MTBE Biodegradation in the Vadose Zone

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ABSTRACT: This study presents an effort to improve the knowledge of biodegradation of MTBE, a recalcitrant VOC, in the vadose zone, by providing a basis for discussing the extent to which MTBE biodegradation can be expected to occur in the vadose zone at LUST sites and potential degradation rates. Soil samples were collected from the vadose zone at five gas stations with gasoline spills containing MTBE, both at the hot-spots and at non-polluted reference locations at each site. The samples were included in a batch screening study, in which we investigated the aerobic MTBE degradation potential (primary and pseudo-cometabolic with addition of 5% propane). Primary MTBE degradation was found in hot-spot samples from four out of five sites, and in non-polluted reference samples from one out of five sites. Pseudo-cometabolic degradation (both primary and cometabolic with 5% propane added) was found in four out of five samples for both hot-spot and unpolluted reference samples. Primary degradation rates were found in the range of (1.order) 0.046– 1.1 d^{-1} and (0.order) 0.16–1.4 µg MTBE/g TS/d. Degradation rates with 5% propane were generally found to be 1.1–3.9 times higher than primary rates. However, samples from one site showed a 55% decrease in the degradation rates with propane added. Addition of toluene (10 mg/L) to hot-spot samples from one site was observed to have a stimulating effect on the primary MTBE degradation with a 10 fold rate increase. Toluene addition had no effect on the pseudo-cometabolic degradation of MTBE.

INTRODUCTION

Often, the effects of degradation of VOCs (e.g., BTEX) are observed at sites where we are conducting risk assessments for the indoor air. The effects of degradation are seen in the form of very low levels of benzene in the soil vapors only a short distance above quite concentrated source zones—levels that cannot be explained by physiochemical processes alone.

Although recent studies have addressed the issue of vadose zone degradation of BTEX in a mechanistic way (e.g., Pasteris et al., 2002; Höehner et al., 2003; and Kjeldsen et al., 2004), present day knowledge does not allow us to include degradation in our risk assessments in Denmark. Furthermore, little is known about degradation of more recalcitrant VOCs like MTBE in the vadose zone, and, according to the authors' knowledge, only one study has focused specifically on vadose zone degradation of MTBE (Moreels et al., 2004).

Hence, this study was initiated in an effort to improve the knowledge of biodegradation of MTBE in the vadose zone, by performing a batch screening study of soil samples from five gas stations in Northern Jutland, Denmark, where site investigations have revealed soil and/or groundwater pollution with gasoline and MTBE.

MATERIALS AND METHODS

Study Soils. Soil samples were collected from the vadose zone or the capillary fringe of five gas stations (labeled A–E) with gasoline spills containing MTBE. At each site, samples were collected from the hot-spot at leaking tanks, pipes or pumps, and from an unpolluted reference area nearby at approx. the same depth. Hence, a total of 10 soil samples have been screened for MTBE degradation potential. Soil samples from the hot-spots were sent for GC-analysis and various parameters were determined for soil characterization.

Table 1 shows the spill age at the five locations, estimated from historical information in the case file and the introduction of MTBE around 1985. Table 1 also shows contaminant concentrations for the study soils collected at the five hot-spots: benzene, toluene, ethylbenzene, xylenes, total hydrocarbon (THC), and MTBE. Due to a very inhomogeneous contaminant distribution, two samples were analyzed for soil B. As seen from the results, MTBE was only detected in one soil sample (at site E), which also has the highest general level of contamination. However, MTBE has been detected in groundwater - samples at all sites as late as 2005/2006.

TABLE 1. Contaminant concentrations for the five hot-spot samples.

Site	Spill age (years)	Depth (m)	BTEX (mg/kg TS)				THC (C6-C35) (mg/kg TS)	MTBE [GV] (mg/kg TS)
			Benzene	Toluene	Ethylbenzene	Xylenes		
A	6 – 22	1.0 – 3.0	0.79	10	2.9	27	950	<0.01
B	21	1.0 – 1.5	3.6 / 9.7	2.4 / 6.7	4.3 / 12	8.3 / 55	150 / 340	<0.2
C	10 – 22	ca. 1.5	4.2	3,8	10	50	460	<0.01
D	12 – 22	1.0 – 1.5	2.5	26	16	120	620	<0.01
E	6 – 22	0.5 – 1.0	54	350	91	440	2,600	0.53

GV = MTBE has been detected in groundwater samples at all sites in 2005/2006.

