TECHNISCHE UNIVERSITÄT DRESDEN

Jens Fahl, Helmut Lorbeer, Peter Werner, Dresden University of Technology, Institute of Waste Management and Contaminated Site Treatment, Pratzschwitzer Str. 15, D - 01796 Pirna, Germany

Report:

Characterisation of MTBE degradation pathways and capacities under groundwater conditions, elucidation of stimulating and inhibiting effects for BTEX and MTBE including favourable electron acceptors (WP 2, Deliverables No. 9, 16)

Content

Summary

This study investigates the degradation capacities of MTBE under groundwater conditions. Degradation experiments were performed with aquifer sediments from 13 different sites. Investigations concentrated on the search for favourable electron acceptors for the biodegradation process and metabolite formation. The enrichments were established under 6 different conditions promoting aerobic degradation, denitrification, sulphate reduction, Fe(III) reduction, Mn(IV) reduction and methanogenesis. In additional experiments, the MTBE degradation behaviour of groundwater samples from a contaminated aquifer at a refinery site was studied under aerobic and sulphate reducing conditions.

Several aerobic microcosms showed a significant decrease in MTBE concentrations, even after multiple readdition of MTBE. No accumulation of GC/FID detectable metabolites (TBA, TBF) was observed. The sediments of all these microcosms came from sites, which had been contaminated by MTBE. Sediments from completely unpolluted sites were unable to biodegrade MTBE in a time scale of 10 months.

Under anaerobic conditions no significant MTBE degradation was observed, in spite of the successful desired anaerobic milieu and ongoing electron acceptor decrease in most microcosms. Only some experiments under iron and sulphate reducing conditions seemed to show a MTBE degrading capacity with very low reaction velocities. It was clearly apparent that oxygen is the preferred electron acceptor for MTBE`s biodegradation.

Additional experiments showed the inhibiting effects of BTEX contaminations on MTBE biodegradation. BTEX, in comparison to MTBE, are more favoured and more rapidly used substrates for the investigated bacteria cultures. The “inhibition” appears as an effect of substrate preference causing consecutive degradation of BTEX and MTBE. Also, TBA was degraded faster by our mixed culture than MTBE; this observation explains why TBA does not accumulate during MTBE biodegradation in all of our experiments.

1. Introduction

In order to estimate the capacity of natural processes on pollutant biodegradation and retardation in laboratory experiments, it was necessary to obtain sediment samples from the groundwater-zone. In the groundwater sediments, a sufficient number of autochthonous bacteria are expected and retardation effects can be investigated only in original soil samples.

Due to the local variation of indigenous microorganisms, significant investigations of naturally occurring biodegradation requires the utilization of sediment samples from sites with different hydrogeological and geochemical conditions. Therefore the TUDRE.IAA group carried out soil sampling in December 2001/ January 2002 on 10 sites in East Germany, some of them with existing MTBE and BTEX contaminations. Soil (sediment) samples were taken from various depths – all in all 21 samples with 5-10 kg of material. They were stored in PolyPropylene-buckets at 4°C, most of them in oxygen-reduced atmosphere by using anaerobic-cartridges (Merck Anaerocult A). Additional sediment samples were collected in April 2002 from 3 sites in Düsseldorf, West Germany.

The MTBE biodegradation was studied under 6 different redox and electron acceptor conditions in these sediment samples: aerobic degradation, nitrate reduction, sulphate reduction, Fe(III) reduction, Mn(IV) reduction and methanogenesis.

Additionally, we investigated the degradation potential of a mixed bacterial culture, obtained from the most active aerobic sediment experiments. While increasing concentration of readded MTBE, we observed the metabolic pathways of the pollutant and monitored the formation and the accumulation of reported metabolites such as TBA (Tert-Butyl alcohol) and TBF (Tert-Butyl formate).

Further experiments should provide knowledge about the influence of typical co-contaminants of MTBE-plumes in groundwater – the BTEX compounds – on the biodegradation of MTBE. Gasoline consists of up to 50% (Vol.) BTEX [BIA REPORT, 1999]. Spills and leakages of gasoline produce a mixture of contamination in soil and groundwater of MTBE and BTEX. Several publications reported the inhibiting effects of BTEX [FAYOLLE ET AL, 2003, DA SILVA AND ALVAREZ, 2002], nevertheless it remained to be clarified whether the BTEX substances continuously inhibit MTBE biodegradation or if they are only preferentially degraded due to a better energy or metabolic efficiency of the BTEX biodegradation.

2. Materials and methods

2.1. Analytical methods

MTBE, BTEX and reported MTBE metabolites were detected by gas chromatography with a flame ionisation detector (GC / FID). Determination and quantification follow the laboratory procedure that TUDRE.IAA group developed in its first project year (deliverable 3, Methodology Report).

The analytical system based on a HEWLETT-PACKARD GC / FID 6890 connected to an autosampler PERKIN-ELMER HS40XL. The method is calibrated to measure concentrations of MTBE and BTEX in 10 ml water samples filled in 20 ml crimp sealed headspace vials. The required volume can be reduced to 1 ml (diluted with 9 ml purified water), if the pollutant concentrations exceed 50 – 100 µg/l. Detection limits in headspace samples were 4 µg/l for MTBE, 40 µg/l for TBA and 1 µg/l for the BTEX-Compounds (benzene, toluene, ethylbenzene, m- and p-xylene, o-xylene).

The anionic electron acceptor concentrations (NO_3^-, SO_4^{2-}) were monitored by ion chromatography (Metrohm IC-System 733) and the converted cationic electron acceptors (Fe^{2+}, Mn^{2+}) were measured via colour-complex-photometry (Photometer WTW MultiLab P5; photometric test kits Spectroquant and the iron test from MERCK). The formation of methane was measured via GC / FID, using the same system and method as described above (detection limit of dissolved methane: 1 µg/l).

2.2. Culture Media

Aerobic: (contains in g/l) NH_4Cl 0.25, Na_2HPO_4 2.8, KH_2PO_4 1.2, $MgSO_4 \times 7H_2O$ 0.2, $CaCl_2 \times 2H_2O$ 0.05; Wolfe´s Trace mineral Solution 10 ml/l (nitrilotriacetic acid 1.5, $MgSO_4 \times 7\ H_2O$ 3.0, $MnSO_4 \times 2H_2O$ 0.5, NaCl 1.0, $FeSO_4 \times 7H_2O$ 0.1, $CoSO_4$ 0.1, $CaCl_2 \times 2H_2O$ 0.1, $ZnSO_4$ 0.1, $CuSO_4 \times 5H_2O$ 0.01, $AlK(SO_4)_2$ 0.01, H_3BO_3 0.01, $Na_2MoO_4 \times 2H_2O$ 0.01); Wolfe´s vitamin Solution 10 ml/l ([contains in mg/l] biotin 2, folic acid 2, pyridoxine hydrochloride 10, thiamine hydrochloride 5, riboflavin 5, nicotinic acid 5, DL-calcium pantothenate 5, vitamin B_{12} 0.1, *p*-aminobenzoic acid 5, lipoic acid 5)

