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Fuel Oxygenates

The Handbook of Environmental Chemistry

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Preface

Environmental Chemistry is a relatively young science. Interest in this subject, however, is growing very rapidly and, although no agreement has been reached as yet about the exact content and limits of this interdisciplinary discipline, there appears to be increasing interest in seeing environmental topics which are based on chemistry embodied in this subject. One of the first objectives of Environmental Chemistry must be the study of the environment and of natural chemical processes which occur in the environment. A major purpose of this series on Environmental Chemistry, therefore, is to present a reasonably uniform view of various aspects of the chemistry of the environment and chemical reactions occurring in the environment.

The industrial activities of man have given a new dimension to Environmental Chemistry. We have now synthesized and described over five million chemical compounds and chemical industry produces about hundred and fifty million tons of synthetic chemicals annually. We ship billions of tons of oil per year and through mining operations and other geophysical modifications, large quantities of inorganic and organic materials are released from their natural deposits. Cities and metropolitan areas of up to 15 million inhabitants produce large quantities of waste in relatively small and confined areas. Much of the chemical products and waste products of modern society are released into the environment either during production, storage, transport, use or ultimate disposal. These released materials participate in natural cycles and reactions and frequently lead to interference and disturbance of natural systems.

Environmental Chemistry is concerned with reactions in the environment. It is about distribution and equilibria between environmental compartments. It is about reactions, pathways, thermodynamics and kinetics. An important purpose of this Handbook, is to aid understanding of the basic distribution and chemical reaction processes which occur in the environment.

Laws regulating toxic substances in various countries are designed to assess and control risk of chemicals to man and his environment. Science can contribute in two areas to this assessment; firstly in the area of toxicology and secondly in the area of chemical exposure. The available concentration ("environmental exposure concentration") depends on the fate of chemical compounds in the environment and thus their distribution and reaction behaviour in the environment. One very important contribution of Environmental Chemistry to

the above mentioned toxic substances laws is to develop laboratory test methods, or mathematical correlations and models that predict the environmental fate of new chemical compounds. The third purpose of this Handbook is to help in the basic understanding and development of such test methods and models.

The last explicit purpose of the Handbook is to present, in concise form, the most important properties relating to environmental chemistry and hazard assessment for the most important series of chemical compounds.

At the moment three volumes of the Handbook are planned. Volume 1 deals with the natural environment and the biogeochemical cycles therein, including some background information such as energetics and ecology. Volume 2 is concerned with reactions and processes in the environment and deals with physical factors such as transport and adsorption, and chemical, photochemical and biochemical reactions in the environment, as well as some aspects of pharmacokinetics and metabolism within organisms. Volume 3 deals with anthropogenic compounds, their chemical backgrounds, production methods and information about their use, their environmental behaviour, analytical methodology and some important aspects of their toxic effects. The material for volume 1, 2 and 3 was each more than could easily be fitted into a single volume, and for this reason, as well as for the purpose of rapid publication of available manuscripts, all three volumes were divided in the parts A and B. Part A of all three volumes is now being published and the second part of each of these volumes should appear about six months thereafter. Publisher and editor hope to keep materials of the volumes one to three up to date and to extend coverage in the subject areas by publishing further parts in the future. Plans also exist for volumes dealing with different subject matter such as analysis, chemical technology and toxicology, and readers are encouraged to offer suggestions and advice as to future editions of "The Handbook of Environmental Chemistry".

Most chapters in the Handbook are written to a fairly advanced level and should be of interest to the graduate student and practising scientist. I also hope that the subject matter treated will be of interest to people outside chemistry and to scientists in industry as well as government and regulatory bodies. It would be very satisfying for me to see the books used as a basis for developing graduate courses in Environmental Chemistry.

Due to the breadth of the subject matter, it was not easy to edit this Handbook. Specialists had to be found in quite different areas of science who were willing to contribute a chapter within the prescribed schedule. It is with great satisfaction that I thank all 52 authors from 8 countries for their understanding and for devoting their time to this effort. Special thanks are due to Dr. F. Boschke of Springer for his advice and discussions throughout all stages of preparation of the Handbook. Mrs. A. Heinrich of Springer has significantly contributed to the technical development of the book through her conscientious and efficient work. Finally I like to thank my family, students and colleagues for being so patient with me during several critical phases of preparation for the Handbook, and to some colleagues and the secretaries for technical help.

I consider it a privilege to see my chosen subject grow. My interest in Environmental Chemistry dates back to my early college days in Vienna. I received significant impulses during my postdoctoral period at the University of California and my interest slowly developed during my time with the National Research Council of Canada, before I could devote my full time of Environmental Chemistry, here in Amsterdam. I hope this Handbook may help deepen the interest of other scientists in this subject.

Amsterdam, May 1980 *O. Hutzinger*

Twenty-one years have now passed since the appearance of the first volumes of the Handbook. Although the basic concept has remained the same changes and adjustments were necessary.

Some years ago publishers and editors agreed to expand the Handbook by two new open-end volume series: Air Pollution and Water Pollution. These broad topics could not be fitted easily into the headings of the first three volumes. All five volume series are integrated through the choice of topics and by a system of cross referencing.

The outline of the Handbook is thus as follows:

1. The Natural Environment and the Biochemical Cycles,
2. Reaction and Processes,
3. Anthropogenic Compounds,
4. Air Pollution,
5. Water Pollution.

Rapid developments in Environmental Chemistry and the increasing breadth of the subject matter covered made it necessary to establish volume-editors. Each subject is now supervised by specialists in their respective fields.

A recent development is the accessibility of all new volumes of the Handbook from 1990 onwards, available via the Springer Homepage springeronline.com or springerlink.com.

During the last 5 to 10 years there was a growing tendency to include subject matters of societal relevance into a broad view of Environmental Chemistry. Topics include LCA (Life Cycle Analysis), Environmental Management, Sustainable Development and others. Whilst these topics are of great importance for the development and acceptance of Environmental Chemistry Publishers and Editors have decided to keep the Handbook essentially a source of information on "hard sciences".

With books in press and in preparation we have now well over 40 volumes available. Authors, volume-editors and editor-in-chief are rewarded by the broad acceptance of the "Handbook" in the scientific community.

Bayreuth, July 2001 *Otto Hutzinger*

Contents

Foreword

Oxygenates like MTBE (methyl tertiary butyl ether) were developed in the 1970s as octane enhancers to replace toxic additives like lead, which have been phased out of gasoline. The presence of oxygenates in gasoline promotes cleaner fuel combustion within the engine, boosts fuel octane values and reduces vehicle air emissions.

Two types of oxygenates are commonly used in gasoline: ethers and alcohols. MTBE is by far the most commonly used ether oxygenate due to its high-octane properties, cost effectiveness and supply flexibility. Other ether oxygenates that can potentially be used are tertiary amyl methyl ether (TAME), tertiary-amyl ethyl ether (TAEE), ethyl-tertiary-butyl ether (ETBE) and diisopropyl ether (DIPE). Ethanol is by far the most commonly used alcohol oxygenate. Other alcohols that can be used as fuel oxygenates are methanol and tertiary-butyl alcohol (TBA). TBA is also the main degradation product of MTBE and a potential impurity from the MTBE manufacturing process.

MTBE has been extensively detected in groundwater supplies and other water reservoirs, especially in the US. The groundwater controversy has generated a vast quantity of reports, scientific studies and media coverage. The adverse effects on human health and the environment are a growing cause of concern. An excellent book entitled *Oxygenates in Gasoline: Environmental Aspects* was edited by A.F. Diaz and D.L. Drogos in 2002 as ACS Symposium Series, volume 799.

Europe has been always behind the US in relation to MTBE groundwater contamination. MTBE studies only started in Europe in the early 1990s. However there still remains a lack of representative monitoring data, especially for the Southern and Eastern European countries. The EU-funded project Water Catchment Areas: Tools for Management and Control of Hazardous Compounds (WATCH), which lasted three years from April 2001 up to April 2004, stimulated EU research in this area.

The idea for this book on fuel oxygenates came on the occasion of the WATCH project and after the Second European Conference on MTBE was held in Barcelona in November 2004. This conference, a follow-up to the first European Conference held in Dresden in September 2003, was a platform of communication and scientific exchange between scientists and stakeholders interested in MTBE. It reflected the European interest and the efforts of the

EU to identify and solve the problems caused by MTBE contamination. The present book covers a comprehensive overview of the problems associated with fuel oxygenates and in particular on contamination caused by MTBE, TBA and to a minor extent by ETBE.

The book is organized into five sections: Analysis of Fuel Oxygenates, Occurrence in the Environment, Transport and Degradation Processes, Treatment Technologies and Health Risks. Written by recognized specialists in the field, this book offers a unique opportunity not only for scientists that want to get more comprehensive information on this topic, but also for policy makers and stakeholders that need to manage real-world environmental problems associated with fuel oxygenates contaminating our groundwater resources.

Overall, the present book is certainly timely since the interest in fuel oxygenates, including biofuels, in the environment has grown considerably during the last few years. The book can be considered, in a way, a follow-up to the Diaz and Drogos book, but in this case with a more European point of view. This book will be of interest to a broad audience of analytical chemists, environmental scientists, toxicologists and technologists already working in the field of fuel oxygenates in the water cycle, or newcomers who want to learn more about this problem. Finally, I would like to thank all the contributing authors of this book for their time and effort in preparing this comprehensive compilation of research papers.

Barcelona, 30 April 2007 Damià Barceló

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Novel Analytical Methods for the Determination of Fuel Oxygenates in Water

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Abstract Fuel oxygenates such as methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE) and *tert*-amyl methyl ether (TAME) today are among the most frequently detected volatile organic compounds in groundwater and, thus, they have become priority groundwater pollutants over the last decade. Thus, their quantitative determination at very low concentrations is routinely required. Methods for this purpose and for compound-specific isotope analysis (CSIA), especially of MTBE and its key degradation intermediate *tert*-butyl alcohol (TBA) in ground and surface water are reviewed in this work. For quantitative determination, fuel oxygenates are almost exclusively analyzed by gas chromatography, mainly with mass spectrometric detection due to selectivity and sensitivity requirements. Sample introduction/enrichment based on membrane introduction mass spectrometry, direct aqueous injection, headspace analysis, purge&trap, solid-phase microextraction (direct immersion or headspace) and other microextraction approaches such as solid phase dynamic extraction and liquid-phase microextraction

are discussed. Furthermore, the use of ion mobility spectrometry for the determination of fuel oxygenates and related compounds is reviewed. Specific advantages and disadvantages of these techniques are compared and criteria for the choice of an appropriate method are given. The application of CSIA nowadays can be used to determine the isotopic composition of MTBE and related compounds in the low $\mu g\,L^{-1}$ range and thus will become an invaluable tool in the characterization of the environmental fate of such pollutants. Therefore, an overview of analytical aspects of this technique is included here.

Keywords Fuel oxygenates · MTBE · TBA · GC · CSIA

Abbreviations

CSIA	compound specific isotope analysis
c_w^{sat}	water solubility
DAI	direct aqueous injection
ETBE	ethyl *tert*-butyl ether
FID	flame ionization detector
HS	headspace
IMS	ion mobility spectrometry
K_{AF}	air–fuel partition coefficients
K_H	Henry's law constant
K_{OW}	octanol/water partitioning coefficient
LOD	limit of detection
LPME	liquid-phase microextraction
MIMS	membrane Introduction Mass Spectrometry
MTBE	methyl *tert*-butyl ether
MS	mass spectrometry
PEG	polyethylene glycole
PVOC	polar Volatile Organic Compounds
p_0	vapor pressure
Ref.	reference
RSD	relative standard deviation
s_d	standard deviation
SDME	single drop microextraction
SIM	single ion monitoring
SPDE	solid-phase dynamic extraction
SPME	solid-phase microextraction
TAME	*tert*-amyl methyl ether
TBA	*tert*-butanol
TBF	*tert*-butyl formate
T_b	boiling point
US EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
% (v/v)	volume percent
% (v/v)	mass percent

1 Introduction

The use of fuel oxygenates such as methyl *tert*-butyl ether (MTBE) in gasoline has raised an intense discussion on its environmental benefits and impacts in the US and, with a time lag of several years, also in Europe. However, the situation in Europe and the US differs substantially with regard to the use and emission of MTBE [1]. In the US, the 1990 Amendments to the Clean Air Act still require a minimum oxygen content of 2.7% (w/w) for oxyfuels and 2.0% (v/v) for reformulated gasoline in CO and ozone non-attainment areas, respectively. In Europe there is no minimum requirement but the addition of maximum 15% (v/v) to gasoline is allowed. Alternative fuel oxygenates include several alcohols and other dialkyl ethers. However, only methanol (in blends with gasoline, e.g., in Brasil), ethanol (mainly in the US Midwest), *tert*-amyl methyl ether (TAME; Finland) and in particular ethyl *tert*-butyl ether (ETBE; replacement for MTBE throughout Europe) are of economic importance today. *Tert*-butyl alcohol (TBA) is of considerable interest mainly because it is the key intermediate in the degradation of MTBE and ETBE [2]. Thus, analytical methods aiming at the determination of fuel oxygenates in the environment need to consider both alcohols and ethers.

Table 1 comprises common fuel oxygenates, their abbreviations used in the text and the physico-chemical properties relevant for their analysis and environmental behavior. Compared with classical fuel-related contaminants such as BTEX, alcohols and ethers have higher water solubilities, lower Henry's law constants and lower sorption constants. These properties make them more difficult to determine at trace levels ($\mu g\,L^{-1}$-range and below) in aqueous samples. In the literature, mainly analytical methods for the determination of MTBE are reported but most of these are also applicable to other dialkyl ethers. The use of fuel oxygenates is a good example for the necessity of multicomponent methods: The quick shift from MTBE to ETBE production in many European refineries within the last few years would lead to a lack of positive findings in the environment if methods dedicated to MTBE analysis alone are used. A general shortcoming is the lack of methods that comprise the simultaneous analysis of ethers and alcohols. For the latter, analytical methods at the trace level are generally scarce.

The aim of this review is to provide a critical evaluation of recently published analytical methods available for (i) the quantification of fuel oxygenates in environmental aqueous samples and (ii) the characterization of the environmental fate of MTBE in groundwater with the use of compound-specific isotope analysis (CSIA). For a detailed evaluation of methods available for air, water and soil analysis of dialkyl ethers and alcohols and their specific advantages and drawbacks, the reader is also referred to previous reviews. [3–6].

Table 1 Environmentally relevant physico-chemical properties of fuel oxygenates (T = 25 °C)

Compound class	Ethers				Alcohols				
Compound	methyl *tert*-butyl ether	ethyl *tert*-butyl ether	*tert*-amyl methyl ether	diiso-propyl ether	methanol	ethanol	isopropyl alcohol	isobutyl alcohol	*tert*-butyl alcohol
Abbreviation	MTBE	ETBE	TAME	DIPE	MeOH	EtOH	IPA	IBA	TBA
CAS No.	1634-04-4	637-92-3	994-05-8	108-20-3	67-56-1	64-17-5	67-63-0	78-83-1	75-65-0
Molecular weight [g/mol]	88.15	102.18	102.18	102.18	32.04	46.07	60.1	74.12	74.12
Boiling point T_b [°C]	55.2	72.2	86.3	68.2	64.6	78.3	82.2	107.9	82.4
Density ρ [kg L^{-1}]	0.744	0.73	0.77	0.72	0.80	0.79	0.79	0.80	0.79
Vapor pressure p_0 [7]	332	203	91	200	168	79	61	14	56
Water solubility C_w^{sat} [mol L^{-1}]	0.54	0.12	0.12	0.019	complete	complete	complete	0.92	complete
Henry's law constant K_H [Pa m^3 mol^{-1}]	85.5	162	116	253	0.466	0.527	0.800	1.22	1.46
Octanol-water partition constant log K_{OW} [–]	1.24	1.74	1.55	1.52	– 0.77	– 0.31	0.05	0.76	0.35
Fuel-water partition coefficient log K_{FW} [–][e]	1.20[b]	1.78[c]	1.78[c]	2.56[c]	– 2.29[b]	– 1.82[f]	– 0.69[d]	– 0.26[d]	– 0.62[d]
Organic carbon-water partition coefficient log K_{OC} [–]	0.55–0.91[g] 1.04–1.09[h] 1.6[j] 1.14[a]	0.95[h] 2.2[h] 1.7[i]	1.3[h] 2.2[h] 1.7[i] 1.8[j]	1.13[g] 1.5[h] 1.8[h] 1.55[a]	0.44[h] 0.92[h] 0.68[i] – 0.15[a]	– 0.14[g] 0.20[h] 1.2[h] 0.71[i] 0.16[a]	1.4 0.37[a]	0.95 0.87[a]	1.57[h] 0.67[a]

Data sources (references given therein): Schmidt et al. [3], and Syracuse Research Corporation Physical Property database (free access under http://www.esc.syrres.com), Henry's law constants for the ethers and TBA: [8]

[a] Calculated using the polyparameter LFER [9] by Nguyenet al., Eq. 9a, [b] exp. values from [10], [c] calculated values based on Eq. 7 in [10], [d] measured by us using the standard addition method described in [1] and the analytical method described in [11], [e] note that K_{FW} values may vary considerably for fuels of different composition, [f] exp. value from [12], [g] [13], [h] [14], [i] [15], [j] [16]

2
Gas-Chromatographic Methods for Quantification

2.1
General

Gas chromatographic methods are used almost exclusively for the analysis of polar volatile organic compounds (PVOC) and mass spectrometric detection has been employed in most studies. The most critical step in the analysis of trace concentrations of MTBE and related fuel oxygenates in environmental samples is the enrichment of the analytes from the likewise polar matrix water. Hence, the part on quantification methods is organized according to the various enrichment methods reported for the sensitive analysis of these compounds. For each method discussed, tables summarizing important experimental settings and benchmark parameters are provided. It is, however, important to note, that a direct comparison of reported limits of detection (LOD) is not feasible because these values strongly depend on the method used for their determination. As far as possible, this information is therefore given in the tables along with the LOD.

Although benefits from the use of internal standards (IS) matching the physico-chemical behavior of the analytes have been known for a long time only in a few of the reported studies were IS employed. The multi-compound EPA and USGS methods [17–19] rely on fluorobenzene as internal standards. However, for the specific analysis of fuel oxygenates, commercially available, isotopically labelled compounds such as MTBE-d_3 are better suited as long as MS detection is used. For the analysis of TBA and other alcohols, TBA-d_{10} might be used as IS although in a recent study this did not improve method performance in comparison with the use of MTBE-d_3 [11]. More recently, Tanabe et al. proposed the use of MTBE-d_{12} instead of MTBE-d_3 in the case of samples that could contain carbon disulfide (m/z = 76) [20]. Another alternative is the use of TAME-d_3. As a result of an interlaboratory comparison, Schuhmacher et al. concluded that the use of an internal standard is highly recommended for the determination of MTBE at concentrations below 1 $\mu g\,L^{-1}$ [21].

2.2
Direct Aqueous Injection

In direct aqueous injection (DAI-GC/MS) aqueous samples are injected into the GC system without any pre-treatment except filtration or centrifugation (if necessary) as well as addition of an internal standard. The lack of pre-treatment steps makes the method attractive because such steps are both time consuming and artefact-prone. Furthermore, it is the method with the least demands on sample volume, with 50 μL or even less being sufficient. A special advan-

tage of the direct injection is that it allows the simultaneous analysis of ethers and alcohols. Nevertheless, the use of direct aqueous injection is still hampered by the perception of many analysts that aqueous injections into a GC will rapidly deteriorate system performance and especially for GC/MS large amounts of water vapor can lead to unstable vacuum conditions in the ion source. In case of GC/MS these disadvantages were partly solved with high performance vacuum pumps and strongly water retaining capillary columns coated with polyethylene glycole (WAX) phases. Even these polar coatings are nowadays rather stable against water. However, the achieved sensitivity in the order of about 0.1 $\mu g\,L^{-1}$ for the dialkyl ethers will not suffice for all applications. Hence DAI is particularly useful for the investigation of contaminated sites where the analysis of alcohols such as TBA is required. Table 2 summarizes experimental settings and benchmark parameters of direct aqueous injection methods for MTBE and related fuel oxygenates. A direct aqueous injection method for MTBE, other dialkyl ethers, their degradation products and TBA was first described by Church et al. [22]. They used splitless injection into a liner held at 130 °C that protruded into the GC oven held at 30 °C, thus allowing recondensation at the bottom of the liner. With 10-μL injections, LODs of 0.1 $\mu g\,L^{-1}$ were achieved for all dialkyl ethers and TBA. One disadvantage of this method was the obligatory use of a MS equipped with large vacuum pumps not found in most laboratories. Zwank et al. [11] have shown that cold on-column injection into a long deactivated pre-column is also successful. An advantage of the used pre-column is that it will act as a guard column in which salts and other insolubles will be retained to protect the analytical column. Thus, a frequent maintenance of the pre-column by cutting suffices to restore performance. With 10-μL injections, LODs for MTBE, other dialkyl ethers, benzene, and toluene were in the range 0.05 to 0.45 $\mu g\,L^{-1}$. In that study, a benchtop MS with a 250 L turbomolecular pump was used, which did not allow the injection of more than 1 μL water for the analysis of alcohols. Thus, the LOD for TBA was one order of magnitude higher than reported by Church et al. [22]. Both methods used a polar polyethylene glycol column for separation that retains water quite strongly and in both papers cross-validation studies of the DAI method with standard methods yielded very good agreement of analytical results. Hong et al. used a Carbofrit filled liner and a 5 m polar guard column (PEG) prior to a nitroterephthalic acid-modified polyethylene glycol (FFAP) column for separation [23]. In that study, limits of detection between 30 to 100 $\mu g\,L^{-1}$ were obtained in single ion monitoring mode. By using an FID as the detector detection limits of only 1 $mg\,L^{-1}$ for MTBE and *tert*-butanol were obtained. A potential problem in GC/MS is that for MTBE quantification typically the signal at m/z 73 is used that may be obscured by degradation products of bleeding polyethylene glycol columns with the same mass to charge ratio.

Environmental applications of DAI-GC/MS methods, including investigations of contaminated sites, have been described in various papers [11, 22, 24–26].

Table 2 Direct aqueous injection methods for determination of fuel oxygenates

Detector	Column type	Injection mode	LOD MTBE [$\mu g\,L^{-1}$]	LOD other oxygenates [$\mu g\,L^{-1}$]	LOD definition	RSD [%] & conc. [$\mu g\,L^{-1}$]	Internal standards	Application	Refs.
FID	6% cyanopropylphenyl-94% dimethylpolysiloxane	hot on-column (165 °C)	50	TBA 50	minimum conc. of linear range	not reported	none	aqueous gasoline extracts	[27]
MS SIM	polyethylene glycol	Splitless	0.1	ETBE 0.1 TAME 0.1 TBA 0.1 TBF [a] 5	S/N 10/1	not reported	none	contaminated groundwater, process studies	[22]
MS SIM	nitroterephthalic acid-modified polyethylene glycol (FFAP)	Splitless	30	TBA 30	minimum conc. of linear range	not reported	none	process studies	[23]
MS SIM	polyethylene glycol	cold on-column	0.10	ETBE 0.16 TAME 0.21 TBA 1.1 TBF [a] 7.9	$3 \times s_d$ of 10 spike samples & 0.59 $\mu g\,L^{-1}$	7.7 & 0.59–2.37	MTBE-d_3 (TBA-d_{10})	contaminated groundwater, runoff, process studies, aqueous gasoline extracts	[11]

[a] *tert*-Butyl formate, the primary atmospheric degradation product of MTBE

DAI-GC with flame ionization detection (FID) has also been reported for the analysis of MTBE in water by Potter et al. [27]. By injection of 1–5 μL aqueous sample into a 165 °C hot injector and using a 6% cyanopropylphenyl 94% dimethylpolysiloxane megabore column, detection limits of 50 μg/L for MTBE and its degradation product TBA were obtained. However, in general, the limited sensitivity and selectivity of FID will often not suffice for environmental analysis.

2.3 Headspace Analysis

Static headspace analysis is based on the partitioning of analytes from an aqueous or solid sample to air in a closed system (headspace vial) as shown in Fig. 1a. This method is suitable for compounds that show sufficiently high air–water partitioning (quantified by the Henry's Law constant). Although the Henry's Law constant for MTBE (see Table 1) is about one order of magnitude smaller than for benzene or toluene, analytical methods based on headspace sampling have been developed. Advantages of headspace analysis are its robustness, its applicability to all sample matrices (including highly contaminated water and soil samples) and its non-destructiveness to the samples that allows multiple analyses. However, the reported LODs are at least one order of magnitude higher than for the other methods. Headspace methods are therefore in particular useful for the investigation of contaminated sites. Furthermore, alcohols can hardly be analyzed with static headspace analysis

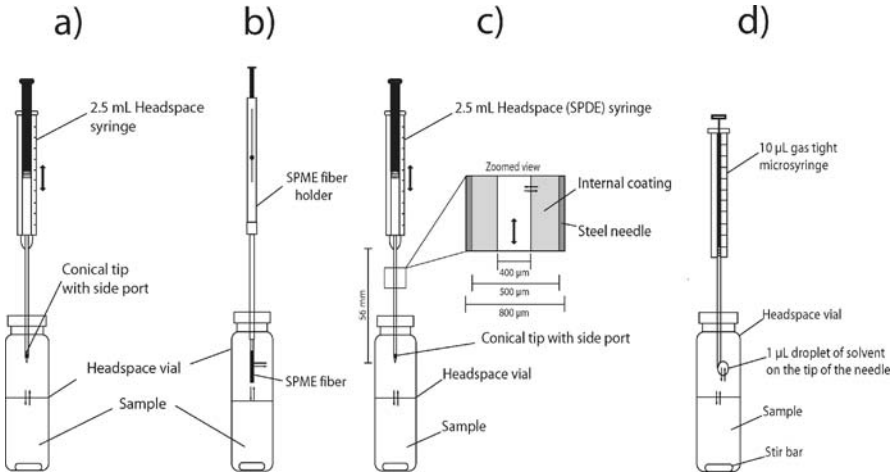

Fig. 1 Headspace analysis and microextraction methods (here only the headspace mode is shown) used for the determination of fuel oxygenates. (**a**) Headspace analysis, (**b**) headspace solid-phase microextraction (HS-SPME) redrawn after [60], (**c**) solid-phase dynamic extraction (SPDE) redrawn after [61] and (**d**) liquid-phase microextraction (LPME) redrawn after [62]

Table 3 Headspace methods for determination of fuel oxygenates

Detector	Salt [g L^{-1}]	Equil. time [min]	Temp. [°C]	Injection volume [mL]	LOD [μg L^{-1}]	LOD other oxygenates [μg L^{-1}]	LOD definition	RSD [%] & conc. [μg L^{-1}]	Internal standards	Application	Refs.
FID	0	60	60	not reported	50	–	not reported	7 & 60	none	contaminated groundwater	[29]
FID	0	12 (incl. 4 min mixing)	70	1	5.7	–	$t_{(N-1,\,1-\alpha=0.99)} \times s_d$ [32]	7.9, conc. not reported	α,α,α-trifluoro-toluene	contaminated groundwater	[30]
MS SIM	250	720	25	0.1	2.0 1.2	–	$t_{(N-1,\,1-\alpha=0.99)} \times s_d$ [32] from calibration plot [33]	4.5 &12	none	contaminated groundwater, river water	[31]
MS Full Scan	200	30	80	1	0.21	Ethanol 18 IPA 5.5 TBA 0.79 ETBE 0.21 TAME 0.17 DIPE 0.14	$t_{(N-1,\,1-\alpha=0.99)} \times s_d$	13.3 & 0.5 (MTBE) 8.6–13 & 0.5 to 50 (other compounds)	fluoro-benzene, bromo-fluoro-benzene	no	[28]

Table 4 Purge & Trap methods for determination of MTBE

Detector	Sample volume [mL]	Purge time [min]	Purge flow [mL min^{-1}]	Temp. [°C]	Trap material	Desorpt. time [min]	Desorpt. temp. [°C]
MS Full Scan	5	11	40	ambient	Tenax-silica gel-charcoal	4	180
MS Full Scan	25	11	40	ambient	Carbo-pack B/ Carboxen 1000	4	250
FID	5	11	40	ambient	Tenax	2.5	200
MS SIM	15[a]	13[a]	35	ambient	Tenax-silica gel-charcoal	4[a]	225
MS SIM	5	30	30	60	Tenax-silica gel-charcoal	4	180

[a] Corrected values provided by the authors
[b] value seems unrealistic because it implies a higher purge efficiency for TBA than for

at the $\mu g\,L^{-1}$ level except when samples are heated to very high temperatures [28]. Table 3 summarizes experimental settings and benchmark parameters of headspace sampling methods for MTBE.