Table 2 shows the soil characterization parameters: pH, water content (w), fraction of organic matter (fom), and the particle size distribution (USDA) in the fractions clay (CL), silt (SI), fine sand (FS), medium sand (MS), coarse sand (CS), gravel and the nutrients ortho-phosphate and inorganic nitrogen (NO_2^-, NO_3^- and NH_4^+).

TABLE 2. Soil characterization parameters for the five hot-spot samples.

Site	Depth (m)	pH* –	w*	fom*	CL + SI	FS	MS	CS	Gravel	ortho-P*	inorg. N*
			(% wt.)		(% wt.)					(mg/kg TS)	
A	1.0–3.0	7.3	7.3	1.7	3.0	59.2	17.6	13.3	6.9	0.62	11
B	1.0–1.5	6.8	32	4.1	100	0.0	0.0	0.0	0.0	0.35	14
C	ca. 1.5	7.3	17	1.3	9.3	53.7	15.3	17.9	3.8	0.024	14
D	1.0–1.5	7.2	18	0.8	10.1	42.3	13.3	16.5	17.8	0.064	2.9
E	0.5–1.0	7.0	20	1.2	8.0	42.1	15.7	20.5	13.7	0.065	2.3

* = Analyses performed in triplicate (fom in duplicate); average shown.

Sample Preparation and Soil Characterization. The collected soil samples were thoroughly homogenized and sub samples were collected for determination of pH, soil water content, organic matter content, particle size distribution and nutrients (ortho-phosphate and inorganic nitrogen).

Soil pH was determined by adding 100 mL demineralized water to a soil sample of 20 grams of wet soil. After shaking for 1 hour and subsequent sedimentation, pH was measured with a pH-probe directly in the solution. pH was determined in duplicate.

Soil water content was determined by weighing and heating 100 grams of soil (at 105°C) until constant weight was achieved (~1-2 days). Soil water content was determined in triplicate. Organic matter content was determined in duplicate from weight loss by further heating (at 550°C) for 6-8 hours.

Soil particle distributions were determined by dry sieving (0.063 mm. 0.2489 mm. 0.5 mm and 2 mm) of approx. 100 gram soil samples.

Ortho-phosphate was determined by adding 100 mL 0.1 M H2SO4 to 20 grams of soil, shaking and subsequent spectrophotometric analysis of the extracted ortho-P by the molybdenum blue-ascorbic acid method. Nitrogen was determined by adding 50 mL 1 M KCl to 10 grams of soil, shaking and subsequent analysis of NH_4 and NO_2+NO_3 on a TRAACS autoanalyzer. Phosphate and nitrogen analyses were performed in triplicate.

Screening Study. For the screening study, the homogenized soils were left spread out for 2–3 days for most volatile compounds to evaporate. Subsequently, the soils were again homogenized and the new water content was determined on sub samples.

For each experiment (performed in duplicate) 50 grams of soil were placed in 580 mL serum bottles. Based on the amount of carbon (MTBE, propane and/or toluene) added to each bottle, an inorganic nutrient solution (NPK 23:3:7 with 50/50 NO_3^-/NH_4^+) was added to prevent nutrient limitation during degradation. Nutrients were added to ensure a C:N:P relationship of 100:20:2.6. The bottles were sealed with gas-tight rubber stoppers. The batch experiments were carried out at 23°C.

MTBE was added to all bottles from a stock solution (100 mg/L) to a final soil water concentration of approx. 10 mg/L. Propane was added to selected bottles as a 90% pure gas to a final gas phase concentration of 5% (vol.). Toluene was added to selected bottles from a stock solution (550 mg/L) to a final soil water concentration of 10 mg/L. Finally, the moisture content was adjusted by addition of demineralized water to the original soil water content (cf. Table 1).

MTBE, propane, and toluene were determined by head space analysis on a gas chromatograph (Chromopack 9000, WCOT CP-select 624-CB column, 30 m length, 0.53 mm i.d.). Headspace concentrations were converted to total concentrations by using Henry's constants for MTBE, propane and toluene of 0.022, 28.8 and 0.27, respectively. Adsorption was considered negligible for MTBE and propane, whereas adsorption of toluene was considered by assuming linear adsorption and a relationship between the fraction of organic carbon (foc) and organic matter (fom) of *foc = fom·0.58* and a K_{oc} of 92.9.

