTEX by microorganisms extracted from aquifer sediments under aerobic condition. After a lag period of 74 days, MTBE started to be degraded slowly to 22.2 mg/L with 57% removal efficiency on day 236. Because MTBE was slowly degraded, the production of TBA was also slow; the relatively small amount of TBA may be immediately biodegraded. Hence, the accumulation of TBA was not observed in this experiment. The complete degradation of BTEX in 12 days shows that in situ microorganisms are well capable of degrading BTEX, and the results also show that MTBE and BTEX can be used as the major carbon sources by the intrinsic microorganisms.

4.1.3. Activated sludge

In this study, activated sludge was used to evaluate the feasibility of performing on-site treatment of polluted groundwater. The degradation of MTBE and BETX by activated sludge under aerobic condition is shown in Fig. 3. During 300 days of incubation, MTBE was not degraded whereas BTEX was completely decomposed in 6 days. These results show that using the current activated sludge will not achieve effective MTBE degradation. Therefore, if activated sludge is considered to be used for treating polluted groundwater on-site in the future, external source of carbon needs to be added to evaluate the feasibility of using cometabolism for MTBE degradation [28]. In addition, the experimental results also indicate that the removal of MTBE in the wastewater process by the activated sludge system may not be effective at this facility.

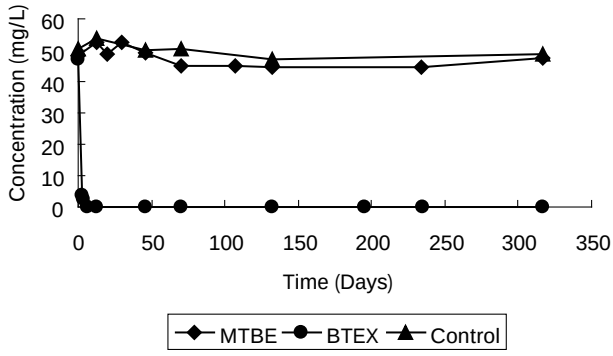

Fig. 3. Degradation of MTBE and BETX by activated sludge under aerobic condition.

4.2. The nitrate reduction group

Adding 100 mg/L of nitrate to MTBE and BTEX solution to carry out the degradation experiment will lead to understanding the degradation of MTBE and BTEX at this site under the condition of nitrate reduction. In this experiment, the initial concentrations were 50 mg/L for MTBE and 20 mg/L for BTEX. Fig. 4 shows the degradation of MTBE and BTEX by aquifer sediments under nitrate reduction condition. MTBE was not decomposed in 315 days whereas 78% of BTEX was decomposed. Toluene had the fastest degradation rate with 100% reduction efficiency whereas benzene had the slowest degradation rate with only 25% removed. Generally, toluene degradation occurs with all terminal electron acceptors whereas benzene is more recalcitrant under nitrate reducing condition [18]. Dou et al. [20,21] have utilized the incubated microorganism species isolated from oil-contaminated soil to degrade BTEX under nitrate reduction condition. Their results show the order of magnitude of BTEX reduction rate as: toluene > ethylbenzene > *m*-xylene > *o*-xylene > benzene > *p*-xylene. Additionally, Schreiber et al. [18] sorted out the first order rate constants for aerobic, nitrate reduction, iron

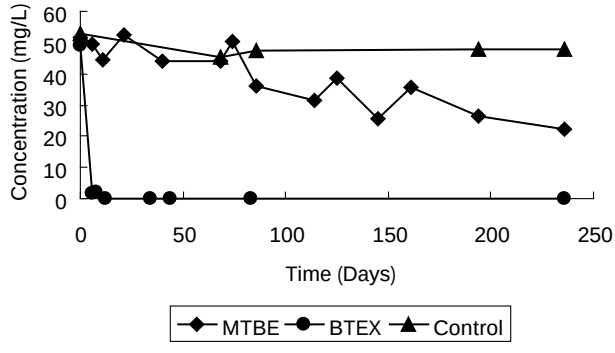

Fig. 2. Degradation of MTBE and BTEX by microorganisms extracted from aquifer sediments under aerobic condition.

