Environ Geol (2006) 50: 439–445
DOI 10.1007/s00254-006-0227-z

ORIGINAL ARTICLE

K. F. Chen
C. M. Kao
T. Y. Chen
C. H. Weng
C. T. Tsai

# Intrinsic bioremediation of MTBE-contaminated groundwater at a petroleum-hydrocarbon spill site

Received: 26 September 2005
Accepted: 15 February 2006
Published online: 10 March 2006


K. F. Chen · C. M. Kao (✉)
T. Y. Chen · C. T. Tsai
Institute of Environmental Engineering,
National Sun Yat-Sen University,
Kaohsiung, Taiwan
E-mail: jkao@mail.nsysu.edu.tw
Tel.: +886-7-5254413
Fax: +886-7-5254449

C. H. Weng
Department of Civil Engineering,
I-Shou University, Kaohsiung, Taiwan

**Abstract** An oil-refining plant site located in southern Taiwan has been identified as a petroleum-hydrocarbon [mainly methyl tert-butyl ether (MTBE) and benzene, toluene, ethylbenzene, and xylenes (BTEX)] spill site. In this study, groundwater samples collected from the site were analyzed to assess the occurrence of intrinsic MTBE biodegradation. Microcosm experiments were conducted to evaluate the feasibility of biodegrading MTBE by indigenous microorganisms under aerobic, cometabolic, iron reducing, and methanogenic conditions. Results from the field investigation and microbial enumeration indicate that the intrinsic biodegradation of MTBE and BTEX is occurring and causing the decrease in MTBE and BTEX concentrations. Microcosm results show that the indigenous microorganisms were able to biodegrade MTBE under aerobic conditions using MTBE as the sole primary substrate. The detected biodegradation byproduct, tri-butyl alcohol (TBA), can also be biodegraded by the indigenous microorganisms. In addition, microcosms with site groundwater as the medium solution show higher MTBE biodegradation rate. This indicates that the site groundwater might contain some trace minerals or organics, which could enhance the MTBE biodegradation. Results show that the addition of BTEX at low levels could also enhance the MTBE removal. No MTBE removal was detected in iron reducing and methanogenic microcosms. This might be due to the effects of low dissolved oxygen (approximately 0.3 mg/L) within the plume. The low iron reducers and methanogens ($< 1.8\times10^3$ cell/g of soil) observed in the aquifer also indicate that the iron reduction and methanogenesis are not the dominant biodegradation patterns in the contaminant plume. Results from the microcosm study reveal that preliminary laboratory study is required to determine the appropriate substrates and oxidation-reduction conditions to enhance the biodegradation of MTBE. Results suggest that in situ or on-site aerobic bioremediation using indigenous microorganisms would be a feasible technology to clean up this MTBE-contaminated site.

**Keywords** Intrinsic bioremediation · Groundwater · Microcosm · MTBE (methyl tert-butyl ether) · TBA (tri-butyl alcohol) · Refinery · Taiwan

## Introduction

Methyl tert-butyl ether (MTBE) has been used as a gasoline additive to improve combustion efficiency and to replace lead since 1978. MTBE is the most commonly used oxygenate now due to its low cost, convenience of transfer, and ease of blending and production. Currently, MTBE has become a prevalent groundwater contaminant because it is widely used and it has been disposed inappropriately. MTBE is a highly water-soluble compound and its biodegradation rate is low in many cases. Consequently, a MTBE plume typically results in longer remediation periods (Squillace et al. 1996; US EPA 1998; US EPA 1999; Fischer et al. 2004). Recent studies indicate that MTBE is a possible human carcinogen. Thus, the US Environmental Protection Agency (US EPA) has set its advisory level for drinking water at 20–40 μg/L (US EPA 1997).

MTBE may release to the environment from aboveground and underground storage tanks, gasoline pipelines and industrial wastewater. Moreover, recreational watercraft using MTBE-containing fuel is also one of MTBE releasing sources (US EPA 1997; Toran et al. 2003; Zuccarello et al. 2003).