RESULTS

Primary Degradation. Figure 1 shows the results from an abiotic control and the screening results for primary MTBE degradation for the five hot-spot samples with fitted first order (K1) or zero order degradation rates (K0). Also, a zero order rate analysis was performed for all soils (results not shown).
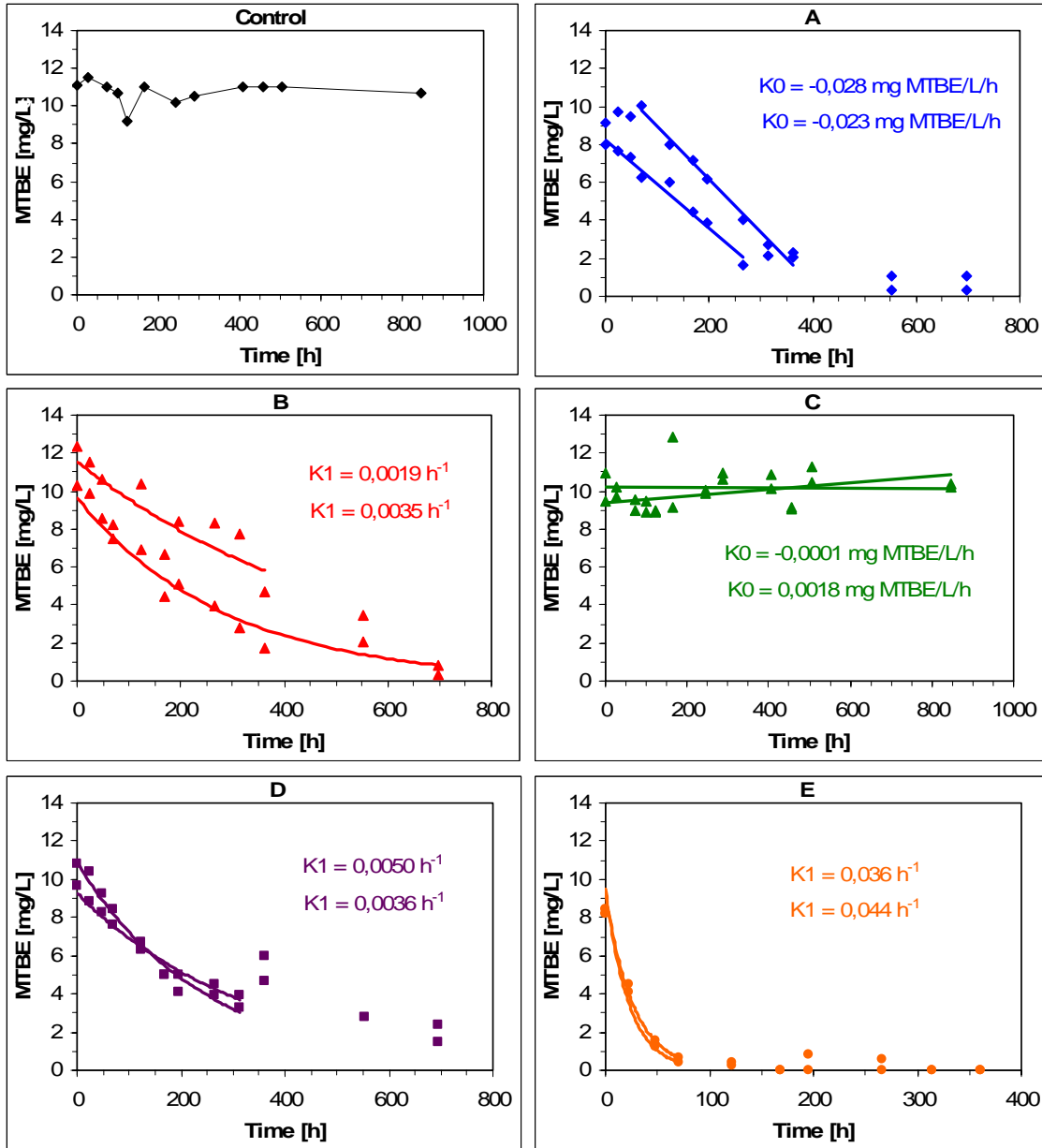
FIGURE 1. Primary MTBE degradation potential for five hot-spot samples, and fitted 1.order (K1) or 0.order (K0) degradation rates.