Nouri et al. [29] and Lacorte et al. [30] compared static headspace and purge&trap methods for the analysis of MTBE. With FID they reported LODs of 50 and $5.7\,\mu g\,L^{-1}$, respectively, for the headspace method. O'Neill [31] reported significantly lower LODs of 1.2 to $2.0\,\mu g\,L^{-1}$ for MTBE with MS detection in the selected ion monitoring (SIM) mode although only a comparably low injection volume of 0.1 mL was used. Partitioning of a compound into the headspace can be enhanced by the addition of salt ("salting-out") and elevated temperatures. Interestingly, two of the mentioned methods used elevated temperatures [29, 30], one used addition of sodium sulfate [31], but

Cryofocusing	LOD [$\mu g\,L^{-1}$]	LOD other oxygenates [$\mu g\,L^{-1}$]	LOD definition	RSD [%] & conc. [$\mu g\,L^{-1}$]	Internal standards	Application	Refs.
– 10 °C	0.09	–	$t_{(N-1,\,1-\alpha=0.99)} \times s_d$, of 7 low level spikes [32]	5.6 & 0.4, 2.5–3.6 & 20 (depend. on matrix)	fluorobenzene	none	[18], [19]
– 20 °C	0.083	ETBE 0.015 TAME 0.032 DIPE 0.021	$t_{(N-1,\,1-\alpha=0.99)} \times s_d$ of 50 low level spikes over 6 months	7 & 1.1	fluorobenzene	surface and groundwater	[17]
– 60 °C	2	–	not reported	2 & 5/60	none	contaminated groundwater	[29]
No	0.001	–	$t_{(N-1,\,1-\alpha=0.99)} \times S_d$, of 7 low level spikes [32]	11 & 1/50/500	MTBE-*d*3	contaminated groundwater	[30]
No	0.0033	TBA 0.0025[b]	not clearly stated	1.9 & 0.006	none	contaminated groundwater	[36]

MTBE

neither method used both approaches to increase sensitivity. By a combination of high temperature and high salt content, Lin et al. have been able to achieve the lowest LODs reported so far for headspace analysis of fuel oxygenates [28]. However, when high temperatures are used for enhancing the phase transfer, care must be taken to avoid the hydrolysis of MTBE, in particular at low pH (see below).

2.4 Purge and Trap Enrichment

Purge&trap (P&T) enrichment comprises a purge step where an inert gas (helium or nitrogen) is bubbled through an aqueous sample in order to transfer the analytes into the gas phase. The analytes in the gas phase are then

trapped and concentrated from the gas phase on a suitable sorbent, which is subsequently desorbed thermally. Frequently, the desorbed compounds are cryofocussed prior to GC analysis. As for static headspace sampling, sufficiently high Henry's Law constants of the analytes are a prerequisite for a sensitive analysis. However, for MTBE and other dialkyl ethers efficient enrichment by purge&trap can be achieved although the Henry's Law constants are much smaller than for classical volatile organic compounds such as BTEX and chlorinated hydrocarbons.

Disadvantages of purge&trap enrichment are susceptibility to contamination from highly polluted samples and the rather complex system necessary for automated analyses. Table 4 summarizes experimental settings and benchmark parameters of published purge&trap methods. Purge&trap enrichment, if available in a laboratory, is in particular recommended for the monitoring of MTBE in rather clean water samples such as drinking, uncontaminated surface and groundwater. At contaminated sites it should only be used for confirmatory analysis after appropriate dilution of samples.

MTBE has been incorporated in various purge&trap standard analytical protocols adopted by the US Environmental Protection Agency (US EPA) [18, 19, 34] and the US Geological Survey (USGS) [17, 35]. These methods have therefore been extensively evaluated and successfully used for the surveillance of MTBE in numerous surface and groundwater samples. The reported LODs (see Table 4, first two rows) are very conservative estimates ($\sim 0.09\ \mu g\,L^{-1}$).

Purge&trap is hardly used for alcohol analysis due to the poor transfer of such compounds to the gas phase. However, Bianchi et al. [36] reported the lowest LOD published for TBA so far ($2.5\ ng\,L^{-1}$) using P&T-GC/MS. This LOD is at least two orders of magnitude lower than all previously reported values for water analysis, therefore confirmation of this value in independent studies and in the measurement of real samples will help to ensure its validity.

Nouri et al. [29] and Lacorte et al. [30] compared purge&trap methods with static headspace analysis of MTBE. Reported LODs for headspace analysis were around $50\ \mu g\,L^{-1}$ and $5.7\ \mu g\,L^{-1}$, for the purge&trap methods much lower LODs of $2\ \mu g\,L^{-1}$ were obtained with FID and $0.001\ \mu g\,L^{-1}$ with MS detection, respectively. In both cases, the agreement of results for headspace and purge&trap analysis of contaminated groundwater samples was poor. Nouri et al. [29] attributed this to the high contamination in the investigated samples that required dilution of samples but still suffered from the presence of coeluting compounds. Lacorte et al. [30] frequently found false negatives with the headspace method and pointed out the difference of both methods if a residual organic phase is present in the samples. In this case, purge&trap can exhaustively extract both phases and thus lead to a measurement of the sum of the analytes in both phases whereas headspace analysis will essentially only determine the concentration in the aqueous phase. Morgenstern et al. used P&T-GC/MS for a study of MTBE occurrence in drinking water in the Nether-

lands [37]. They were able to measure 40 samples per day and achieved detection limits of 2 ng L^{-1}. A P&T-GC/AED method was developed by Mezuca et al. [38]. The method had higher method detection limits (10 μg L^{-1} for MTBE) and a relatively high relative standard deviation of 17% in comparison with the detection with MS in SIM mode in the same study (LOD 0.04 μg L^{-1} and an RSD of 3%). Nevertheless, the P&T-GC/AED method was used in a subsequent study of the effectiveness of Fenton's reagent as MTBE oxidant in slurries and water [39].

2.5 Microextraction Techniques

2.5.1 Solid-Phase Microextraction

A dominant trend in sample preparation and extraction is miniaturization and for more than a decade now various solventless or solvent-reduced extraction methods based on a micro scale approach have been developed.

Solid-phase microextraction (SPME) was the first microextraction method that was introduced by Pawliszyn and co-workers in the early 1990s [43, 44]. Over the last few years, solid-phase microextraction (SPME) has become the most widely used enrichment method for the trace analysis of PVOC and fuel oxygenates in particular. This is mainly due to the ease of operation and automation at rather low cost and the achieved high sensitivities for such compounds. Disadvantages of SPME comprise susceptibility to matrix effects that hamper accurate quantification (see below), and the limited lifetime of the fiber, in particular in direct immersion mode. For highest extraction yields, extraction and desorption parameters including desorption temperature, extraction time and temperature, as well as salting out have to be optimized. Extraction times of the reviewed methods were between 8 min and 60 minutes. The used extraction temperatures ranged from 10 °C to 50 °C. Achten et al. obtained best results when using an extraction temperature of 18/19 °C while simultaneously cooling the SPME fiber by a home-made device to 5 °C [45]. Dron et al. used a fractional factorial design for screening and a central composite design for optimizing the significant variables for the best response of a headspace SPME method [46]. It was found that extraction temperature and ionic strength were the most pronounced parameters that should be optimized for a maximal extraction yield. Table 5 summarizes experimental settings and benchmark parameters of published SPME methods. Both direct immersion and headspace SPME have been successfully utilized. SPME with PDMS fibers that enrich analytes only based on partitioning was not successful. To achieve good extraction yields for polar compounds it is necessary to use other fiber types instead that enable both adsorption and partitioning such as PDMS/DVB or PDMS/Carboxen. With such fibers, LODs

in the range of $10\,ng\,L^{-1}$ are feasible. Achten et al. reported such low LODs even using full scan mass detection that allows confirmation of compound identity via the mass spectrum [45]. By using a FID as detector, LODs were typically 30–50 times higher than with MS. Internal standards were utilized only in a few studies although for quantitative measurements with SPME this is highly recommended [47] (see also general comments above). Cassada et al. [48] have shown the applicability of the developed SPME method for other fuel oxygenates such as ETBE, TAME, TBA and ethanol. For these compounds, method detection limits of 0.025, 0.038, 1.8 and $15\,\mu g\,L^{-1}$, respectively, have been reported. A comparison between a headspace SPME method and US EPA method 5030/8260B was done for MTBE and showed that both methods are in good agreement over three orders of magnitude in concentration [49].

A limitation of adsorption/partitioning fibers is their susceptibility to sorption competition. Thus, less polar analytes might well replace the PVOC from the fiber [50]. Black and Fine [47] indeed recently reported this effect for MTBE and TBA with various fibers in the presence of high concentrations of BTEX. SPME with adsorption/partitioning fibers such as PDMS/DVB or PDMS/Carboxen should therefore only be used for less contaminated samples. Typical examples are surface water, precipitation, road runoff, and background concentrations in groundwater, all of which have been successfully analyzed with SPME-GC/MS for MTBE. Another possible limitation of SPME was reported by Lin et al. [51]. They found a significant decrease of the measured MTBE (up to 27%) concentration in drinking water with increase of the residual chlorine concentration. They recommended therefore dechlorination of drinking water before analysis. Their experimental data set for MTBE, however, was very limited and only comprised PDMS/Carboxen fibers. Furthermore, no explanation for the observed effect was given. Thus, no final conclusions seem to be justified at the moment. However, several problems result from the construction of the SPME device itself. The most common practical problems facing SPME are mechanical damage of the coating due to scraping, needle bending and fiber ruption caused by the fragility of the fused silica support. Several attempts to overcome these mechanically related drawbacks have been reported, such as the introduction of bendable StableFlex fibers with an alloy core [52]. At the same time, several new microextraction approaches have been developed to overcome such problems of SPME and have been applied in fuel oxygenate analysis (see below).

2.5.2 Solid-Phase Dynamic Extraction

As shown in Fig. 1c, solid-phase dynamic extraction (SPDE) utilizes a 2.5 mL headspace syringe with a needle that is coated on the inside similar to a fused silica GC column with an immobilized extraction phase. SPDE needle coat-

Table 5 SPME methods for determination of fuel oxygenates

Fiber	Mode	Detector	NaCl [g L^{-1}]	Extraction time [min]	Temp. [°C]	LOD [µg L^{-1}]	LOD other oxygenates [µg L^{-1}]	LOD definition [µg L^{-1}]	RSD [%] & conc.	Internal standard	Application	Refs.
75 µm PDMS/ Carboxen	HS	FID	250	10	40	0.27	ETBE 0.44	intercept of calibration plot + 3 × s_d of 7 blanks	7.7 & 28.7	none	none	[53]
75 µm PDMS/ Carboxen	Direct	MS Full Scan	100	60	18–19	0.01	–	S/N 10/1	12 & 0.01	MTBE-d_3 water	surface	[45]
50/30 µm DVB/ Carboxen/ PDMS	Direct	MS SIM	250	25	not report.	0.008	ETBE 0.025 TAME 0.038 TBA 1.8 ethanol 15	from s_d of 8 spike samples & 40 ng L^{-1}	5 & 0.84	isopropyl alcohol or *n*-propyl alcohol	non-contaminated groundwater	[48]
75 µm PDMS/ Carboxen	HS	MS SIM	0	8	50	0.1	–	not reported	11.5 & 1.04	MTBE-d_{12}	tap water	[54]
75 µm PDMS/ Carboxen	HS	MS Full Scan	100	30	35	0.01	–	S/N 10/1	11 & 0.02	MTBE-d_3	surface and groundwater, precipitation	[55]
50/30 µm DVB/ Carboxen/ PDMS	HS	MS SIM	250	15	ambient	0.014	–	S/N 3/1	8 & 0.1	none	surface water, snow	[56]
65 µm PDMS/DVB	HS	FID	300	5	20	0.45	–	S/N 3/1	6.3 & 250	none	none	[46]
85 µm PDMS/ Carboxen	HS	MS SIM	340	30	40	0.007	–	$t_{(N-1,\,1-\alpha=0.99)} \times s_d$, of 7 low level spikes	4.5 & 0.047	MTBE-d_3	tap water, precipitation	[57]

Table 5 (continued)

Fiber	Mode	Detector	NaCl [g L^{-1}]	Extraction time [min]	Temp. [°C]	LOD [µg L^{-1}]	LOD other oxygenates [µg L^{-1}]	LOD definition [µg L^{-1}]	RSD [%] & conc.	Internal standard	Application	Refs.
75 µm PDMS/ Carboxen	Direct	MS Full Scan	250	30	not report.	0.5	TAME 1 TBA 2	S/N 5/1	1.6 & 1707	MTBE-d_3 TBA– d_{10} (for TBA)	contaminated groundwater	[58]
65 µm PDMS/DVB	HS	FID	300	10	10	1.1	ETBE 0.3 TAME 0.5	intercept of calibration plot + 3 × s_d of 5 blanks	between 2.6 and 8.5 & 500	none	ground- and surface water	[7]
100 µm PDMS	HS	MS	300	30	60	0.01		S/N 3/1	6.6 & 0.1	1,4-Dioxane-d_8	tap- and river water	[59]
75 µm PDMS/ Carboxen	HS	MS	–	30	not report.	0.03	TBA < 1.98	Standard method 1010C and 1030E (APHA 1995)	–	fluorobenzene	groundwater	[49]

ings possess around 4–6 times larger extraction phase volumes compared with a 100-μm SPME fiber [63]. For the extraction, the needle is immersed in the headspace above the aqueous phase. The syringe plunger is moved up and down several times for a dynamic extraction of the sample, and the analytes are sorbed in the internal coating. After several extraction cycles (aspirating and dispensing) the analytes are thermally desorbed from the coating in the GC injector. A SPDE method for the trace analysis of 15 alcohols and ethers in water, including some of the fuel oxygenates discussed here (MTBE, ethanol, IPA, IBA, TBA) was recently developed by the authors. The method achieves similar LODs to headspace SPME methods for the ethers and very low LODs for most alcohols. Furthermore, robustness was much improved with one SPDE syringe being used for more than 1000 injections even with a typically rather fragile PEG coating [61]. With the PEG phase, method detection limits for MTBE, ethanol, IPA, IBA and TBA were 0.06, 2.3, 0.3, 1.9 and 0.15 $\mu g\,L^{-1}$, respectively.

2.5.3 Liquid-Phase Microextraction (LPME)

Liquid-phase microextraction (LPME) or headspace solvent microextraction (HSME) is a simple extraction approach that combines classical liquid-liquid extraction with microextraction by greatly reducing the solvent to sample phase ratio. As shown in Fig. 1d, a very small drop of a water immiscible solvent or in the case of headspace measurements, a high boiling solvent, is applied for analyte extraction from water samples. Drop volumes are in the micro- to picoliter range, and the technique can be categorized by the used sample volumes. LPME has some distinct advantages over other microextraction techniques. LPME with its very low solvent amounts is very inexpensive compared with SPME, SBSE and other microextraction techniques. In the case of thermal desorption into the GC injector, the method does not lead to peak broadening and tailing by slow analyte desorption as might be the case for desorption from polymer coatings and no carry over effects can occur due to renewal of the solvent after each extraction. In Table 6 an overview of the extraction conditions of HSME for the determination of MTBE is given. Two Iranian groups developed methods that apply $\sim 2\,\mu L$ drops of benzyl alcohol as extraction solvent. Yazdi et al. reported a detection limit of $7\,\mu g\,L^{-1}$ and a precision of 5.5% at a concentration of $1\,mg\,L^{-1}$ [64]. Bahramifar et al. cooled the needle to – 6 °C during the extraction and achieved a detection limit of $0.06\,\mu g\,L^{-1}$ which is in the region between detection limits for headspace SPME-GC/FID and headspace SPME-GC/MS [65].

3
Other Methods for Quantification

3.1
Membrane Introduction Mass Spectrometry

Membrane introduction mass spectrometry (MIMS) of MTBE applies a flow injection module with a silicone membrane that allows the diffusion of MTBE from water into the ion source of a mass spectrometer. The direct analysis of MTBE in water was reported by Lopez-Avila et al. [66]. They reported a limit of detection of 0.1 $\mu g\,L^{-1}$ and a dynamic linear range of more than two orders of magnitude. However, a major drawback of MIMS, in particular for the analysis of small organic compounds, is the poor selectivity because of the lacking chromatographic separation prior to analysis. Hence, so far MIMS can only be recommended for laboratory studies with very low levels of co-contaminants or degradation products. Interestingly, within the past years no further study utilizing MIMS for MTBE determination has been reported.

3.2
Ion Mobility Spectrometry

Baumbach et al. were the first to show that IMS can be used to quantify MTBE with ion mobility spectrometry (IMS) both in gasoline and in aqueous samples [67]. They coupled the IMS with a 25-cm multi-capillary column (MCC) in order to separate BTEX compounds and MTBE. Sensitivity depended very much on the ionization source used (see Table 7). Aqueous samples were introduced into the MCC by a membrane inlet but no further enrichment step has been applied.

Pozzi et al. used a dynamic headspace method in combination with a mobile ion mobility spectrometer for the determination of MTBE in drinking water and groundwater [68]. The analytes were sorbed on a Tenax trap cooled with liquid nitrogen, placed between the sample bottle and the IMS. An on-line method for the determination of MTBE and BTEX from groundwater samples was developed by Borsdorf et al. [69]. In this study, an extraction chamber with a membrane was used for the extraction of MTBE from the aqueous phase with a detection limit of 12 $\mu g\,L^{-1}$. Neither inorganic compounds nor humic substances affected the peak intensity of MTBE significantly.

IMS could well become a simple and cost-effective tool for a rapid on-site analysis of volatile organic compounds in water but so far it does not reach the detection limits of SPME or Purge and Trap. However, for a first screening at contaminated sites IMS already seems to be well suited.

Table 6 Other microextraction methods for fuel oxygenate determination

Enrichment method	Detector	Experimental settings	LOD [$\mu g\,L^{-1}$]	LOD other oxygenates [$\mu g\,L^{-1}$]	LOD definition	RSD [%] & conc. [$\mu g\,L^{-1}$]	Internal standard	Application	Refs.
Headspace solvent microextraction (HSME)	FID	Extraction solvent: 2 µL benzyl alcohol, 6 mL sample, HS ratio 1.7, 4 M NaCl, 35 °C, 7.5 min, stirring at 1000 rpm, needle cooling to – 6 °C	0.06		S/N 3/1	4.8 & 0.1–500	toluene	tap and groundwater	[65]
Headspace solvent microextraction (HSME)	FID	Extraction solvent: 1.8 µL benzyl alcohol, 4 mL sample, HS ratio 2.3, 0.8 g NaCl, 35 °C, 10 min, stirring at 300 rpm	7		$3 \times s_d$	5.5 & 1000	none	none	[64]
Solid-phase dynamic extraction (SPDE)	MS Full Scan	10 mL sample (+ salt), HS ratio 0.75, 3.33 g NaCl, 70 °C extraction strokes, aspiration flow rate 125 µL/s,desorption gas volume 1000 µL, desorption flow rate 50 µL/s	0.06[a]	ethanol 2.3[a] IPA 0.15[b] IBA 0.09[b] TBA 0.15[a]	$t_{(N-1,1-\alpha=0.99)} \times s_d$ of 7 low low level spikes	3 & 0.6	TBA-d_{10}	alcoholic beverages and spirits	[61]

[a] using a 50 µm ×56 mm PEG coating
[b] using a 50 µm ×80 mm PDMS/AC coating

3.3 Compound-Specific Isotope Analysis

Compound-specific isotope analysis (CSIA) is a rather new tool in environmental analysis. It is not used for quantitative analysis of organic compounds but rather for the characterization of environmental fate by monitoring changes in the isotopic composition of organic molecules. *Analytical aspects* are covered here because this rather new area of research is expected to mature to a standard tool in environmental analysis that will complement the quantitative analysis in the environment [70].

As shown in Fig. 2, in CSIA, organic compounds are separated with gas chromatography and on-line fed to a combustion unit where they are totally converted to simple gases such as CO_2 or H_2 (for isotope analysis of carbon and hydrogen, respectively). The combustion gases are transferred to an isotope-ratio mass spectrometer (IRMS) that measures precisely the isotopic composition of these gases. For example, in the case of carbon, the element by far most frequently determined today, the ratio of masses 44 ($^{12}CO_2$) and 45 ($^{13}CO_2$) is measured.

Several reviews of CSIA principles, techniques and important application areas have been published in the past years [70–72]. A current problem of CSIA is the rather poor sensitivity of the IRMS that frequently limits potential applications to highly contaminated samples [70]. Improvements of this situation are directly related to the appropriate use of enrichment techniques as discussed in detail above for MTBE and related compounds.

In combination with GC/IRMS, static headspace analysis for fuel containing compounds such as BTEX was applied in different studies [73, 74]. Headspace injection does not fractionate significantly for MTBE [74, 75]. Method detection limits for $\delta^{13}C$ static headspace-GC/IRMS applications are between 4000–5000 μg/L for MTBE [74, 75].

A few studies have been published on the use of CSIA in combination with SPME or purge&trap to characterize MTBE biodegradation in laboratory microcosms and in the field. Table 8 summarizes experimental settings and benchmark parameters of the utilized methods. The reported LODs demonstrate that it is nowadays indeed possible to use CSIA in the low $\mu g\,L^{-1}$ range. Hunkeler et al. [76] observed a reproducible depletion of ^{13}C in MTBE and TBA extracted with SPME, both in the headspace and the direct immersion mode. In contrast, they observed a small enrichment in ^{13}C for water–air partitioning, which implies that there will be a reverse trend in $\delta^{13}C$ results determined by headspace analysis compared with SPME. However, in both cases the observed effects were much smaller than the fractionation found in degradation studies. Zwank et al. [77] reported a thorough evaluation of several enrichment/injection techniques (on-column, splitless, split, direct SPME, purge&trap) coupled to GC/IRMS. For SPME they found a somewhat higher but reproducible depletion of ^{13}C in extracted MTBE. For purge&trap

Table 7 Ion mobility spectrometry for fuel oxygenates

Detector	Ionization source	Sample volume [mL]	Flow into IMS [$L\,h^{-1}$]	Sample introduction	LOD [$\mu g\,L^{-1}$]	RSD [%] & conc. [$\mu g\,L^{-1}$]	Application	Refs.
IMS	UV	200	20–50	Membrane inlet to multi capillary column	20 000	5.3 & 74 000	none	[67]
	^{63}Ni	200	25		1	4.6 & 46		
IMS	^{63}Ni	100	25	Enrichment on Tenax (70 °C desorption temperature)	0.5	4.7% & 50 intra-day 8.4% & 50 inter-day	groundwater, tap water	[68]
IMS	^{63}Ni (UV and Corona discharge also tested)	1000	25	Membrane separation of water and gas stream	12	No data	groundwater	[69]

a very small enrichment of ^{13}C in extracted MTBE was found, which corroborates previous results by Smallwood et al. [78]. The isotopic shift did not depend on the extracted amount of MTBE if the extraction efficiency exceeded 20%. Recently, Elsner et al. tried to improve quantification limits of headspace analysis by freezing out the aqueous samples [79]. They found a 35-fold and a 14-fold concentration enhancement for TBA and MTBE, respectively, over the frozen sample compared with the headspace concentration at 25 °C. The obtained standard deviations were too high for using this method for quantification, but no measurable carbon isotopic effect for TBA and MTBE was observed, thus it could be used to increase sensitivity in CSIA.

In summary, these results demonstrate that for CSIA, in addition to the method validation required for quantitative determinations, the methods

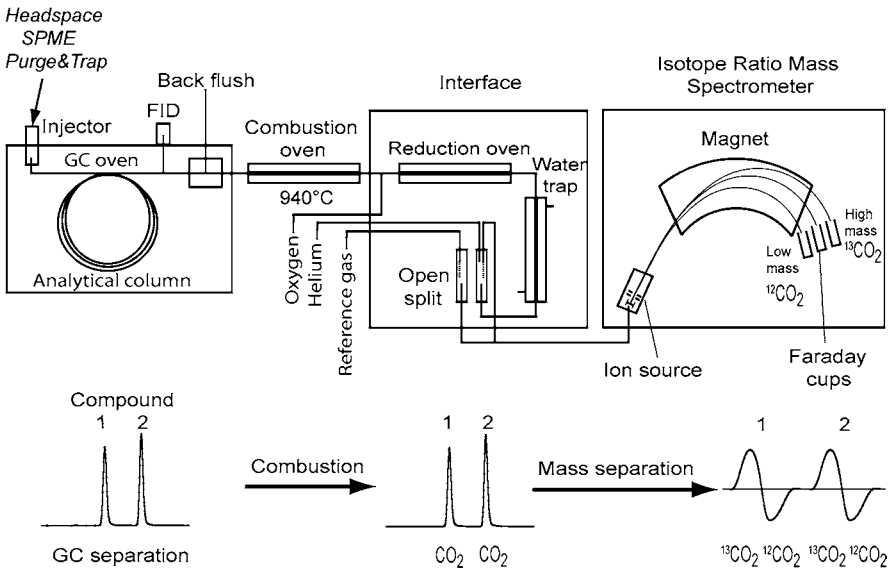

Fig. 2 Schematic overview of a GC/C/IRMS for determination of $\delta^{13}C$ values. Following headspace injection or enrichment with SPME or purge & Trap the analytes were separated by GC. After separation the target analytes were completely combusted to CO_2 and H_2O by using a PT/NiO/CuO catalyst containing combustion oven. Water is removed by a Nafion™ membrane to prevent formation of $^{12}CO_2H^+$ (m/*z* 45) during ionization. Following combustion the CO_2 is ionized in the ion source of the mass spectrometer. After ionization the formed isotopologues $^{12}CO_2$ (m/*z* 44), $^{13}CO_2$ (m/*z* 45) and $^{12}C^{18}O^{16}O$ (m/*z* 46) are diverted according to their masses in the magnetic field and detected in separate Faraday cups. The signal output of the Faraday cups is amplified according to the natural isotope abundances of the detected isotopes. Mass 46 is used for correction of the relative abundance of ^{17}O by determination of ^{18}O in the mass 46 isotopologue and an empirical correlation between ^{17}O and ^{18}O content

used for enrichment and injection need to be evaluated with regard to their influence on the original isotopic composition. Otherwise, artifacts may be obtained in situations where the isotopic fractionation is not very pronounced

4
Potential Problems in Fuel Oxygenate Analysis

4.1
Hydrolysis of Dialkyl Ethers

In contrast to the standard procedure for sampling and storage of volatile organic compounds, samples should *not* be preserved with acid since the hydrolysis of MTBE to TBA has been described under moderately acidic conditions [82]. Furthermore, preservation seems not necessary if samples are stored in the dark at 4 °C because biodegradation of MTBE usually is very slow. In a long-term storage study conducted by the USGS National Water Quality Laboratory for MTBE, ETBE, TAME and di-isopropyl ether over 216 days no loss of the analytes was found at pH 2 and 4 °C [17], so under these conditions MTBE hydrolysis is obviously negligibly slow. Most problematic is acidic preservation when using analysis techniques at high sample temperatures because hydrolysis may be accelerated sufficiently to cause losses as has been reported for MTBE, ETBE and TAME [28]. Only DIPE seems to be hardly affected at a pH of 2. Therefore, if there is the need for preservatives due to regulations or co-contaminants, addition of trisodiumphosphate (TSP) to a pH of 11.5–12.0 is recommended [28, 83].

