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Long term studies on the anaerobic biodegradability of MTBE and other gasoline ethers

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ABSTRACT

Anaerobic biodegradation of methyl *tert*-butyl ether (MTBE) using electron acceptors such as nitrate, Fe(III), sulfate and bicarbonate, may be more cost effective and feasible compared to aerobic treatment methods, for dealing with the MTBE problem. Currently, there are a few reports in the literature which have documented anaerobic biodegradation of MTBE in batch studies. However, some of the reports have been controversial, additionally many other studies have failed to document anaerobic biodegradation. Experiments were conducted over a long term period in both batch and continuous reactors to investigate the anaerobic biodegradability of MTBE and other gasoline ethers. Inoculums collected from various environments were used, along with different electron acceptors. Only one set of the batch experiments showed a 30–60% conversion of MTBE to *tert*-butyl alcohol under Fe(III)-reducing conditions, using complexed Fe(III). The use of complexed Fe(III) created an initial low pH of 1–2 in these batches due to its acidic nature, therefore, the removal may be due to acid hydrolysis rather than biological processes. Based on the findings obtained, caution should be applied in the interpretation of experimental data in which complexed Fe(III) is used for bioremediation of MTBE.

1. Introduction

Methyl *tert*-butyl ether (MTBE) was developed in the 1970s as an octane enhancer to replace toxic additives such as lead in gasoline [1]. Since the 1990s many brands of gasoline sold in Europe and the United States contained MTBE, used both for octane and oxygen enhancement. Its use as an oxygenate results in a cleaner burning fuel with reduced ozone forming smog, carbon monoxide, particulates, unburnt hydrocarbons as well as other toxic air pollutants. In Europe, the typical content in gasoline is 1–5% (v/v); however, it may be as high as 15% in some countries [2,3]. MTBE producers predict its use will remain stable [4].

Due to the widespread usage of MTBE, and its mobility and persistence, it has become an important contaminant in groundwater. The most severe forms of MTBE contamination of groundwater occur through leaking underground storage tanks and by accidental releases [5,6]. Its presence in drinking water causes tastes and odor problems, and it can be detected at concentrations as low as 2 μg/L [7]. In the state of California, USA, a drinking water guideline limit of 5 μg/L has been set [8]. In Denmark, the guideline limit has also been set at 5 μg/L, but preferable below 2 μg/L [9]. The application of biodegradation is considered an important option for removing this contaminant from groundwater.

Currently, there are numerous studies on the aerobic biodegradation of MTBE [10–13]. Comparatively, documentation of positive results on anaerobic biodegradation has been rather sketchy. Some of the results purporting anaerobic degradation have even been controversial [14]. Table 1 shows a summary of major reports so far on the removal of MTBE under anaerobic conditions in batch studies. The list illustrates that there have been reports of degradation under the most common terminal electron acceptors found in anaerobic groundwater. The removal rates are shown to be mostly $\ll$1 mg/(L d), these are very low compared to mineralization rates for aerobic degradation in reactors, which are in the range of 500–1500 mg/(L d) [10,15–17].

Anaerobic degradation of MTBE still remains an important challenge, which will require considerable research in order to be considered as a remediation option for contaminated groundwater. Its observation is rather a rarity than a norm; there are several studies that have documented no degradation under anaerobic conditions using different electron acceptors [18–21]. In many of the reports on anaerobic MTBE degradation the percentage removed was low.