As shown in Figure 1, only the sample from site C did not exhibit any primary MTBE degradation, whereas the samples from all other sites exhibited varying degrees of primary degradation. In the samples from site B, D and E, degradation started immediately, and the degradation pattern could be fitted with 1.order degradation rates. Of the samples from site A, one replicate showed signs of a lag phase, whereas degradation started immediately in the other sample. Both replicate samples from site A exhibited 0.order behavior. Rates of duplicate batches varied with a factor of 1.2–1.8. For the unpolluted reference areas, only the sample from site E exhibited primary MTBE degradation potential (graph not shown).

The 1.order degradation rates (K1) of Figure 1 are summarized in Table 3 together with the fitted 0.order degradation rates (K0) and the results from the unpolluted reference sample from site E.

TABLE 3. Rates of primary MTBE degradation for hot-spot samples and for unpolluted reference (ref.) samples (site E only).

Site		1.order rate (K1)		0.order rate (K0)	
		(h^{-1})	(d^{-1})	(mg MTBE/L/h)	(µg MTBE/g TS/d)
hot-spot samples	A	not 1.order degradation		0.023	0.16
				0.028*	0.19*
	B	0.0019	0.046	0.015	0.24
		0.0035	0.084	0.023	0.37
	C	n.d.	n.d.	n.d.	n.d.
		n.d.	n.d.	n.d.	n.d.
	D	0.0050	0.12	0.035	0.29
		0.0036	0.086	0.025	0.20
	E	0.036	0.87	0.146	1.4
		0.044	1.1	0.146	1.3
ref	E	0.0012	0.029	0.020	0.18
		0.0021	0.049	0.028	0.22

* = Approx. 70 hours of lag time. n.d. = no degradation within 35 days.

For the hot-spot samples, 1.order MTBE degradation rates (K1) vary between 0.046 and 1.1 d^{-1}. The 0.order rates (K0) vary between 0.16 and 1.4 µg MTBE/g TS/d.

For the unpolluted reference areas, the degradation rates from site E are at the low end of the rates for the hot-spot samples, at (K1) 0.029-0.049 d^{-1} and (K0) 0.18-0.22 µg MTBE/g TS/d, respectively.

Toluene addition (10 mg/L) to two parallel hot-spot samples from site B showed an average 10 fold rate increase, cf. Figure 2.
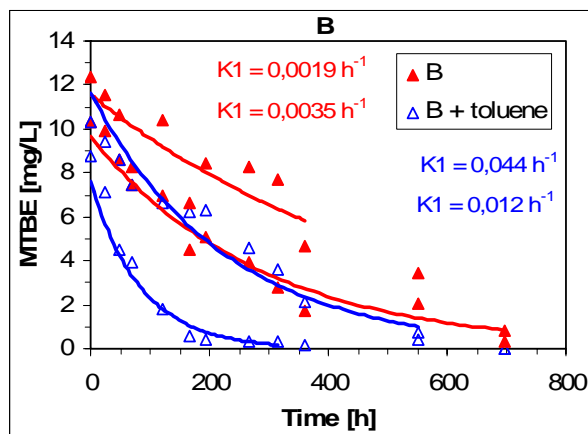

FIGURE 2. Results of toluene addition (10 mg/L) for site B hot-spot samples.

Pseudo-Cometabolic Degradation with Propane. Figure 3 shows the screening results of MTBE degradation (with addition of 5% vol. propane) for the five hot-spot samples with fitted zero order degradation rates (K0). It is important to keep in mind that the degradation patterns shown in Figure 3 could be a result of both cometabolic and primary degradation for the soils exhibiting primary MTBE degradation (site A, B, D, and E), hence the term "pseudo-cometabolic" degradation.

The pseudo-cometabolic data are not suited for 1.order rate analysis, since the degradation patterns are clearly not of 1.order. Also, the degradation patterns of the samples from three of the four sites (A, D and E) show signs of microbial growth; i.e., have a

lower degradation rate at first than at later times, which is not in accordance with 1.order theory. Hence, only zero order rates are shown and reported for these data.