4.2
Blank Contamination

Achten et al. [55] reported on the presence of MTBE in blank samples in the low ng L^{-1} range and attributed this to contaminations of the GC/MS system. In our own investigations we frequently found small concentrations of MTBE and benzene in Nanopure water blanks and therefore used tap water for preparation of standards instead. Tap water collected after 10 min flushing consistently showed the lowest background contamination levels of volatile organic compounds [11]. Similarly, Arambarri et al. used organic free spring mineral water because of blank contamination in bidistilled and deionized water [7]. In a USGS study, reagent blank water was prepared by boiling deionized water for one hour and subsequent purging with nitrogen for at least one hour. Cardinali et al. used blank water that had previously been helium sparged, distilled, and stored in flame-sealed ampoules [84]. In both waters, no MTBE or other dialkyl ethers

Table 8 CSIA methods for the trace measurement of the carbon isotopic composition of fuel oxygenates

Enrichment method	Experimental settings	LOD [$\mu g\,L^{-1}$]	LOD definition	Application	Refs.
SPME 75 µm PDMS/Carboxen	Extraction time: 20 min & 23 °C, desorption with split ratio 2 : 1 HS: 42-mL vial filled with 36 mL solution, 250 $g\,L^{-1}$ NaCl Direct: 2-mL vial filled with 1.7 mL solution, 250 $g\,L^{-1}$ NaCl	HS: 11 (TBA: 860) Direct: 90 (TBA: 370)	Peak height > 0.75 V (m/z 44)	aerobic laboratory micocosms	[76]
Purge&trap	Purge: 40 °C, 11 min, (Trap type, purge flow, and sample volume not given) Desorption: 180 °C, 4 min, no cryofocusing	15	not reported	none	[78]
HS-SPME 75 µm PDMS/Carboxen	Extraction time: 20 min (temp. and sample volume not given); Desorption: 2 min & unknown temp.	350 (δ^2H: 1000)	not reported	aerobic laboratory microcosms, pure culture studies (PM1)	[74]
Purge&trap	Tenax-silica gel-charcoal trap, purge: 5 mL sample, 40 °C, 8 min (purge flow not given), Desorption: 180 °C, 4 min, no cryofocusing	5.0 (TBA: 60)	not reported	anaerobic laboratory microcosms, field site with anaerobic (presumably methanogenic) conditions	[80]

Table 8 (continued)

Enrichment method	Experimental settings	LOD [$\mu g\,L^{-1}$]	LOD definition	Application	Refs.
direct SPME 75 μm PDMS/Carboxen	Extraction: 30 min & 30 °C, 2-mL-vial filled with 1.3 mL solution plus 0.3 g NaCl Desorption: 1 min & 270 °C, splitless time 1 min, split 50 ml min^{-1}	16	Peak height > 0.5 V (m/z 44)	none for MTBE	[77]
Purge&trap	Carbopack B/Carboxen 1000 trap, purge: 25 mL sample, 23 °C, 30 min, purge flow 40 mL min^{-1} N_2, Desorption: 250 °C, 1 min, cryofocusing @ – 120 °C	0.63	Peak height > 0.5 V (m/z 44)	none for MTBE	[77]
headspace injection	–	4000 (TAME: 6000)	not reported	groundwater, surface water	[75]
Purge&trap	Vocarb3000, purge: 25 mL sample, 50 °C, 14 min purge flow 40 mL min^{-1} N_2, 3 min dry purge Desorption: 250 °C, 1 min, liquid N_2 cryofocusing (temp. not given)	5 (TBA: 25) 20 (δ^2H)	not reported	contaminated groundwater at various field sites	[81]

were found. Cardinali et al. also reported on contamination of water samples with MTBE by using polypropylene tubes as sample transit containers [84]. In general, the use of plastics in the handling of samples and standards for fuel oxygenate analysis should be minimized, in particular to avoid cross contamination by carry-over effects. In conclusion, if measurements in the low ng L^{-1} range are required, one should carefully check the presence of blank values.

4.3
False Positive Detection with Non-Selective Detectors

Halden et al. [34] have carried out round-robin and split-sample studies to evaluate EPA standard methods for MTBE analysis. They have shown that EPA method 8020A/8021B utilizing photoionization detection (PID) is susceptible to false positive misidentifications and inaccurate results of MTBE and TBA at high concentrations of total petroleum hydrocarbons frequently encountered at contaminated sites. The authors therefore recommend using EPA method 8240B/8260B with MS detection for the investigation of such sites. Similar problems as with PID are expected using FID. The use of other detectors than MS is therefore not feasible except for laboratory studies because either sensitivity (background concentrations) or selectivity (point sources) is generally not sufficient.

5
Choice of an Appropriate Method

The concentrations of MTBE and other fuel oxygenates in water differ by several orders of magnitude from environmental background to sites affected by point sources. Thus, different analytical strategies may be required. Important differences between diffuse and point sources are summarized in [4]. Today, MTBE is a nearly ubiquitous contaminant in atmospheric and surface water [85, 86] and is also one of the most frequently detected VOC in groundwater at levels well below 2 μg L^{-1} [87, 88]. If higher concentrations than 2 μg L^{-1} are found this is an indication of an unknown point source and further investigations should be initiated. The same holds true for other dialkyl ethers.

The choice of an appropriate method depends on the individual compounds and matrix to be investigated, concentration ranges to be analyzed, the available laboratory equipment and compliance with regulations. As a rule of thumb, for analyses of background concentrations in the range below 1 μg L^{-1} SPME-GC/MS or purge&trap-GC/MS are recommended. If properly set up, both methods are able to detect MTBE at concentrations as low as 10 ng L^{-1}. In the concentration range below 1 μg L^{-1}, Schuhmacher et al. have

reported on an interlaboratory comparison study. They concluded that in the investigated concentration range static headspace methods lack in inter- and intra-laboratory precision and recommended the use of SPME or purge&trap for enrichment [21]. Results of the few laboratories that used FID for detection were not acceptable. A further method comparison by Stringfellow and Oh, although performed at a higher concentration range (3 to 300 $\mu g\,L^{-1}$), has shown that SPME-GC/MS and purge&trap-GC/MS yield well comparable results [49].

Both methods, however, have their weaknesses for the investigation of contaminated sites. In the case of SPME, sorption competition by frequently found co-contaminants needs to be considered. Purge&trap systems are susceptible to long-lasting contaminations if highly polluted samples are analyzed without previous dilution. For the investigation of contaminated sites, HS-GC/MS or DAI-GC/MS are therefore recommended. The latter allows the simultaneous determination of TBA, the key intermediate in MTBE and ETBE degradation, which is often abundant at such sites. Method validations have shown that results are well comparable among various methods [11, 22]. DAI-GC/MS is also well suited for laboratory studies if only small volumes of sample are available.

An attractive on-site method to complement the laboratory-based GC methods for the investigation of contaminated sites is ion mobility spectrometry. However, so far its applicability has only been shown for MTBE but neither for other ethers nor alcohols in aqueous samples.

While the quantitative determination of MTBE and other dialkyl ethers even in the $ng\,L^{-1}$ range is possible on a routine base, the determination of alcohols in water at such low concentrations is still an analytical challenge.

If research aims at the characterization of origin and fate of PVOC in the environment, compound-specific isotope analysis is a powerful complement of classical quantification methods and will become a more widely employed analytical tool in the future.

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Occurrence and Fate of MTBE in the Aquatic Environment Over the Last Decade

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Abstract In the last decade, it became increasingly evident that the fuel oxygenate methyl tertiary butyl ether (MTBE) is nearly ubiquitous in the worldwide environment. The detection frequency of MTBE rivals other volatile organic compounds (VOCs) that have been produced and used for a much longer period of time. Its mere presence in water bodies used as drinking water reservoirs (rivers, lakes, or groundwater tables) has aroused concern about its potential sources, persistence, or possible adverse effects (aesthetic or toxic implications) for end-users and aquatic life. The purpose of this chapter is to provide an updated overview of the current environmental concentrations, the occurrence of the pollutant in the different aquatic compartments, the relevance of diffuse and point sources, and the different alternatives for remediation of MTBE contaminated sites.

Keywords Diffuse and point sources water · Environmental levels · ETBE · MTBE

Abbreviations

AED	Atomic emission detection
ATD	Automated thermal desorption sampler

BP	British Petroleum
BTEX	Benzene, toluene, ethylbenzene and xylenes
CAA	Clean Air Act
DAI	Direct aqueous injection
DIPE	Diisopropyl ether
EFOA	European Fuel Oxygenates Association
ETBE	Ethyl tertiary butyl ether
EU	European Union
FID	Flame ionization detector
GAC	Granular activated carbon
GC	Gas chromatography
GW	Groundwater
HS	Headspace
IS	Internal standard
MMA	Methyl methacrylate
MS	Mass spectrometry
MTBE	Methyl tertiary butyl ether
P&T	Purge and trap
PID	Photoionization detector
P-THREE	"Removal of persistent polar pollutants through improved treatment of wastewater effluents"
RFG	Reformulated gasoline
RON	Research octane number
SMCL	Secondary maximum contaminant level
SPME	Solid-phase microextraction
$t_{1/2}$	Half-life time
TAME	Tertiary amyl methyl ether
TBA	Tertiary butyl alcohol
TBF	Tertiary butyl formate
tert-	Tertiary
USEPA	US Environmental Protection Agency
VOC	Volatile organic compound
WATCH	"Water catchment areas: tools for management and control of hazardous compounds"
WHO	World Health Organization
WWTP	Wastewater treatment plant
LUST	Leaking underground storage tank

1 Introduction

Commercial production of methyl tertiary (*tert-*) butyl ether (MTBE) started in Europe in 1973 and in the USA in 1979, but it was in the 1990s when the MTBE market grew strongly (in 1999 the annual consumption was about the double that of 1992). Since then, large amounts (around 20 million t) of this chemical has been produced worldwide each year. Ninety-eight percent of this chemical production is used as an additive in petrol. Its low cost,

easy production, favorable transfer and blending features turned MTBE into the most commonly used fuel oxygenate. Worldwide MTBE consumption has been dominated by the USA (about 60% in front of 15% in Europe) mainly to meet the oxygen requirements mandated in 1990 by the Clean Air Act (CAA) Amendments in areas where certain air-quality standards (related to CO or O_3) have not been attained. In contrast, MTBE was incorporated in European gasoline as an octane enhancer to replace banned tetraalkyl lead compounds and increasing restrictions on aromatics content.

Specific chemical and physical properties of MTBE are compared to other common fuel oxygenates and aromatic hydrocarbons in Table 1. In general, alcohols and ethers have higher water solubilities, lower Henry's law constants, and lower sorption constants than do aromatics. Among fuel additives, MTBE is the ether with more extreme values, which favored its higher mobility (nearly as fast as the groundwater rate) and harder removal from water by aeration or degradation processes [1].

With such production, use, and properties, it is not surprising that MTBE has been released into the environment and has adversely affected the quality of water. It has been responsible for documented taste and odor problems in drinking water, and there are also concerns about possible adverse human health effects. With this alarm, several environmental, health, and government institutions have prepared their own risk-assessment studies, but up till now, new MTBE regulation is required in Europe [2]. Since 1997, the US Environmental Protection Agency (EPA) has established an MTBE drinking water advisory at 20–40 $\mu g\,L^{-1}$ based on aesthetic (taste and odor) criteria [3], however, for a long time it was expected that the EPA will adopt a federal secondary maximum contaminant level (SMCL) probably at 15 $\mu g\,L^{-1}$ for MTBE according to lower consumer acceptance [4].

However, the World Health Organization (WHO) decided not to establish an MTBE health-based guideline value because any guideline value based on any adverse effects would be significantly higher than the concentration at which it would be detected by odor [5].

MTBE toxicity effects on freshwater and marine organisms have been found at higher concentrations ($mg\,L^{-1}$), which seldom happen in the environment [6, 7]. However, the presence of MTBE can substantially enhance the toxicity of other pollutants such as pesticides, which are often present in the same waters [8].

For these reasons as well its omnipresence in water samples globally during the last decade, the environmental behavior of MTBE has been considered as an international concern. Knowledge of MTBE levels and distribution in natural water (groundwater and surface) and soil became a challenging task because conventional analytical methods such as liquid–liquid extraction were not feasible. Moreover, the concentrations of MTBE in water differ by several orders of magnitude between environmental background ($ng\,L^{-1}$) and sites affected by point sources ($mg\,L^{-1}$), thus requiring different analytical

Table 1 Physicochemical properties of MTBE, its main degradation products, and other common gasoline additives/octane enhancers

Gasoline additive/ substance	Abbreviation	CAS no.	Molecular Weight (g/mol)	Blending RON	Boiling point (°C)	Solubility in water (mg/L)	Henry's law Constant ($atm \cdot m^3$)/($g \cdot mol$)	Vapor Pressure (mm Hg at 25 °C)	Log K_{ow}
ETHER OXYGENATES									
Methyl *tert*-butyl ether	MTBE	1634-04-4	88	116[a]–118[b]	55	51 000	5.87E-04	250.00	0.94
Ethyl *tert*-butyl ether	ETBE	637-92-3	102	118[ab]	73	12 000	1.39E-03	124.00	1.92
tert-amyl methyl ether	TAME	994-05-8	102	109[b]–111[a]	86	2640	2.68E-03	75.20	1.92
Diisopropyl ether	DIPE	108-20-3	102	nd	69	8800	2.28E-03	149.00	1.52
ALCOHOL OXYGENATES									
Methanol	MeOH	67-56-1	32	125[b]–133[a]	65	Complete	4.55E-06	127.00	– 0.77
Ethanol	EtOH	64-17-5	46	129[a]–130[b]	78	Complete	5.00E-06	59.30	– 0.31
DEGRADATION PRODUCTS									
tert-butyl alcohol	TBA	75-65-0	74	105[b]	82	Complete	9.05E-06	40.70	0.35
tert-butyl formate	TBF	762-75-4	102	nd	83	11 200	6.90E-04	86.40	1.19
AROMATIC HYDROCARBONS									
Benzene	B	71-43-2	78	98[a]	80	1790	5.55E-03	94.80	2.13
Toluene	T	108-88-3	106	124[a]	111	526	6.64E-03	28.40	2.73
Ethylbenzene	E	100-41-4	106	124[a]	136	169	7.88E-03	9.60	3.15
m-xylene	X	108-38-3	106	162[a]	138	161	7.18E-03	8.29	3.20
p-xylene	X	106-42-3	106	155[a]	139	162	6.90E-03	8.84	3.15
o-xylene	X	95-47-6	106	126[a]	144	178	5.18E-03	6.61	3.12

All data at 25 °C, obtained from Syracuse Research Corporation PhysProp Database (free access under www.syrres.com/esc/physdemo.htm) except the Research Octane Number (RON) values obtained from:

[a] Department of Information and Computing Sciences (University of Utrecht, The Netherlands): http://www.cs.uu.nl/wais/html/na-dir/autos/gasoline-faq/part2.html

[b] European Fuel Oxygenates Association (EFOA): www.efoa.org/EFOA_Pages/02_What/02b_Propertie.html

nd: no data available

strategies. As discussed in Chap. 1 of the book (*Novel Analytical Methods for the Determination of Fuel Oxygenates in Water*) and in previous comprehensive reviews [9–11], there are several enrichment and injection techniques almost exclusively coupled to gas chromatography (GC) and mainly with mass spectrometric (MS) detection, which can be selected depending on current requirements.

The purpose of this chapter is to provide an updated overview of the state of the art of the environmental occurrence and fate of MTBE over the last decade studies and to compare them with some of our results from previous EU projects: WATCH ("Water Catchment Areas: Tools for Management and Control of Hazardous Compounds") and P-THREE ("Removal of Persistent Polar Pollutants through Improved Treatment of Wastewater Effluents").

2 Production, Usage, and Source Characterization

Detailed knowledge of the oxygenate type and fraction in gasoline is essential in any attempt to estimate the potential local or regional environmental impact from using oxygenated fuel [12].

Reformulated gasoline (RFG) represented almost 30% of all gasoline sold in the USA. The CAA required 2.0% (w/w) minimum oxygen content, which was mainly accomplished by an MTBE content about 11% (v/v), but also by a mixture of other oxygenates such as *tert*-amyl methyl ether (TAME), ethyl *tert*-butyl ether (ETBE) or diisopropyl ether (DIPE). In a study conducted in 2000 in the state of New Hampshire, TAME was found in 88% of all gasoline samples at a mean volume of 1.2% and ETBE in 51% of the samples at lower amounts, 0.5% [13]. However, in the last years, MTBE was phased-out in many states and substituted by ethanol. In 2004, total US MTBE demand fell 26.5% (6.5 million t) versus the prior year. In addition, with the passing of the new US Energy Policy Act in July 2005, the oxygen content mandate was removed, and a provision adopted which set the annual use of 4 billion gallons of renewable fuels in 2006 rising to 7.5 billion in 2012. Although there has been no federal ban on the use of MTBE (derived mainly from natural gas), this mandate will undoubtedly reduce the amount of MTBE used in the USA while increasing the switch to ethanol obtained by a fermenting process from corn and other agricultural products.

In contrast, the maximum oxygen content of 2.7% (w/w) and up to 15% ethers with ≥ 5 carbon atoms (v/v) are allowed in EU [14]. Thus, the average content of MTBE in European gasoline has been quite low (about 2%), but great differences can be found between countries and different gasoline research octane number (RON) grades [15]. As an illustrative example, Achten et al. [16] measured average MTBE content in German regular (0.4%), eurosuper (0.4–4.2%), super premium unleaded (9.8%), and Optimax (11.9%)

gasoline. Similar percentages were also available from the two main Spanish petrol companies in the year 2000. Repsol used an MTBE content in volume from 4.3 to 10.0% (RON 95 and 98, respectively) whereas in Cepsa gasolines, it was slightly lower (from 2.8 up to 6.9% for the same grades) (Pérez Pascual MA (2001) Personal communication from: Director Centre Investigació de CEPSA, January 2001). However, at that time, both companies estimated an increment (up to 12%) for the year 2005. Later on, in January 2003, the analysis of different gasoline grades sold by British Petroleum (BP) in Barcelona, Spain, showed MTBE contents from 1% (RON 95) to 7% (RON 97 and 98), but lower amounts were detected in March 0.06–4% accompanied with higher ETBE (up to 14%) and toluene (15%) percentages [17]. These discrepancies were explained as a progressive change of gasoline composition. In fact, in Spain, as in other European countries such as France, Portugal or Poland, MTBE is already being substituted by ETBE due to tax incentives for the application of biomass-derived ethanol, which is synthesized to produce the ethyl ether group of ETBE (Directive 2003/30/EC) [18]. The directive proposed a proportion of biofuels in all gasoline and diesel fuels sold on all Member States market at 2% minimum by the end of 2005, and 5.75% by 2010. Currently there are more than 50 production plants for ether oxygenates in Europe, the majority of them still produce MTBE, but ETBE and TAME are indeed gaining market share (see Fig. 1). In fact, over 20 refineries switched production to ETBE, which rep-

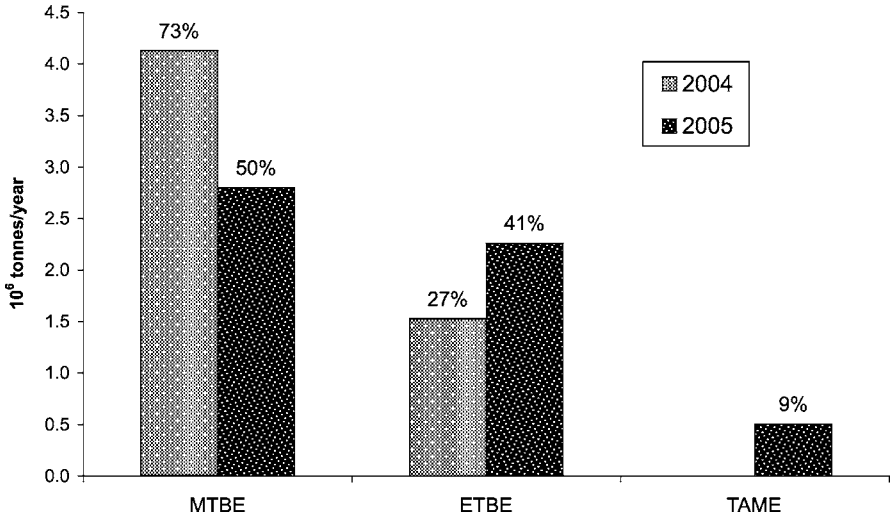


Fig. 1 European production capacities for ether oxygenates (MTBE, ETBE, and TAME) in 2004 and 2005. The total annual production was about 5.6 million t and the relative contribution of each additive is given in % (Data from European Fuel Oxygenates Association (EFOA) Web site: http://www.efoa.org/)

resented about 41% of the EU ether oxygenates production capacity in 2005 (http://www.efoa.org/supply_demand.html). Due to their similar blending features, analogous percentages of ETBE should be expected in current gasoline.

On the other hand, most countries in Asia-Pacific have chosen MTBE to replace lead additives and the demand is still growing. Nevertheless, MTBE has also received much attention as a chemical requiring serious investigation in Japan [19] and the Japanese oil industry is currently exploring the possibility of blending ETBE into gasoline to contribute to the mitigation of CO_2 emissions from road transport sector (Hara H (2006) Personal communication from: representative of Japan Petroleum Energy Center (JPEC) Brussels office, Brussels, Belgium. April 2006). Japanese official specifications continue to allow a 7% MTBE volume limit, whereas Australia is more restrictive, fixing a maximum of 1% of any ether oxygenate since 2004 (http://www.ea.gov.au).

In addition to its use as an octane enhancer, MTBE is increasingly being used as a feedstock for methyl methacrylate (MMA) in some countries such as Japan, South Korea, and Singapore. The demand for MMA is growing worldwide due to the increased popularity of flat-screen displays. So, in conclusion, the total consumption of MTBE might be expected to remain fairly stable over the next few years.

Accidental spills and tank corrosion leakage from gasoline stations and refineries are the main sources of MTBE entering the environment. From an RFG gasoline release, the MTBE solubility was estimated one order of magnitude lower than from the pure compound, but still 200–1600 times higher than BTEX [20]. Given the varying levels of MTBE in different types of gasoline and countries, the risk MTBE poses to the environment should be regarded as crucial. However, the occurrence of MTBE has been the focus of studies in the USA and Europe (mostly carried out in Germany). MTBE levels in background environmental samples are generally below 2 $\mu g\,L^{-1}$; when higher concentrations are found, it is an indication of an unknown point source.

3 Environmental Fate

3.1 Precipitation

Partitioning between air and water is normally assumed to be the primary process affecting the occurrence of VOCs in precipitation samples. Due to the characteristics of MTBE, stormwater runoff and atmospheric transport are low contributors to the water concentrations of this pollutant, as shown in several occurrence, transport and modelling studies (see Fig. 2). In the

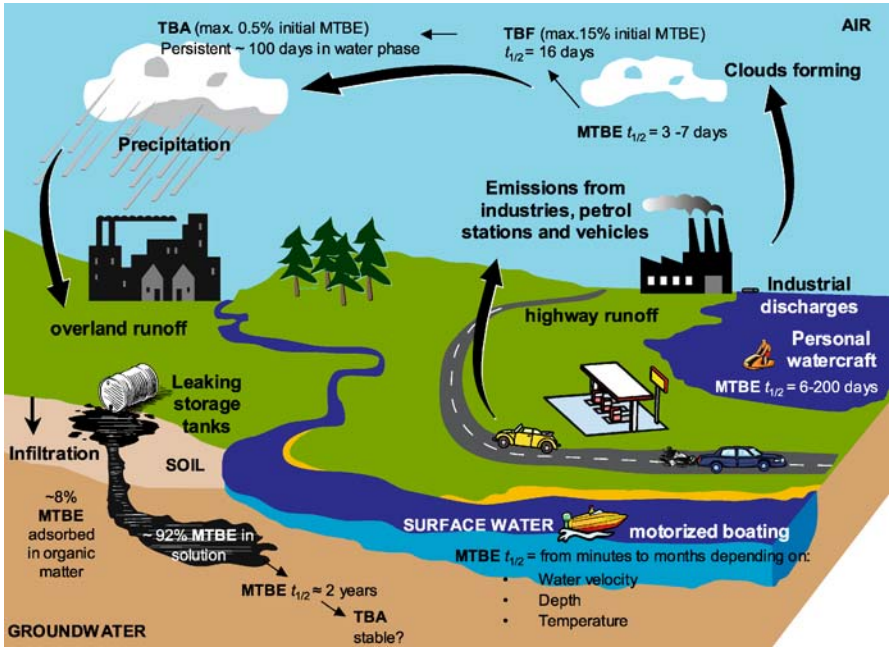


Fig. 2 Review of MTBE behavior and fate in the environment: diffuse and point sources, degradation products and its reported half-life times ($t_{1/2}$) at the different environmental compartments

air, MTBE degradation is expected to be fast (half-life, $t_{1/2}$ between 3 and 7 days [1, 21]) depending mainly on hydroxyl radical (OH) concentration, which is considered much more determining than photolysis or reaction with ozone or other radicals [1]. In all cases, *tert*-butyl formate (TBF) was observed to be the major degradation product and its maximum concentration was predicted to be 15% of the initial MTBE emission after 4 days [22]. Once TBF hydrolyzes to *tert*-butyl alcohol (TBA), traces of this compound can persist in the water phase at low temperature (5 °C) for around 100 days, having a mass of 0.2% of the original MTBE emission [22].

In 1991–1995, Delzer et al. [23] detected MTBE in 7% of US municipal stormwater samples (up to 8.7 $\mu g\,L^{-1}$), which represented the seventh VOC most frequently found. The reporting level for MTBE in that study was of 1 $\mu g\,L^{-1}$, thus it was likely that lower detection limits might result in a higher occurrence.