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Table 1
Summary of the major reports of MTBE degradation under anaerobic conditions in batch reactors

Inoculum	Redox	Initial con. (mg/L)	Final con. (mg/L)	Rate (mg/(L d))	Lag time (days)	Ref.
Fuel impacted river sediment	HCO_3^-	48	22	0.51	152	[19]
Petroleum impacted aquifer	HCO_3^-	1	0.1	0.003	175	[26]
Surface water sediments (oasis)	SO_4^{2-}	1.5	1.38	0.00072		[27]
Petroleum impacted estuary	SO_4^{2-}	100	0	0.8	1160	[20]
Fe(III)-reducing reactor	Fe(III)	5	0	0.012	3	[28]
Surface water sediments (oasis)	Fe(III)	1.5	1.32	0.0011		[27]
Petroleum impacted aquifer	Fe(III)/HS[a]	50	5	1.13	275	[29]
Surface water sediments (oasis)	Mn(IV)	1.5	1.08	0.0025		[27]
Surface water sediments (oasis)	NO_3^-	1.5	0.525	0.006		[27]
Petroleum impacted stream	NO_3^-	1.76	1.32	0.006		[30]

[a] Humic substances.

The degradation was also mostly partial, with *tert*-butyl alcohol (TBA) being the dead end metabolite [22,23]. TBA is considered just as undesirable in groundwater as MTBE [14].

Anaerobic bioremediation of MTBE either under in situ conditions in the subsurface or in engineered reactors could be the most convenient method of removing it from groundwater. Gasoline impacted plumes are normally anaerobic, since aerobic degradation process in the aquifer quickly depletes the oxygen present [24]. Adding oxygen to groundwater can be expensive; in contrast, the electron acceptors used under anaerobic conditions are often already present. For example, ammonium and Fe(II) oxidized in aquifers, whether naturally or by engineered remediation activities, could become available for denitrification and Fe(III) reduction, with MTBE as the electron donor. Furthermore, electron acceptors such as nitrate and sulfate, which have very high solubilities, can be easily injected into aquifers to promote anaerobic MTBE removal. For these reasons, research into this topic is worthwhile conducting.

The ethers ethyl *tert*-butyl ether (ETBE), diisopropyl ether (DIPE) and *tert*-amyl methyl ether (TAME) can all be used as substitutes for MTBE [1]; they also have a similar fate and behavior in the environment. Kharoune et al. [25] and a preliminary undocumented study by us have shown that under aerobic conditions all ethers tested can be degraded. The degradation rates are in the following order: ETBE > MTBE, TAME > DIPE. Due to the similar chemical and physical properties of these ethers with MTBE they have also been included in the anaerobic degradation studies.

Both batch and continuous experiments were carried out to investigate the anaerobic biodegradability of the ethers MTBE, ETBE, DIPE and TAME. The experiments were conducted using inoculums from various sources with the likelihood of containing ether degraders, and by using different terminal electron acceptors.

2. Materials and methods

2.1. Analytical methods and chemicals

The analysis of sulfate and nitrate were conducted using Spectroquant® measuring kits (Merck, Germany) and a spectrophotometer (Spectroquant® NOVA 60). Methane was measured by gas chromatography (GC) with flame ionization detector (GC–FID) (Shimadzu GC-14A; Koyoto, Japan). The pH was measured using electrodes (WTW, Germany). MTBE, ETBE, DIPE, TAME and TBA were measured using the Purge and Trap method. A Tekmar LSC 2000 instrument coupled to a Shimadzu GC 14B instrument with flame ionization detector was used, according to US EPA method 5030C [31]. The GC was initially set to 40 °C, and ramped at 10 °C/min to 140 °C. The detector was set at 340 °C, and nitrogen was used as the carrier gas, set to 50 KPa. The GC was fitted with an Agilent Technologies HP-5 column of length 50 m, internal diameter 0.2 mm, and film thickness of 0.11 μm. Samples were normally stored at −18 °C, prior to analysis. Chemicals used were purchased from Merck (Darmstadt, Germany) or Sigma–Aldrich.