Many physical and chemical technologies such as air sparging, chemical oxidation or pump and treat have been used to treat MTBE-contaminated groundwater (US EPA 1997; Sutherland et al. 2004). However, bioremediation is a more attractive remediation option because of its economic benefit. Recently, intrinsic bioremediation or natural bioremediation has been considered as one of the potential methods for the cleanup of petroleum-hydrocarbon contaminated sites. Compared to other traditional technologies, intrinsic bioremediation has a cost-effective advantage (Hooker and Skeen 1996; Suthersan 1997; Alexander 1999). If the intrinsic bioremediation rate is limited by in situ environmental factors (e.g., oxygen, nutrients and electron acceptors), enhanced in situ or on-site bioremediation can be applied to stimulate contaminants biodegradation (Borden et al. 1997; Van Cauwenberghe and Roote 1998; Bradley et al. 1999; Langwaldt and Puhakka 2000; Finneran and Lovley 2001; Kharoune et al. 2001). Therefore, biological systems, including intrinsic and enhanced approaches, offer the possibility of a cost-effective destruction technology for groundwater remediation (Mo et al. 1997; Somsamak et al. 2001; Fiorenza and Rifai 2003; Schirmer et al. 2003; Schmidt et al. 2004).

MTBE is biodegradable under both aerobic and anaerobic conditions. Nevertheless, rates of MTBE biodegradation under aerobic conditions are higher than those under anaerobic conditions (Deeb et al. 2000; Prince 2000; Kern et al. 2002; Wilson et al. 2002; Deeb et al. 2003; Moreels et al. 2004). Aerobic bioremediation generally proceeds more quickly than anaerobic bioremediation. Organic compounds are removed completely under aerobic biodegradation (Van Cauwenberghe and Roote 1998; Langwaldt and Puhakka 2000). Further degradation of MTBE results in the production of tri-butyl alcohol (TBA). TBA has been used as an indicator of MTBE biodegradation (Mo et al. 1997; Deeb et al. 2000; Finneran and Lovley 2001; Kharoune et al. 2001; Somsamak et al. 2001; Kern et al. 2002; Fiorenza and Rifai 2003; Schirmer et al. 2003; Moreels et al. 2004; Schmidt et al. 2004).

The main objectives of this study were to (1) evaluate the occurrence of intrinsic bioremediation of MTBE and BTEX (benzene, toluene, ethylbenzene and xylenes) at an oil-refining plant site through field investigation and microbial enumeration, (2) conduct microcosm experiments to evaluate the biodegradability of MTBE using indigenous microorganisms under aerobic, aerobic cometabolic, iron reducing and methanogenic conditions and (3) evaluate the feasibility of using alternative primary substrates (e.g., propane, ethanol and BTEX) to enhance the cometabolism of MTBE.

## Study site description

The selected site in this study is an oil-refining plant located in southern Taiwan. Main products manufactured by the plant are gasoline, diesel, jet fuel, kerosene and lubricating oils. For years, inappropriate operation and storage have resulted in groundwater contamination. Results from previous studies reveal that MTBE and total BTEX concentrations were approximately 200 mg/L in collected groundwater samples from the highly contaminated zone, respectively. In the downgradient monitoring well, MTBE and BTEX concentrations were approximately 10 and 1.2 mg/L in collected groundwater samples, respectively (CPC 2001).

Soils at the site consist of browny silty sand and clayey silt. A thickness of 5–10 m clay layer is located at 40 m below land surface. Depth of the seasonal high water table is 2–5 m and the seasonal low water table is 3–7 m. The average hydraulic conductivity of the host geological material is 0.1 cm/sec and the groundwater slope is approximately 0.25%. The groundwater flows at a velocity of 0.2–1.4 m/day from southwest to northeast. Figure 1 shows the contaminant source area, groundwater flow direction and the soil and groundwater sampling locations used in this study.

## Materials and methods

### Field investigation and microbial enumeration

Monitor wells MW1, MW2 and MW3, which were located near the source zone, downgradient area and background area, respectively, were selected as the

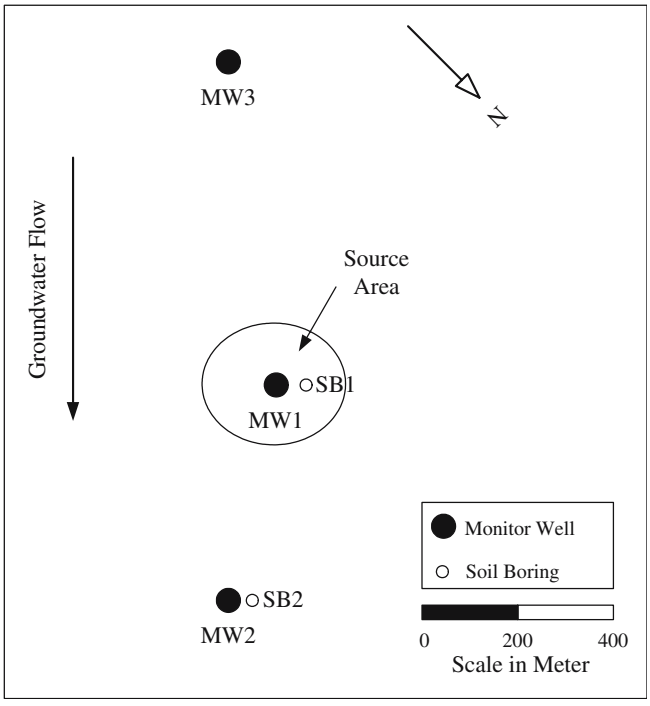


**Fig. 1** Site map showing the contaminant source area, groundwater flow direction and the soil and groundwater sampling locations