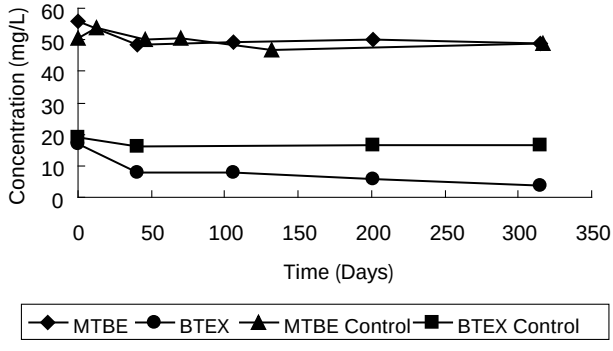

Fig. 4. Degradation of MTBE and BTEX by aquifer sediments under nitrate reduction condition.

reduction, sulfate reduction and methanogenesis of BTEX degradation. The results reveal that toluene and ethylbenzene had fast degradation rate, and benzene had the slowest degradation rate under nitrate reducing conditions. Kao and Wang [29] also reported that toluene had very rapid degradation while benzene removal was slowest within the denitrifying zone at a gasoline spill site. Hence, the results obtained in this study are similar to those reported in literature. Results of the experiments show that under the condition of nitrate reduction, MTBE cannot be removed while BTEX can be partially decomposed by in situ microorganisms.

4.3. The mathanogenesis group

The objective of this experiment was to evaluate the degradation of MTBE and BTEX at the polluted site under methanogenesis. Degradations of 50 mg/L MTBE and 20 mg/L BTEX by aquifer sediments under methanogenesis condition are shown in Fig. 5. During 300 days of reaction, MTBE was not degraded; BTEX was about 98% degraded. For BTEX, benzene had the slowest degradation rate; it was not completely degraded with 90% removed while toluene, ethylbenzene, and xylenes were totally degraded. These results conform to those reported by Johnson et al. [30] and Schreiber et al. [18] that degradation of benzene is highly site-specific since the structure and shape make it difficult to be degraded. Under methanogenesis condition, although the majority of BTEX can be degraded, MTBE cannot be biodegraded by in situ microbes.

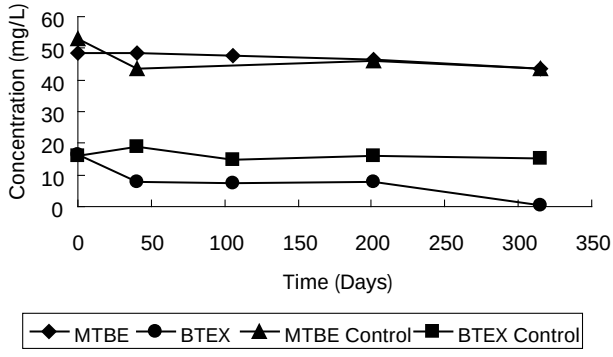

Fig. 5. Degradation of MTBE and BTEX by aquifer sediments under methanogenesis condition.

4.4. Evaluating the feasibility of bioremediation at the contaminated site

Aquifer sediments, the extracted supernatant of aquifer sediments and activated sludge were used to investigate the feasibility of degrading MTBE and BTEX under various conditions. The results of batch studies as listed in Table 2 show that in situ microorganisms are capable of degrading MTBE and BTEX under aerobic condition using MTBE and BTEX as the major source of carbon. Under nitrate reduction and methanogenesis, BTEX can be biodegraded by in situ microorganism whereas MTBE cannot be decomposed. Until now, aerobic biodegradation of MTBE has been successfully demonstrated while anaerobic biodegradation of MTBE has not been well documented [11,14,31–33]. Although MTBE biodegradation under anaerobic conditions has been reported, many studies have failed to demonstrate the occurrence of anaerobic MTBE biodegradation [13,31]. Recently, Waul et al. [13] conducted a long-term study of MTBE anaerobic biodegradation in batch and continuous reactors. MTBE removal was observed in only one set of the batch experiments due to acid hydrolysis of low pH values cased by complexed Fe(III) rather