2.2. Batch reactors setup

Batch experiments were conducted using 200 mL serum vials capped with 1 cm thick butyl rubber septum and aluminum crimped caps. The liquid content of most of the batches were made with a nutrient media containing trace elements, vitamins, reductants and $NaHCO_3$ [32]. Only normal tap water (non-chlorinated) from Lyngby near Copenhagen, Denmark, was used in preparing two sets of the batches under Fe(III)-reducing conditions, which are discussed. Batches were inoculated with different types of inoculums (Table 2). Generally, the volume of the innoculum was one third to one half of the volume of the entire liquid. The vials were

Table 2
Inoculum source and type for batch experiments

Inoculum source	Inculum type	Comments
Petroleum refinery (Kalundborg, Denmark)	Primary sludge	Samples obtained from the primary pond at the wastewater treatment plant (WWTP). The pond had a depth of ca. 2 m, retention time of about 10 years and was anaerobic.
	Secondary sludge	Samples collected from an activated sludge unit down stream of the primary pond; the biomass retention time was about 9–10 months. This unit may have been anaerobic in some sections.
	Contaminated soil	Sampled at a depth of 10 cm from a field used to landfarm oily sludges which were removed from the primary pond at the WWTP.
Biogas plant	Digested manure	Samples obtained from a farm anaerobic digester in Jutland, Denmark.
Upflow anaerobic sludge bed reactor (UASB)	Granular sludge	Samples obtained from an industrial UASB reactor treating the effluent from a paper mill.
Forest	Manure	Samples obtained from a deer park in Lyngby, Denmark.
Membrane bioreactor	Biomass	Samples obtained from an MTBE degrading Fe(III)-reducing reactor [28]. The samples were a gift from Amy Pruden at the Colorado State University, Department of Civil Engineering, Colorado, USA.
Packed bed reactor	Biofilm	Samples obtained from a reactor fed with MTBE under aerobic conditions for over 3 years.

Table 3a
Results: summary of the investigations conducted on the anaerobic degradation of ethers in batch reactors

Inoculum source	Inoculum type	Incubation time	Oxygenate(s)	Initial con. (mg/L)	Electron acceptor	Rep.	Temp. (°C)	Degr.	Status summary
Petroleum refinery	P.S., S.S., K.S.	2.5 years	MTBE, ETBE	50, T	NO_3^-, SO_4^{2-}, HCO_3^-	3* (A), 3* (K)	15	No	Significant removal of NO_3^-, SO_4^{2-} and production of CH_4 in respective batches
Petroleum refinery	K.S.	2.5 years	MTBE, ETBE, DIPE	50, T	Fe(III)/HS	3 (A), 3 (K)	15	No	Significant reduction of Fe(III) to Fe(II) in active batches
Packed bed reactor	biofilm sample	2 months	MTBE	100	NO_3^-	6 (A), 4 (K)	10	No	Insignificant removal of NO_3^-
Packed bed reactor	Biofilm sample	2 months	MTBE	50	Fe(III)	4 (A), 4 (K)	10	Yes	Low pH of 1–2 in (active) batches; ≈ 30% MTBE removal, and TBA production
Membrane bioreactor	Biomass sample	2 months	MTBE	50	Fe(III)	4 (A), 4 (K)	10	Yes	Low pH of 1–2 in (active) batches; ≈ 60% MTBE removal, and TBA production
Forest	Manure	2 years	MTBE, ETBE	200, S	HCO_3^-	9 (A), 4 (K)	37	Yes	Significant production of CH_4 in active batches
UASB Reactor	G.S.	2.5 years	MTBE, DIPE, ETBE	250, S	HCO_3^-	3 (A), 3 (K)	37	No	Significant production of CH_4 in active batches
Biogas plant	Digested manure	2 years	MTBE, DIPE, ETBE	50, T	HCO_3^-	3 (A), 3 (K)	37	No	Significant production of CH_4 in active batches

Degr.: Degradation; G.S.: granular sludge; K.S.: Kalundborg soil; P.S.: primary sludge; Rep.: replicates; S.S.: secondary sludge; Initial con. 50, T: ethers spiked together at 50 mg/L each; 200, S: –ethers spiked in separate vials at 200 mg/L; A: active batch; K: killed batch; 3*: 3 batches per electron acceptor condition.