representative wells for the investigation of intrinsic bioremediation. During the investigation period of one and a half years, groundwater samples from the three monitor wells were collected and analyzed for organic compounds and geochemical indicators, including MTBE, BTEX, TBA, methane ($CH_4$), $CO_2$, inorganic nutrients, anions, pH, redox potential (Eh) and dissolved oxygen (DO). Organic compound analyses were performed in accordance with US EPA Method 502.2, using a Varian 3800 gas chromatograph (GC). Methane was analyzed on a Shimadzu GC-9A GC using headspace techniques. Ion chromatography (Dionex) was used for inorganic nutrients and anions ($NO_3^-$, $NO_2^-$, $SO_4^{-2}$ and $PO4^{-3}$) analyses. Total iron and ferrous iron were analyzed by Hach DR/400 spectrophotometer using EPA Method 8008 and Method 8146, respectively. DO, Eh, pH, $CO_2$ and temperature were measured in the field. Two MP120 pH/Eh meters (Mettler-Toledo) were used for pH and Eh measurements; a WTW DO meter (Oxi 330) was used for DO and temperature measurements and a Hach digital titrator cartridge was used for $CO_2$ measurements.

Aquifer sediments were collected from soil borings SB1 and SB2, which were located near MW1 and MW2, respectively (Fig. 1). Collected aquifer sediments were used for microbial enumeration and microcosm study. Microbial enumeration was performed to determine the number of total heterotrophs, total heterotrophic anaerobes (total anaerobes), iron reducers, sulfate reducers and methanogens. Total plate counts were conducted using plate count agar (Difco) to assess the approximate size of the total heterotrophic bacterial in soil samples SB1 and SB2 using the spread plate method (APHA 1995). Prepared plates were incubated at 30°C for 48 h, then counted for colony forming unit (CFU). The analytical methods for total heterotrophic anaerobes (total anaerobes), iron reducers and sulfate reducers are described by Kota (1998) and Kao and Wang (2000). Total anaerobes and methanogens were enumerated using five-tube MPN assay. The total anaerobe tubes contained media described by Kota (1998) and were score positive based on optical density. The methanogen tubes contained 20% $H_2$ and 80% $CO_2$ in the headspace and were score positive based on production of methane. Total iron reducers and sulfate reducers were determined by a ten-tube multiple probable number (MPN) assay using media individually selective for ferric iron reducers and sulfate reducers as described by Kao and Wang (2000).

## Microcosm study

Microcosm experiments were conducted to examine the feasibility of MTBE biodegradation under aerobic, aerobic cometabolic and anaerobic conditions. The inocula used in this microcosm study were aquifer sediments collected from SB1 and SB2. In the aerobic cometabolic microcosms, propane and ethanol were added as the primary substrates (or primary carbon sources). Because BTEX was also detected at this site, BTEX was also applied as the primary substrate for the cometabolic microcosms. Iron reducing and methanogenic microcosms were prepared in an anaerobic glovebox to preclude intrusion of oxygen. Hungate techniques were used to prepare anaerobic solutions (Kota 1998; Kao and Wang 2000). A redox indicator (0.0002% of resazurin) and reducing agent (1 mM of sodium sulfide) were added to each bottle. Sodium sulfide was chosen because it would not serve as a carbon source and it has a redox potential (−571 mV) low enough to completely reduce resazurin. In the iron reducing microcosms, amorphous Fe(III) oxide was used as the source of ferric iron (electron acceptor). The sodium sulfide was prepared by neutralizing 0.4 M of $FeCl_3$ solution with 1 M of NaOH and washing the suspension with deionized water to remove NaCl (Kota 1998; Kao and Wang 2000).