Nitrate reducing: (contains in g/l) NH_4Cl 0.25, NaH_2PO_4 0.6, $MgSO_4 \times 7H_2O$ 0.2, KCl 0.1, $CaCl_2 \times 2H_2O$ 0.05, $NaHCO_3$ 2.5; KNO_3 0.25 (Nitrate 5 mM); Wolfe´s trace mineral solution 10 ml/l; Wolfe´s vitamin solution 10 ml/l; na-acetate 5 µM; 100 µg/l yeast extract

Manganese reducing: (contains in g/l) NH_4Cl 0.25, NaH_2PO_4 0.6, KCl 0.1, $NaHCO_3$ 2.5; MnO_2-suspension 12.5 ml/l; Wolfe´s trace mineral solution 10 ml/l; Wolfe´s vitamin solution 10 ml/l; na-acetate 5 µM; 100 µg/l yeast extract, reducing agent solution A 10 ml/l (ascorbic acid 10 g/l oxygen free H_2O)

Iron reducing: (contains in g/l) NH_4Cl 0.25, NaH_2PO_4 0.6, $NaHCO_3$ 2.5; KCl 0.1; Fe(III)-OOH-suspension 4 ml/l; FeNTA-solution 10 ml/l ($NaHCO_3$ 16.4, nitrilotriacetic acid -Na_3 25.6, $FeCl_3 \times 6H_2O$ 27.0); Wolfe´s trace mineral Solution 10 ml/l; Wolfe´s vitamin solution 10ml/l; na-acetate 5 µM; 100 µg/l yeast extract; reducing agent solution A 10 ml/l

Sulfate reducing: (contains in g/l) NH_4Cl 0.25, KH_2PO_4 0.5, MgSO4 $\times$ $7H_2O$ 1, $CaSO_4$ 0.5; $FeSO_4$-solution 10 ml/l (0.5 g $FeSO_4 \times 7\ H_2O$ to 10 ml H_2O, acification with 1 ml 1 M H_2SO_4); resazurin-solution 1 ml/l (0,2%); Wolfe´s Trace mineral Solution 10 ml/l; Wolfe´s vitamin solution 10ml/l; na-acetate 5 µM; 100 µg/l yeast extract; reducing agent solution B (na-thioglycolate 100 mg, ascorbic acid 100 mg, na-dithionite 10 mg, oxygen free H_2O 10 ml)

Methanogenic: (contains in g/l) KH_2PO_4 0.3, $(NH_4)SO_4$ 0.3, NaCl 0.6, $MgSO_4 \times 7\ H_2O$ 0.13, $CaCl_2 \times 2\ H_2O$ 0.008, $FeSO_4 \times 7\ H_2O$ 0.002, K_2HPO_4 0.3; resazurin-solution 1 ml/l (0,2%); Wolfe´s trace mineral solution 10 ml/l; Wolfe´s vitamin Solution 10ml/l; na-acetate 5 µM; 100 µg/l yeast extract; reducing agent solution C (Cysteine-HCl $\times$ H_2O 0.5 g, $Na_2S \times 9\ H_2O$ 0.5 g, 1 M NaOH 1 ml, H_2O 19 ml)

2.3. Batch-tests set up

Because the scheduled anaerobic degradation experiments should have a defined low redox-potential - which is often found in a few zones in groundwater – some reducing agent (they react with remaining oxygen) had to be added. To avoid and preclude interactions between these reducing agents and MTBE, preliminary tests were performed on possible reactions between MTBE and na-thioglycolate, na-dithionite and ascorbic acid.

To obtain the data about MTBE degradation, 74 batch-tests were examined under aerobic and anaerobic conditions, with sediment samples from 13 different sites. All experimental vessels were stored in darkness at 20°C. The anaerobic experiments were performed in 250 ml-duran-bottles, sealed with screw-caps and butyl rubber / PTFE-septum (sampling with cannulas and syringes), filled with 100 g sediment and the special “nutrient”-medium optimised for the individual group of bacteria. Aerobic experiments were run in 500 ml-duran-bottles, only half filled providing oxygen-containing air volume. MTBE was added to all experiments in order to start concentrations of approx. 800-1000 µg/l. Each experiment ran parallel with a sterile (killed) control (indicated by suffix “St”). Sodium azide (NaN_3) was added to a concentration of 1 g/l as biostaticum. In most cases, the inhibited biological processes were reliable. After a few months, the biostatic effect diminished in several bottles and bioactivity also occurred in killed controls (see effect in Fig. 2, 3).

Additional experiments with groundwater samples were performed in 120 ml injection bottles, sealed with crimp seals and butyl rubber / PTFE-septum, the aerobic bottles filled with 20 ml groundwater and 40 ml aerobic-media (as described above), here no additional was MTBE added, and the sulphate reducing experiments with 30 ml groundwater and 90 ml sulphate reducer media (as described above), MTBE was added to initial concentration of 23 mg/l.

120 ml injection bottles were also used for the experiments performed to investigate inhibiting and stimulating effects of BTEX and added co-substrates on MTBE biodegradation. They were filled with 20 ml inoculum from a mixed culture enrichment, which originated from the most active aerobic batch test LA 4 O. We added 40 ml Aerobic-media (as described above), including amendments of toluene, ethylbenzene, TBA, or of co-substrates sodium acetate, sodium succinate or methanol. Each bottle contained MTBE in ranges of 15 mg/l and was accompanied by one replicate experimental bottle and one sterile control. The following experiments were investigated:

MTBE 1, MTBE 2, MTBE St	– only MTBE added (St = sterile control)
TBA 1, TBA 2, TBA St	– MTBE + TBA (C_{TBA}= 16 mg/l)
Tol 1, Tol 2, Tol St	– MTBE + Toluene ($C_{Toluene}$= 700 µg/l)
Eb 1, Eb 2, Eb St	– MTBE + Ethylbenzene ($C_{Ethylbenzene}$= 700 µg/l)
Suc 1, Suc 2, Suc St	– MTBE + Sodium succinate ($C_{Sodium\ succinate}$= 1 mg/l)
Ace 1, Ace 2, Ace St	– MTBE + Sodium acetate ($C_{Sodium\ acetate}$= 1 mg/l)
MeOH1, MeOH2, MeOH St	– MTBE + Methanol ($C_{Methanol}$= 1 mg/l)
BTEX 1, BTEX 2, BTEX St	– Toluene + Ethylbenzene ($C_{Tol.}$=4 mg/l, $C_{Ethylb.}$=7 mg/l)

3. Results

3.1. Degradation capacity and pathways under groundwater conditions

Aerobic experiments

During the first 6 – 8 months several aerobic microcosms (8 of 15 soil samples) showed a significant decrease in MTBE concentration. These 8 samples came from MTBE contaminated sites - 7 well-known sites with MTBE-plume, 1 close to a highway, where MTBE-influence is plausible.