This was the challenge of Achten et al. [24] who developed HS-SPME-GC/MS to detect MTBE in water from 0.01 $\mu g\,L^{-1}$. Following, a sampling campaign was carried out in winter 2000/2001 at several German locations. Rainwater collectors were placed on the top of buildings to avoid direct vehicle emissions. MTBE varied according to spatial distribution (higher oc-

currence in urban (86%) than in rural (18%) precipitation samples) and depending on climatic conditions (detectable at temperatures lower than 10–15 °C) [25]. However, the highest values (0.03–0.085 $\mu g\,L^{-1}$), which were detected in the center of Frankfurt am Main, were two orders of magnitude lower than values previously measured in the USA. These differences were mainly explained by the lower and constant year-round MTBE percentage in German gasoline. In addition, the analysis of urban runoff and corresponding rainwater samples revealed that about 20% of MTBE originated from atmospheric (air and precipitation) transport, whereas about 80% may be attributed to direct uptake from vehicle emissions and leakage near the road during precipitation.

Extending this investigation and applying the same analytical method, Kolb and Puttmann [26] measured MTBE levels in snow samples (up to 0.6 $\mu g\,L^{-1}$) in the same locations during the following two winter seasons. Since only 4 g of snow was required for the analysis, the collection time could be held short to avoid post-depositional processes and the melting snow was transferred as soon as possible to vials to minimize volatile losses. Comparison to the previous rainwater samples indicated atmospheric transfer of MTBE from urban to rural areas preferentially in winter due to lower atmospheric degradation rates and suggested that MTBE is more effectively scavenged from the atmosphere by snow than by rain.

In the framework of the WATCH project, a 2-year monitoring program was carried out in the vicinity of an airport located in the southern Iberian Peninsula. MTBE and related compounds were analyzed through seven sampling campaigns (from April 2002 to August 2004) to check the ubiquity of such gasoline additives in different environmental water bodies. A total of 25 runoff rainwater samples from the airport platforms showed a MTBE mean value of 0.15 $\mu g\,L^{-1}$ with higher values, up to 1.40 $\mu g\,L^{-1}$, in summer periods when a higher density of passengers was assumed. This seasonal trend was also observed for BTEX especially in July 03 with levels ranging from 0.9 to 26 $\mu g\,L^{-1}$ while in January the maximum was 0.5 $\mu g\,L^{-1}$. Although TBA presented more variable behavior, higher values were found in both October campaigns (02/03) just after more active period, which may originate from MTBE and TBF atmospheric degradation.

3.2 Groundwater

Infiltration of precipitation and dispersion from urban atmosphere can act as a non-point source transport of MTBE and other VOCs into shallow groundwater [27]. One of the first studies that pointed out the potential MTBE occurrence and persistence in groundwater tables was a survey conducted from 1993–1994 as part of the US Geological Survey's National Water-Quality Assessment Program [28]. Among 60 VOCs analyzed by P&T-GC/MS,

Table 2 Overview of maximum reported concentrations (expressed in $\mu g\,L^{-1}$) of MTBE and related compounds in different environmental water bodies by different analytical methods over the last decade in comparison with EU project (WATCH and P-THREE) results

Location	Country	Sample type	Analytical method	MTBE	ETBE	TAME	DIPE	TBA	BTEX	Refs.
Precipitation										
Several locations	Germany	Precipitation	HS-SPME-GC/MS	0.09	na	na	na	na	na	[25]
Frankfurt am Main	Germany	Road runoff water	HS-SPME-GC/MS	1.17	na	na	na	na	na	[25]
Airport vicinity	Southern Iberian Peninsula	Platform runoff water	P&T-GC/MS	1.40	0.15	0.50	0.02	0.83	35	WATCH
Several locations	US	Stormwater	P&T-GC/MS	8.70	na	na	na	na	15	[23]
Several locations	Germany	Snow	HS-SPME-GC/MS	0.60	na	na	na	na	na	[26]
GW										
Petrol station, Salzburg	Austria	Groundwater	P&T-GC/MS	3.32	0.04	nd	0.01	0.41	0.45	WATCH
Petrol station, Girona	Spain	Groundwater	P&T-GC/MS	48	nd	nd	0.03	8.86	1.43	[33]*
Petrol station, Düsseldorf	Germany	Groundwater	P&T-GC/MS	645	nd	0.08	0.17	440	0.2	[34]*
Petrol station	Germany	Groundwater	P&T-GC/ion-trap MS	730	na	na	na	na	na	[30]
Refinery site, Tarragona	Spain	Groundwater	P&T-GC/MS	666	0.68	nd	1.53	62	4121	[33]*
Refinery site, east Germany	Germany	Groundwater	P&T-GC/MS	215 000	nd	nd	nd	37 000	920	WATCH
Several GW sites (maximum in UK)	EU countries	Groundwater	Several	830 000	na	na	na	na	na	[15]
Santa Monica, CA	US	Groundwater	Unknown	230 000	na	na	na	na	na	[31]
LUST sites in Los Angeles, CA	US	Groundwater	P&T-GC/MS	1.6×10^7	7500	12 000	4700	4.4×10^6	4.2×10^7	[32]
Niigata Prefecture	Japan	Groundwater	P&T-GC/MS	5.90	na	na	na	na	na	[19]

Table 2 (continued)

Location	Country	Sample type	Analytical method	MTBE	ETBE	TAME	DIPE	TBA	BTEX	Refs.
Surface water										
Rhine River (in Düsseldorf)	Germany	River	P&T-GC/MS	0.12	nd	nd	0.08	0.51	0.1	WATCH
Rhine River (in Cologne)	Germany	River	P&T-GC/MS	0.15	< 0.01	< 0.01	< 0.01	0.4	< 0.01	P-THREE
Rhine River (median)	Germany	River	HS-SPME-GC/MS	0.25	na	na	na	na	na	[35]
Rhine River (median)	Germany	River	P&T-GC/ion-trap MS	0.26	na	na	na	na	na	[36]
Several rivers	Germany	river	HS-SPME-GC/MS	2.36	na	na	na	na	na	[35]
Several rivers	Germany	river	P&T-GC/ion-trap MS	14	na	na	na	na	na	[36]
Rivers in northern Italy	Italy	River	HS-SPME-GC/MS	0.15	na	na	na	na	na	[55]
Niigata Prefecture rivers	Japan	River	P&T-GC/MS	5.30	na	na	na	na	na	[19]
San Gabriel River, CA	US	Stream	P&T-GC/MS	52	na	na	na	na	na	[45]
Tegeler See	Germany	Lake	P&T-GC/MS	0.16	< 0.01	< 0.01	< 0.01	0.21	< 0.01	P-THREE
Lake Zurich	Switzerland	Lake	HS-SPME-GC/FID	1.40	na	na	na	na	3.90	[41]
Donner Lake, CA	US	Lake	P&T-GC/MS	12	na	na	na	na	na	[38]
Cranberry Lake, NJ	US	Lake	P&T-GC/MS & FID	31	na	na	na	na	na	[40]
Waste water										
3 Catalonian WWTP	Spain	Influent	P&T-GC/MS	0.40	0.04	0.04	0.02	200	30	P-THREE
		Effluent	P&T-GC/MS	6.34	1.32	< 0.01	nd	1.79	2.50	P-THREE
3 German WWTP	Germany	Influent	P&T-GC/MS	0.18	< 0.01	< 0.01	nd	1.62	0.75	P-THREE
		Effluent	P&T-GC/MS	0.17	< 0.01	< 0.01	nd	0.66	< 0.01	P-THREE
1 Austrian WWTP	Austria	Influent	P&T-GC/MS	121	nd	nd	< 0.5	215	705	P-THREE
		Effluent	P&T-GC/MS	5.60	nd	nd	5.43	0.39	0.20	P-THREE
1 Belgian WWTP	Belgium	Influent	P&T-GC/MS	0.11	< 0.01	< 0.01	nd	0.95	0.01	P-THREE
		Effluent	P&T-GC/MS	0.08	< 0.01	< 0.01	nd	0.51	< 0.01	P-THREE

Table 2 (continued)

Location	Country	Sample type	Analytical method	MTBE	ETBE	TAME	DIPE	TBA	BTEX	Refs.
Niigata Prefecture ($n = 2$)	Japan	Influent	P&T-GC/MS	0.03	na	na	na	na	na	[19]
		Effluent	P&T-GC/MS	0.02	na	na	na	na	na	[19]
Frankfurt/M-Niederrad & Sindlingen	Germany	Influent	HS-SPME-GC/MS	1.27	na	na	na	na	na	[35]
Southern California	US	Effluent	P&T-GC/MS	123	na	na	na	na	na	[45]
Drinking water										
Unknown	Italy	Mineral water	HS-SPME-GC/MS	< 0.01	na	na	na	na	na	[55]
Unknown	Italy	Tap water	HS-SPME-GC/MS	0.40	na	na	na	na	na	[55]
Frankfurt/M.	Germany	Tap water	HS-SPME-GC/MS	0.07	na	na	na	na	na	[35]
Leuna/Spergau	Germany	Tap water	HS-SPME-GC/MS	0.70	na	na	na	na	na	[54]
Big German city (1)	Germany	Tap water	P&T-GC/MS	0.09	< 0.01	< 0.01	< 0.01	nd	< 0.01	P-THREE
Big German city (2)	Germany	Tap water	P&T-GC/MS	0.01	< 0.01	< 0.01	< 0.01	nd	< 0.01	P-THREE
Small Belgian city (rural area)	Belgium	Tap water	P&T-GC/MS	0.01	nd	< 0.01	< 0.01	nd	< 0.02	P-THREE
Unknown	The Netherlands	Drinking water Well	P&T-ATD-GC/MS	2.90	na	na	na	na	na	[52]
Santa Monica, CA	US	Production well	unknown	610	na	na	na	na	na	[31]

Table 2 (continued)

Location	Country	Sample type	Analytical method	MTBE	ETBE	TAME	DIPE	TBA	BTEX	Refs.
Sea water										
Airport vicinity Iberian Peninsula	Southern	coastal water	P&T-GC/MS	40	0.09	0.19	0.02	12	55	WATCH
Almeria/Malaga	Spain	coastal water	P&T-GC/AED & MS	1842	na	na	na	600	na	[47]
Tamar Estuary (harbours/marinas)	UK	coastal water	HS-SPME-GC/MS	0.19	na	na	na	na	na	[48]
Marina del Rey harbour, CA	US	coastal water	direct-SPME-GC/MS	18	na	na	na	na	na	[46]
Mission Bay, CA	US	coastal water	P&T-GC/MS	34	na	na	na	na	1.9	[45]
Santa Monica Bay (Chevron), CA	US	refinery discharge	P&T-GC/MS	1878	na	na	na	na	na	[45]

nd not detected, *na* not analyzed or no data available

* These publications were also part of WATCH project

MTBE (at a reporting level of 0.2 $\mu g\,L^{-1}$) was the second most frequently detected chemical (after chloroform) in shallow ambient groundwater samples collected in urban areas. Recently, an updated data compilation from the first 10-year cycle of this study has been statistically examined by Moran et al. [29]. MTBE showed a total detection frequency of 7.6% (higher than trichloroethylene at 4.5%, which has a much longer production history) and a median concentration around 0.3 $\mu g\,L^{-1}$. Only 0.3% of the groundwater samples exceeded the MTBE lower limit of EPA drinking water advisory levels (20 $\mu g\,L^{-1}$). The probability of detecting MTBE in groundwater was strongly associated with urban land use, population density, use of MTBE in gasoline, and recharge rates. Other ether oxygenates such as TAME or DIPE were less frequently detected (0.25 and 0.19%, respectively) and ETBE has not yet been detected.

In Germany, a similar groundwater monitoring program was undertaken in 1999/2000, but the use of P&T-GC with ion-trap MS allowed a lower limit of determination (0.05 $\mu g\,L^{-1}$) [30]. The study concluded that MTBE was regularly present (almost 50%) in groundwater under urban areas although the median concentration was low (0.17 $\mu g\,L^{-1}$).

But the most important MTBE groundwater contamination events resulted from point sources such as accidental spills during transport and manipulation of gasoline or leaking underground storage tanks (LUST) in petrol stations or refineries. Once there, MTBE moves at velocities similar to local groundwater, suffer of slow biodegradation (abiotic processes are considered negligible) and low sorption. In the USA, the city of Santa Monica, CA, lost 50% of its total water supply in 1996 as a result of high MTBE groundwater LUST contamination (up to 230 mg/L) [31]. The annual cost for water replacement was estimated to be $US 4 million and culminated in the ban of MTBE in California. Johnson et al. [20] estimated MTBE $t_{1/2}$ in LUST sites in at least 2 years whereas 10 years might be necessary to reduce concentrations below cleanup levels in the USA. Later on, Shih et al. [32] evaluated the impact of fuel hydrocarbons and oxygenates at over 868 LUST sites in Los Angeles, CA. MTBE was detected in 83% with a median concentration of 1200 $\mu g\,L^{-1}$ (benzene and TBA showed similar findings).

A wide range of MTBE concentrations from 120 $\mu g\,L^{-1}$ to 830 mg/L have also been reported in polluted groundwater tables in European countries [15]. Similar results were obtained from our study sites in Spain, Austria, and Germany by applying a P&T-GC/MS methodology, as shown in Table 2. After 4 years of a gasoline release in Girona Spain, MTBE levels were still higher than US EPA drinking water advisory levels (40 $\mu g\,L^{-1}$) [33] while in the spill of Düsseldorf, Germany, MTBE concentration did not appreciably decrease during a 2-year monitoring program and reached maximum spot values above the Danish suggested toxicity level (350 $\mu g\,L^{-1}$) [34]. This last study also revealed the high variations of MTBE and TBA concentration in the vertical profile, thus the need of multilevel wells for a better risk assessment.

3.3 Surface Water

3.3.1 Rivers

The multifunctional use of rivers (source of drinking water, sewage disposal, or ship carrier) has aroused concern about MTBE potential sources, persistence, and removal rates before the water arrives to end-users. MTBE $t_{1/2}$ in rivers is highly variable (from 30 min to 52 days), mainly affected by volatilization processes that depend on water velocity, depth, temperature and wind speed [1].

Higher MTBE concentrations in rivers in Germany were detected at urban agglomerations (maximum of 2.36 $\mu g\,L^{-1}$) compared to rural areas [35]. These results correlated with previously analyzed precipitation samples [25]. Similar findings were obtained in another sampling along the Rhine River carried out by Baus et al. [36]. Although MTBE concentration tends to be balanced in the course of the river (by dilution and evaporation), illegal tank ship releases during tank washings and industrial discharges have been reported as major MTBE inputs, which generate punctual "waves" of the pollutant (e.g., 14 $\mu g\,L^{-1}$ were detected by chance) in time and space [36]. In addition, higher levels were detected in the riverbank side where the industries are located than in the cross section of the river [37].

Additionally, in Japan, the MTBE levels were analyzed in some rivers by using an improved P&T-GC/MS (40 °C optimized purging temperature and – 180 °C cryo-focussing) allowing for a low limit of detection (0.003 $\mu g\,L^{-1}$) [19]. MTBE increases from the upper course of the rivers to its mouths as well as higher levels in winter than in summer were observed, which was consistent with other studies.

3.3.2 Lakes

The discovery of MTBE in lakes used for recreational boating and reservoirs has raised concerns over the potential impact on drinking water quality from such water bodies. Multiple-use lakes in the USA such as Donner Lake, located in the Sierra Nevada Mountains, California [38, 39] or Cranberry Lake in New Jersey [40] have been analyzed mainly by P&T-GC/MS and in Europe, Lake Zurich, that supplies drinking water for the largest Swiss city [41] have been investigated by applying HS-SPME-GC/FID. In general, MTBE levels detected in these studies were between 0.03 and 31 $\mu g\,L^{-1}$, similar to rivers. The use of motorized watercraft was, in all cases, the major contributor of MTBE, whereas neither highway runoff nor precipitation contributed significantly. Different MTBE $t_{1/2}$ were reported, mainly on the order of 10 days [38, 40,

42], but up to 193 days during the boating season [38] have been reported. The major loss of MTBE appeared to be volatilization at the air–water interface, which depends on the wind speed and water surface temperature. However Heald et al. [39] found that volatilization alone was inadequate to fully describe the loss of volatile fuel additives from Donner Lake. In particular, under low wind conditions, additional degradation processes (likely microbial processes) dominate the removal of MTBE. Spatial and temporal variations of MTBE concentrations in the lakes were observed associated to the thermal stratification phenomenon during the boating season (summer), which retards MTBE exchange/transport. Schmidt et al. [41] concluded that no risk is expected for the drinking water supply from such lakes if water is extracted from well below the thermocline; but in order to further reduce emissions of unburned fuel into surface water, restrictions of the highly MTBE emitting two-stroke engines of the type used in motorboats should be considered.

3.3.3 Waste Water

Most abiotic elimination techniques, which are normally used in wastewater treatment plants (WWTP) such as ozonation or adsorption on granular activated carbon, are not very effective for MTBE or its main degradation product, TBA removal [36, 43]. These limitations may generate additional problems for water suppliers and regulators since TBA may be considered even more toxic than its parental compound [44].

Achten et al. [35] estimated that roughly 30–35% of MTBE was eliminated in two German sewage plants. This value was slightly lower than the EU risk-assessment calculation (43%) [2], which was mainly attributed to evaporation and dilution much more than adsorption to the sludge or biodegradation processes (considered negligible). In fact, the influent of the sewage plant, which collected mostly industrial discharges, was characterized by receiving some exceptionally high MTBE concentrations (e.g., 1.27 $\mu g\,L^{-1}$) and spot samples during these events showed higher amounts in the effluent than in the influent [35].

In the framework of the P-THREE project, two sampling campaigns were carried out in February and May 2003 with the aim of screening the presence and removal of different organic pollutants in a total of eight European WWTP and some related tap waters from close cities (two large German cities with more than 100 000 inhabitants and one small rural city in Belgium). Despite the limited number of samples, MTBE was detected in 15 out of 16 wastewater samples at median values of 0.12 and 0.08 $\mu g\,L^{-1}$ for influent and effluent waters, respectively. This data demonstrated no evident removal of the compound, which was in accordance with a study carried out in Japan [19]. These estimations excluded the highest values of MTBE, TBA,

and aromatic hydrocarbons, which were detected in WWTP in Austria and were likely due to the proximity of a refinery (see Fig. 4 and Table 2).

Although refinery effluents generally contained the highest MTBE concentrations, discharges from WWTP accounted for the greatest proportion (78%) of the daily mass emission to bays and coastal waters in southern California [45].

3.3.4 Sea Water

Limited data is available on the extent of MTBE contamination in coastal waters, as well as on the persistence of the pollutant in the marine environment to assess potential toxic effects on marine life.

Brown et al. [45] calculated that large point sources (WWTP and refineries) throughout southern California discharged 214 kg/day of MTBE to coastal waters of which 98% arrived in Santa Monica Bay, whereas stream input was considered trivial (< 0.5%). Marinas and areas used intensively for recreational boating had the highest average MTBE concentration (8.8 $\mu g\,L^{-1}$). Later on, Zuccarello et al. [46] focussed on one of these zones, Marina del Rey harbor, where personal watercraft are allowed. As expected, the highest concentration of MTBE (18 $\mu g\,L^{-1}$) was found at the boat launching ramp and the lowest (0.2 $\mu g\,L^{-1}$) near the harbor entrance (2.3 km away). Despite the volatility of MTBE, similar concentrations along the depth profile (0–6 m) suggested that vertical mixing in the water column is more efficient than volatilization.

For the first time in Europe, Mezcua et al. [47] determined MTBE and TBA in coastal water samples from various marinas in the south of Spain (Almeria and Malaga) involving P&T-GC and comparing two detectors AED and MS. AED was not sensitive enough to current environmental concentrations (MTBE detection limit of 10 $\mu g\,L^{-1}$), but validated alarm points. GC-MS allowed detecting MTBE in all the samples at levels generally ranging from 0.033 to 2.20 $\mu g\,L^{-1}$, but occasionally higher (up to 1842 $\mu g\,L^{-1}$) in the vicinity of gasoline stations or boat launching facilities.

Much lower levels were measured by Guitart et al. [48] with HS-SPME-GC/MS in pre-selected potential contaminated harbors and marinas throughout Tamar Estuary in the UK. However, the highest levels (up to 0.19 $\mu g\,L^{-1}$) were generally associated with motor vehicle and boating activities. Road and rail bridges runoff were identified as MTBE major inputs in the lower estuary.

From our study in the southern Iberian Peninsula, four points along the coast were sampled at low and high tide through seven campaigns for getting more representative data. The MTBE median value from a total of 38 samples was 0.37 $\mu g\,L^{-1}$ and a comparable level (0.23 $\mu g\,L^{-1}$) was found for TBA. Lower amounts of BTEX were usually detected (0.09 $\mu g\,L^{-1}$ as median). Exceptionally high values of all gasoline additives (see Table 2) were detected in

one point in July 2003, likely associated with recreational boating activities. In fact, slightly higher values of MTBE were found during the summer compared to winter or spring (as shown in Fig. 3B).

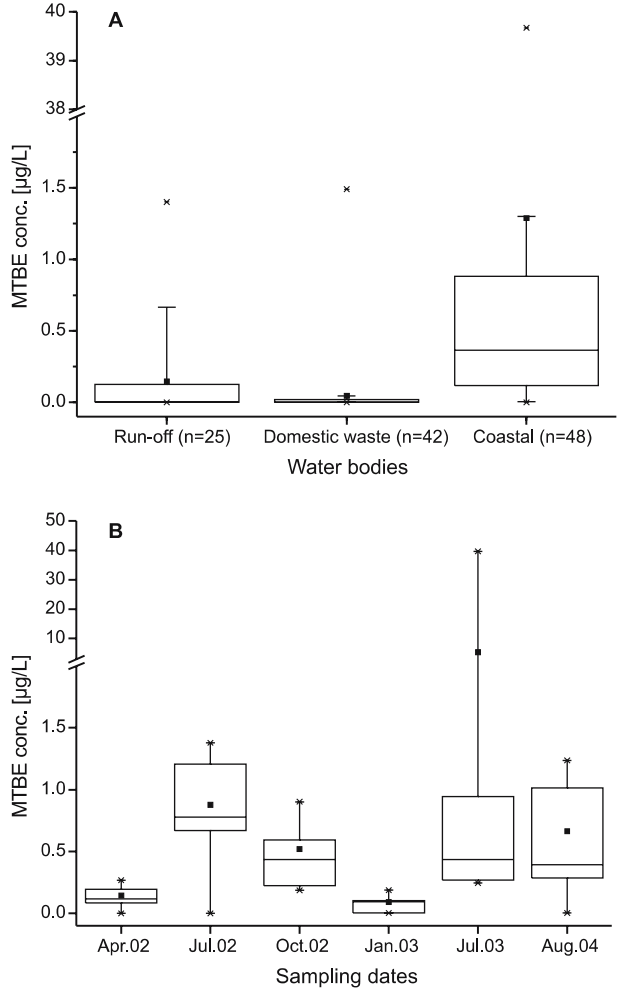


Fig. 3 Box-plot of MTBE concentrations found in the vicinity of an airport (**A**) at different water bodies and (**B**) detailed for coastal water samples ($n = 8$) through seven sampling campaigns. For each variable, the box has lines at the lower quartile (25%), median (50%), and upper quartile (75%) values. The whiskers are the lines extending from each end of the box to show the extent of the data up to 1.5 times the interquartile range (IQR). The mean value is marked with (**a**) and outliers with (x) symbols. Each sample (n) was analyzed in triplicate, and the average value was considered for calculations. Non-detected levels were expressed as half of instrumental limit of detection (5×10^{-4} µgL^{-1})

4
Human Exposure via Drinking Water

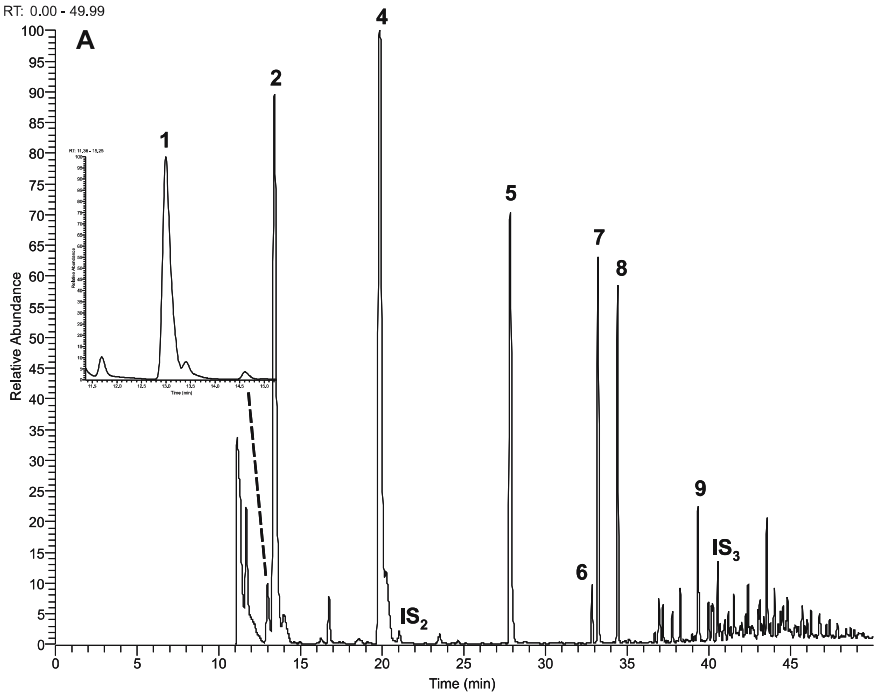
Several studies have attempted to estimate human uptake when MTBE-contaminated water is used to drink, prepare food or shower. For instance, 1% of drinking water supplies in the USA contain MTBE above 20 $\mu g\,L^{-1}$ [31] and it was estimated that via potable water, 5% of the population of the USA may be exposed to higher than 2 $\mu g\,L^{-1}$ levels of MTBE [49]. Williams [50] reported results from a survey of MTBE in drinking waters in California for 1995–2000. In the state that is supposed to be the most impacted by MTBE, this pollutant was detected in about 1.3% of all drinking-water samples and 27% of them above the state's primary health-based standard of 13 $\mu g\,L^{-1}$.

In Europe, some studies have been carried out to check the presence of MTBE in drinking water and corresponding sources. In the UK, Dottritge et al. [51] reported detectable concentrations ($> 0.1\ \mu g\,L^{-1}$) at 13% of studied locations. However, MTBE levels were predominantly low ($< 1\ \mu g\,L^{-1}$) and the study concluded that the presence of less than 1% MTBE (v/v) on average in British gasoline was not a major threat to public water supplies in England and Wales.

A similar survey was carried out in The Netherlands in 2001. Morgenstern et al. [52] developed an off-line P&T coupled to a GC/MS equipped with an automated thermal desorption sampler (ATD), which enabled the analysis of at least 40 samples per day and a MTBE quantification limit of 0.02 $\mu g\,L^{-1}$. MTBE concentrations ranged < 0.01–$0.42\ \mu g\,L^{-1}$ in Dutch drinking water sources with a median below 0.01 $\mu g\,L^{-1}$. The highest value 2.9 $\mu g\,L^{-1}$ was associated with a point source contamination of groundwater.