Each microcosm was constructed with 35 mL of mineral medium or in situ groundwater (collected from MW2), 20 g of sediments and 10 mg/L of MTBE in a 70-mL serum bottle sealed with thick butyl rubber septa. In the cometabolic microcosms, primary substrates (either 1 mL of propane, 10 mg/L of ethane or 1 mg/L of each BTEX compound, respectively) were added in the bottles. The mineral medium contained the specified concentrations (units are in grams per litter of water): $Na_2HPO_4$, 71; $KH_2PO_4$, 68;

**Table 1** Characteristics of microcosms

| Microcosm and control | Treatment | Inocula | Constituents |
|---|---|---|---|
| A1 and A1-C[a] | Aerobic biodegradation | Aquifer sediments (SB2) | Sediments (SB2) + mineral medium + MTBE |
| A2 and A2-C | Aerobic biodegradation | Aquifer sediments (SB2) | Sediments (SB2) + groundwater + MTBE |
| A3 and A3-C | Aerobic biodegradation | Aquifer sediments (SB1) | Sediments (SB1) + mineral medium + MTBE |
| B1 and B1-C | Cometabolism | Aquifer sediments (SB2) | Sediments (SB2) + mineral medium + MTBE + propane |
| B2 and B2-C | Cometabolism | Aquifer sediments (SB2) | Sediments (SB2) + mineral medium + MTBE + ethanol |
| B3 and B3-C | Cometabolism | Aquifer sediments (SB2) | Sediments (SB2) + mineral medium + MTBE + BTEX |
| C1 and B1-C | Iron reduction | Aquifer sediments (SB2) | Sediments (SB2) + mineral medium + MTBE + Fe(III) |
| D1 and D1-C | Methanogenesis | Aquifer sediments (SB2) | sediments (SB2) + mineral medium + MTBE |

[a]Control group, $HgCl_2$ + $NaN_3$ were added into each control microcosm

$(NH_4)_2SO_4$, 33; $CaCl_2 \bullet 2H_2O$, 3.3; $MgCl_2 \bullet 6H_2O$, 12.4; $FeSO_4 \bullet 7H_2O$, 5; $ZnSO_4 \bullet 7H_2O$, 4; $MnCl_2 \bullet 4H_2O$, 0.2; $CoCl_2 \bullet 6H_2O$, 0.5; $NiCl_2 \bullet 6H_2O$, 0.1; $H_3BO_3$, 0.15; EDTA, 2.5. The medium solution was autoclaved before use. Table 1 lists the components of each microcosm. The groundwater and aquifer sediments were purged with air before use. Control bottles containing 250 mg/L of $HgCl_2$ and 500 mg/L of $NaN_3$ and inocula, used for control groups, were autoclaved before use. Duplicate microcosms were scarified at each time point for the analysis of MTBE, BTEX and TBA during the 170-day incubation period.

## Results and discussion

### Field investigation and microbial enumeration

Groundwater samples were collected from two monitor wells MW1 and MW2, which were located near the spill location and downgradient area of the plume, respectively. Groundwater samples were analyzed for organic compounds and geochemical indicators to evaluate the occurrence of the intrinsic bioremediation of MTBE. Table 2 shows the results of groundwater analyses. Results are the averaged values of five sampling events during the investigation period of one and a half years. Aquifer sediments collected from soil borings SB1 and SB2 were used for microbial enumeration. Results of the bacterial population assessment are also shown in Table 2.

High $CO_2$ concentrations were observed in MW1 and MW2. This indicates that significant microbial activity and intrinsic bioremediation occurred in this area. Methane was also detected in MW1 and MW2, indicating that methanogenesis might also be occurring within the contaminated zone. Higher ferrous concentrations were detected in MW1 and MW2. Ferric iron might have been used as the electron acceptor during the low oxygen conditions. The low nitrate concentration