typically filled to two thirds of the total volume. Anaerobic conditions were ensured by gassing each vial for 20 min with an 80:20 nitrogen and carbon dioxide mix. Resazurin was used as the redox indicator in all batch experiments. The ethers were spiked through the septum of the vials from concentrated stock solutions. For batches set up under nitrate and sulfate-reducing conditions, KNO_3 and Na_2SO_4 were used. Complexed $FeCl_3{\cdot}4H_2O$ was used for Fe(III)-reducing conditions. The humic substance (HS) added to some of the Fe(III)-reducing batches was 9, 10 antraquinone, 2, 6 disulfonic acid. The initial concentrations of the ethers in the batches ranged from 50 to 200 mg/L. Whenever depletion of the electron acceptors occurred they were constantly re-spiked. The killed control batches were prepared similar to the active batches, except, 4 g/L sodium azide was added. The batches were incubated at 10–37 °C. The inoculum sources and types are shown in Table 2. Further descriptions of the different setups are shown in Section 3. Analysis of the batches was done by withdrawing a mixed sample using a glass syringe through the septum of the vials.

2.3. Continuous reactors setup

2.3.1. Upflow anaerobic sludge bed reactor

The upflow anaerobic sludge reactor (UASB) used was constructed of glass and had a volume of 200 mL. The internal diameter was 4 cm and the height was 16 cm. This reactor has been described in more detail elsewhere [33]. The reactors were operated with a hydraulic retention time of 2 days, an influent flow rate of 100 mL/day and a recycle ratio of 5. The influent concentration of ethers in the feed stock solution was 10 mg/L for each of the 4 ethers: MTBE, ETBE, DIPE and TAME. The stock solutions were prepared from a nutrient media containing trace elements, vitamins, reductants and $NaHCO_3$ [32]. In order to maintain low redox conditions in the reactors a gasbag containing 80:20 nitrogen and carbon dioxide mix was attached to its feed bottles. Four reactors were operated simultaneously as follows: (1) killed control reactor operated under methanogenic conditions with 4 g/L sodium azide added to its stock solution; (2) a reactor operated under strict methanogenic conditions; (3) a reactor operated under sulfate-reducing conditions by addition of K_2SO_4 to its stock solution and; (4) a reactor operated under nitrate/Fe(III)-reducing conditions by the addition of complexed $Fe(NO_3)_3{\cdot}9H_2O$ to its nutrient solution. All electron acceptors were supplied 3 times in excess of the stoichiometric quantity required for total mineralization of the influent ethers. The reactors were seeded with granular sludge obtained from an industrial UASB reactor, used for treating the effluent from a paper mill. Reactors were operated at 18 °C and at a pH of 7–8.

2.3.2. Packed bed reactor

The packed bed reactor (PBR) used was constructed of Plexiglas®, with a diameter of 0.11 m and a depth of 1 m. It was fully packed with an expanded clay material (Filtralite® MC 2.5–4; Optiroc Rælingen, Norway) with an effective particle diameter of 2.6 ± 0.15 mm. The retention time was 23 min, determined by tracer studies. Biomass inoculation was accomplished by adding MTBE degraders originally obtained from an MTBE contaminated aquifer in Leuna, Germany [34]. The reactor used in this work was similar to the one described in a previous study [35]. In addition, it was connected in series to the outlet of the previously described reactor. The influent of the reactor used for the anaerobic degradability studies contained no dissolved oxygen since this was totally consumed in the first serial reactor. Typically, 5 mg/L MTBE, 1.5 mg N/L nitrate and 13–14 mg/L sulfate was present at the inlet. It was normally operated at 18 °C and at a pH of 7–8.