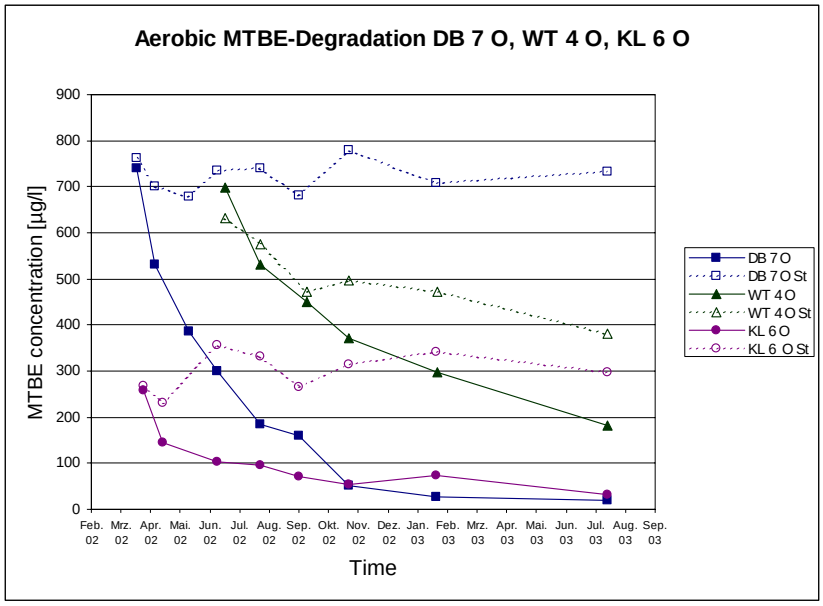

Fig. 1: Degradation of MTBE in 3 aerobic microcosms (with aquifer sediment) over 16 months

(St = sterile control)

None of the reported intermediates (TBA, TBF, formaldehyde, etc.) were detected in the aerobic microcosms. Sediments without MTBE pre-contamination were not able to biodegrade MTBE over a period of 10 months.

Very rapid MTBE decay was observed in a groundwater sediment sample taken from a refinery site. This site is characterised by existing MTBE contamination. The microbes in this LA 4 O batch test were able to degrade MTBE in less than 4 weeks down to below the detection limit (safe quantification above 4 µg/l, MTBE occurrence can be detected down to 0.8 µg/l). Re-addition of MTBE showed the same results. The re-added concentrations were gradually increased, finally up to 5300 µg/l. After 3 weeks, 99.6% of the MTBE had been degraded.

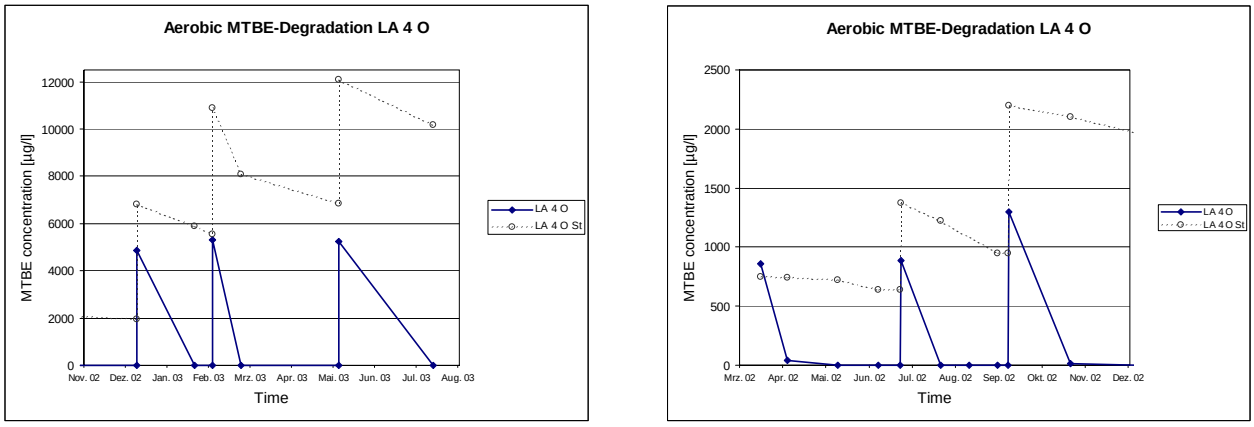

Fig. 2, 3: Diagram of MTBE-concentrations in the refinery sediment microcosm LA 4 O (St = sterile control).

In order to investigate the MTBE metabolism the isolation of MTBE-degrading strains from the most active aerobic experiment LA 4 O was started, by performing enrichments in separate batch cultures. The liquid batch tests without soil matrix showed rapid MTBE decay at the same rate. Re-added MTBE up to 38000 µg/l could be degraded (fig. 4).

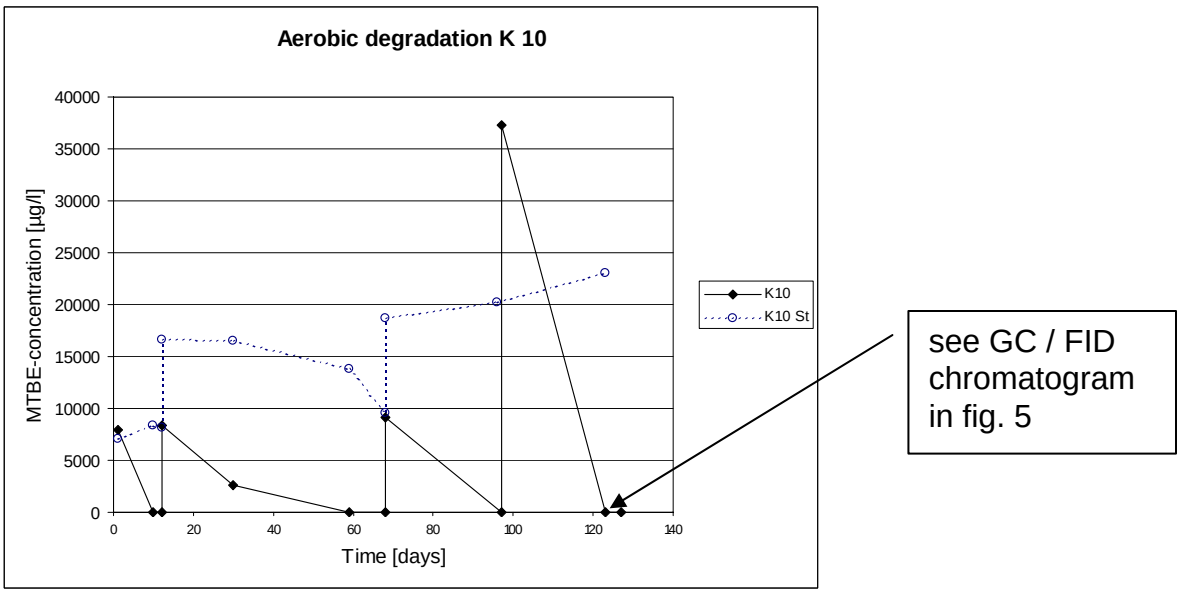

Fig. 4: Liquid batch test K 10 based on inoculum from LA 4 O microcosm

In all MTBE degrading aerobic microcosms and in the LA 4 O – daughter batch tests no accumulation of GC/FID detectable metabolites was found. Especially the bacteria consortium in the LA 4 O sediment seems to be able to mineralise MTBE completely.