In Germany, about 15% of the drinking water used is produced by river-bank filtration or artificial infiltration, thus its quality is directly dependent on the state of the rivers (primarily the Rhine and Elbe Rivers). Some studies pointed out that MTBE is not totally removed by this sand-filtration technique and at least 40% of the pollutant is passing through the subsoil unchanged [36]. MTBE was found at an average concentration of 0.09 $\mu g\,L^{-1}$ in recovering well water and riverbank filtered waters, and at a maximum level of 0.07 $\mu g\,L^{-1}$ in tap water from the metropolitan Frankfurt area [53]. A comparable value (0.09 $\mu g\,L^{-1}$) was detected in tap water collected in Berlin from the P-THREE survey. Later, Kolb et al. [54] found one order of magnitude MTBE higher concentration in tap water from Leuna and Spergau (Saxony-Anhalt), likely influenced by the nearer well-known gasoline contaminated aquifer at Leuna chemical industrial zone.

Some data is also available from Italy where MTBE was not detected in 12 commercial mineral water samples ($< 0.01\ \mu g\,L^{-1}$), whereas five tap water samples from different groundwater sources ranged 0.05–0.40 $\mu g\,L^{-1}$, all measured by HS-SPME-GC/MS [55].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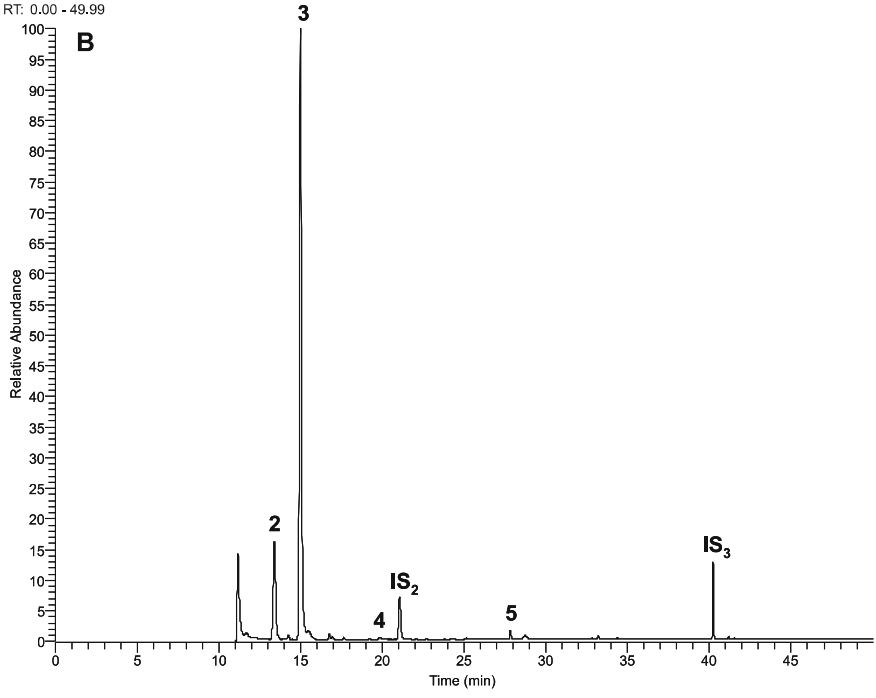
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◀ **Fig. 4** Total ion chromatograms (TIC, 10^8) of the (**A**) influent and (**B**) effluent from a waste water treatment plant (WWTP) in Austria analyzed in February 2003 by P&T-GC/MS. The high gasoline additives concentrations detected were assumed to be originated from a nearby refinery. Compound identification number: 1 = TBA ($m/z = 59$), 2 = MTBE-d_3 (IS_1, $m/z = 76$) + MTBE ($m/z = 73$), 3 = DIPE, 4 = benzene, IS_2 = fluorobenzene, 5 = toluene, 6 = ethylbenzene, 7 = *m* + *p*-xylene, 8 = *o*-xylene, 9 = dicyclopentadiene (DCPD) and IS_3 = 1,2-dichlorobenzene-d_4

It can therefore be concluded that no aesthetic implications (taste and odor) or health risks are likely to be associated with chronic and subchronic human exposure to MTBE in tap water. However, in the case of point sources, risk-assessment studies are needed, especially because consumers may find unacceptable the mere presence of gasoline components in their drinking water.

5 Remediation Actions

Although remediation procedures are often difficult and time-consuming, several methods have been proposed for the removal of MTBE from contaminated sites. These include physical removal such as granular activated carbon (GAC), soil vapor extraction, air-stripping, selected zeolites, ultrasonic irradiation combined with ozonation or ozone/hydrogen peroxide treatment; and biological treatments by mean of microbial consortia or plants (phytoremediation). However, the special properties of this chemical make the success of any of these techniques more complicated as well as costly. For example, MTBE's Henry's constant is several times lower than that of other organic compounds commonly treated through air-stripping, such as trichloroethylene or benzene. In addition, the effectiveness of GAC for the treatment of MTBE has been limited by its poor physical and chemical adsorption characteristics and its high solubility.

At the beginning of the 1990s, MTBE was classified as recalcitrant to biodegradation processes because its removal took much longer than conventional gasoline hydrocarbons [1]. However, during the last decade, the potential of microbial and fungi communities to degrade MTBE has been demonstrated under oxic and nearly all anoxic conditions as summarized in several reviews [56–58]. When MTBE and TBA removal by conventional technologies is not easily achieved [36, 43], new, simpler and less-expensive alternatives such as ex-situ reactors, natural attenuation, and bioaugmentation are envisaged and can be successfully applied for remediation of MTBE-contaminated aquifers (refer to reviews [59, 60].

6 Data Treatment and Modelling Studies

The behavior of MTBE through the different environmental compartments has been investigated using various modelling approaches. For example, the EU risk assessment used the simplest type of fugacity models (a Level I model) and concluded that from diffuse sources 93.9% of MTBE is in the air phase, 6.0% in the water phase, and 0.05% in the soil phase [2]. However, another study by Environment Canada for Southern Ontario [61] used the Level III model and predicted 56% of MTBE in the air, 42% in surface water, and 0.5% in soil and sediment. As can be observed, models developed so far differed in their predictions of relative MTBE concentrations for relevant environmental compartments and of seasonal concentration variations; further, they have hardly considered the formation of transformation products [62]. Moreover, limitations in pollutant environmental data or key physicochemical parameters often make it difficult to validate model predictions.

Achten et al. [21] simulated a German environment using the equilibrium criterion (EQC) model. MTBE concentrations of 0.02 $\mu g\,L^{-1}$ in surface water and 0.17 $\mu g/m^3$ in air were estimated from the year-round scenario at 10 °C. Lower MTBE concentrations in atmospheric and aqueous compartments in summer were explained by higher degradation rates at higher temperatures.

More accurate analysis taking into account the MTBE two major degradation products, TBA and TBF, was performed recently by Arp et al. [62] and it was used for predicting their concentrations in various environmental compartments in Europe. Water and air concentrations of MTBE predicted from this innovative multispecies transformation model were considered in good agreement with measurements of environmental samples. For example, the predicted average MTBE concentration in surface water (0.25 $\mu g\,L^{-1}$) at 10 °C corresponded exactly with the median found in river Rhine [35]. MTBE concentrations were found to be strongly influenced by temperature (in water and air) or hydroxyl (OH) radical levels (only in air). Although MTBE is emitted in large amounts, it is fairly rapidly removed, having an overall $t_{1/2}$ of 4–7 days (excluding groundwater emissions) [62]. However, the lack of MTBE background information in soils in Europe and the scarce data on degradation products prevented further validation of the model.

7 Future Perspective

MTBE Empire at the top of gasoline additives seems close to expire; but how long it will be detected in the environment? Have the responsible authorities learned from past errors? In the absence of a completely new design and construction of underground storage tank systems, the extent of potential human

and environmental exposure should be an important criterion in determining the amount of information needed before making an environmental policy decision. Alternatives to MTBE, such as ETBE, are quite similar in structure. Although ETBE is less well studied, preliminary results from using the Level III fugacity approach model [63] showed that despite the differences in the partitioning properties (refer to Table 1) in general, both ethers have similar behavior in the environment when same emission rates are evaluated. However, less-evaporative ETBE emissions would be expected due to its relatively lower vapor pressure. In water, ETBE taste and odor thresholds (47 and 13 $\mu g\,L^{-1}$, respectively) are almost identical to for MTBE [31]. Thus, due to tax incentives for the application of biomass-derived ethanol, ETBE will be the next emerging fuel-derived contaminant in the future. At least ETBE has a higher Henry's Law constant than does MTBE (up to two to three times higher), indicating that the air-stripping removal technique would be at least slightly more effective [64] and ETBE biodegradation has also been demonstrated with several strains [65]. But its main degradation product is TBA as well. Site groundwater concentrations and plume length data have already indicated TBA contamination at a scale similar to MTBE in LUST sites [32]. Since this product can be stoichiometrically formed from MTBE, ETBE and TBF degradation and may be considered as recalcitrant as MTBE; TBA concentrations in water bodies could pose the greatest problem in the future. So, due to its widespread use, further investigation will soon be required for ETBE as well as TBA.

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Occurrence of Methyl *tert*-Butyl Ether and Other Fuel Oxygenates in Source Water and Drinking Water of the United States

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Abstract The National Water-Quality Assessment Program of the United States (US) Geological Survey conducted surveys of the occurrence of methyl *tert*-butyl ether (MTBE) and other fuel oxygenates in ground water used as a source of drinking water and in drinking water in the United States (USA) from 1993 to 2001. MTBE was detected in about 4% of samples of source water collected from private and public supply wells located throughout the USA and in about 9% of samples of drinking water from 12 Northeastern states. Other fuel oxygenates were detected very infrequently. Few samples of source water or drinking water had concentrations of MTBE greater than the US Environmental Protection Agency drinking-water advisory or state-level benchmarks.

As many as five million people in the USA may potentially be exposed to MTBE through source water derived from ground water. Public wells appear to be more vulnerable to contamination by MTBE than private wells, and more people in the USA rely on drinking water from public wells than private wells. Because of the uncertainty in the long-term health effects of MTBE in drinking water, it is important to monitor for MTBE in ground water used as a source of drinking water, especially ground water from public wells. Better understanding of the sources of MTBE to ground water, the intrinsic susceptibility of aquifers to contamination, and the behavior and fate of MTBE in ground water would aid in adequately protecting ground-water resources from contamination by MTBE.

Keywords MTBE · Source Water · Drinking Water · Occurrence · Fuel Oxygenates

1 Introduction

From about 1992 to 2006, large volumes of fuel oxygenates were used in gasoline in certain areas of the United States (USA). Fuel oxygenates are organic compounds that contain oxygen and are used to increase oxygen content in fuels. Most fuel oxygenates that were used in the USA are alkyl ethers such as methyl *tert*-butyl ether (MTBE), the most commonly used oxygenate.

Oxygenates have been blended into gasoline in the USA for a variety of reasons. MTBE was first used in gasoline in the USA in 1979 as an octane enhancer. In 1990, reauthorization of the Clean Air Act required the use of oxygen in gasoline in areas of the USA with air-quality problems, such as the densely populated Northeastern States and California [1]. This greatly expanded the use of MTBE, especially in reformulated gasoline. The Energy Policy Act of 2005 removed the oxygen requirement in gasoline, and by September 2006, MTBE use in gasoline had declined [2, 3].

MTBE has been detected in ground water and surface water in the USA and other countries such as Great Britain, Spain, and Germany [4–7]. In addition to its occurrence in ground-water resources, MTBE has been detected in ground water used as a source for drinking water and in finished drinking water in the USA, Germany, and Great Britain [4, 8, 9]. The widespread occurrence of MTBE in source water and drinking water around the world has raised serious concern regarding the negative taste and odor aspects of MTBE in water and the potential for negative human-health effects from this compound in water used for human consumption.

The National Water-Quality Assessment (NAWQA) Program of the United States Geological Survey (USGS) has conducted surveys of the occurrence of MTBE and other fuel oxygenates in ground water used as a source of drinking water (hereafter referred to as source water) and in drinking water distributed to consumers. The data from these surveys provide insights into the quality of water used for human consumption with regard to the occurrence of MTBE.

To determine the occurrence of MTBE in source water on a broad scale, samples of ground water were collected from drinking-water supply wells throughout the conterminous USA, as well as Alaska and Hawaii. The wells sampled included domestic wells (hereafter referred to as private wells), which are privately owned wells and are used to supply water for household use, and public wells that supply water for a public water system. Data on MTBE in treated drinking water were available from a 12-state region in the northeast USA and represent samples collected by the water utilities for compliance with US federal drinking-water-quality regulations.

2
Usage and Production of MTBE

MTBE was first used commercially in gasoline in Italy in 1973 [10]. In the USA, MTBE was first added to gasoline in 1979 as an octane booster to replace tetraethyl lead, which was used in gasoline as an antiknock compound. Like tetraethyl lead, MTBE increased octane and prevented engine knocking. However, the amount of MTBE used for this purpose was relatively small, typically only 1–8% by volume [10]. Consequently, MTBE use as an octane enhancer in gasoline resulted in only modest production of the compound through the early 1990s.

In 1990, the Clean Air Act (CAA) Amendments mandated that oxygen be added to gasoline in areas of the USA where certain air-quality standards were not attained. Oxygen allows more complete and clean burning of gasoline in engines. Two areas of oxygenate use in gasoline were required under the CAA Amendments: (1) the Oxygenated Fuels (OXY) Program, where gasoline must contain 2.7% oxygen by weight during the cold season in areas that fail to meet National Ambient Air Quality Standards (NAAQS) for carbon monoxide; and (2) the Reformulated Gasoline (RFG) Program, where gasoline must contain 2% oxygen by weight year-round in areas that fail to meet NAAQS for tropospheric ozone [11]. As of 1990, 40 metropolitan areas of the USA were required to participate in the OXY Program, and 28 metropolitan areas were required to participate in the RFG Program [10].

Although the CAA Amendments did not specify which oxygenate must be added to gasoline to achieve the oxygen requirement, MTBE was the most widely and frequently used oxygenate. To meet the oxygen requirements of the CAA Amendments, OXY gasoline contained 15% MTBE by volume, whereas RFG contained 11% by volume. In addition to the areas mandated for oxygen use by the CAA Amendments, some areas of the USA chose to voluntarily use RFG or had additional local laws requiring oxygenate use. In 1998, MTBE was used in more than 80% of oxygenated gasoline in the USA, and in 1999 about 30% of gasoline sold in the USA was oxygenated [12].

With increased usage of MTBE after 1990, US production also increased. In 1998, almost 12 billion liters of MTBE were produced in the USA [13]. From 1993 to 1998, MTBE was the second most produced organic chemical in the USA [12].

Figure 1 shows the production of MTBE in the USA from 1980 to 2005. Production of MTBE increased from 1985 to 2000, but has declined since 2000. The period when MTBE was produced in the greatest volumes, from about 1993 to 2003, corresponded to the time of sampling of source water by the NAWQA Program and to collection of samples of treated drinking water by public water utilities.

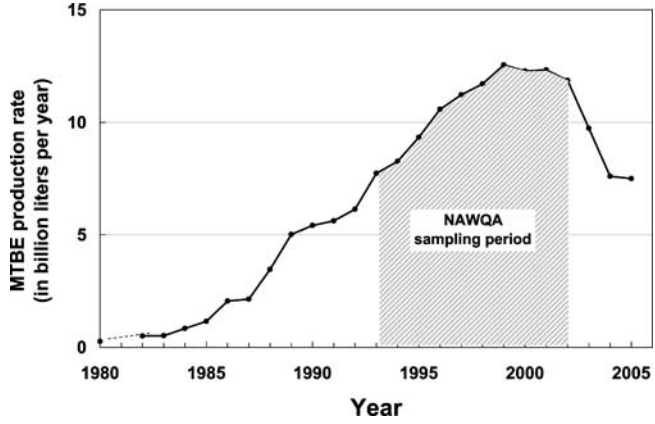

Fig. 1 Production rate of MTBE in the USA from 1980 to 2005

3 Physical Properties and Human-Health Effects of MTBE

Relative to other gasoline hydrocarbons, MTBE has high water solubility. For gasoline that contains 10% MTBE by weight, MTBE has a solubility of 5000 milligrams per liter (mg/L) at room temperature. In comparison, the total hydrocarbon water solubility for a non-oxygenated gasoline is about 120 mg/L. In addition, the percent volume of MTBE in oxygenated gasoline generally is higher than the percent volume of most gasoline hydrocarbons. As a result, MTBE can be found at high concentrations relative to other gasoline hydrocarbons in ground water near point-source release sites [14].

MTBE tends to partition to organic carbon much less strongly than gasoline hydrocarbons. The organic carbon partitioning coefficient for common gasoline hydrocarbons like BTEX (benzene, toluene, ethylbenzene, xylenes) is 50–100% higher than the organic carbon partitioning coefficient for MTBE. Therefore, relative to gasoline hydrocarbons, MTBE has much greater mobility in the subsurface and can move at nearly the same velocity as ground water. This property can result in MTBE traveling farther and faster in ground water than gasoline hydrocarbons [15].

Most studies indicate that MTBE is not biodegraded easily in the environment. In fact, the aerobic half-life of MTBE in ground water has been estimated to be approximately an order of magnitude longer than the average aerobic half-life of most gasoline hydrocarbons in ground water [16]. However, some studies have indicated that MTBE can biodegrade quickly under certain environmental conditions. For example, substantial aerobic biodegradation of MTBE was observed in stream-bed sediments in South Carolina, USA [17].

The occurrence of MTBE in gasoline has been associated with a number of acute human-health effects including headache, nausea, eye irritation, vomiting, and dizziness. However, these symptoms were mainly observed through anecdotal reports in areas of MTBE use in gasoline. Consequently, these symptoms may not be specific to MTBE exposure but could have resulted from a variety of environmental hazards. To date, no large-scale, carefully planned epidemiologic studies on the effects of human exposure to MTBE have been conducted.

Few studies on the effects of MTBE exposure to animals have been conducted. Intake of MTBE by gavage has been associated with acute and carcinogenic effects in laboratory rats [18, 19]. However, the validity of some of the interpretations from these studies has been questioned. The US National Toxicology Program does not recommend listing MTBE in its Report on Carcinogens, whereas the International Agency for Research on Cancer classifies MTBE as a group 3 carcinogen (not classifiable as to its carcinogenicity in humans) [20, 21].

MTBE can be smelled and tasted in water at relatively low concentrations [14]. This has caused concern regarding the aesthetic acceptability of drinking water containing MTBE. In 1997, the US Environmental Protection Agency (USEPA) issued a drinking-water advisory suggesting that concentrations of MTBE in drinking water be less than 20–40 micrograms per liter (μg/L) [22]. This level was believed to provide protection against taste and odor concerns in drinking water as well as provide a large margin of safety against potential human-health effects. However, taste and odor studies found that MTBE can be detected in water at concentrations as low as 5 μg/L [15]. The USEPA advisory was not based on potential human-health effects of MTBE and does not represent a federally enforceable drinking-water standard. Some states have established health-based standards and other benchmarks for MTBE in drinking water. For example, California has designated a primary drinking-water standard of 13 μg/L for MTBE in drinking water from public water systems, and a secondary drinking-water standard of 5 μg/L to address taste and odor concerns.

4
Occurrence of MTBE and Other Fuel Oxygenates in Source Water

As part of NAWQA occurrence surveys, samples of ground water from private and public supply wells were collected during 1993–2001 and were analyzed for MTBE and other volatile organic compounds (VOCs). These samples were collected prior to any treatment or distribution and prior to any pressure or holding tanks, and are believed to accurately represent the quality of ground water used as a source of drinking water. Detailed discussion of the design of the NAWQA Program, field methods, sampling and analytical protocols, and

quality assurance plans is beyond this scope of this chapter and is covered elsewhere [23–25].

Samples from 1931 private wells and 913 public wells were analyzed for MTBE. In source water from both well types, MTBE was detected in about 4% of samples at or above a concentration of 0.2 μg/L. A common concentration was necessary for comparison of detection frequencies between private and public wells, because the effective laboratory reporting level for samples from most public wells was 0.2 μg/L while the effective laboratory reporting level for samples from private wells was lower. The detection frequency of MTBE by well type is shown in Fig. 2. MTBE was detected in about 3% of private wells and about 5% of public wells. The detection frequency of MTBE was higher in public wells compared to private wells, and the difference in detection frequencies was statistically significant at the 95% confidence interval. The higher detection frequency of MTBE in public wells compared to private wells may be the result of the higher pumping rate of public wells. Higher pumping rates for public wells result in a larger area of the aquifer contributing water to the well, which is believed to draw MTBE from more sources and from longer distances than private wells.

The NAWQA Program conducted three types of ground-water surveys: (1) aquifer studies designed to characterize the quality of water in regional-scale aquifers used as an important source of drinking water, (2) agricultural land-use studies designed to characterize the quality of shallow ground water underlying areas of primarily agricultural land use, and (3) urban land-use studies designed to characterize the quality of shallow ground water underlying areas of primarily urban land use. In source water from private wells, MTBE was more frequently detected in aquifer studies than in agricultural land-use studies. The number of samples from private wells in urban land-

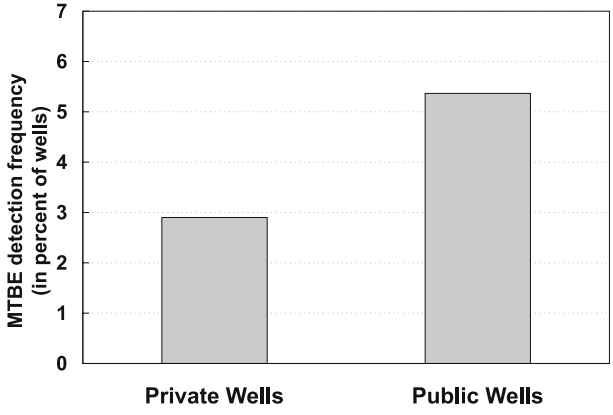

Fig. 2 Detection frequency of MTBE in source water from private and public wells at or above a concentration of 0.2 μg/L

use studies was too small to compute a detection frequency for MTBE. The number of samples from public wells in agricultural and urban land-use areas was too small to compute detection frequencies of MTBE by NAWQA study type.

Quantified concentrations of MTBE in source water from private and public wells are shown in Fig. 3. The concentrations shown in this figure are those that were greater than 0.2 μg/L, the effective reporting level for public wells. Many quantified concentrations of MTBE in samples from private or public wells were less than 1 μg/L. Only one sample from a private well had a concentration of MTBE greater than the lower limit of the USEPA drinking-water advisory of 20 μg/L. No samples from any public well had concentrations of MTBE greater than 20 μg/L. Median quantified concentrations of MTBE in samples from private and public wells were 0.67 and 0.61 μg/L, respectively. When the distributions of all concentrations of MTBE in source water from private and public wells were compared, including non-detect values, the concentrations in public wells were higher than the concentrations in private wells.

Three other fuel oxygenates were analyzed in source water from private and public wells: *tert*-amyl methyl ether (TAME), diisopropyl ether (DIPE), and ethyl *tert*-butyl ether (ETBE). Detection frequencies of these oxygenates were less than 1% in source water from either private or public wells, and ETBE was not detected in source water from private wells. All quantified concentrations of these fuel oxygenates were less than 2 μg/L with the exception of DIPE, which had a concentration of 22 μg/L in one private well sample.

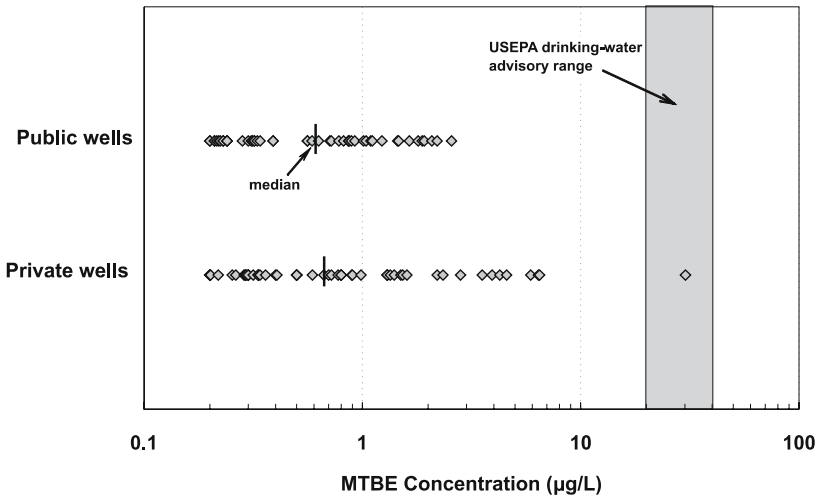

Fig. 3 Concentrations of MTBE in source water from private and public wells at or above a concentration of 0.2 μg/L

5
Occurrence of MTBE in Drinking Water

The USGS conducted an assessment of MTBE in drinking water for the period 1993–1998. Samples were collected by community water systems for compliance with federally enforceable drinking-water regulations. In all cases, the samples were collected after treatment, if any, and prior to distribution to consumers.

Data on MTBE in drinking water were compiled by the USGS from 12 states in the northeast USA. These states were chosen because they are densely populated, have a long history of urbanization, and were areas of substantial use of MTBE in gasoline. Details of the design of the data compilation, data characteristics, and results of the drinking-water survey can be found elsewhere [26, 27]. In order to compare data on MTBE in drinking water with data on MTBE in source water, only data on MTBE in drinking-water systems supplied exclusively by ground water were examined here. In addition, only data on MTBE in drinking water were examined because fuel oxygenates other than MTBE are rarely analyzed in drinking water, as most states do not have drinking-water standards for these compounds.

Samples from 985 community water systems supplied exclusively by ground water were analyzed for MTBE and the results are summarized by system. MTBE was detected in about 9% of systems. MTBE was detected nearly twice as frequently in systems supplied exclusively by ground water compared to systems supplied exclusively by surface water (about 4.5%). The higher detection frequency of MTBE in community water systems served by ground water probably is a result of the greater persistence of MTBE in ground water compared to surface water, especially in ground water that is strictly anaerobic [17].

Figure 4 shows the detection frequency of MTBE in drinking water supplied by ground water by size category of the system. Size categories for drinking-water systems were as follows: small—serving 25 to 500 people; medium—serving 501 to 3000 people; large—serving 3301 to 50 000 people; very large—serving greater than 50 000 people. The detection frequency of MTBE was higher in large and very large community water systems compared to small and medium systems. The higher detection frequency of MTBE in larger systems probably is the result of their location in or near large metropolitan areas and their use of large pumping-capacity wells. Large metropolitan areas have more potential sources of MTBE, and large-capacity wells have a larger area of the aquifer contributing water to the well, which draws MTBE from more sources and from longer distances than small-capacity wells.

Concentrations of MTBE in drinking water ranged from 0.3 to 210 μg/L. Median concentrations of MTBE by system ranged from 0.5 to 52 μg/L. Five systems had median MTBE concentrations greater than the lower limit of the

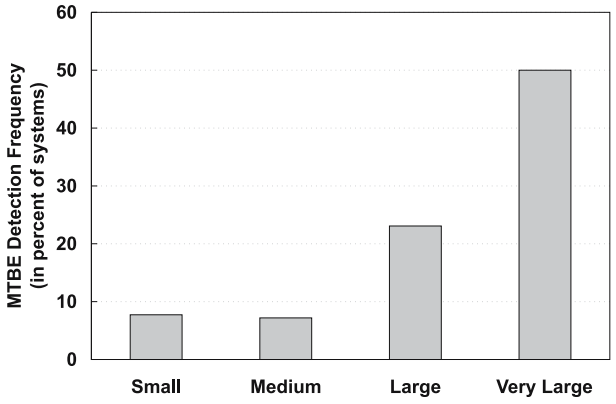

Fig. 4 Detection frequency of MTBE in drinking water from public water systems, by size of system

USEPA drinking-water advisory of 20 μg/L. The five systems were located in Connecticut, New York, and Virginia.