Table 2
Results of batch biodegradation experiments

Trial	Redox condition	Microorganism	Pollutant	Initial concentration (mg/L)	Incubation time (day)	First-order rate constant (d^{-1})	Removal efficiency (%)
1	Aerobic	Aquifer sediments	MTBE	50	200	0.0276	>99.9
2	Aerobic	Extracted supernatant of aquifer sediments	MTBE	50	236	0.0031	57
			BTEX	50	12	0.4529	100
3	Aerobic	Activated sludge	MTBE	50	317	0	0
			BTEX	50	6	0.8086	100
4	Nitrate reduction	Aquifer sediments	MTBE	50	315	0	0
			BTEX	20		0.006	78
5	Methanogenesis	Aquifer sediments	MTBE	50	315	0	0
			BTEX	20		0.01070	98

than biological processes. The rate of anaerobic degradation of BTEX was much slower than aerobic BTEX degradation. Additionally, under methanogenesis condition, BTEX was biodegraded more effectively and had a better degradation than under nitrate reduction condition (Table 2).

The experimental results indicate that MTBE cannot be removed under anaerobic condition, and that benzene has slower degradation than other BTEX components. Because the DO at this site is smaller than 1 mg/L, natural attenuation or natural biodegradation will not be effective in controlling the MTBE and benzene pollution plume so that they are not suitable for remediating this site. If the natural attenuation is implemented for this site, a detailed survey of the site must be carried out to understand whether non-biological mechanisms (e.g. evaporation, adsorption and dilution factors) can control the pollution plume effectively. The experimental results reveal that the enhanced aerobic biodegradation may be practical for remediating this site. Directly injecting pure oxygen or air, or adding oxygen-releasing substances to raise the groundwater DO for enhancing the pollutant removal rate could be considered for the site remediation.

The aerobic activated sludge method is effective in degrading BTEX but is not applicable for removing MTBE. Because the extracted supernatant of aquifer sediments has the ability of degrading MTBE, on-site renovation of the polluted groundwater using biological method can be carried out by: (1) inoculating the activated sludge system with the extracted supernatant of aquifer sediments, and (2) incubating a large quantity of the extracted supernatant of aquifer sediments to directly treat the pollutants in a reactor.

5. Conclusions

In this study, a gasoline-contaminated site was selected to evaluate the biodegrading potential of MTBE and BTEX under various conditions at the site. In addition, feasible remediation options based on the results of batch experiments were developed for the contaminated site. Conclusions of this study are described as follows:

1. Under aerobic condition, in situ microorganisms have capabilities of degrading MTBE and BTEX using MTBE and BTEX as major carbon sources.
2. Under conditions of nitrate reduction and methanogenesis, BETX could be decomposed by in situ microorganism; but MTBE was not decomposed. The decomposition of BTEX under anaerobic condition was much slower than aerobic condition. Benzene had the slowest degrading rate.
3. BTEX was decomposed faster and more completely under methanogenesis condition than nitrate reduction condition.
4. The on-site activated sludge system is effective in decomposing BTEX but not effective in decomposing MTBE.
5. The enhanced aerobic bioremediation method is more feasible for renovating this site. Injecting air, pure oxygen or oxygen-releasing chemicals will raise the removal rate of pollutants.
6. If on-site biological systems are used to remove pollutants from groundwater, the system can be applied by: (1) inoculating the activated sludge system with the extracted supernatant of aquifer sediments, and (2) incubating a large quantity of the extracted supernatant of aquifer sediments to directly treat the pollutants in a reactor.
7. If this site is to be renovated using natural attenuation, detailed survey of the site must be done in order to understand whether non-biological mechanisms (e.g. evaporation, adsorption and dilution factors) can control the pollution plume effectively.