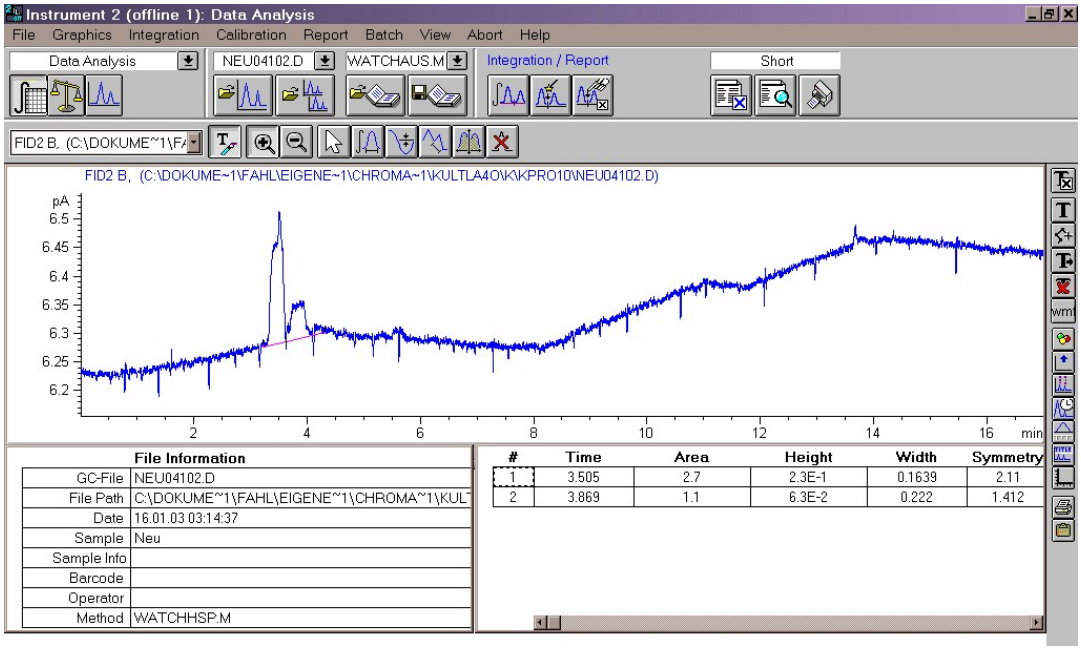

Fig. 5: GC/FID chromatogram of K 10 batch test after degradation of 38000 µg/l MTBE. The two peaks are: 3.5min capillary column and system break through peak (compounds without column retention) and the 3.87min peak, caused from the biostaticum Sodium azide, which we used for conservation after sampling to prevent further degradation in the headspace vessels. The most commonly reported metabolite TBA (retention time 4.93min, detection limit 40µg/l) was not detected. (retention times [min]: Acetone - 4,47; Isopropanol - 4,69; Formaldehyde - 4,85; TBA - 4.93; MTBE - 5.63; TBF - 6,8)

Groundwater samples collected in a contaminated aquifer near a second refinery site showed efficient MTBE degradation capacities. The groundwater of these wells had a MTBE contamination of 45 mg/l and no BTEX content. Initial MTBE concentrations of 13 mg/l in the batch experiments (after dilution with aerobic media) could be degraded completely within 2 months. Readded MTBE (up to 80 mg/l) was completely consumed in less than 2 weeks.

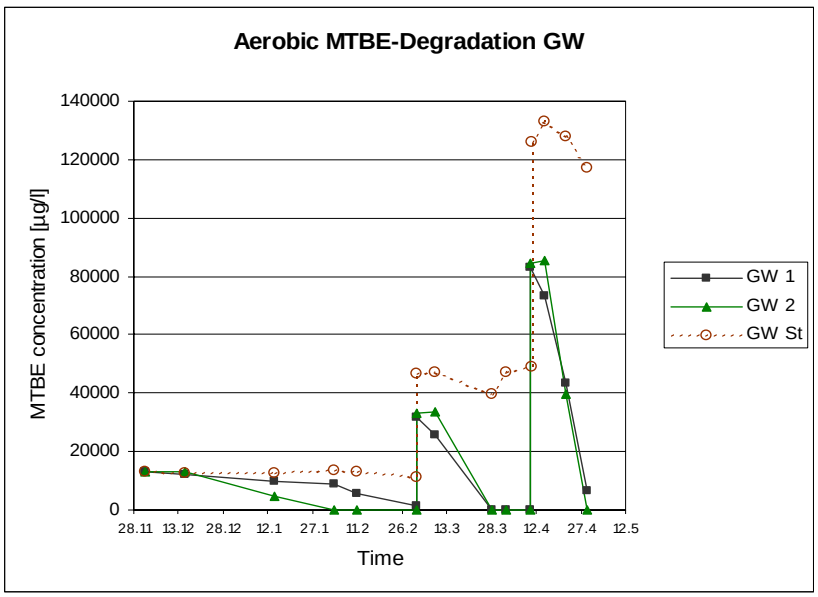

Fig.6: Refinery groundwater experiments GW 1, GW 2 (parallel control) and GW St (sterile control).

Anaerobic experiments

The initial electron acceptor concentrations amounted to 150 mg/l [NO_3^-], sulphate-reducing to 950 mg/l [SO_4^{2-}], Fe(III)-reducing to 280 mg/l [Fe^{3+}], Mn(IV)-reducing to 130 mg/l [Mn^{4+}] in the nitrate-reducing media and to 1300 mg/l [CO_2] in the methanogenic (CO_2-reducing) media.

Contrary to aerobic experiments, the anaerobic microcosms have shown no evidence of MTBE degradation for more than 10 months. In most cases the desired redox milieu was reached and ongoing electron acceptor decrease could be observed.

Slightly decreasing MTBE concentrations (max. 50% reduction) occurred in only a few microcosms with iron-reducing conditions (DL 5 F, RB 5 F, PY 2 F, WT 4 F) and, to a lower extent, in some sulphate-reducing microcosms (LW 5 S, DL 5 S). Traces of up-coming TBA [observ. C_{max} < 100 µg/l] do not correspond stoichiometrically with MTBE-decrease.