States in the northeast have varying types of benchmarks for allowable concentrations of MTBE in drinking water. Table 1 lists benchmarks for MTBE in drinking water in each of 12 Northeastern states. The benchmark values listed in Table 1 represent a variety of enforceable and unenforceable guidelines for MTBE in drinking water. Some states have primary drinking-

Table 1 Benchmark values for MTBE in drinking water in 12 Northeastern states, USA [28]

State	Benchmark concentration (in μg/L)	Type of benchmark
Delaware	10	Primary health-based drinking-water standard
New Hampshire	13	
New Jersey	70	
New York	10	
Vermont	40	
Connecticut	70	Drinking-water guideline, health advisory or action level
Massachusetts	70	
Rhode Island	40	
Maine	35	Drinking-water cleanup level
Maryland	20	
Pennsylvania	20–40	Follows USEPA drinking-water advisory
Virginia	None	–

water benchmarks that require treatment of the water prior to human consumption if concentrations of MTBE exceed the benchmark. Public water systems that distribute water with concentrations of MTBE greater than these benchmarks could be subject to fines or legal sanctions. However, benchmarks in some states are only used as guidelines for protection of drinking-water quality and do not carry legal responsibility for the water system.

Median concentrations of MTBE exceeded the state benchmarks in only one state, New York. Four community water systems in New York supplied exclusively by ground water had median concentrations of MTBE greater than the drinking-water standard of 10 μg/L. It is not known how many community water systems in the USA treat source water to remove MTBE contamination.

6 Comparison of MTBE Occurrence in Source Water and Drinking Water

The detection frequency of MTBE in source water is for combined private and public wells sampled by the NAWQA Program. The detection frequency of MTBE in drinking water is for community water systems in 12 Northeastern states. To make comparisons between source water and drinking water most equitable, data were used from source water wells located in the same 12-state area as the drinking water samples ($n = 578$). In addition, only detections of MTBE in source water at or above a concentration of 0.5 μg/L were examined. This concentration is equivalent to the effective reporting level in the drinking water data of 0.5 μg/L.

The detection frequency of MTBE in source water and drinking water in 12 Northeastern states is shown in Fig. 5. The detection frequency of MTBE

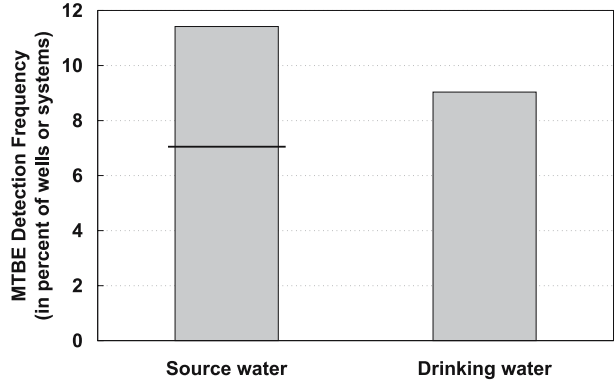

Fig. 5 Detection frequency of MTBE in source water and drinking water in 12 Northeastern states, at or above a concentration of 0.5 μg/L

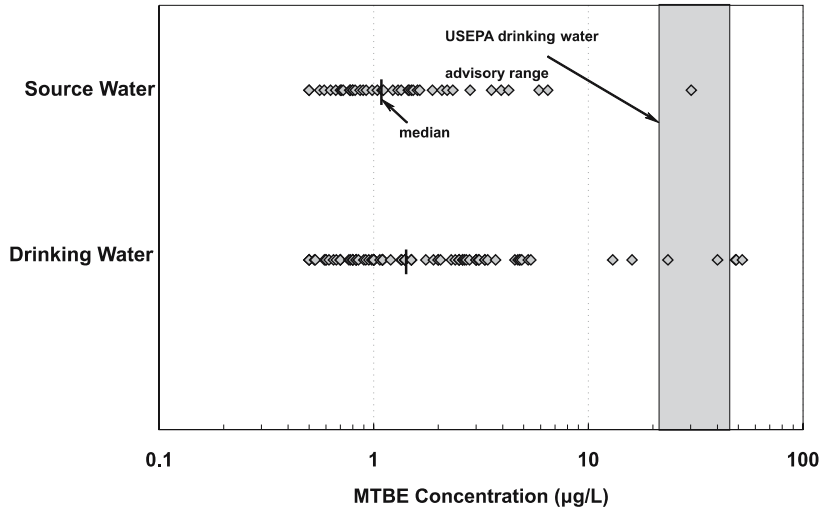

Fig. 6 Concentrations of MTBE in source water and drinking water in 12 Northeastern states

in drinking water was about 9% while the detection frequency of MTBE in source water was about 7%. The higher detection frequency of MTBE in drinking water compared to source water is probably because the detection frequency of MTBE is higher in samples from public wells, which entirely comprise the drinking water data, compared to samples from private wells, which dominate the source water data. Figure 6 shows the range in quantified concentrations of MTBE in source water and drinking water. Only quantified concentrations of MTBE at or above 0.5 μg/L were examined. The concentrations in drinking water represent the median values of all quantified concentrations in each system. The median quantified concentrations of MTBE in source water and drinking water were 1.1 and 1.4 μg/L, respectively.

When the distributions of all concentrations of MTBE in source water and drinking water were compared, including non-detect values, the concentrations in drinking water were higher than the concentrations in source water. The higher concentrations of MTBE in drinking water compared to source water probably are the result of a number of factors, which are explained in more detail in the discussion and implications section.

7 Variables Associated with the Occurrence of MTBE in Source Water

The occurrence of MTBE in source water was evaluated with respect to associations with various natural and anthropogenic variables. The identification of variables associated with the occurrence of MTBE in source water may aid

in understanding the sources and pathways of MTBE to ground water and the susceptibility of aquifers to contamination by MTBE. Only source water was chosen for this evaluation because the data for drinking water were summarized by system, and the locations of individual sources of ground water in each system were not known. The source water data used in this analysis consisted primarily of samples of ground water from private wells collected by the NAWQA Program as part of the aquifer studies. Natural and anthropogenic variables were selected that might facilitate understanding of the sources of MTBE in ground water or the transport and fate of MTBE in the environment. A list of all natural and anthropogenic variables included in these analyses can be found elsewhere [4].

Logistic regression was used to determine associations between the occurrence of MTBE in source water and the anthropogenic and hydrogeologic variables. The results of the logistic regression analyses are presented in Table 2, including the explanatory variables significantly associated with the probability of occurrence of MTBE, the unstandardized slope estimate of each variable, and the standardized slope estimate of each variable. Variables strongly associated with the probability of occurrence of MTBE in source water have standardized slope coefficients greater than 0.1 (absolute value) and are shaded in gray.

The probability of occurrence of MTBE in source water was strongly associated with water temperature and precipitation. The probability of occurrence of MTBE in ground water decreased as water temperature increased. This relation is believed to represent temperature regulation of MTBE biodegradation. As temperature increases, the rate of biodegradation of MTBE also

Table 2 Results of logistic regression analyses of MTBE occurrence in source water

Explanatory variable	Type of variable	Unstandardized slope estimate	Standardized slope estimate
Anthropogenic variables			
Leaking underground storage tanks (number within 1 km of well)	Source	+ 1.383	+ 0.07
Hydrogeologic variables			
Water temperature (°C)	*Fate*	– 0.187	– 0.13
Precipitation (in.)	*Transport*	+ 0.05	+ 0.11
Dissolved oxygen (mg/L)	Fate	+ 0.106	+ 0.05
Aquifer consolidation	Transport	– 1.121	– 0.08

Italics indicate a relatively strong relation
+ indicates positive relation between MTBE occurrence and variable
– indicates negative relation between MTBE occurrence and variable

increases [29]. The probability of occurrence of MTBE in ground water increased as precipitation near the well increased. Precipitation is believed to be a surrogate for recharge. As recharge increases, the movement of MTBE from the surface or unsaturated zone to the water table increases [30].

The probability of occurrence of MTBE in source water was weakly associated with the number of leaking underground storage tanks within 1 kilometer of the sampled well, and aquifer consolidation. The probability of occurrence of MTBE in source water increased as the number of leaking underground storage tanks within 1 km of the sampled well increased. Leaking underground storage tanks are a likely source of MTBE detected in source water. The probability of occurrence of MTBE in source water was higher in aquifers composed of consolidated geologic materials than in aquifers composed of unconsolidated geologic materials. Ground water can move quickly through fracture porosity in most types of consolidated aquifer materials. The highly interconnected nature of fracture porosity allows movement of contaminants through the aquifer system with less time available for biodegradation, dispersion, and diffusion.

The probability of occurrence of MTBE in ground water also increased as dissolved oxygen content of the water increased. The dissolved oxygen content of ground water is believed to regulate biodegradation of MTBE. This result suggests that MTBE is biodegraded under low dissolved-oxygen conditions or conditions where dissolved oxygen is absent. Some studies indicate that MTBE may be biodegrading under anaerobic conditions [31, 32]. However, most studies indicate that the fastest, and most complete, MTBE biodegradation occurs under aerobic conditions [17, 33]. A previous study on the occurrence of MTBE in ground water of the USA at a national scale suggested that dissolved oxygen had little effect on MTBE occurrence or concentrations [4]. Thus, for source water data, dissolved oxygen may be acting as a surrogate for another variable which is controlling or influencing the occurrence of MTBE.

8
Discussion and Implications

The occurrence frequency of MTBE in source water from private wells was about 3% and the occurrence frequency of MTBE in source water from public wells was about 5%. As of 2000, approximately one half of the US population relied on ground water as a source of drinking water [34]. In terms of population, about 44 million people use ground water from private wells for water supply and about 90 million people use ground water from public wells for water supply. By extrapolating the detection frequency of MTBE in source water from the NAWQA survey to the entire USA, about one million people may potentially be exposed to MTBE through source water from

private wells, while about four million people may potentially be exposed to MTBE through source water from public wells. Thus, a total of about five million people may potentially be exposed to MTBE through source water derived from ground water. The actual number of people exposed to MTBE through drinking water is likely smaller than this because many public systems, and some private systems, treat water before it is consumed, although few likely treat specifically for MTBE. Nevertheless, the magnitude of this number is high enough to warrant concern regarding human exposure to MTBE in drinking water.

The detection frequency and concentrations of MTBE were higher in source water from public wells than from private wells. Public wells appear to be more vulnerable to contamination by MTBE. The increased vulnerability of public wells to MTBE contamination relative to private wells probably is a result of several factors including: (1) compared to private wells, public wells tend to be higher capacity and extract ground water from large areas in an aquifer, which can integrate ground water from multiple contaminated sites and from contaminated sites at longer distances; (2) unlike private wells, public wells generally are screened throughout an entire aquifer or throughout multiple aquifers and can draw ground water from multiple flow paths of both long and short residence time; and (3) most private wells sampled by the NAWQA Program are located in rural settings, whereas most public wells are generally located in areas of higher population density and urban development that often have more potential sources of MTBE.

The implications of the increased vulnerability of public wells to contamination by MTBE are twofold. About twice as many people in the USA derive their drinking water from public systems that rely exclusively, or partly, on ground-water sources than those that derive their drinking water from private wells. Consequently, more people in the USA may potentially be exposed to MTBE through drinking water from public systems than from private wells. Second, many ground-water resource managers may perceive public wells to be less vulnerable to contamination by MTBE because public wells generally are deeper, are screened in multiple aquifers, and are often protected from contamination by regulations such as wellhead protection areas. This perception may result in less concern for monitoring of unregulated contaminants like MTBE in public wells and therefore less awareness of important ground-water-quality issues.

Control of sources or potential sources of MTBE to ground water is important in protecting ground-water resources used to supply water for human consumption. Because of the uncertainty in the long-term health effects of MTBE in drinking water, it is important to monitor for MTBE in ground water used as a source of drinking water, especially ground water from public wells. Low-level analytical methods are best for determining trends in the occurrence of MTBE in ground water and for providing an early warning of new sources of contamination.

The detection frequency of MTBE in drinking water from community water systems supplied exclusively by ground water in 12 Northeastern states was about 9%. By extrapolating this detection frequency to the population of the 12-state area that derives drinking water from ground water, as many as one million people may potentially be exposed to MTBE through drinking water. MTBE was detected more frequently in large- and very large-size community water systems than in small- and medium-size community water systems. The higher detection frequency of MTBE in large systems probably is a result of their location in or near more developed areas with higher population densities, and of their use of larger pumping-capacity wells.

The detection frequency and concentrations of MTBE were higher in drinking water compared to source water in 12 Northeastern states. This is likely because samples from public wells comprise the entire drinking water data while samples from private wells dominate the source water data. Public wells used by community water systems generally have higher pumping rates and can integrate MTBE from more sources than smaller pumping-rate private wells.

Few community water systems in 12 Northeastern states had concentrations of MTBE greater than state benchmarks for MTBE in drinking water. In addition, concentrations of MTBE generally were low enough so as not to cause taste and odor concerns in drinking water. However, the potential long-term effects from exposure to low concentrations of MTBE in drinking water are not known. Different conclusions have been drawn from the relatively few studies that have been conducted on the toxicity of MTBE, and different public health agencies have classified the carcinogencity of MTBE differently. The uncertainty regarding the long-term health effects of MTBE, especially at low concentrations, has raised concerns about its presence in drinking water and warrants that MTBE continue to be monitored in ground water used to supply drinking water, especially ground water from public wells.

The probability of occurrence of MTBE in source water was strongly associated with transport and fate variables including water temperature, precipitation, and dissolved oxygen. The transport variable aquifer consolidation and the source variable proximity of leaking underground storage tanks were weakly associated with the probability of occurrence of MTBE in source water. It appears that fate and transport are most important in determining the presence of MTBE in source water and that leaking underground storage tanks may contribute at least some of the MTBE detected in source water. However, the nature of the source water data and the limitations of the national-scale ancillary data need to be further understood in order to put the results of the relational analyses in the correct context.

All of the wells used to determine relations with anthropogenic and hydrogeologic variables were private wells and many of these were located in rural areas. Although many rural private wells probably draw water from the shallowest depth possible in an aquifer, the depths of aquifers that are tapped

by private wells and the resultant flow-path lengths probably vary considerably across the USA. Some private wells may draw water from long flow paths whose recharge areas are far removed from the well location. In these wells, little relation would be expected between MTBE occurrence and source variables near the well. In addition, many types of intense sources, such as accidental spills or releases, especially those that might be encountered near rural private wells, are not represented in the ancillary data. Other variables, such as hydrologic properties, may be poorly or inaccurately represented or only represented by surrogates in the national-scale ancillary data.

Understanding the occurrence of MTBE in source water would be enhanced by more extensive and higher-resolution ancillary data on the sources of MTBE, the hydrogeologic properties of aquifers, and the fate of MTBE in aquifers. Better understanding of the sources of MTBE to ground water, the intrinsic susceptibility of aquifers to contamination, and the fate of MTBE in ground water would aid in adequately protecting ground-water resources from contamination by MTBE.

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Biodegradability of Oxygenates by Microflora from MTBE-Contaminated Sites: New Molecular Tools

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Abstract Ethers, such as methyl *tert*-butyl ether (MTBE) and ethyl *tert*-butyl ether (ETBE) are added to gasoline to enhance the octane index and to improve the air emission quality. MTBE, especially, has been found in several aquifers as a contaminant after accidental releases of ether-supplemented gasoline. The presence of these ethers in groundwater is considered to be the consequence of their persistence in the environment, due to their high water solubility and poor biodegradability. Herein, we will summarize the results of studies that have been carried out to investigate the actual capacity of indigenous microflora sampled from a variety of contaminated sites under different conditions (oxic and anoxic), and present the results of a survey to evaluate both the biodegradation capacity of ethers and *tert*-butyl alcohol (TBA) and the presence of catabolic genes that have been shown to be involved in fuel-ether degradation pathways. The aim of this study was to assess the correlation between the indigenous biodegradation capacity and the presence of these specific genes, so as to provide a basis for the use of genetic tools, such as microarrays, for the management of this environmental issue.

Keywords MTBE · ETBE · TBA · biodegradability · genes · microarray

Abbreviations

ETBE	ethyl *tert*-butyl ether
EU	European Union
HIBA	hydroxyisobutyric acid
MNA	monitored natural attenuation
MTBE	methyl *tert*-butyl ether
2M1,2PD	2-methyl 1,2-propanediol
PCR	polymerase chain reaction
RFG	reformulated gasolines
TAA	*tert*-amyl alcohol
TAME	*tert*-amyl methyl ether
TBA	*tert*-butyl alcohol
TBF	*tert*-butyl formate
ThOD	theoretical oxygen demand
USEPA	U.S. Environmental Protection Agency

1 Introduction

Fuel oxygenates have been added to gasoline since the 1980s in order to obtain the high octane index required by automobile manufacturers. The compounds that fulfill this requirement are the ethers, MTBE, ETBE or TAME, or the alcohols, methanol, ethanol or TBA. MTBE has been used worldwide, and ETBE has been used in Europe (Spain, France and, more recently, Germany) where it was produced by using ethanol from biomass and so considered as part of the effort to replace gasoline by biofuels. TBA, a key intermediate of the MTBE or ETBE catabolic pathway, is found in MTBE and/or ETBE-impacted aquifers as the result of the partial biodegradation of the ethers under limiting conditions; it can also be used as an additive itself or be present as a production impurity of MTBE or ETBE [1].

These compounds all have a high octane index and high water solubility (Table 1).

The use of these additives was shown to have a positive effect on emission quality in large urban areas [2]. Nevertheless, the use of MTBE in gasoline which is one of the most frequent pollutants of groundwater, led to its detection in gasoline-impacted aquifers [3]. MTBE accounted for 4% (vol/vol) of all gasoline in the U.S.A. in 2002 [4] and its use has now been banned in several US states. MTBE is used without major restrictions in Europe and producers expect its utilization to remain stable [4]. The concern regarding the use of these compounds is due to their environmental impact on water quality. They are more soluble in water than the monoaromatics present in gasoline. After several years of use of these compounds, it appeared from several stud-

Table 1 Chemical characteristics of gasoline and oxygenates

Characteristics	Gasoline	MTBE	ETBE	TAME	TBA
Molecular formula	–	$CH_3OC(CH_3)_3$	$CH_3CH_2OC(CH_3)_3$	$CH_3OC(CH_3)_2CH_3CH_2$	$(CH_3)_3COH$
Molecular weight ($g\,mol^{-1}$)	–	88.15	102.18	102.18	74.12
Boiling point (°C)	30–190	55.3	72.8	86.3	82.8
Density at 20 °C ($Kg\,l^{-1}$)	0.72–0.77	0.74	0.74	0.77	0.79
Solubility in water ($g\,l^{-1}$)	–	48	12	12	∞
Research octane index (RON)	95	118	117	114	113
Motor octane index (MON)	85	101	101	100	100

ies, first in the United States [5] that MTBE contamination of aquifers was common when reformulated gasoline has been spilled in soils. The plumes created by MTBE were larger than the plumes created by benzene. This is attributed to a combination of the high water solubility of these compounds and their low biodegradability. In the case of MTBE, the retardation factor (R) of 1.1 indicates that MTBE migrates nearly as rapidly as the water front (R for water = 1) [6]. The presence of MTBE in groundwater was first demonstrated in Santa Monica (Ca, USA) in 1996 [5]. To study the extent of MTBE contamination, a number of studies, first in the USA then in Europe, were undertaken to characterize the level of MTBE contamination in the different environmental compartments (for a review see [3, 7]). According to Squillace et al. [8], MTBE was the second most frequently detected compound among volatile organic compounds in the USA and its presence was detected in 20% of the samples collected in urban areas using reformulated gasolines (RFGs) [5]. In European countries, the presence of MTBE in the environment was also reported [9–11].

To our knowledge, the state of contamination of groundwater by ETBE has not yet been documented in the countries that use it.

The presence of MTBE (and of ETBE) in aquifers was, and remains, an issue mainly because of the two following points that remain controversial: (i) the impact of the oxygenates to humans and animals when present in drinking water at low concentrations and ingested over long periods of time was not clearly determined and (ii) their comparative biodegradability, when released into the environment, has not been thoroughly documented.

In this work, we summarize what has been previously reported on the biodegradability of these compounds, and present the results of a recent survey of several contaminated sites to establish a correlation between indigenous microbial biodegradation potential and the presence of genes known to be involved in the biodegradation of the fuel oxygenates.

2
Previous Studies on the Biodegradability of Oxygenates

Since MTBE can be a contaminant of aquifers, it is necessary to assess the efficiency of remediation technologies. MTBE (and possibly ETBE) was initially considered to be completely recalcitrant and therefore persistent in contaminated aquifers. The size and extent of MTBE plumes is also a concern since this will impact directly on the amount of water requiring treatment. A variety of technologies have been used to clean-up MTBE contaminated aquifers (for a review, see [12]). The potential to use aerobic in situ bioremediation to clean up sites contaminated by MTBE [13] originated from reports indicating the occurrence of aerobic MTBE biodegradation in several contaminated sites. In order to have some level of confidence that bioremediation of MTBE

is possible in contaminated sites, it is necessary to obtain data on the intrinsic biodegradation potential of MTBE, i.e. the frequency of the presence of indigenous microorganisms with MTBE/ETBE and TBA degradation capabilities in samples from different origins, tested under specific and standardized conditions.

The studies were mostly undertaken under oxic conditions, although several studies have also examined the biodegradation of MTBE under anoxic conditions.

2.1 Biodegradability under Oxic Conditions

Studies of intrinsic biodegradation can be performed using samples from different sites tested under aerobic conditions.

A survey for the presence of indigenous MTBE biodegradation capacity was carried out by Salanitro et al. [14] who reported that two sites out of ten from different parts of the United States showed the presence of MTBE-degrading activity.

Kane et al. [15] showed that MTBE degradation occurred in two microcosms from four MTBE-contaminated sites characterized by oxygen-limited in situ conditions. Under these conditions, TBA accumulated transiently and its level of accumulation was increased in the presence of gasoline, possibly due to oxygen-limitation.

Bradley et al. [16] incubated the sediments from surface-water systems collected in eleven different American locations, statically under an air atmosphere. The microorganisms present in the sediments were able to mineralize MTBE to various extents. The authors concluded from their results that the persistence of MTBE in water systems is more likely due to ongoing MTBE contamination than to environmental recalcitrance. Oxygen availability is clearly a very important factor as previously shown by Salanitro et al. [17].

In Europe, a similar study was carried out with soil samples from seven different locations in Belgium [18]. Only samples of a soil from one site with a history of MTBE contamination exhibited MTBE biodegradation capacity. These results clearly indicated that intrinsic aerobic degradation potential towards MTBE is rare in Belgium and that selective pressure is probably undergoing in such cases of ancient contamination. The authors proposed that this result could possibly be attributed to the relatively recent use of MTBE in Belgium (after 1988).

A number of site studies evaluating different methods to clean up aquifers contaminated by MTBE, have been conducted, mainly in the United States (for a review, see [12]). The results demonstrated that bioremediation was a possible option in 57 of the 244 cases studied [19, 20].

In parallel to the on site and laboratory microcosm studies, different groups have tried to isolate microorganisms able to grow on MTBE or ETBE.

A few strictly aerobic strains able to grow on MTBE or ETBE have been isolated (for a review, see [7]) and characterized to various extents. These studies have facilitated the elucidation of the aerobic biodegradation pathways for these compounds [21].

2.2 Biodegradability in the Presence of Various Electron Acceptors

Biodegradability studies have been carried out using samples from sites in the presence of different electron acceptors to identify the conditions that enable anaerobic biodegradation. Natural conditions in groundwater are often anoxic, or oxygen can be locally depleted when large amounts of gasoline are discharged to groundwater leading to an excess of electron donors, i.e. carbon substrates [22].

Finneran and Lovley [23] and Schmidt et al. [24] have discussed the thermodynamics of the MTBE and TBA degradation process in the presence of different terminal electron acceptors. Schmidt et al. [24] calculated the free energy ΔG^0 yield from MTBE or TBA under different redox conditions from oxic to methanogenic, where this value ranged from –3172 to –88.6 kJ mol^{-1} or from –2499 to –32.1 kJ mol^{-1}, respectively. This showed that although less favorable, anaerobic conditions could allow the biodegradation of MTBE and TBA. Bradley et al. [25] tested the mineralization potential of [^{14}C]-MTBE in the presence of all the predominant electron acceptors with lake or stream sediments sampled at three different sites in the United States. Mineralization was observed in all cases but to different extents.

This conclusion was supported by several reports showing a decrease in MTBE concentrations under denitrifying conditions in various locations [13]. Other experimental results [26] showed anaerobic degradation of MTBE in aquifer sediments in the presence of Fe(III) as the electron acceptor and that unamended aquatic sediments produced $^{14}CO_2$ and $^{14}CH_4$ from [^{14}C]-MTBE. These results were supported by a report of natural MTBE biodegradation under iron-reducing conditions [6].

Nevertheless, as mentioned by Schmidt et al. [24], the degradation pathways under anoxic conditions have yet to be elucidated and MTBE/ETBE/TBA degradation rates under all these different conditions are still not available.

3 Comparison of Indigenous MTBE, ETBE and TBA Degradation Capacities in MTBE-Contaminated Sites

Most of the previous studies have focused on MTBE biodegradability, although it would be of considerable interest to compare the biodegradation

potential of MTBE and ETBE to determine if there is an environmental advantage in using one fuel oxygenate over the other. Such a result would be very interesting at the moment since ETBE is used in some European countries to incorporate bioethanol in the gasoline pool.

Here, we summarize the results that we obtained by comparing the intrinsic biodegradation potential of MTBE, ETBE and TBA in MTBE-contaminated samples from sites of different geographical origins. In addition, since information on the genes involved in the biodegradation of MTBE, ETBE or TBA is now available, we examined the same samples for the presence of these specific target genes.

Soil and groundwater samples from fuel oxygenate contaminated sites were obtained from different geographical locations, transferred into sterile, airtight flasks and transported to the laboratory in refrigerated containers. Samples were used immediately upon arrival for biodegradation potential assessment or for chemical analysis of fuel oxygenate concentrations (Table 2). Samples for molecular analysis were maintained frozen until used.

3.1 Biodegradability Analysis

Biodegradation tests were carried out in air-tight Schott flasks using a medium modified from François et al. [27] containing NH_4NO_3 ($1.5\,g\,l^{-1}$) as the nitrogen source, $FeSO_4$, $7H_2O$ ($1\,mg\,l^{-1}$) as the iron source and supplemented with $0.1\,mg\,l^{-1}$ of yeast extract (YE) since small amounts of YE were previously shown to improve the growth rate of *Hydrogenophaga flava* ENV735 [28] and of *Mycobacterium austroafricanum* IFP 2012 [27] during cultivation on MTBE. MTBE, ETBE and TBA were used as the carbon sources (final concentration of $200\,mg\,l^{-1}$). The headspace in the flasks was calculated so that oxygen was in large excess with regard to the theoretical oxygen demand (ThOD) of the carbon substrate. The flasks were incubated under agitation at 30 °C. Liquid samples were regularly withdrawn and the residual fuel oxygenate concentrations were measured by GC/FID as previously described [29]. When degradation occurred, the flasks were re-spiked with the same substrate to confirm the biodegradation capacity.