Results of this study will be helpful in designing a practical system for bioremediation of this and other similar sites.

References

[1] EIA (Energy Information Administration), Petroleum: An Energy Profile, DOE/EIA-0545(99), 1999.
[2] M.L. Davis and D.A. Cornwell, Introduction to Environmental Engineering,4th Ed, ISBN: 9780071259224, McGraw-Hill, 2008, pp. 21.
[3] TEPA (Taiwan Environmental Protection Administration). www.epa.gov.tw, 2011.
[4] ATSDR (Agency of Toxic Substances and Disease Registry), Interaction Profile for Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX), US Department of Health and Human Services, Public Health Service, Atlanta, GA, USA, 2004.
[5] US EPA (US Environmental Protection Agency), Drinking Water Advisory: Consumer Acceptability Advice and Health Effects Analysis on Methyl Tertiary-Butyl Ether (MTBE), EPA-822-F-97-009, Washington, DC, 1997.
[6] M.J. Day, R.F. Reinke and J.A.M. Thomson, Fate and transport of fuel components below slightly leaking underground storage tanks. Environ. Forensics, 2 (2009) 21–28.
[7] K.F. Chen, C.M. Kao, J.Y. Wang, T.Y. Chen and C.C. Chien, Natural attenuation of MTBE at two petroleum-hydrocarbon spill sites. J. Hazard. Mater., 125 (2005) 10–16.
[8] M. Rosell, S. Lacorte and D. Barceló, Analysis, occurrence and fate of MTBE in the aquatic environment over the past decade. Trends Anal. Chem., 25 (2006) 1016–1029.
[9] H. Shim, E. Shin and S.T. Yang, A continuous fibrous-bed bioreactor for BTEX biodegradation by a co-culture of *Pseudomonas putida* and *Pseudomonas fluorescens*. Adv. Environ. Res., 7 (2002) 203–216.
[10] M.L.B Da Silva and P.J.J. Alvarez, Enhanced anaerobic biodegradation of benzene–toluene–ethylbenzene–xylene–ethanol mixtures in bioaugmented aquifer columns. Appl. Environ. Microb., 70 (2004) 4720–4726.