The hot-spot samples from site C did not exhibit any MTBE degradation within the 35 day experiment. This was also seen in primary degradation experiment (Figure 1, site C). The other samples show MTBE degradation rates (K0) between 0.024–0.13 mg MTBE/L/h. For the unpolluted reference areas, only the sample from site A did not show any MTBE degradation potential (graphs not shown), and the samples from site C and D only showed MTBE degradation after a considerable lag/growth phase of approx. 8 days (195 hours).
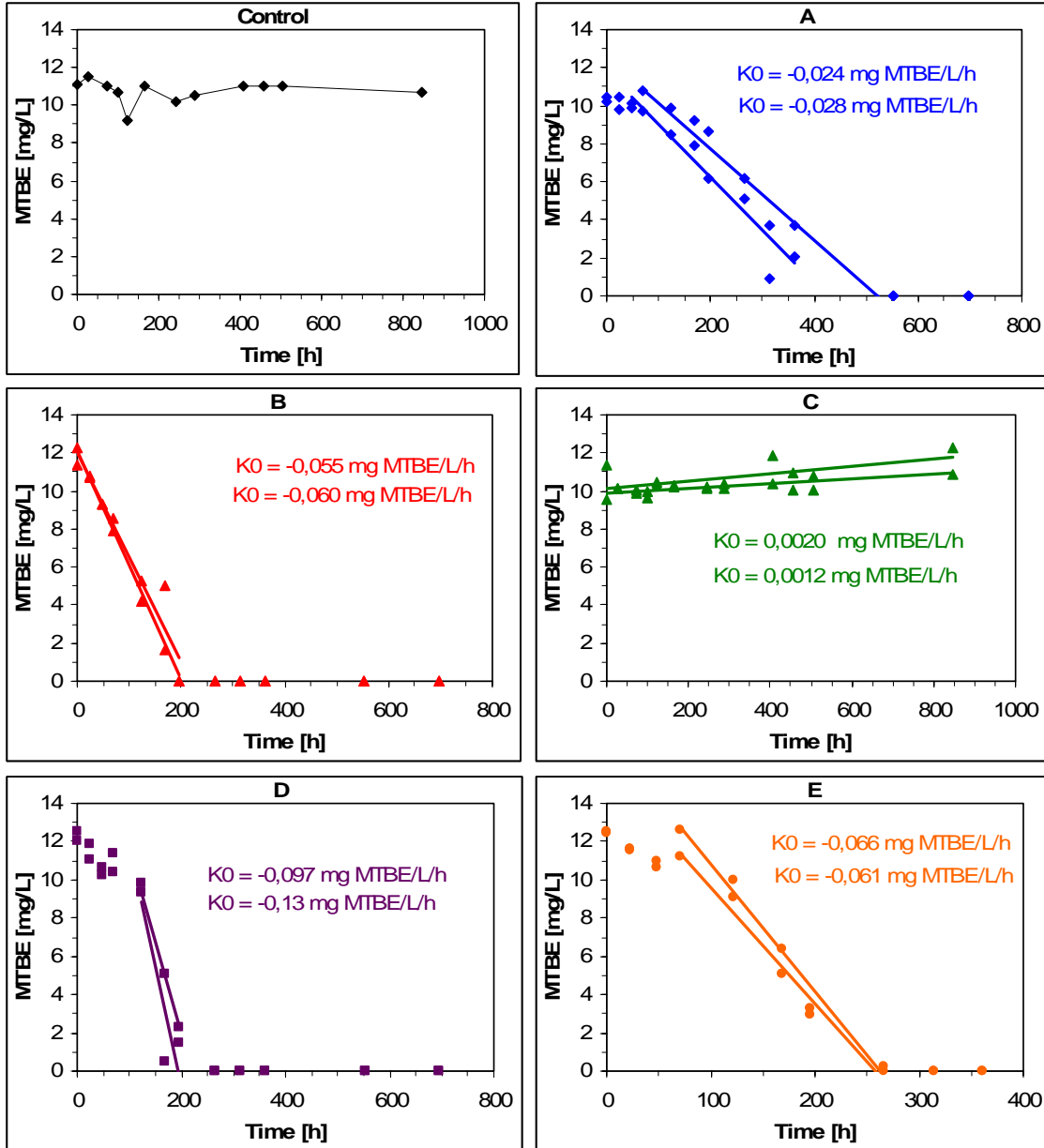

FIGURE 3. MTBE degradation potential with 5% propane for five hot-spot samples, and fitted 0.order (K0) degradation rates.

A visual comparison between the degradation patterns in Figures 1 and 3 indicate that propane addition, i.e., presence of an easily degradable primary substrate, might affect MTBE degradation in various ways. Hence, the degradation patterns for hot-spot samples for site A are almost identical, indicating an absence of cometabolic activity, whereas the overall degradation rate for the samples from site B is higher with propane addition. In contrast, MTBE degradation for the hot-spot samples from site E appears to be inhibited by propane addition. For site D, the results are mixed since there is no lag/growth phase associated with the primary degradation experiment, whereas the addition of 5% propane induces a lag/growth phase of approx. 3–5 days (70–123 hours,) where after MTBE is quickly degraded. The results from site D and E indicate that addition of 5% propane might inhibit the primary MTBE degraders.