The results of this biodegradation analysis, which was carried out under non-limiting conditions (nutrients, nitrogen sources, oxygen) are summarized in Table 3.

3.1.1 MTBE Biodegradability

There were three sites located in the USA (10, 11 and 14) where no biodegradation took place even after a long period of incubation (about one year). Three of the sites (2, 7 and 8) showed an efficient biodegradation of MTBE.

Table 2 Characteristics of the different contaminated samples

Sample	Origin	Oxygenates in the sample						Bacterial Count (bacteria ml^{-1})
		MTBE	ETBE	TAME	TBF	TBA	TAA	
1	USA [a]	0.6*	n.d.	n.d.	0.9*	0.4*	n.d.	–
2	Germany [b]	29.3**	n.d.	n.d.	0.7**	0.4**	n.d.	7.37E+04
3	Belgium [a]	0.46*	n.d.	n.d.	n.d.	n.d.	n.d.	–
4	France [b]	113.5**	4.2**	8.1**	n.d.	16.4**	1.7**	1.28E+05
5	France [b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.52E+05
6	France [b]	4.54**	n.d.	n.d.	n.d.	0.68**	n.d.	9.53E+05
7	USA [b]	4.72**	n.d.	n.d.	n.d.	0.35**	n.d.	3.52E+05
8	USA [b]	19.3**	n.d.	n.d.	0.7**	0.56**	n.d.	1.11E+05
9	USA [b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.97E+04
10	USA [b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.28E+05
11	USA [b]	1.95**	n.d.	n.d.	0.7**	0.57**	n.d.	4.63E+04
12	UK [a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–
13	UK [a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–
14	USA [b]	164.8**	n.d.	n.d.	n.d.	24.32**	n.d.	–

[a] soil
[b] aquifer
– not performed
* expressed in $\mu g\, g^{-1}$ of soil
** expressed in $mg\, l^{-1}$ of aquifer
n.d. not detected (not present or below the quantification limit)

Table 3 Biodegradation of MTBE, ETBE and TBA in the 14 different sites tested

Site	Oxygenate being used as the carbon substrate:		
	MTBE	ETBE	TBA
1	9% biodegraded in 345 days (transient TBA production = 1.5 mg l^{-1})	8% biodegraded in 345 days (transient TBA production = 2.6 mg l^{-1})	Efficient biodegradation [a]
2	Efficient biodegradation [a]	Efficient biodegradation [a]	Efficient biodegradation [a]
3	Slow biodegradation (100% in 300 days)	Efficient biodegradation [b]	Efficient biodegradation [a]
4	18% biodegraded in 310 days	36% biodegraded in 310 days	Efficient biodegradation [b]
5	1st addition: 100% in 27 d. No biodegradation of the 2nd addition	Efficient biodegradation [a]	1st addition: 100% in 27 d. 2nd addition: only 60% biodegraded
6	1st addition: 100% in 27 d. 2nd addition: 81% biodegraded in 242 d.	Efficient biodegradation [b]	1st addition: 100% in 15 d. No biodegradation of the 2nd addition
7	Efficient biodegradation [b]	No biodegradation [c]	Efficient biodegradation [a]

Table 3 (continued)

Site	Oxygenate being used as the carbon substrate:		
	MTBE	ETBE	TBA
8	Efficient biodegradation[a]	No biodegradation[c]	Efficient biodegradation[a]
9	21% biodegraded in 277 days	22% biodegraded in 277 days	Efficient biodegradation[a]
10	No biodegradation[c]	No measurable biodegradation (but TBA production = 3 mg l^{-1})	Degradation in 275 days (long lag phase = 121 d.)
11	No biodegradation[c]	47% biodegraded in 275 days (long lag phase = 182 d.)	Efficient biodegradation[a]
12	4% biodegraded in 271 days	8% biodegraded in 271 days	1st addition: 100% in 116 d. No biodegradation of the 2nd addition
13	24% biodegraded in 271 days	No biodegradation[c]	1st addition: 100% in 116 d. No biodegradation of the 2nd addition
14	No biodegradation[c]	8% biodegraded in 261 days (TBA production = 3.9 mg l^{-1})	No biodegradation

[a] three successive additions
[b] two successive additions
[c] no TBA production

Six intermediate cases displayed a very low biodegradation of MTBE, even under non-limiting conditions, which was not very promising for natural attenuation to occur (sites 1, 3, 4, 9, 12, 13). There were also two cases with an ambiguous result (sites 5 and 6): MTBE was utilized rather rapidly after the first addition (27 days) but after the second addition, MTBE was either not used or only slowly utilized. This result suggests that biodegradation of MTBE is via a cometabolic mechanism, the primary substrate being provided together with the inoculum at the seeding step. As a result, no more MTBE biodegradation would be observed after the consumption of the primary substrate.

3.1.2
ETBE Biodegradability

There were three sites (7, 8 and 13) where no degradation of ETBE was observed, even after a long period of incubation (about one year). Two of these sites were in the USA and showed efficient degradation of MTBE (sites 7 and 8). Four sites, all of which were in Europe, showed efficient degradation of ETBE (2, 3, 5 and 6). There were seven intermediate cases with a very low biodegradation of ETBE, even under non-limiting conditions, suggesting these were poor candidates for natural attenuation (sites 1, 4, 9, 11, 8, 14).

3.1.3
TBA Biodegradability

Only one site showed no TBA degradation capacity (site 14). The degradation of TBA was efficient in eight of the 14 sites (1–4, 7–9, 11).

In five sites, TBA biodegradation was very slow (site 10) or not confirmed after a second addition of TBA (sites 5, 6, 12, 13). The case of sites 5 and 6 is interesting because the same behavior was observed in the presence of MTBE and a similar explanation involving a cometabolic process could be proposed (see above, under MTBE biodegradability).

In all the cases where biodegradation occurred, the biodegradation capacities were confirmed by subsequent cultures. It was thus possible to get stable microcosms with high degradation capacities (i) towards MTBE, as in the case of microcosms obtained from site 2 (Fig. 1a), and microcosms obtained from sites 7 and 8 (Figs. 2a and 2b, respectively) or (ii) towards ETBE, as in the case of microcosms obtained from site 2 (Fig. 1b), and microcosms obtained from sites 3 and 6 (Figs. 3a and 3b, respectively).

3.2
Detection of Genes Involved in MTBE, ETBE and TBA Biodegradation

When DNA sequences for the specific detection of microorganisms (16S rRNA gene) or for the specific detection of functional genes involved in

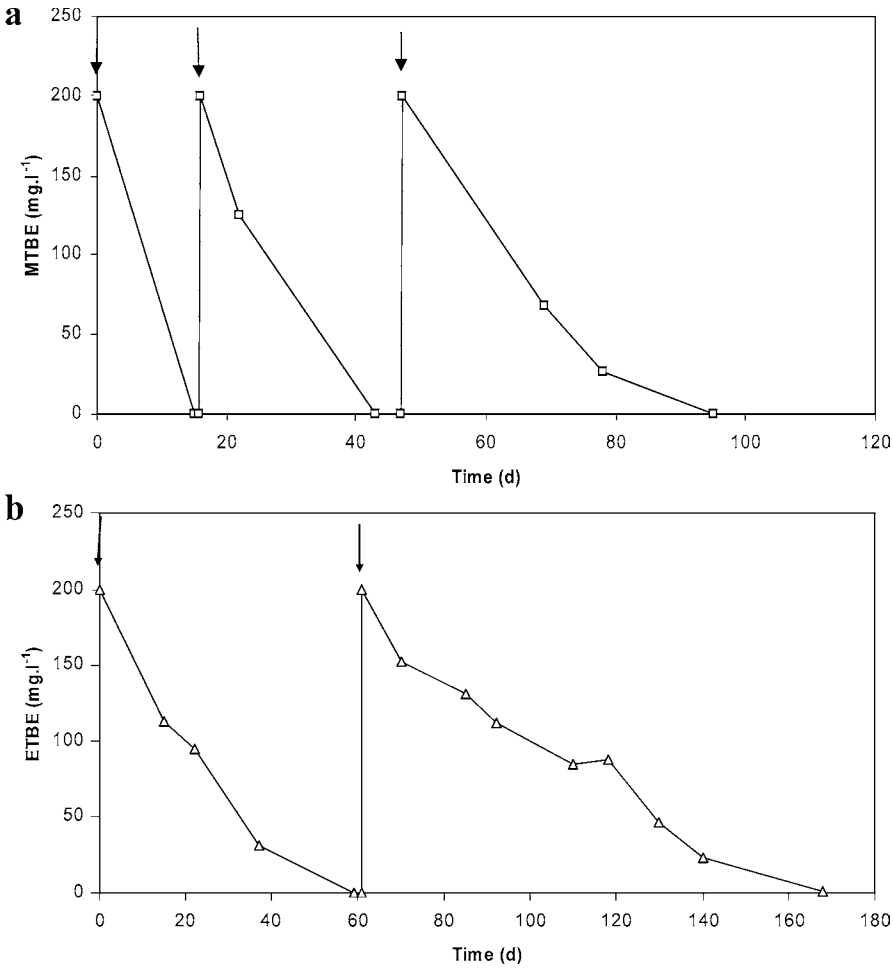

Fig. 1 Biodegradation of MTBE (**a**) and ETBE (**b**) in the microcosms obtained from site 2. Residual MTBE (□ – □) or ETBE (△ – △) were measured by GC/FID as previously described [29]. When all the ether had been utilized, new additions were performed (*arrow*)

a biodegradation pathway are known, it is possible to look for similar DNA sequences in polluted environmental samples by using the polymerase chain reaction (PCR) with oligonucleotide primers designed to specifically amplify the DNA sequences of interest. The amplified DNA fragment can then be visualized by agarose gel electrophoresis. When the reaction is specific, one single DNA band is visualized that is the size of the expected fragment. The amplified DNA fragments can be sequenced and compared to the expected sequence and against the sequence databases to look for homologous target genes.

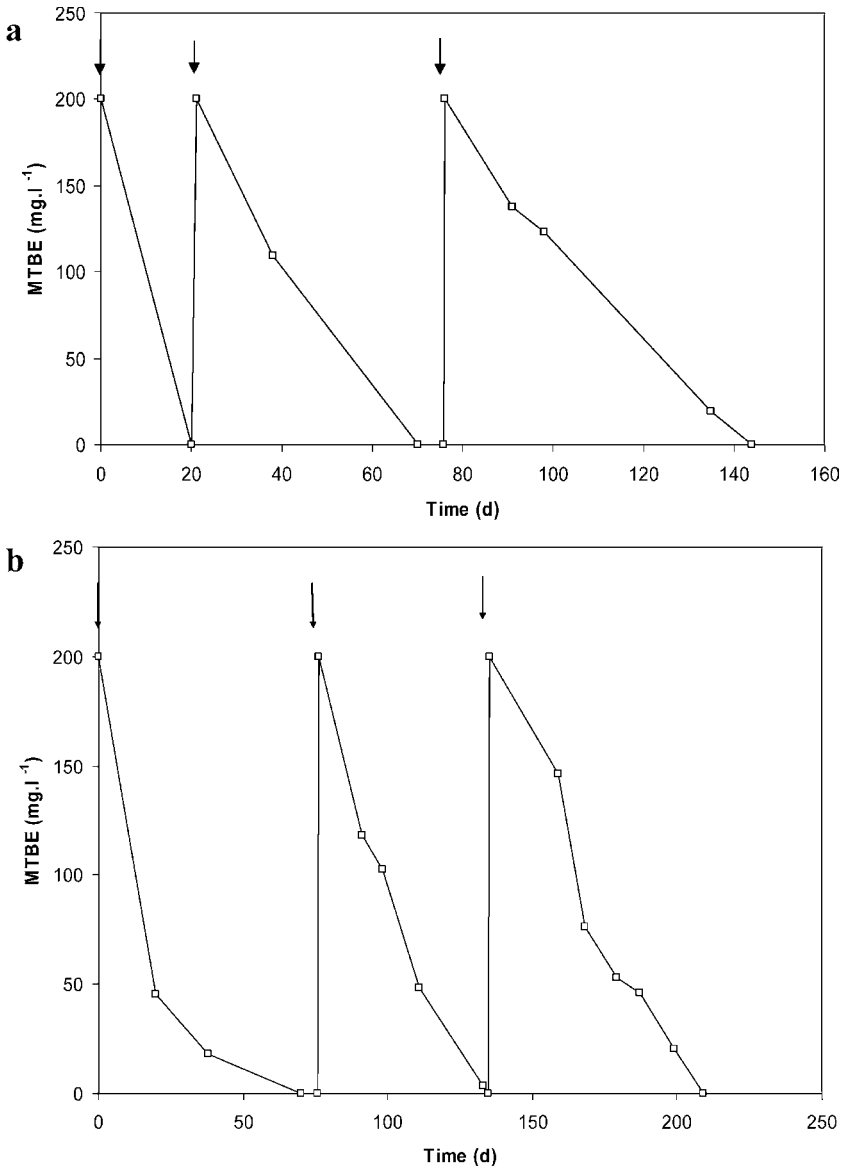

Fig. 2 Biodegradation of MTBE in the microcosms obtained from sites 7 (**a**) and 8 (**b**). Residual MTBE (□ – □) was measured by GC/FID as previously described [29]. When all MTBE had been utilized, new additions were performed (*arrow*)

Another method to characterize the DNA fragment obtained by PCR amplification is by DNA/DNA hybridization. This method compares a specific target gene to the DNA extracted from an environmental sample in order to determine its presence or absence. This technique can be very specific and

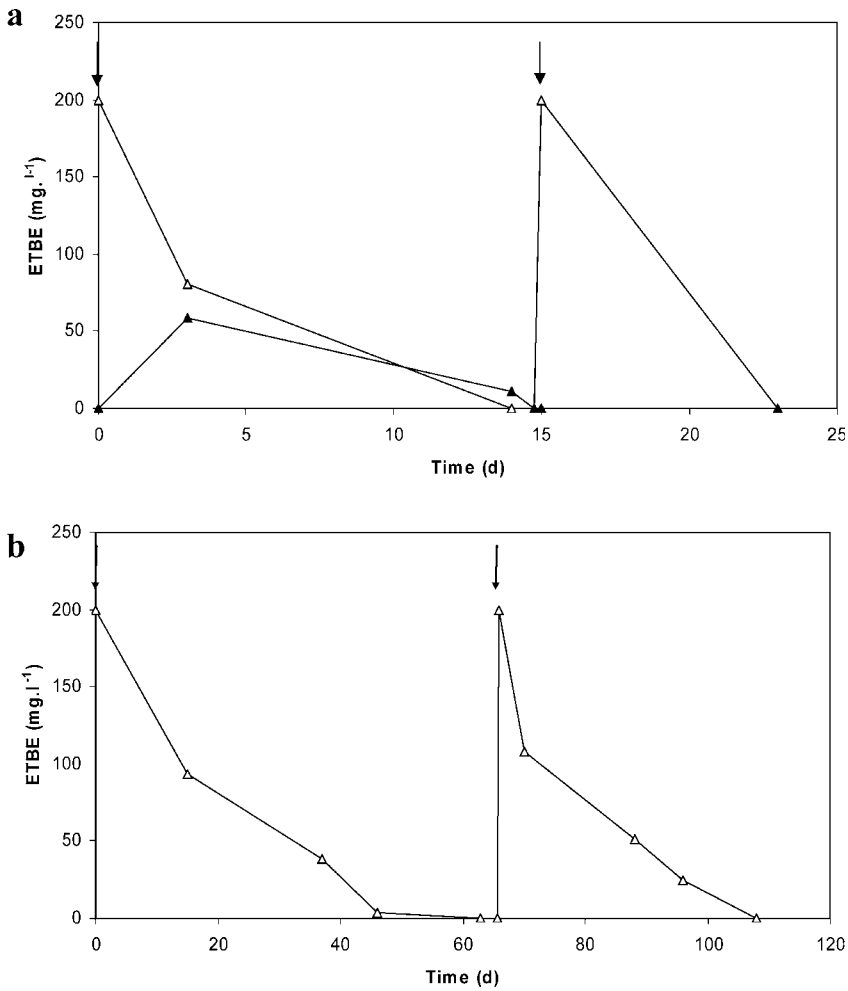

Fig. 3 Biodegradation of ETBE in the microcosms obtained from sites 3 (**a**) and 6 (**b**). Residual ETBE (△ – △) was measured by GC/FID as previously described [29]. When all ETBE had been utilized, new additions were performed (*arrow*). Production of TBA (▲ – ▲) was transiently observed

sensitive. The stringency can also be lowered to search homologous, but not identical, target genes.

3.2.1 Tools for Phylogenetic Microarrays

The phylogenetic characterization of microorganisms with MTBE or ETBE degradation capabilities is of great interest because it can facilitate detection

of these microorganisms in MTBE/ETBE-contaminated sites. A phylogenetic tree of the microorganisms able to grow on MTBE was constructed using partial 16S rRNA gene sequences [21]. It showed that the following four groups of microorganisms, isolated to date, were able to grow on MTBE:

- Group 1: related to *Methylibium petroleiphilum* PM1 [30, 31],
- Group 2: related to the new species, *Aquincola tertiaricarbonaris*, including strains L10 and I-2052 (IFP 2003) [32],
- Group 3: related to *Hydogenophaga flava* ENV735 [28],
- Group 4: related to *Mycobacterium austroafricanum* strains, among them, strains IFP 2012 [27] and IFP 2015 [29].

The detection of these microorganisms in contaminated aquifers using molecular techniques could provide indications of the potential for natural attenuation under aerobic conditions. Kane et al. [15] and Hristova et al. [33] reported the amplification of 16S rRNA gene sequences closely related to that of *Methylibium petroleiphilum* PM1, the first strain isolated for its ability to grow on MTBE [34] from samples of MTBE-contaminated aquifers.

DNA primers that have been used to detect the presence of one of these groups of microorganisms are presented in Table 4.

3.2.2 Tools for Catabolic Microarrays

To design microarrays for detecting MTBE or ETBE biodegradation capacities, a second, possibly more efficient, approach would be to use genes known to be involved in the early steps of MTBE and/or ETBE biodegradation. Such tools could help in determining the potential for ether biodegradation in MTBE or ETBE contaminated sites and help to select and optimize monitored natural attenuation (MNA) processes on such sites under oxic conditions. Lopes Ferreira et al. [21] recently summarized the present state of knowledge for these target genes:

- The genes encoding a cytochrome P450 monooxygenase system involved in the initial attack of ETBE and of MTBE by *Rhodococcus ruber* IFP 2001 were isolated and characterized [35, 36]. *Eth* genes were also found to be highly conserved in two other strains able to grow on ETBE [37].
- The *mpd* genes involved in the early steps of the TBA catabolic pathway were recently isolated and characterized in *M. austroafricanum* IFP 2012 [38]. The enzymes encoded by these genes catalyzed the transformation of 2-methyl 1,2-propanediol (2M1, 2PD), the product of TBA oxidation, into HIBA.
- The possible involvement of an alkane hydroxylase was recently shown in MTBE oxidation by *Methylibium petroleiphilum* PM1 [30] and in TBA oxidation by *Mycobacterium austroafricanum* IFP 2012 able to grow on

Table 4 Primers used in this study for PCR amplification

DNA sequence amplified	DNA primers used for PCR amplification	Size of the amplification product	Refs.
16S rDNA- Group 1	613F/988R	203	[33]
16S rDNA- Group 2	IFP 2003-F1/IFP 2003-R1	403	This study
16S rDNA- Group 3	ENV735-F1/ENV735-R1	399	This study
16S rDNA- Group 4	MaFV2/MaRV6	331	[29]
ethB (encoding the cytochrome P450)	ethB-F2/ethB-R2	881	C. Malandain, personal communication
alkB (encoding the alkane hydroxylase)	alkB2012_for/alkB2012-rev	334	N. Lopes Ferreira, personal communication
mpdC (encoding the hydroxyisobutyraldehyde dehydrogenase)	mpdC-F2/mpdC-R2	590	This study
mpdB (encoding the 2M 1,2PD dehydrogenase)	mpdB-f1/mpdB-r1	437	[38]

MTBE [39]. The involvement of the alkane hydroxylase in MTBE oxidation was also previously demonstrated in *Pseudomonas putida* Gpo1 [40].

This panel of genes involved in the early steps of ETBE and MTBE metabolism was used to design specific DNA primers (Table 4) to detect these genes in environmental samples.

3.2.3 Detection of Genes in Active Microcosms

Samples from the MTBE-contaminated sites (Table 2) were screened by PCR and DNA/DNA hybridization analysis for the presence of the genes involved in the MTBE metabolic pathway using the primers presented in Table 4.

One of the primer sets (613F/988R) was previously used by Hristova et al. [33] to check the presence of strains similar to *M. petroleiphilum* PM1 in contaminated aquifers. A unique band of the expected size after gel electrophoresis could not be obtained using these primers, possibly because the primers were non-specifically amplifying sequences with no relationship to *M. petroleiphilum* PM1. With all the other primer sets, we were able to generate a unique PCR fragment of the expected size (see Table 4). The results obtained from the sites that had demonstrated MTBE or ETBE degradation capacity are presented in Table 5.

The results obtained confirmed the relevance of the panel of designed primers, especially when targeting the catabolic genes. The *alkB*, *ethB* and *mpdB* genes were amplified after one growth step in MTBE or ETBE of the samples displaying biodegradation activities, confirming the involvement of these genes in the degradation pathway of these compounds and demonstrating that these genes are good candidates for application on a catabolic microarray.

Concerning the 16S rRNA gene primers for the different groups of microorganisms, there is a lack of information for strains related to *M. petroleiphilum* PM1 (Group 1) in our samples, suggesting that more specific primers would be required. The presence of strains related to *Hydrogenophaga flava* (Group 3) was detected in all cases after culture on MTBE or ETBE. The presence of *Aquincola tertiaricarbonis* (Group 2) was confirmed after DNA/DNA hybridization in three of the four sites. The presence of *Mycobacterium austroafricanum* IFP 2012 (Group 4) was confirmed after DNA/DNA hybridization in two of the four sites.

4 Conclusions and Perspectives

From previous studies and from the present results, several conclusions can be drawn regarding the capacity for MTBE or ETBE biodegradation by in-

Table 5 PCR amplifications and DNA/DNA hybridizations using target DNA from sites with MTBE or ETBE degradation capacities

Site	DNA extracted from the aquifer*	DNA extracted after culture on MTBE*		DNA extracted after culture on ETBE*	
		PCR amplification	DNA/DNA hybridization	PCR amplification	DNA/DNA hybridization
2	*mpdB* (+)	*alkB* (+) *ethB* (+) *mpdB* (+) *mpdC* (+) 16S rDNA/Group 2 (+) 16S rDNA/Group 3 (+) 16S rDNA/Group 4 (–)	*alkB* (+) *ethB* (+) *mpdB* (+) *mpdC* (+) 16S rDNA/Group 2 (+) Nd 16S rDNA/Group 4 (–)	*alkB* (+) *ethB* (+) *mpdB* (+) *mpdC* (+ / –) 16S rDNA/Group 2 (+) 16S rDNA/Group 3 (+) 16S rDNA/Group 4 (–)	*alkB* (+) *ethB* (+) *mpdB* (+) *mpdC* (–) 16S rDNA/Group 2 (+) Nd 16S rDNA/Group 4 (–)
5	*ethB* (+)	No culture obtained		*alkB* (+) *ethB* (+) *mpdB* (+) *mpdC* (+) 16S rDNA/Group 2 (+) 16S rDNA/Group 3 (+) 16S rDNA/Group 4 (+)	*alkB* (+) *ethB* (+) *mpdB* (+) *mpdC* (–) 16S rDNA/Group 2 (+) Nd 16S rDNA/Group 4 (+)

Table 5 (continued)

Site	DNA extracted from the aquifer*	DNA extracted after culture on MTBE*		DNA extracted after culture on ETBE*	
		PCR amplification	DNA/DNA hybridization	PCR amplification	DNA/DNA hybridization
7	*mpdB* (+) 16S rDNA/Group 3 (+)	*alkB* (+) *ethB* (+) *mpdB* (+) *mpdC* (–) 16S rDNA/Group 2 (–) 16S rDNA/Group 3 (+) 16S rDNA/Group 4 (+)	*alkB* (+) *ethB* (+) *mpdB* (+) *mpdC* (–) 16S rDNA/Group 2 (–) Nd 16S rDNA/Group 4 (+)	No culture obtained	
8	*ethB* (+)	*alkB* (+) *ethB* (+) *mpdB* (–) *mpdC* (+ / –) 16S rDNA/Group 2 (+) 16S rDNA/Group 3 (+) 16S rDNA/Group 4 (–)	*alkB* (+) *ethB* (+) *mpdB* (–) *mpdC* (+) 16S rDNA/Group 2 (+) Nd 16S rDNA/Group 4 (–)	No culture obtained	

Nd: not determined (the sequence of the 399-bp fragment was not available for DNA/DNA hybridization studies)

* DNA was extracted according to Fortin et al. [43]

digenous microorganisms present in contaminated soils or aquifers and the presence of genes involved in the degradation pathways.

Our study confirms what had previously been reported by several authors regarding the capacity of indigenous microflora to degrade MTBE [14, 15, 18]. In our study, several sites exhibited either no or very low capacities for MTBE biodegradation in 64% of the cases or for ETBE biodegradation in 71% of the cases, even under optimal aerobic growth conditions. Without a larger number of study sites, it is necessary to be cautious before drawing conclusions, nevertheless ETBE does not seem to be more easily biodegraded than MTBE.

Obviously in cases where no biodegradation capacity was detected, the use of MNA would not be appropriate. On the contrary, the use of efficient and well-adapted strains could be suitable for cleaning-up the sites. Bioaugmentation was a valuable option in some cases of aquifers contaminated with MTBE [41, 42]. The present study showed that microcosms that efficiently degraded MTBE and/or ETBE could be obtained following enrichment culture of material from contaminated sites, emphasizing the potential for bioaugmentation processes in contaminated sites.

Rather surprisingly, there is no strict relationship between the capacity to degrade MTBE and the capacity to degrade ETBE. The indigenous microflora of two sites with very efficient MTBE biodegradation capacities (sites 7 and 8) were unable to degrade ETBE. There are two explanations that could explain this: (i) the monooxygenase responsible for the initial attack on the methyl group of MTBE is not able to attack the ethyl group of ETBE, (ii) ETBE can not induce or derepress the synthesis of the monooxygenase responsible for MTBE oxidation. A similar result was previously reported by Lopes Ferreira et al. [29] who showed that *M. austroafricanum* IFP 2012 and IFP 2015, able to grow on MTBE, were rather poor degraders of ETBE.

The capacity to degrade ETBE efficiently (sites 3, 5, 6) was correlated to a capacity to degrade MTBE, but less efficiently. There was only one case (site 2) where the capacities to degrade MTBE and ETBE were similar.

TBA was clearly more easily biodegraded than MTBE or ETBE. Nevertheless, there was a case where no TBA degradation occurred (site 14). Even if partial biodegradation of MTBE or ETBE takes place, TBA would accumulate in the aquifer and the use of adapted microorganisms or microcosm material with TBA degradation capacity would be required to clean up the site.