[11] C.W. Lin, Y.W. Cheng and S.L. Tsai, Multi-substrate biodegradation kinetics of MTBE and BTEX mixtures by *Pseudomonas aeruginosa*. Process Biochem., 42 (2007) 1211–1217.
[12] A. Volpe, G.D. Moro, S. Rossetti, V. Tandoi and A. Lopez, Enhanced bioremediation of methyl tert-butyl ether (MTBE) by microbial consortia obtained from contaminated aquifer material. Chemosphere, 75 (2009) 149–155.
[13] C. Waul, E. Arvin and J.E. Schmidt, Long term studies on the anaerobic biodegradability of MTBE and other gasoline ethers. J. Hazard. Mater., 163 (2009) 427–432.
[14] S. Fiorenza and H.S. Rifai, Review of MTBE biodegradation and bioremediation. Bioremediation J., 7 (2003) 1–35.
[15] J.V. Littlejohns and A.J. Daugulis, Kinetics and interactions of BTEX compounds during degradation by a bacterial consortium. Process Biochem., 43 (2008) 1068–1076.
[16] C. Holliger and A.J.B. Zehnder, Anaerobic biodegradation of hydrocarbons. Curr. Opin. Biotech., 7 (1996) 326–330.
[17] T.C. Schmidt, M. Schirmer, H. Weiβ and S.B. Haderlein, Microbial degradation of methyl tert-butyl ether and tert-butyl alcohol in the subsurface. J. Contam. Hydrol., 70 (2004) 173–203.
[18] M.E. Schreiber, G.R. Carey, D.T. Feinstein and J.M. Bahr, Mechanisms of electron acceptor utilization: Implications for simulating anaerobic biodegradation. J. Contam. Hydrol., 73 (2004) 99–127.
[19] M.K. Jahn, S.B. Haderlein and R.U. Meckenstock. Anaerobic degradation of benzene, toluene, ethylbenzene, and *o*-xylene in sediment-free iron-reducing enrichment cultures. Appl. Environ. Microb., 71 (2005) 3355–3358.
[20] J. Dou, X. Liu, Z. Hu and D. Deng, Anaerobic BTEX biodegradation linked to nitrate and sulfate reduction, J. Hazard. Mater., 151 (2008) 720–729.
[21] J. Dou, X. Liu and Z. Hu, Anaerobic BTEX degradation in soil bioaugmented with mixed consortia under nitrate reducing conditions. J. Environ. Sci., 20 (2008) 585–592.
[22] H.X. Corseuil, C.S. Hunt, R.C.F. Dos Santos and P.J.J. Alvarez, The influence of the gasoline oxygenate ethanol on aerobic and anaerobic BTX biodegradation. Water Res., 32 (2008) 2065–2072.
[23] G.M.L. Ruiz-Aguilar, J.M. Fernandez-Sanchez, S.R. Kane, D. Kim and P.J.J. Alvarez, Effect of ethanol and methyl-tert-butyl ether on monoaromatic hydrocarbon biodegradation: Response variability for different aquifer materials under various electron-accepting conditions. Environ. Toxicol. Chem., 21 (2002) 2631–2639.
[24] G. Emtiazi, A.H. Abari and F. Rahehagh, Removal of MTBE by novel *Exiguobacterium* in seawater. Desalin. Water Treat., 28 (2011) 8–11.
[25] J. Dou, X. Liu and Z. Hu, Substrate interactions during anaerobic Biodegradation of BTEX by the mixed cultures under nitrate reducing conditions. J. Hazard. Mater., 158 (2008) 264–272.
[26] D.A. Souza, F.A. Chinalia, E. Foresti and M. Zaiat, Bioremediation of gasoline-contaminated groundwater in a pilot-scale packed-bed anaerobic reactor. Int. Biodeterior. Biodegrad., 63 (2009) 747–751.
[27] K.F. Chen, C.M. Kao, T.Y. Chen, C.H. Weng and C.T. Tsai, Intrinsic bioremediation of MTBE-contaminated groundwater at a petroleum-hydrocarbon spill site. Environ. Geol., 50 (2006) 439–445.
[28] C.M. Kao, C.T. Chai, J.K. Liu, T.Y. Yeh, K.F. Chen and S.C. Chen, Evaluation of natural and enhanced PCP biodegradation at a former pesticide manufacturing plant. Water Res., 38 (2004) 663–672.
[29] C.M. Kao and C.C. Wang, Control of BTEX migration by intrinsic bioremediation at a gasoline spill site. Water Res., 34 (2000) 3413–3423.
[30] S.J. Johnson, K.J. Woolhouse, H. Prommer, D.A. Barry and N. Christofi, Contribution of anaerobic microbial activity to natural attenuation of benzene in groundwater. Eng. Geol., 70 (2003) 343–349.
[31] E.A. Seagren and J.G. Becker, Review of natural attenuation of BTEX and MTBE in groundwater. Prac. Period. Haz. Tox. Rad. Wst. Mgt., 6 (2002) 156–172.
[32] S.C. Chen, C.S. Chen, K.V. Zhan, K.H. Yang, C.C. Chien, B.S. Shieh and W.M. Chen, Biodegradation of methyl tert-butyl ether (MTBE) by *Enterobacter* sp. NKNU02. J. Hazard. Mater., 186 (2011) 1744–1750.
[33] J. Purswani, B. Juárez, B. Rodelas, J. Gónzalez-López and C. Pozo, Biofilm formation and microbial activity in a biofilter system in the presence of MTBE, ETBE and TAME. Chemosphere, 85 (2011) 616–624.