The 0.order degradation rates (K0) of Figure 3 are summarized in Table 4 together with the fitted 0.order degradation rates for the unpolluted reference samples and observed lag/growth periods.

TABLE 4. Zero order MTBE degradation rates (K0) and lag/growth phases with 5% propane addition for both hot-spot samples and unpolluted reference samples.

Site	Hot-spot samples (K0)			Unpolluted reference samples (K0)		
	(mg MTBE/L/h)	(µg MTBE/g TS/d)	Lag (d)	(mg MTBE/L/h)	(µg MTBE/g TS/d)	Lag (d)
A	0.024	0.17	3	n.d.	n.d.	>35
	0.028	0.20	2	n.d.	n.d.	>35
B	0.055	0.88	0	0.056	0.78	1
	0.060	0.94	0	0.064	0.90	3
C	n.d.	n.d.	>35	0.056	0.55	11
	n.d.	n.d.	>35	0.043	0.41	11
D	0.097	0.81	3-5	0.086	0.92	8
	0.13	1.1	3-5	0.098	1.0	8
E	0.061	0.57	3-5	0.065	0.59	0
	0.066	0.61	3-5	0.071	0.72	2

The 0.order pseudo-cometabolic degradation rates (K0) for the hot-spot samples vary between 0.17 and 1.1 µg MTBE/g TS/d. Hence, they are of the same order of magnitude as the primary MTBE degradation rates shown in Table 3. The 0.order pseudo-cometabolic rates (K0) for the unpolluted reference samples range between 0.41-1.0 µg MTBE/g TS/d.

Addition of toluene (10 mg/L) to two parallel hot-spot samples from site B showed no effect of the toluene addition (results not shown).

DISCUSSION AND CONCLUSIONS

The results of the present study indicate that aerobic degradation of MTBE (primary as well as cometabolic) might be quite wide spread in vadose zone hot-spots at LUST sites. However, the present study was performed on soils collected at shallow depths (max 1.5 meters), where oxygen supply is not expected to have been limited by diffusion. Also, we chose to investigate sites with more than 6 year old spills, so that significant populations of slow growing MTBE degraders might have formed. Under these conditions, a significant primary MTBE degradation potential (not limited by oxygen or nutrient supply) was found at four out of five sites. The samples from the same four sites also

exhibited MTBE degradation with a 5% propane addition, although the effect of an easily degradable primary substrate had varying effects on the degradation rates. We found no MTBE degradation potential in the hot-spot samples collected at one of the sites (site C), even though this sample varies in no discernible way from the other sites with regard to a range of soil parameters (cf. Tables 1 and 2).

In a comparable study, Moreels et al. (2004) found MTBE degradation in soil samples collected at one out of four sites, and for this one site further investigated MTBE degradation at four depths, both with propane and benzene addition. In another similar study (although for groundwater sediments), Loll et al. (2003) found primary degradation potentials in two out of six sediments and a pseudo-cometabolic degradation potential (with propane) in five out of six sediments.

Moreels et al. (2004) found primary 1.order MTBE degradation rates (for one site; four depths) on the order of 0.004-0.097 d^{-1}, which is about 10 times lower than the rates for the hot-spot samples found in the current study (0.046 and 1.1 d^{-1}). Moreels et al. (2004) found rates with propane addition (5 mg/L, liquid) were between 5 and 10 times higher than primary rates, where we found rates on the same order of magnitude. Loll et al. (2003) found primary 0.order degradation rates of 0.10-1.8 µg MTBE/g TS/d and pseudo-cometabolic degradation rates of 0.35-2.8 µg MTBE/g TS/d. Hence, the primary degradation rates (0.16-1.4 µg MTBE/g TS/d) and pseudo-cometabolic rates (0.17-1.1 µg MTBE/g TS/d) found in this study are comparable.

For hot-spot samples from site B, toluene addition (10 mg/L) was observed to have a stimulating effect on the primary MTBE degradation with a 10 fold rate increase, and no effect on the pseudo-cometabolic degradation of MTBE. Moreels et al. (2004) found that benzene addition (5 mg/L) increased the MTBE degradation rates 2-9 fold in three out of four samples (one decreased by 50%). In the study of Loll et al. (2003) toluene addition (10 mg/L) inhibited primary MTBE degradation in groundwater sediment from site B.

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