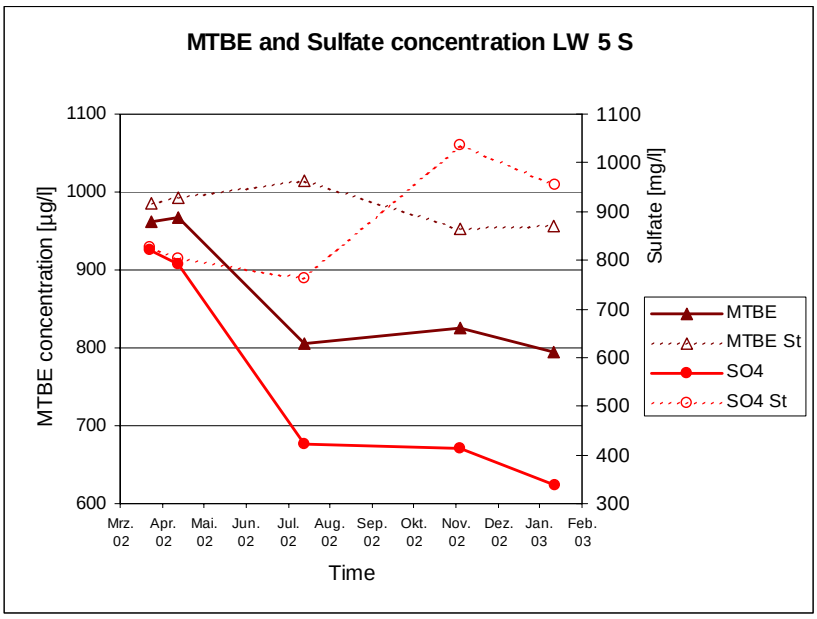

Fig. 7: Sulphate reducing microcosm LW 5 S, MTBE and sulphate concentrations (St = Sterile control)

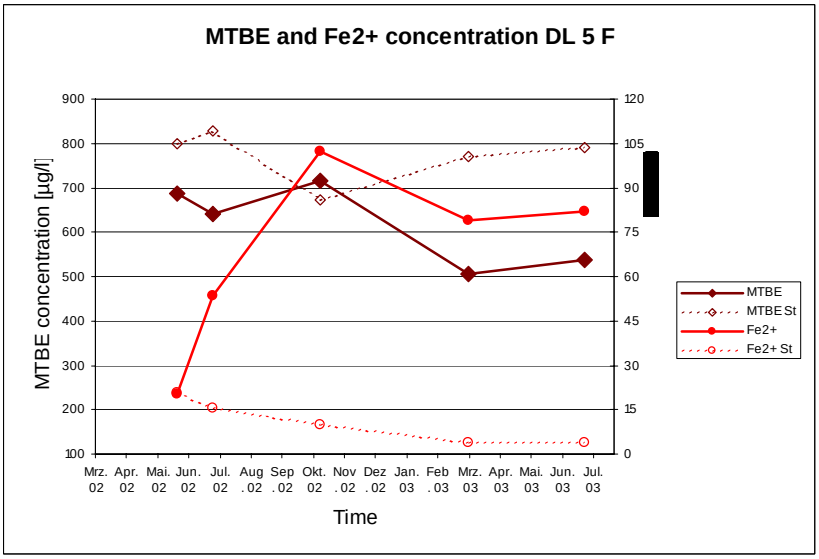

Fig. 8: Iron reducing microcosm DL 5 F, MTBE concentrations and formed Fe^{2+} (St = Sterile control)

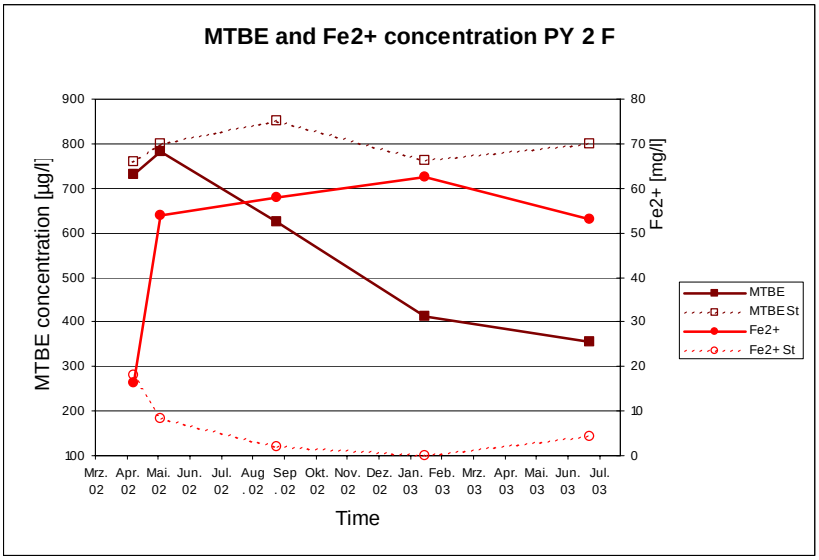

Fig. 9: Iron reducing microcosm PY 2 F, MTBE concentrations and formed Fe^{2+} (St = Sterile control)

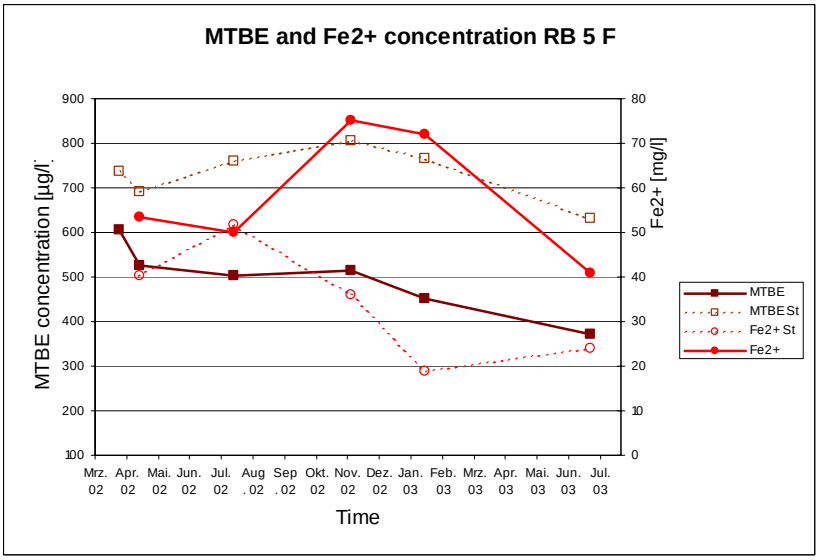

Fig. 10: Iron reducing microcosm RB 5 F, MTBE concentrations and formed Fe^{2+} (St = Sterile control)

In batch-tests with groundwater from the second refinery site, we were not able to find any MTBE degradation under sulphate reducing conditions over a period of 5 months. Influence of other electron acceptors has not been investigated until now. The groundwater of this well had contaminations of MTBE with 1700 µg/l and benzene with 1800 µg/l. After dilution with the sulphate-reducer media the benzene content amounted to initial concentrations of 250 µg/l, however the MTBE was spiked to initial values of around 22000 µg/l. The MTBE concentrations remained nearly constant for over 5 months of observation, but the benzene concentrations decreased to 40 µg/l in the active (not killed) bottles.