**Table 2** Field investigation results

| | MW1 | MW2 | SB1 | SB2 |
|---|---|---|---|---|
| Location | Source zone | Downgradient | –[a] | – |
| MTBE (mg/L) | 0.2[b] ± 0.05[c] | 0.075 ± 0.012 | – | – |
| TBA (mg/L) | 0.92 ± 0.23 | 0.01 ± 0.007 | – | – |
| Benzene (mg/L) | 116.68 ± 31.26 | 0.52 ± 0.28 | – | – |
| Toluene (mg/L) | 47.63 ± 17.45 | 0.30 ± 0.14 | – | – |
| Ethylbenzene (mg/L) | 10.14 ± 2.59 | 0.10 ± 0.07 | – | – |
| m, p-Xylene (mg/L) | 14.30 ± 3.77 | 0.16 ± 0.10 | – | – |
| o-Xylene (mg/L) | 8.93 ± 2.19 | 0.18 ± 0.12 | – | – |
| PH | 6.67 ± 0.31 | 7.00 ± 0.19 | – | – |
| DO (mg/L) | 0.36 ± 0.10 | 0.34 ± 0.13 | – | – |
| Redox potential (mV) | −84 ± 25 | −1 ± 31 | – | – |
| Carbon dioxide (mg/L) | 286 ± 73 | 102 ± 51 | – | – |
| Temperature (°C) | 29.0 ± 1.5 | 31.3 ± 1.4 | – | – |
| Nitrate (mg/L) | 0.56 ± 0.22 | 0.37 ± 0.18 | – | – |
| Total iron (mg/L) | 9.12 ± 2.0 | 3.46 ± 1.5 | – | – |
| Ferrous iron (mg/L) | 8.28 ± 3.7 | 3.28 ± 2.9 | – | – |
| Sulfate (mg/L) | 6.6 ± 1.8 | 27 ± 3.5 | – | – |
| Phosphate (mg/L) | 5.82 ± 1.77 | 7.02 ± 1.56 | – | – |
| Methane (mg/L) | 12.1 ± 3.2 | 46.2 ± 11.9 | – | – |
| Total heterotrophs (cell/g) | – | – | $1.2\times10^6$ | $3.0\times10^7$ |
| Iron reducers (cell/g) | – | – | $1.2\times10^2$ | $3.8\times10^2$ |
| Sulfate reducers (cell/g) | – | – | $4.4\times10^1$ | $1.3\times10^2$ |
| Methanogens (cell/g) | – | – | $8.0\times10^2$ | $1.8\times10^3$ |

[a]"-" Not available
[b]Arithmetic mean
[c]Standard deviation

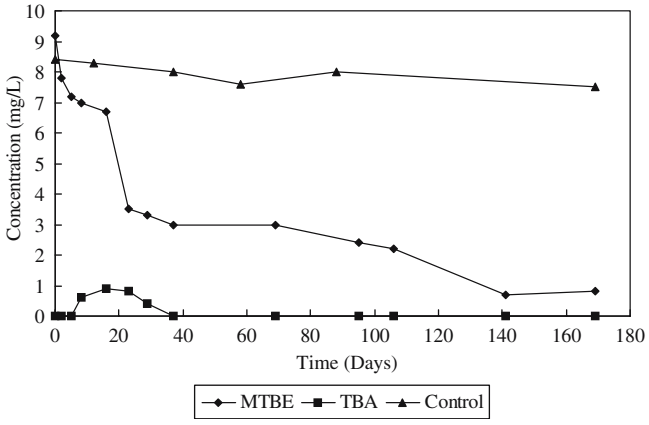


**Fig. 2** Results of MTBE biodegradation in Microcosm A1 under aerobic conditions

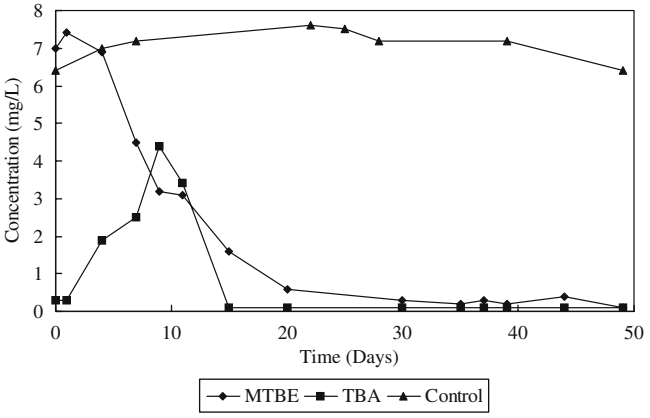


**Fig. 3** Results of MTBE biodegradation in Microcosm A2 under aerobic conditions

reveals that denitrification was not the dominant biodegradation pattern in the site. The decreases in MTBE and BTEX concentrations from MW1 to MW2 indicate the occurrence of natural attenuation processes. The detected biodegradation byproduct (TBA) also suggests the occurrence of MTBE biodegradation.

Results show that significant amount of total heterotrophs ($>10^6$ cell/g of soil) was detected in the collected SB1 and SB2 samples. Approximately, only $10^1$–$10^3$ of iron reducers, sulfate reducers and methanogens per g of soil were observed in SB1 and SB2. Iron reduction, sulfate reduction and methanogenesis might not be the dominant biodegradation processes around soil sampling locations. Thus, aerobic biodegradation process plays a more important role in MTBE removal.