Table 3b
Results: summary of the investigations conducted on the anaerobic degradation of ethers in continuous reactors

Reactor	Inoculum type	Operation time	Oxygenate(s)	Inlet con. (mg/L)	Electron acceptor	Rep.	Temp. (°C)	Degr.	Status
UASB	G.S.	2 months	MTBE, DIPE, ETBE, TAME	10, T	NO_3^-/Fe(III), SO_4^{2-}, HCO_3^-	3 (L), 1 (K)	18	No	Trace production of CH_4 in active methanogenic reactor
Packed bed	Aquifer sediments	3.5 years	MTBE	≈ 5	NO_3^-, SO_4^{2-}	1 (L)	18	No	–

3. Results and discussion

Tables 3a and 3b show the description and results of the batch and continuous screening experiments on the degradation of MTBE under anaerobic conditions. The degradation results are based on the ether analysis of both the active and killed experiments. With the exception of the (active) batches under Fe(III)-reducing conditions, with the inoculum taken from the PBR and the membrane bioreactor (MBR), all other batches showed no ether removal. The active batches that showed no removal of MTBE, however, had a significant removal of the electron acceptors indicating that anaerobic conditions were well established. Overall, the lack of MTBE biodegradation in the experiments is indicative of the rarity of anaerobic MTBE degraders in the environment.

3.1. TBA accumulation in Fe(III)-reducing batches

The (active) Fe(III)-reducing batches with the PBR and MBR inoculums showed a build up of the metabolite TBA, while no TBA was found in the killed batches. An average of 30 and 60% removal of MTBE, respectively, was found. The precise quantity of TBA detected could not be ascertained due to deficiencies in the analytical system during the measurements. Mineralization of MTBE could not be confirmed either. These results appeared very promising initially because of the short time required for the degradation of MTBE (2 months). However, at the same time, they were somewhat inconsistent since most other reports on anaerobic removal of MTBE have documented long lag times, up to 1000 days [20,21,29]. Furthermore, it was also initially unclear, why the other batches under Fe(III)-reducing conditions using contaminated soil, taken from the refinery, and the Fe(III)-reducing UASB reactor had all not shown any removal of the ethers. Upon further investigations, it was found that all the active Fe(III)-reducing batches (PBR and MBR innoculums) which had shown an accumulation of TBA had a pH of about 1–2. On the other hand, the parallel killed batches had a neutral pH. The low pH was due to insufficient buffering in these batches. The complexed Fe(III) used in the batches was found to acidify them down to a pH of 1–2 [36]. These two sets of batches (PBR and MBR innoculums) contained normal tap water as the stock solutions. The reason for doing this was to include variations in the experimental setups; furthermore, the PBR had always been operated with normal tap water. The killed batches, however, with normal pH, had been buffered by the presence of the sodium azide. The NaN_3 (Na^+ $N^- = N^+ = N^-$) can act as a Lewis base, neutralizing or complexing the free Fe(III) ions. In the other batches in which Fe(III) reduction was observed, a neutral pH was present, these were well buffered, since they were prepared with the nutrient solution of Angelidaki et al. [32], which contained $NaHCO_3$.

It is known that MTBE can be hydrolyzed to the metabolite TBA under low pH conditions [37–39]. In the light of these findings, it is likely that the removal of MTBE found in the Fe(III)-reducing batches was due only to acid hydrolysis, rather than biological removal. Unlike the other batches under the Fe(III)-reducing condition, which changed color from orange/brown to green, this was not observed in the batches with low pH. Furthermore, the other Fe(III)-reducing batches and the UASB reactor were found to have a normal pH, as a result of buffering by the $NaHCO_3$ in the nutrient media.

Although it has been reported that complexed Fe(III) has a high bioavailability and can possibly stimulate Fe(III) reduction and MTBE oxidation [24], its usage will lead to low pH conditions due to its acidic properties. Based on the findings obtained here, caution should be applied in the interpretation of results in which complexed Fe(III) is used for anaerobic MTBE biodegradation. The removal mechanisms under these conditions may actually be due to abiotic processes, caused by acid hydrolysis, rather than biological.