3.2. Stimulating and inhibiting effects on MTBE biodegradation

First signs of inhibiting effects of BTEX co-contaminations on MTBE degradation were observed in LW 5 O microcosm, a groundwater sediment sample from a highly contaminated refinery site. During the first 4 months, the BTEX concentrations decreased, MTBE degradation started only after disappearance of the BTEX compounds toluene and m-/p-

xylenes (fig. 6, fig. 7). The readdition of oxygen on 11. September 2002 could also be a reason for the beginning MTBE degradation, but the calculation of oxygen consumption during microbial BTEX degradation did not yield any lack of oxygen. (Initial BTEX-conc. were only in the µg/l- range)

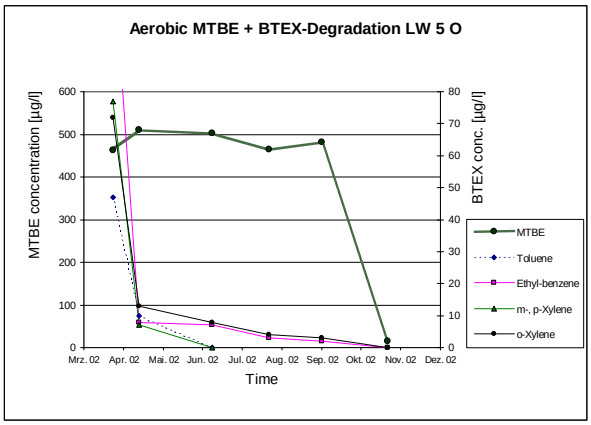

Fig. 11: Decay of BTEX contents in LW 5 O microcosm. (Initial Ethylbenzene conc. was 123 µg/l). Start of MTBE degradation after September 2002. (Readdition of Oxygen at 11. Sept. 2002)

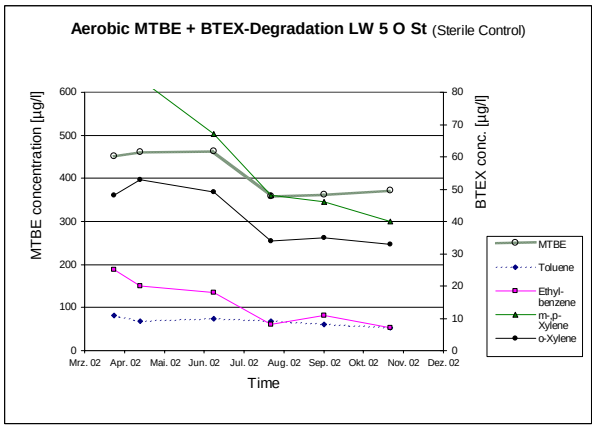

Fig. 12: Sterile control LW 5 O, BTEX and MTBE concentrations (Readdition of Oxygen at 11. Sept. 2002)

A second row of experiments concentrated on a comparison of MTBE biodegradation between batch tests containing different amendments of carbon sources.

Clarity was sought on inhibition caused by BTEX compounds and its sustainability/longevity and on the cometabolic MTBE degradation during a consumption of other organic Carbon sources. We tested the effect of the known, well degradable, co-substrates sodium acetate, sodium succinate and methanol on biodegradations velocity of MTBE. Several environmental pollutants which are not effectively useful as the sole C-source can vanish during a cometabolic degradation of microorganisms, which degrade other available primary substrates. The effect can be explained by an unspecific activity of enzymes, which are produced in the metabolism of the primary substrates. BTEX amended batch test should provide more detailed knowledge of toluene and ethylbenzene influences on the MTBE consumption by our mixed culture.

All amendments, except methanol, decelerated the degradation of MTBE significantly compared to the batch test without any addition of other C-sources.

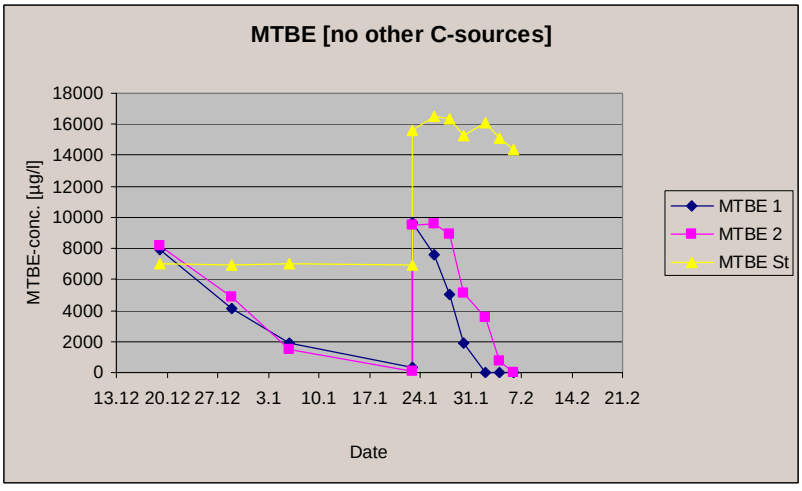

Fig. 13: Decrease of MTBE concentrations in batch test without other organic C-sources (St = Sterile control)

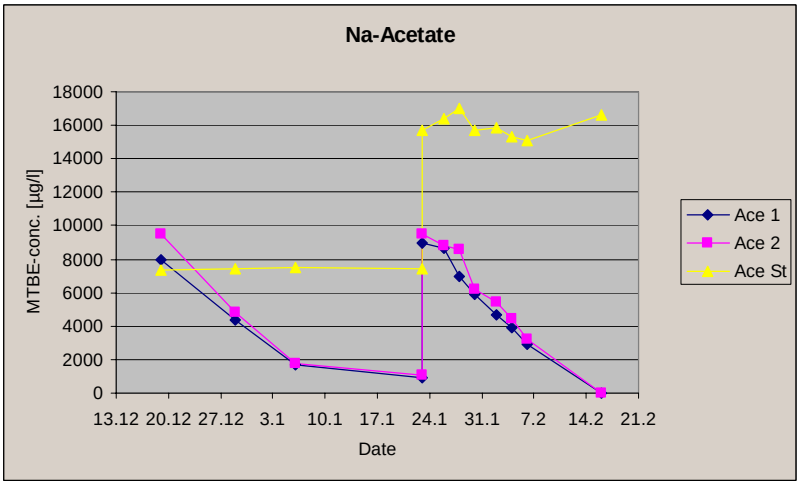

Fig. 14: MTBE concentration in batch tests with co-substrate Na-Acetate (St = Sterile control)

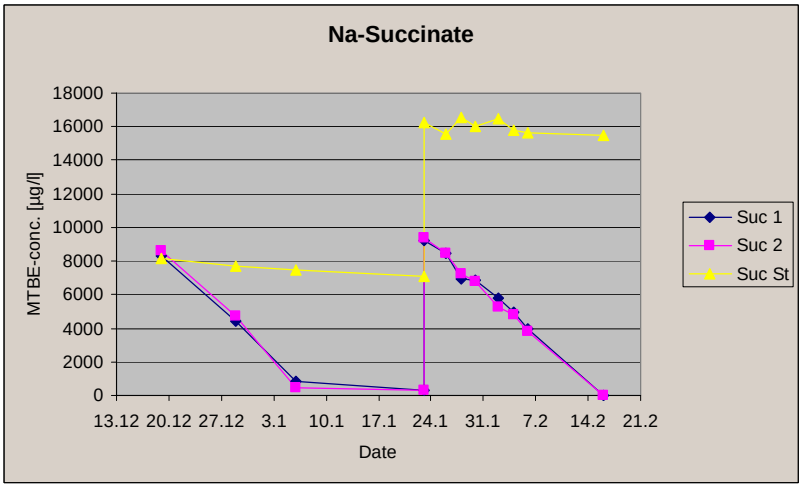

Fig. 15: MTBE concentration in batch tests with co-substrate Na-Succinate (St = Sterile control)