## Microcosm study

Under aerobic conditions, a total of eight groups of microcosms were prepared including three groups of aerobic (A1–A3) microcosm, three groups of cometabolic microcosms (B1–B3), one group of iron reducing microcosm (C1) and one group of methanogenesis (D1) (Table 1). Figures 2–4 show the MTBE biodegradation results in Microcosms A1, A2 and A3, respectively. Because no extra carbon sources were added in Microcosm A1, results from A1 indicate that MTBE can serve as the sole carbon source (primary substrate) under aerobic conditions (Fig. 2). More than 90% of MTBE removal was observed in A1 after a 175-day incubation period. Therefore, the studied MTBE-contaminated site contained bacteria which can biodegrade MTBE without the supplemental second carbon source. MTBE was biodegraded completely in A2 within a 50-day incubation period (Fig. 3). In contrast to microcosms inoculated with mineral medium (A1), microcosms with site groundwater as the medium solution (A2) show higher MTBE biodegradation rate (Figs. 2, 3). Site groundwater might contain some trace minerals or organics which could enhance MTBE biodegradation. Less MTBE biodegradation rate was observed in microcosms inoculated with highly BTEX-contaminated sediments (SB1) (Fig. 4). The toxicity of BTEX in SB1 might inhibit microbial activity. Results from all aerobic microcosms reveal that MTBE was biodegraded by aquifer sediments and resulted in the formation of the detected biodegradation byproduct, TBA. Moreover, TBA can also be biodegraded completely by the indigenous microorganisms.

In Microcosm B1 and B2, propane and ethanol were added as the extra carbon sources, respectively. Results from B1 and B2 reveal that decrease in MTBE concentrations in B1 and B2 were not observed during the experimental period (data not shown). This might be due to the preferential utilization of more favorable carbon substrates by microorganisms. The presence of alternative carbon sources caused competitive inhibition of

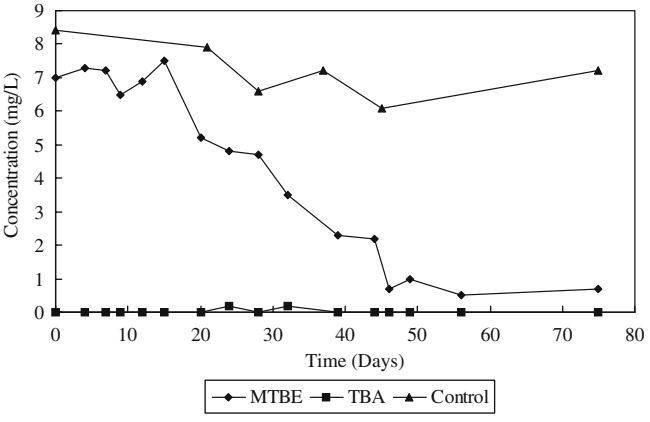


**Fig. 4** Results of MTBE biodegradation in Microcosm A3 under aerobic conditions

MTBE biodegradation (Bradley et al. 1999; Kern et al. 2002). Results also indicate that the cometabolism mechanism in microcosms with propane and ethanol addition was not effective enough to enhance MTBE degradation. Because BTEX was also detected at this site, BTEX was applied as the primary substrate in the cometabolic microcosms (B3) to evaluate BTEX-influence on MTBE biodegradation. Figure 5 shows the MTBE biodegradation results in Microcosms B3. The addition of BTEX enhanced MTBE removal (Fig. 5). This might be due to the cometabolic process causing the removal of MTBE cometabolicly. Furthermore, BTEX compounds were also completely depleted in this experiment within 25 days of incubation (data not shown). TBA was detected in the experiment and was degraded subsequently without accumulation. Results from cometabolic microcosm study reveal that the application of an appropriate primary substrate could significantly enhance the biodegradation rate of MTBE. However, no MTBE removal was observed in anaerobic microcosms prepared under iron reducing and methanogenic conditions during the 170-day incubation period. Because low DO (approximately 0.3 mg/L) was observed in monitor wells within the MTBE plume, this might cause a longer acclimation period to initiate MTBE biodegradation processes under anaerobic conditions. The limited populations of iron reducers and methanogens ($< 1.8\times10^3$ cell/g of soil) observed in the aquifer may also have caused insignificant anaerobic biodegradation.