The usefulness of complexed Fe(III) may be limited in actual groundwater conditions, if bioremediation of MTBE is desired. It may immediately lower the pH of the groundwater if the buffering capacity is insufficient and impact negatively on the microbial life. Buffering of complexed Fe(III) on the other hand may lead to precipitation.

3.2. Redox conditions in the experiments

By degassing the feed for the batch and UASB reactors with 80:20 nitrogen and carbon dioxide mixed gas as well as the Na_2S contained in the BA media created a low initial redox potential in the reactors. This was indicated by a color change of the redox indicator resazurin from red to colorless. In the setups where Fe(III)-reducing conditions where created, the reductant $NaSO_3$ was used instead of Na_2S to avoid precipitation of iron by Na_2S.

Batch reactors which the inoculums originated from the petroleum refinery, forest, UASB reactor or biogas plant showed a significant removal of nitrate and sulfate in the batches (Tables 2 and 3a). There was also Fe(III) reduction based on a color change from orange/brown to green in Fe(III)/HS amended batches. The electron acceptors were re-added several times after the initial setup of these batches. Significant CH_4 production in the methanogenic batches was also measured. The target redox conditions were established within a week of the initial setup. The inoculums stated in this paragraph all had easily degradable organic matter and a high microbial activity.

In batches where the innoculum was taken from the packed bed reactor and amended with nitrate, only an insignificant quantity was removed. This indicated that easily degradable organic matter was hardly present in these batches. Nitrate reducing conditions may not have been well established.

Other batches in which the packed bed or membrane reactor inoculums were used and were amended with Fe(III) showed a low pH and accumulation of TBA (Section 3.1).

In the continuous reactors no removal of the electron acceptors was measured. Therefore, it is uncertain whether or not the target redox conditions were achieved. The exception, however, was the UASB reactor operated under methanogenic conditions; a small quantity of CH_4 was detected in its headspace.

3.3. Choice of inoculum

The choice of inoculums used in these experiments was done such that the best conditions existed for the detection of anaerobic

MTBE degradation (Table 2). The samples collected from the petroleum refinery are best suited since this facility handles huge volumes of MTBE. Therefore, an acclimatization of microorganisms to anaerobic MTBE degradation was thought to be possible.

The –C–O–C– bond found in ethers also occurs in lignocelluloses materials [40], which are the primary diets of ruminant animals such as deer and cows. These animals have anaerobic bacteria in their rumen, which can breakdown lignocelluloses contained in the plant material they consume. Therefore, the manure samples collected from the forest and the biogas plant used as inoculums were expected to contain a high proportion of microorganisms capable of attacking ether molecules.

Granular sludge samples contain different groups of anaerobic microorganisms in high numbers; hence, the assumption was that some organisms present may be capable of degrading ethers.

4. Conclusions

This research has shown that despite a few past reports having documented anaerobic biodegradation of MTBE, it is still a rare occurrence. Not even inoculums from environments that were exposed to MTBE for very long or likely to contain ether degraders showed any biodegradation potential. This shows that acclimatization of organisms to biodegrade MTBE under anaerobic conditions is very difficult.

Removal of 30 and 60% MTBE under Fe(III)-reducing conditions was found with the membrane bioreactor (Amy Pruden) and the packed bed reactor innoculums (own reactor). This, however, was likely due only to acid hydrolysis, caused by insufficient buffering, and not from a biological process. The batches which showed MTBE removal all had a low pH of about 1–2; this was attributed to the acidic nature of complexed Fe(III).

Based on the findings obtained here caution should be applied in the interpretation of experimental data in which complexed Fe(III) is used for anaerobic MTBE biodegradation. The removal mechanisms under these conditions may actually be due to abiotic processes, caused by acid hydrolysis, rather than biological.

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