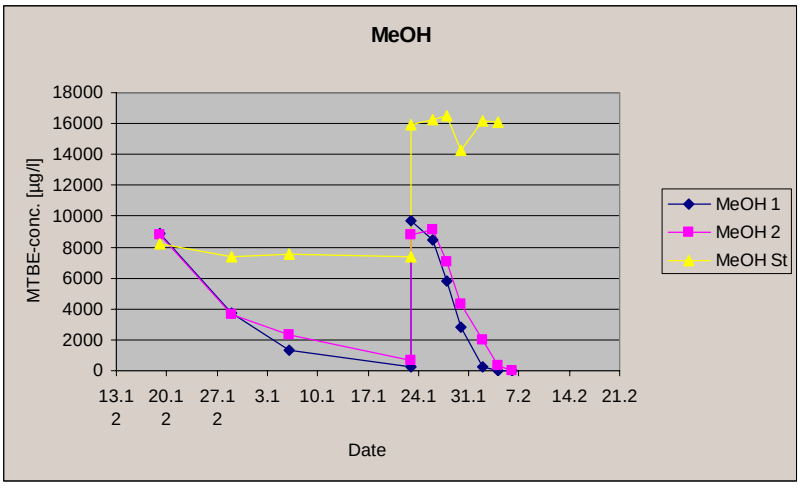

Fig. 16: MTBE concentration in batch tests with co-substrate Methanol (St = Sterile control)

Both BTEX compounds were degraded in less than 3 days to concentrations below detection limit (1 µg/l).

Date	Tol 1	Tol 2	Tol St
19.12.03	697	329	309
29.12.03	n.d.	n.d.	663
06.01.04	n.d.	n.d.	624
23.01.04	n.d.	n.d.	600
23.01.04	734	744	1226
26.01.04	n.d.	n.d.	1409
28.01.04	n.d.	n.d.	1249
30.01.04	n.d.	n.d.	1285
02.02.04	n.d.	n.d.	1380
04.02.04	n.d.	n.d.	1394

Tab. 1: Decay of Toluene in batch test Tol 1, 2 and St (St = Sterile control; Readdition of Toluene at 23. 01.2004)

Date	Eb 1	Eb 2	Eb St
19.12.03	573	615	510
29.12.03	n.d.	n.d.	581
06.01.04	n.d.	n.d.	554
23.01.04	n.d.	n.d.	530
23.01.04	655	674	893
26.01.04	1	n.d.	1272
28.01.04	n.d.	n.d.	1167
30.01.04	n.d.	n.d.	987
02.02.04	n.d.	n.d.	1160
04.02.04	n.d.	n.d.	1069

Tab. 2: : Decay of Ethylbenzene in batch test Eb 1, 2 and St (St = Sterile control; Readdition of Ethylbenzene at 23. 01.2004)

Date	BTEX 1		BTEX 2		BTEX St	
	C Tol.	C Ethylbenz.	C Tol.	C Ethylbenz.	C Tol.	C Ethylbenz.
19.12.03	2724	1855	7834	4712	5934	4534
29.12.03	n.d.	n.d.	n.d.	n.d.	6200	5950
06.01.04	n.d.	n.d.	n.d.	n.d.	5840	4800
23.01.04	n.d.	n.d.	n.d.	n.d.	4950	4700
23.01.04	3425	6079	3490	6307	8413	10206
26.01.04	304	1	48	n.d.	7918	9594
28.01.04	n.d.	n.d.	n.d.	n.d.	7233	8555
30.01.04	n.d.	n.d.	n.d.	n.d.	8537	10407
02.02.04	n.d.	n.d.	n.d.	n.d.	8716	10607
04.02.04	n.d.	n.d.	n.d.	n.d.	7640	9208

Tab. 3: Decay of toluene and ethylbenzene in batch test BTEX 1, 2 and St
(St = Sterile control; readdition of toluene on 23. 01.2004, n.d. = not detected, detection limit = 1 µg/l)

BTEX represent favoured and more useful substrates compared to MTBE for the investigated bacteria cultures. After the depletion of BTEX and a brief lag-phase of 3-5 days, MTBE could also be completely degraded. Toluene and ethylbenzene did not cause any permanent inhibition of MTBE biodegradation.

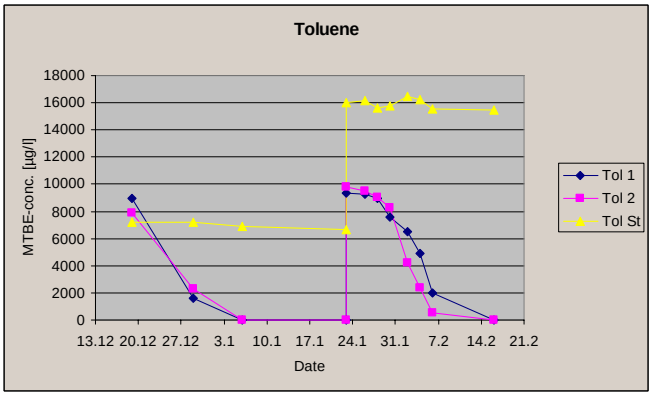

Fig. 17: MTBE concentration in toluene amended batch tests (St = Sterile control)

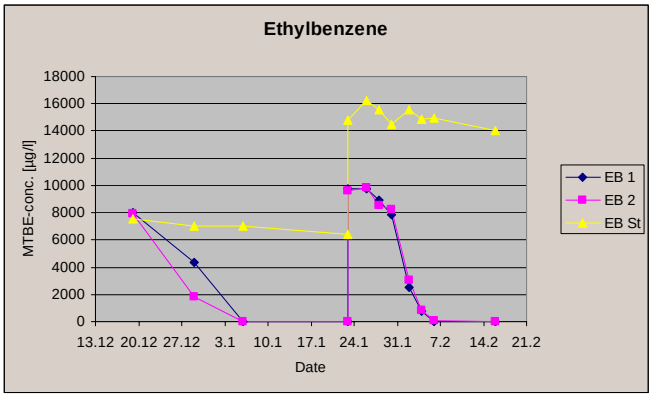

Fig. 18: MTBE concentration in ethylbenzene amended batch tests (St = Sterile control)

The mixed culture, which we used as inoculum for all these stimulation/inhibition experiments, was cultivated over a period of more than 20 months with MTBE as the sole carbon-source. It is not very probable, that many vitally and specialized BTEX-degraders should remained in that culture.

The use of monoaromatic hydrocarbons as energy- and C-source for microbial growth is a widespread capability in the natural environment [FRITSCHE, 1998]. The observed ability of our mixed culture to degrade BTEX-compounds immediately after the first supplement in December 2003 suggests that this degradation was brought about by MTBE-degrading microorganisms.