## Conclusions

An oil-refining plant located in southern Taiwan was contaminated by MTBE and other components of fuel-oil. MTBE is one of the resistant compounds in the environment and a possible human carcinogen. In general, intrinsic biodegradation is an important process for contaminant mass reduction and plume containment. In this study, soil and groundwater samples collected from the MTBE-contaminated site were analyzed to assess the occurrence of intrinsic MTBE biodegradation. The field investigation results show that the intrinsic bioremediation mechanisms are occurring which cause the removal of MTBE and BTEX through mixed biodegradation processes. Evidences of intrinsic biodegradation include the following:

1. Depletion of DO within the plume
2. Production of dissolved iron, $CO_2$ and methane within the plume
3. Decreased MTBE and BTEX concentrations along the transport path
4. Production of MTBE degradation byproduct, TBA
5. Sufficient microorganisms in the subsurface.

Microcosm study was conducted to evaluate the feasibility of using bioremediation technology to clean-up MTBE-contaminated sites. Conclusions from the microcosm study include the following:

1. Indigenous microorganisms can biodegrade MTBE under both aerobic and aerobic cometabolism conditions.
2. MTBE could be used as the sole carbon source by the indigenous microorganisms.
3. Microcosms with in situ groundwater as the medium solution had a more effective MTBE biodegradation compared to the microcosms inoculated with the laboratory mineral medium. This indicates that the site groundwater might contain some trace minerals or organics which could enhance the MTBE biodegradation.
4. Microcosms inoculated with highly BTEX-contaminated sediments in the aerobic microcosm study had a less MTBE biodegradation rate probably due to the toxicity of BTEX.
5. Enhanced MTBE removal was observed in microcosms with low levels of BTEX added. However, microcosms with propane and ethanol addition could not enhance the degradation of MTBE. No MTBE removal was observed in anaerobic microcosms. Thus, preliminary laboratory study is required to determine the appropriate primary substrates and oxidation-reduction conditions to significantly enhance the biodegradation of MTBE.

Based on the results from the field investigation and laboratory microcosm studies, MTBE could be biodegraded by natural microbial populations at the studied site under both aerobic and cometabolic conditions. Results from this study could be useful in determining

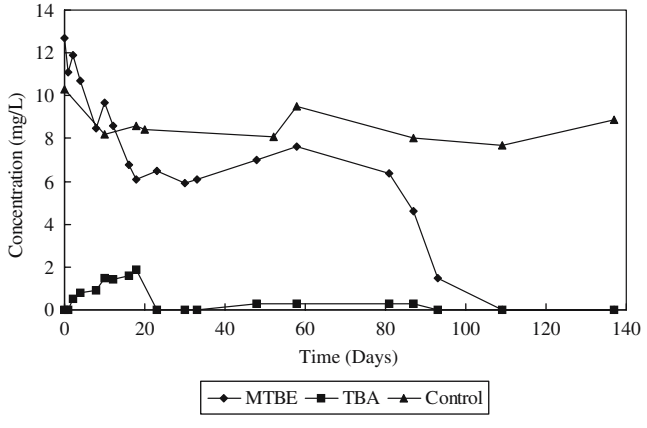


**Fig. 5** Results of MTBE biodegradation in Microcosm B3 under cometabolic aerobic conditions

favorable bioremediation conditions and designing an efficient and cost-effective bioremediation system, such as monitored natural attenuation or in situ or on-site MTBE bioremediation.

**Acknowledgments** This project was funded in part by National Science Council in Taiwan. Additional thanks to Professor S.C. Chen of National Kaohsiung Normal University and Professor C. K. Liu of National Sun Yat-Sen University for their support and assistance throughout this project.

## References

Alexander M (1999) Biodegradation and bioremediation, 2nd edn. Academic, San Diego, USA

APHA (American Public Health Association) (1995) Standard methods for the examination of water and wastewater, 19th edn. APHA-AWWA-WEF, Washington, DC, USA

Borden RC, Daniel RA, LeBrun LE IV (1997) Intrinsic biodegradation of MTBE and BTEX in a gasoline-contaminated aquifer. Water Resour Res 33:1105–1115

Bradley PM, Landmeyer JE, Chapelle FH (1999) Aerobic mineralization of MTBE and tert-butyl alcohol by stream-bed sediment microorganisms. Environ Sci Technol 33:1877–1879

CPC (Chinese Petroleum Corp) (2001) Cleanup fuel-oil-contaminated soil and groundwater using intrinsic bioremediation technology. Technical Report, Taiwan

Deeb RA, Scow KM, Alvarez-Cohen L (2000) Aerobic MTBE biodegradation: an examination of past studies, current challenges and future research directions. Biodegradation 11:171–186

Deeb RA, Chu KH, Shih T, Linder S, Suffet IH, Kavanaugh MC, Alvarez-Cohen L (2003) MTBE and other oxygenates: environmental sources, analysis, occurrence and treatment. Environ Eng Sci 20:433–447