Also, TBA was degraded faster by our mixed culture than MTBE; this observation explains why TBA does not accumulate during MTBE biodegradation in all of our experiments.

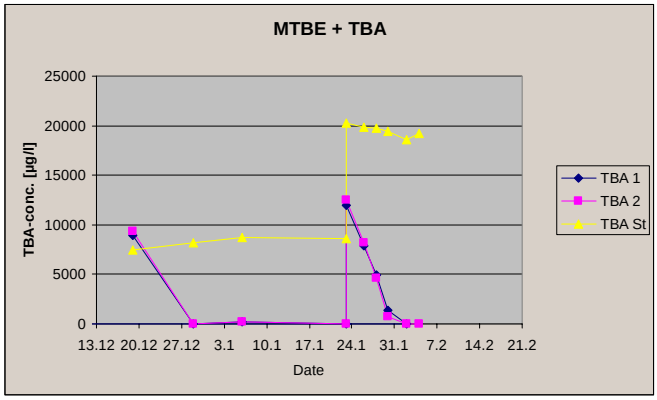

Fig. 19: TBA concentration in TBA amended batch tests (St = Sterile control)

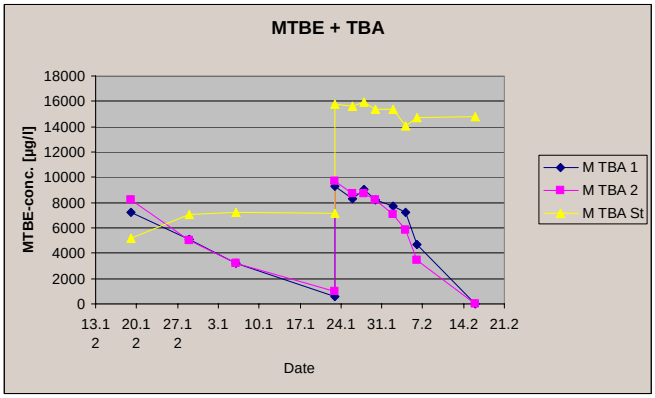

Fig. 20: MTBE concentration in TBA amended batch tests (St = Sterile control)

A comparison of MTBE concentration decreases in the different experiments is presented in fig. 21:

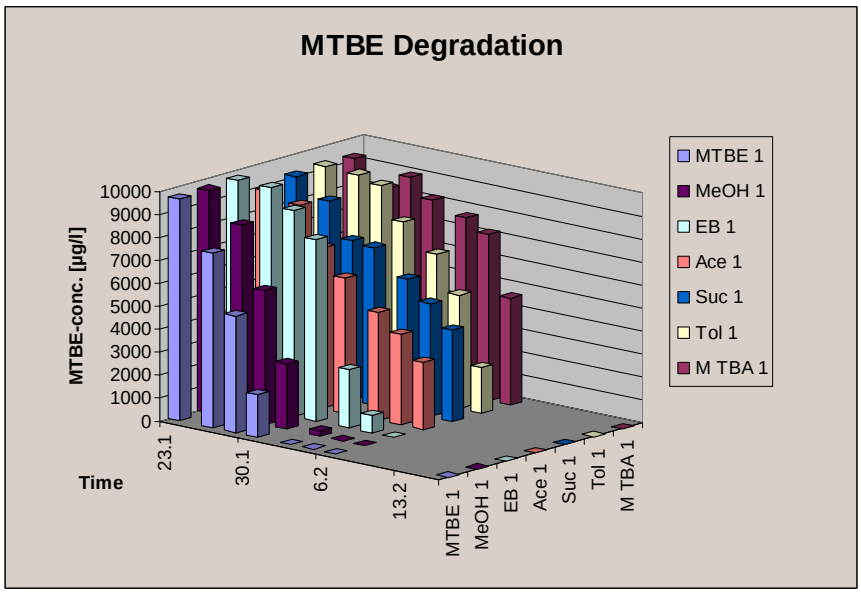

Fig. 21: Decrease of MTBE concentration in replicates 1 (values after readdition of compounds at 23.01.2004)

4. Discussion

A lot of environmental data shows the gasoline additive MTBE as a substance, which has the potential to seriously endanger the quality of groundwater reservoirs in subsurface sediments. The ether linkage and tertiary carbon structure are two features of MTBE that render it relatively recalcitrant to microbial degradation . Experimental results of the WATCH project Work package 2 confirmed the recalcitrant and persistent character of MTBE under groundwater conditions. An efficient natural attenuation of this hazardous compound on each site cannot be expected.

Physical data, like the high water solubility (up to 60 g/l) and the HENRY-constant (0,02 – dimensionless form, $C_{MTBE\ air}$ / $C_{MTBE\ water}$, at 20 °C) [FISCHER ET AL 2004, CALLENDER ET AL 2001] predetermine an enrichment of MTBE in groundwater. The effect is intensified by the low adsorption affinity of MTBE in the soil matrix [SUFFET 2001].

Literature and scientific reports demonstrate, that only under the presence of oxygen (aerobic conditions) the biodegradation can reduce MTBE pollutions significantly [EUROPEAN UNION 2002, FAHL ET AL 2003, MORMILE ET AL 1994, SCHIRMER ET AL 1998]. But our experiments provided evidence of a non-ubiquitous distribution of the aerobic degradation potential. A presence of a potently MTBE degradation was only observed in aerobic microcosms with sediments from MTBE contaminated sites. Populations of adapted microorganisms, which have the ability to biodegrade MTBE, can be expected there.

The aerobic experiments didn't show any accumulation of metabolites such as TBA and TBF.

Results of our anaerobic experiments are in accordance with literature, where only a few hints of anaerobic MTBE degradation with low decay rates were reported. LOVLEY (2001) found some evidence for iron-reducing conditions. He reported experiments based on incubated aquifer sediments, where he found MTBE reduction from 80 mg/l to contents below the detection limit (1mg/l) in 60 days, but only in bottles amended with humic acids (HS). Bottles without HS amendments did not degrade MTBE over a period of 275 days. SOMSAMAK ET AL. (2001) observed a complete MTBE depletion under sulphate-reducing conditions – but combined with a stoichiometric formation of TBA. The enrichments were incubated for 1,160 days with initial concentrations of 88 mg/l. Further investigations with the same enrichments supplied degradation times from 130 days. MORMILE ET AL. saw a decrease of MTBE from 48 mg/l to 22 mg/l after 157 days in one of three methanogenic replicate experiments.

Investigation of competitive and inhibiting effects of BTEX co-contaminations on MTBE biodegradation provided data about the consecutive degradation of BTEX and MTBE and the preference of the BTEX compounds toluene and ethylbenzene as substrates by our mixed bacteria culture. A biodegradation of MTBE is not very probable if BTEX are present in the MTBE contaminated groundwater plume.

Several authors [BORDEN ET AL 1997, STRAND 2001] reported significantly longer plumes of MTBE than BTEX plumes in the field.

A result of all the attributes, which have been described about MTBE and the facts presented in this paper should lead to greater awareness of MTBE as a serious threat to ground and drinking water.

5. Literature

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