Finneran KT, Lovley DR (2001) Anaerobic degradation of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA). Environ Sci Technol 35:1785–1790

Fiorenza S, Rifai HS (2003) Review of MTBE biodegradation and bioremediation. Bioremediation J 7:1–35

Fischer A, Müller M, Klasmeier J (2004) Determination of Henry's law constant for methyl *tert*-butyl ether (MTBE) at groundwater temperatures. Chemosphere 54:689–694

Hooker BS, Skeen RS (1996) Intrinsic bioremediation: an environmental restoration technology. Curr Opin Biotech 7:317–320

Kao CM, Wang CC (2000) Control of BTEX migration by intrinsic bioremediation at a gasoline spill site. Water Res 34:3413–3423

Kern EA, Veeh RH, Langner HW, Macur RE, Cunningham AB (2002) Characterization of methyl tert-butyl ether-degrading bacteria from a gasoline-contaminated aquifer. Bioremediation J 6:113–124

Kharoune H, Pauss A, Lebeault JM (2001) Aerobic biodegradation of an oxygenates mixture: ETBE, MTBE and TAME in an upflow fixed-bed reactor. Water Res 35:1665–1674

Kota S (1998) Biodegradation in contaminated aquifers: influence of microbial ecology and iron bioavailability. Ph.D. Dissertation, North Carolina State University, Raleigh, NC

Langwaldt JH, Puhakka JA (2000) On-site biological remediation of contaminated groundwater: a review. Environ Pollut 107:187–197

Mo K, Lora CO, Wanken AE, Javanmardian M, Yang X, Kulpa CF (1997) Biodegradation of methyl t-butyl ether by pure bacterial cultures. Appl Microbiol Biotechnol 47:69–72

Moreels D, Bastiaens L, Ollevier F, Merckx R, Diels L, Springael D (2004) Evaluation of the intrinsic methyl tert-butyl ether (MTBE) biodegradation potential of hydrocarbon contaminated subsurface soils in batch microcosm systems. FEMS Microbiol Ecol 49:121–128

Prince RC (2000) Biodegradation of methyl tert-butyl ether (MTBE) and other fuel oxygenates. Crit Rev Microbiol 26:163–178

Schirmer M, Butler BJ, Church CD, Barker JF, Nadarajah N (2003) Laboratory evidence of MTBE biodegradation in Borden aquifer material. J Contam Hydrol 60:229–249

Schmidt TC, Schirmer M, Weib H, Haderlein SB (2004) Microbial degradation of methyl tert-butyl ether and tert-butyl alcohol in the subsurface. J Contam Hydrol 70:173–203

Somsamak P, Cowan RM, Häggblom MM (2001) Anaerobic biotransformation of fuel oxygenates under sulfate-reducing conditions. FEMS Microbiol Ecol 37:259–264

Squillace PJ, Pankow JF, Korte NE, Zogorski JS (1996) Environmental behavior and fate of methyl tert-butyl ether (MTBE). Fact Sheet, FS-203-96, US Geological Survey (USGS)

Sutherland J, Adams C, Kekobad J (2004) Treatment of MTBE by air stripping, carbon adsorption, and advanced oxidation: Technical and economic comparison for five groundwaters. Water Res 38:193–205

Suthersan SS (1997) Remediation engineering: Design concepts. CRC/Lewis Publishers

Toran L, Lipka C, Baehr A, Reilly T, Baker R (2003) Seasonal and daily variations in concentrations of methyl-tertiary-butyl ether (MTBE) at Cranberry Lake, New Jersey. Water Res 37:3756–3766

US EPA (US Environmental Protection Agency) (1997) Drinking water advisory: consumer acceptability advice and health effects analysis on methyl tertiary-butyl ether (MTBE). EPA 822-F-97-009

US EPA (US Environmental Protection Agency) (1998) MTBE fact sheet #2, Remediation of MTBE contaminated soil and groundwater. EPA 510-F-97-015

US EPA (US Environmental Protection Agency) (1999) Achieving clean air and clean water: The report of the Blue Ribbon Panel on oxygenates in gasoline. EPA 420-R-99-021

Van Cauwenberghe L, Roote DS (1998) In situ bioremediation. TO-98-01, Ground-Water Remediation Technologies Analysis Center (GWRTAC)

Wilson RD, Mackay DM, Scow KM (2002) In situ MTBE biodegradation supported by diffusive oxygen release. Environ Sci Technol 36:190–199

Zuccarello JL, Ganske JA, Green DB (2003) Determination of MTBE in a recreational harbor using solid-phase microextraction. Chemosphere 51:805–810