The results obtained with the panel of selected target genes was a very promising approach to build molecular tools for the detection of ETBE and/or MTBE biodegradation capacities in a contaminated site. This is especially true for the catabolic genes, *alkB* and *ethB* encoding the monooxygenases responsible for the initial attack on MTBE and/or ETBE. These genes were present (PCR amplification and DNA/DNA hybridization) after a growth step on MTBE or ETBE in sites 2, 5, 7 and 8. The *mpdB* gene encoding the 2M1,2PD dehydrogenase which is an important step in the TBA assimilation pathway was also detected in sites 2, 5 and 7. The sequences of the corresponding

genes could be used on microarrays dedicated to the detection of catabolic genes. The principle of microarrays is depicted in Fig. 4. The extent of the contamination by ethers [3, 5, 7–11] and the lack of biodegradation capabilities in a number of contaminated sites demonstrate the need for tools to estimate the capacity of the indigenous microorganisms in a contaminated site to degrade MTBE or ETBE.

Our results also showed that a phylogenetic microarray would be interesting to detect ether degrading microorganisms. It could allow the detection of microorganisms belonging to one of the groups of microorganisms known to grow on MTBE. The ability to detect strains similar to *M. petroleiphilum* PM1, which was previously proposed as a way to identify MTBE degradation potential in the environment [15, 33], is too limited. Nevertheless, a phylogenetic microarray containing a broader variety of strains capable of MTBE

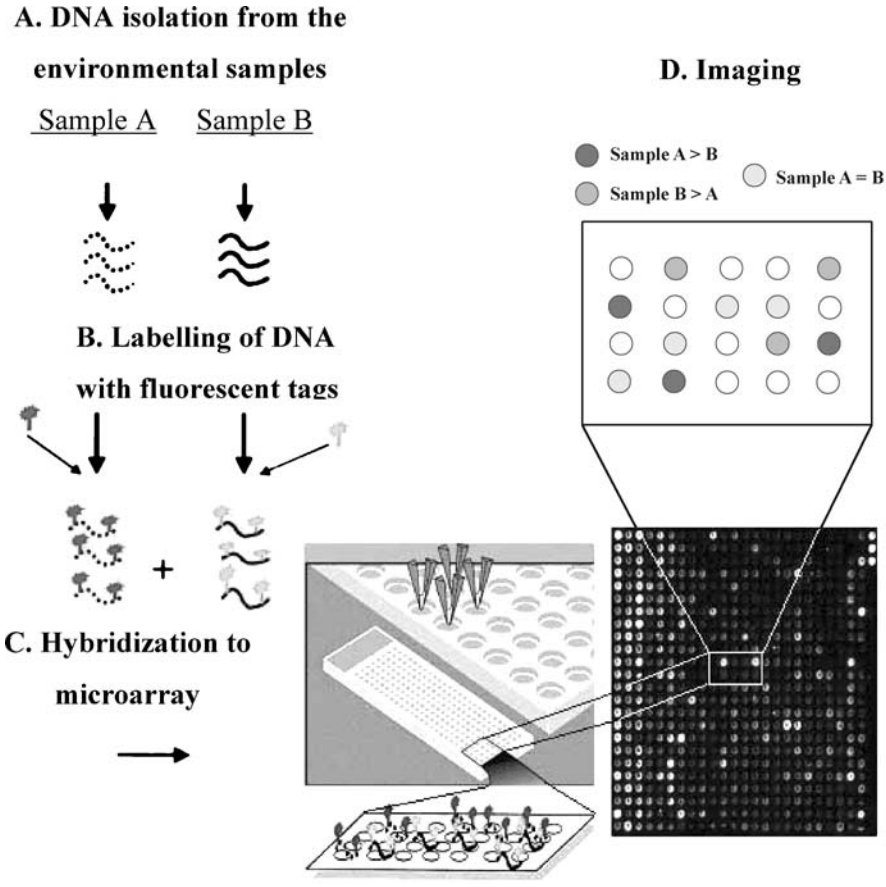

Fig. 4 Principle of a microarray

degradation could provide some important information on the biodegradation potential towards fuel oxygenates on a contaminated site.

From an ecological perspective, the different consortia exhibiting biodegradation capacities towards MTBE and/or ETBE (sites 2, 3, 6, 7 and 8) deserve more detailed characterization. This could be performed using the appropriate molecular tools (Denaturating Gel Gradient Electrophoresis or DGGE). A comparison of the microbial composition of these consortia from their different geographical origins could bring new insight on the distribution of ether-degrading microorganisms. It would also be interesting to compare the microbial composition after growth on MTBE/ETBE or on TBA and to detect the changes induced in the consortium composition by the change of growth substrate.

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Compound-Specific Isotope Analysis (CSIA) to Characterise Degradation Pathways and to Quantify In-Situ Degradation of Fuel Oxygenates and Other Fuel-Derived Contaminants

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Abstract Isotope fractionation of fuel oxygenates has been employed as an indicator for monitoring in-situ degradation in the field. For quantification of in-situ degradation, the Rayleigh concept can be applied. The selection of an appropriate isotope enrichment factor (ε) that is representative of the biogeochemical conditions governing the microbial

degradation process in the field is crucial for quantification. Therefore, the biogeochemistry of contaminated aquifers has to be taken into account in the development of isotope strategies in assessment and monitoring operations. In addition, controlled microcosms studies are needed to determine the extent of isotope fractionation under different conditions. The simultaneous analysis of carbon and hydrogen isotope composition of fuel oxygenates in a two-dimensional isotope approach opens opportunities for analysis of the predominant degradation process in the field and can be used to select an appropriate fractionation factor. In this contribution we summarise the concept of isotope fractionation of fuel oxygenates to assess in-situ degradation with respect to analytical techniques, recent progress on isotope fractionation in laboratory studies, the concept of two-dimensional isotope analysis, and experience from field studies.

Keywords MTBE · ETBE fuel oxygenates ·
CSIA (compound-specific stable isotope analysis) · In-situ biodegradation

Abbreviations

B	Biodegradation extent
BTEX	Benzene, toluene, ethylbenzene and xylenes
C	Concentration of pollutant
CSIA	Compound-specific isotope analysis
DIN	Deutsches Institut für Normung or German Institute for Standardization
DIPE	Diisopropyl ether
ETBE	Ethyl tertiary butyl ether
GC	Gas chromatography
HS	Direct headspace
IRMS	Isotope ratio mass spectrometry
MNA	Monitored natural attenuation
MS	Mass spectrometry
MTBE	Methyl tertiary butyl ether
P&T	Purge and trap
R	Isotopic ratio of the heavy isotope to the light isotope
S_N1	Nucleophilic substitution, acidic hydrolysis reaction
SPME	Solid-phase microextraction
TAME	Tertiary amyl methyl ether
TBA	Tertiary butyl alcohol
USEPA	US Environmental Protection Agency
VAFB	Vandenberg Air Force Base
VOC	Volatile organic compound
V-PDB	Vienna Pee Dee Belemnite standard
V-SMOW	Vienna Standard Mean Ocean Water
α	Isotopic fractionation factor
δ	Isotopic composition reported as delta notation
ε	Isotopic enrichment factor

1 Introduction

Methyl tertiary butyl ether (MTBE) has been by far the most commonly used fuel oxygenate for more than two decades because of its high-octane properties, cost effectiveness and supply flexibility. As a result of its intense production, use and physico-chemical properties, MTBE has become one of the most frequently detected volatile organic compounds (VOCs) in drinking water reservoirs and groundwater tables [1, 2]. The highest MTBE concentrations in the environment are related to accidental spills and tank corrosion leakage from gasoline stations and refineries. Aquifers heavily contaminated with fuel oxygenates have been identified in several countries [3–6] and natural attenuation is currently discussed as a remediation strategy [7, 8]. At present, ethyl *tert*-butyl ether (ETBE) and *tert*-amyl methyl ether (TAME) are progressively replacing MTBE in European gasoline due to tax incentives for the application of biofuels. Due to high production data it is expected that ETBE will be one of the emerging fuel contaminants in groundwater.

Biodegradation is the major process leading to a decrease of MTBE and other fuel oxygenate concentrations in groundwater, coupled to a sustainable reduction of its mass. Therefore, the evaluation of in-situ biodegradation is essential to monitor the fate of fuel oxygenates. Of particular interest is the assessment of in-situ biodegradation for the implementation and validation of groundwater management strategies such as monitored natural attenuation (MNA).

2 Concept of Isotope Fractionation to Assess In-Situ Degradation

In recent years, compound-specific stable isotope analysis (CSIA) has become a tool for characterising and assessing in-situ biodegradation of organic pollutants in contaminated aquifers [9, 10]. This concept relies on the fractionation of stable isotopes occurring during the microbial degradation of contaminants, leading to an enrichment of heavier stable isotopes in the residual fraction of a pollutant. Thus, the observation of isotope ratio shifts for carbon, hydrogen or other elements that are involved in the breakage or generation of chemical bonds during the initial step of microbial transformation can be used as an indicator for in-situ biodegradation. CSIA makes use of kinetic isotope fractionation processes upon biodegradation and uses the enrichment of heavy isotopes (^{13}C and ^{2}H) in the residual fraction as an indicator for in-situ biodegradation. For quantitative assessment of in-situ degradation the compound-specific isotope fractionation factor (α) is needed, which is obtained in controlled laboratory studies [9, 10].

Stable isotope fractionation studies of fuel compounds initially focussed on aromatic hydrocarbons, in particular regarding the ratio of $^{13}C/^{12}C$ [11–13], but also of D/H [14, 15]. However, in the last few years, MTBE has also received special attention. One of the first uses of MTBE stable isotopes was reported by Smallwood et al. [16] who attempted to distinguish between manufacturers and consequently to identify the contamination source in a case site. Surprisingly, the MTBE $\delta^{13}C$ values for several gasolines were in a relatively narrow range (–28.3 to –31.7‰), taking into account that MTBE is manufactured by three different processes [16, 17]. Up to now, the highest MTBE $\delta^{13}C$ value reported is –27.4±0.4‰ [18], which has been used as an estimate of the $\delta^{13}C$ of the MTBE originally spilled in a field site study in California [19].

The purpose of this chapter is to provide a summary of the different isotope enrichment factors for MTBE and related fuel oxygenates from biotic and abiotic reactions. Furthermore, the chapter shows how the current CSIA state of the art can help in the characterisation of MTBE degradation pathways and the quantification of in-situ degradation.

3
Compound-Specific Isotope Analysis (CSIA)

3.1
Stable Isotope Calculations and Definitions

CSIA yields data of the isotopic composition of a single compound relative to an international standard that is usually expressed as delta notation (δ) values in parts per thousand (‰) according to Eq. 1. The most common ones, the carbon and hydrogen isotopic compositions (R), are reported as $\delta^{13}C$ and $\delta^{2}H$ relative to Vienna Pee Dee Belemnite standard (V-PDB) and Vienna Standard Mean Ocean Water (V-SMOW), respectively [20]:

$$\delta\,[‰] = \left(\frac{R_x}{R_{reference}} - 1\right) \times 1000\,, \tag{1}$$

where R_x and $R_{reference}$ are the ratios of the heavy isotope to the light isotope ($^{13}C/^{12}C$ or D/H) in compound x and the international standard, respectively. Calculation of the isotopic fractionation factor (α) is based on the Rayleigh equation [21] simplified for a closed system [22]:

$$\ln\left(\frac{R_t}{R_0}\right) = \left(\frac{1}{\alpha} - 1\right) \times \ln\left(\frac{C_t}{C_0}\right)\,, \tag{2}$$

where R is the isotope ratio, C is the concentration at times $t = 0$ and t. The isotope fractionation factor relates changes in concentration to changes in isotope composition in a closed system and is used to express the extent of the

isotope fractionation process. In reference experiments in controlled laboratory systems, the isotope fractionation factor is determined preferentially in experiments with pure cultures and a known degradation pathway if possible. The index (0 and t) in Eq. 2 describes the incubation time at the beginning (0) and during the reaction time of the experiment (t). When $\ln\left(R_t/R_0\right)$ is plotted versus $\ln\left(C_t/C_0\right)$, the isotopic enrichment factor (ε) within the 95% confidence interval (95% CI) can be determined from the slope (b) of the linear regression of each data set, with $b = \left(1/\alpha\right)-1$ and $\varepsilon = 1000 \times b$. Both the enrichment factor (ε) and the isotope fractionation factor (α) can be used to describe isotope fractionations, whereas the enrichment factor (ε) has been frequently used in recent literature on environmental chemistry.

In field studies, the extent of biodegradation (B [%]) along the contaminated aquifer can be calculated by a modified Rayleigh equation (Eq. 3). A fractionation factor (α) or enrichment factors (ε) must be selected to reflect the environmental conditions in the aquifer. Commonly, a laboratory-derived fractionation factor α (or ε) that has been obtained in controlled laboratory experiments is used to quantify the microbial in-situ degradation (Eq. 3):

$$B\,[\%] = 100 \times \left[1 - \frac{C_t}{C_0}\right] = \left[1 - \left(\frac{R_t}{R_0}\right)^{\left\{\frac{1}{\frac{1}{\alpha}-1}\right\}}\right] \times 100$$

$$= \left[1 - \left(\frac{R_t}{R_0}\right)^{\left\{\frac{1000}{\varepsilon}\right\}}\right] \times 100\,. \tag{3}$$

B [%] represents the concentration decrease expected along a theoretical streamline plug flow without mixing and a single degradation process with a constant isotope fractionation factor. C_0 is the concentration of contaminants in the source area and C_t is the concentration of contaminants along the flow path. R is the isotope ratio calculated:

$$\frac{R_t}{R_0} = \frac{\left(\delta_t + 1000\right)}{\left(\delta_0 + 1000\right)}\,, \tag{4}$$

using the isotopic composition of the pollutant expressed as delta notation (δ_t). Commonly, the isotope composition is analysed from different monitoring wells along the plume and the initial value (δ_0) is usually assumed to be the groundwater well with the most negative value or located closer to the source.

Once an appropriate α or ε has been selected from the literature or determined through controlled microcosms studies, B can be calculated, and the corresponding residual substrate concentration (C_t) can be obtained by Eq. 3. For practical reasons, the highest concentration in the area of the source of contaminants is used as the initial concentration, C_0. The C_t is the expected concentration that should be present if biodegradation was the only process

leading to reduction of the pollutant concentration. Therefore, when C_t values are compared to the measured concentrations on the field site, the difference should give an estimate of the contribution of other processes such as dilution or sorption. If multiple plumes from different sources and, therefore, potentially different isotope ratios commingle at a site, the changes in isotope composition can be related to different sources and may not reflect biodegradation. In this case, hydrogeological knowledge is necessary to relate the plumes to sources with different isotope ratios. The correlation of concentration and the isotope data can be tested by a plotting $\ln\left(R_t/R_0\right)$ versus $\ln\left(C_t/C_0\right)$ according to Eq. 2. The slope and the quality of correlation of the regression curve may show whether biodegradation affects concentration on a water flow path between source and monitoring wells [13, 23, 24]. This may be further used to test whether other sources with varying isotope composition may be present at a field site and may prevent a quantitative assessment of biodegradation.

3.2 Sampling, Extraction and Isotope Measurement of Fuel Oxygenates

Groundwater samples for the isotope measurements of fuel oxygenates should be taken according to standard groundwater sampling practice (such as German Institute for Standardization, DIN, norm [25] or US Environmental Protection Agency, EPA, methods [26]) and sampling techniques for VOCs or fuel oxygenates in particular [27, 28]. No specific requirements are needed to take groundwater samples of fuel oxygenates for isotope analysis, which implies that monitoring of isotope composition as an indicator for in-situ degradation can be easily incorporated into groundwater monitoring strategies.

For the determination of the isotopic composition of individual components in mixtures of organic compounds, gas chromatography-isotope ratio mass spectrometry (GC-IRMS) has been developed into a mature analytical method over the last decade. Carbon isotope analyses are available in commercial laboratories, whereas measurements of the other elements amenable to CSIA (hydrogen, nitrogen, oxygen) are much less routine [10]. CSIA principles and technical aspects [29] as well as its application as a tool to monitor biodegradation in contaminated sites are summarised in recent reviews [9, 10]. The instrument consists of a GC system to separate mixtures of organic analytes, which is connected via an interface with an isotope mass spectrometer. For carbon and nitrogen isotope analysis, the analytes are oxidised to CO_2 or N_2, which are used to determine the isotope composition of the target compound [29–31]. For the determination of hydrogen and oxygen isotopes, the analytes are pyrolysed to single compounds such as H_2 or CO, which are used for determination [32, 33]. A good gas chromatographic separation of target compounds is an absolute requirement to determine a reliable isotope composition.

CSIA have in general a much lower sensitivity than common GC and GC-MS techniques used for concentration measurements, which has to be taken into account when planning extraction and isolation strategies to measure the isotope composition of fuel oxygenates. According to information given by suppliers of CSIA systems the standard error associated with the instrument (precision calculated by means of the reference gas) is about ±0.06‰ for CO_2 (^{13}C), ±0.06‰ for N_2 (^{15}N), ±0.50‰ for H_2 (^{2}H) and ±0.15‰ for CO (^{18}O) [34]. However, in most cases, reproducibility values for samples may be considered one order of magnitude higher for each element.

The relatively high concentration of analytes can limit the application of CSIA techniques for environmental applications where the values are generally low [10]. In these cases, sophisticated enrichment and isolation techniques for the analysis of fuel oxygenates may be required.

Several enrichment and injection techniques previously discussed for the analysis of MTBE [35, 36] as well as for other VOCs have been also coupled to CSIA systems.

The direct headspace (HS) technique has been used to determine VOCs in water samples. This method overcomes complications associated with the sample matrix and can be applied to a wide range of concentrations. HS requires little sample preparation. Salt is usually added to improve the partitioning into the gas phase and the sample is heated to temperatures of about 50–60 °C, which enhances the volatilisation of the analyte, increasing the efficiency of the extraction process and consequently the sensitivity. Typically, the HS is directly sampled with a μL-lock valve-gastight syringe and injected into the GC. This method has been used for the determination of the isotope composition of MTBE, ETBE and TAME, reporting detection limits of 3–6 mg L^{-1} for $\delta^{13}C$ and 8–20 mg L^{-1} for the $\delta^{2}H$ [37–40].

To improve detection of isotope composition in samples with concentrations $< 5\,mg\,L^{-1}$ other techniques have been employed. For example, headspace solid-phase microextraction (HS-SPME) has determined $\delta^{13}C$ [37, 41] and $\delta^{2}H$ [37] of MTBE in aqueous samples, reaching up to one order of magnitude lower detection limits (11 μg L^{-1}) for carbon mode [41]. For hydrogen isotope analysis, concentrations down to 1 mg L^{-1} have been measured [37]. The small isotopic fractionations caused by these extraction techniques were evaluated by Zwank et al. [42] and were negligible and highly reproducible.

Purge-and-trap (P&T) techniques are characterised by higher reproducibility and smaller isotopic fractionations than SPME and exhibit a higher sensitivity. The isotope composition of MTBEs were determined by the P&T method to as low as 0.63 μg L^{-1} as compared to the values reported previously by Smallwood et al. [16] (15 μg L^{-1}) and Kolhatkar et al. [23] (5 μg L^{-1}). Longer purge times (30 min) resulting generally in higher extraction efficiency (∼ 70%) and the use of a larger sample volume (25 mL) can enhance the mass of analytes subjected to the CSIA system.

P&T extraction techniques tend to show slight enrichments (+ 0.38‰) of $^{13}C/^{12}C$ ratios, but were reported to be reproducible with a shift of + 0.66‰ for carbon isotopes [16]. Recently, a commercially available P&T system has been evaluated for several VOCs, confirming its good reproducibility, high linearity and small isotopic fractionation. This technique was found to be very sensitive for field studies [43].

4 Microbiology of Fuel Oxygenate Degradation

Traditionally, microcosm studies have been used to demonstrate that microorganisms at a site can degrade a pollutant. Despite the fact that MTBE was classified in the 1990s as recalcitrant [1], during the last decade, laboratory degradation studies have demonstrated that fuel oxygenates are degradable under oxic and nearly all anoxic conditions by microbial and fungal communities [44–46]. However, strains using oxygenates as the sole source of carbon and energy are rarely found. Bacterial isolates capable of aerobic growth on MTBE belong to the β-proteobacterial phylum such as *Methylibium petroleiphilum* PM1 [47] *Hydrogenophaga flava* ENV 735 [48], *Variovorax paradoxus* CL-8 [49], strains UC1 and UC2 [50] and strain L108 [51]; as well as to the *Actinobacteria phylum* (gram-positive) *Mycobacterium austroafricanum* IFP 2012 [52] IFP 2015 [53] and UC3 [50]. The present state of knowledge about MTBE degradation pathways as well as the phylogeny of the microorganisms capable for MTBE degradation has been summarised recently by Ferreira et al. [54]. In addition, growth on other oxygenates such as ETBE or TAME has been also demonstrated with several strains capable of growing on MTBE (e.g. L108, IFP 2012 or IFP 2015). However, other strains such as *Rhodococcus ruber* (IFP 2001 and IFP 2007) able to grow on ETBE are not capable of growth on MTBE or TAME [55].

Anaerobic MTBE degradation and transformation has been demonstrated under methanogenic [38, 39, 56–59], sulfate-reducing [39, 57], denitrifying [60, 61], manganese(IV)-reducing [61], and iron(III)-reducing [61, 62] conditions. However, pure anaerobic strains have not been isolated and the degradation pathways under anaerobic conditions have not yet been described.

5 Laboratory Degradation Studies to Assess the Isotope Enrichment Factor

The present-day state as well as the history of isotope organic geochemistry has been extensively reviewed by Galimov [63]. Basically, the kinetic isotope fractionation of a substrate depends on rate-limitation due to the difference

in activation energy upon cleavage of chemical bonds substituted by isotope species. Due to a preferential transformation of the lighter isotope species, the heavy isotopes will accumulate in the non-reacted residual fraction. The mass-dependent stability of a chemical bond leads to higher reaction rates of light isotope species upon a (bio)chemical reaction and affects to a significant extent only the atoms involved in the cleavage reaction (primary isotope effect). Compared to primary isotope effects, changes in bonding are much smaller in the case of secondary isotope effects, where positions adjacent to the reacting bond are only slightly affected by the proximity to the reaction centre. However, particularly in the case of hydrogen, secondary isotope effects can, of course, not be neglected if primary effects are absent [64]. The reaction type governs the extent of isotope fractionation and may allow for determination of the biochemical reaction pathway [64, 65]. Multi-isotope analysis can be used to analyse which atoms are involved in the degradation reaction and, therefore, the simultaneous analysis of hydrogen, carbon and possibly oxygen may open perspectives for characterisation of the biochemical degradation reaction of fuel oxygenates. Since the degradation mechanisms depend on the geochemistry and reaction pathway, the variability of the isotope enrichment factor in controlled laboratory experiments must be analysed for the selection of the appropriate fractionation factor for quantification. Therefore, knowledge of the variability of isotope fractionation for distinct pathways is crucial.

Published MTBE carbon and hydrogen isotope enrichment factors detected under oxic [37, 40, 41] and anoxic conditions [23, 38, 39, 66] in microcosm experiments up to 2007 are summarised in Table 1. Isotope fractionation studies of other ether oxygenates are still very scarce. For example, TAME carbon fractionation has been studied under methanogenic conditions [38] and recently carbon and hydrogen fractionation of ETBE under oxic conditions has been reported [40].

5.1 Aerobic Biodegradation

Enrichment cultures from Borden aquifer (Ontario, Canada) showed a low variability in isotope fractionation of –1.52 to –1.97‰ upon aerobic biodegradation of MTBE, which is comparable to two enrichment cultures (–1.5 to –1.8) from Vandenberg Air Force Base (VAFB, CA, USA) [37, 41]. The strain *Methylibium petroleiphilum* PM1 and a close relative *Methylibium* sp. R8 gave a carbon enrichment factors of about –2.4‰ [37, 40]. The small variability, less than 1‰, suggested that carbon isotope analysis was appropriate for providing quantitative assessment of the extent of in-situ biodegradation and an average enrichment factor (ε) of –1.82‰ was used for this purpose [65]. Recently, strain L108 was isolated from a highly MTBE-contaminated site in Leuna, Germany [51] and exhibited a much lower enrichment factor (–0.48),

Table 1 Comparison of carbon and hydrogen isotopic enrichment factors (ε) with 95% confidence intervals (95% CI) for aerobic and anaerobic biodegradation of fuel oxygenates

Fuel oxygenate	Scale	Assumed redox conditions	Culture	εC_{bulk} [‰]	±95% CI [‰]	εH_{bulk} [‰]	±95% CI [‰]	Refs.
MTBE	Batch	Oxic	Enrichment culture (Borden aquifer)	– 1.52 to – 1.97	0.06	na		[41]
MTBE	Batch	Oxic	VAFB mixed consortium	– 1.5 to – 1.8	0.1	– 29 to – 66	3–4	[37]
MTBE	Batch	Oxic	Strain PM1	– 2.0 to – 2.4	0.1–0.3	– 33 to – 37	4–5	[37]
MTBE	Batch	Oxic	Strain L108	– 0.48	0.05	nd (– 0.2)	8	[40]
MTBE	Batch	Oxic	Strain IFP2001 (resting cells)	– 0.28	0.06	nd (+ 5)	17	[40]
MTBE	Batch	Oxic	Strain R8	– 2.4	0.1	– 42	4	[40]
MTBE	Batch	Methanogenic	Sediment enrichment culture (NJ)	– 9	5	na		[23]
MTBE	Field	Methanogenic	Field	– 8.1	0.85	na		[23]
MTBE	Batch	Methanogenic	Enrichment culture (Arthur Kill)	– 15 to – 16	4–5	na		[38]
MTBE	Batch & field	Methanogenic	Enrichment culture (NJ)	– 13	1	– 16	5	[66]
MTBE	Batch	Sulfate-reducing & methanogenic	Several enrichments cultures (Arthur Kill and Coronado Cays)	– 14.4	0.7	na		[39]
TBA	Batch	Oxic	Enrichment culture (Borden aquifer)	– 4.21	0.07	na		[41]
ETBE	Batch	Oxic	Strain L108	– 0.68	0.06	– 14	2	[40]
ETBE	Batch	Oxic	Strain L108 (resting cells)	– 0.8	0.1	– 11	3	[40]
ETBE	Batch	Oxic	Strain IFP2001	– 0.8	0.1	– 11	4	[40]
TAME	Batch	Methanogenic	Enrichment culture (Arthur Kill)	– 11 to – 14	3 to 5	na		[38]

na not analysed;
nd not significant enrichment detected

similar to strain IFP 2001 (–0.28). As the latter organism was not able to grow on MTBE, cometabolic degradation experiments with resting cells and glucose as a cosubstrate were performed to get the isotope fractionation [40].

Hydrogen isotope fractionation analysis using *Methylibium* sp. PM1 and R8 revealed enrichment factors for MTBE degradation between –33 and 42‰, respectively, which was in the order of previous studies (–29 to –66‰) using mixed consortia from VAFB [37]. Similar to carbon isotope fractionation of the β-*Proteobacterium* L108 (–0.48±0.05‰) and *Rhodococcus ruber* IFP 2001 (–0.28±0.06‰) the hydrogen isotope fractionation was negligible ($\varepsilon H \leq$ –0.2‰) if present at all [40].

The low fractionation factors indicate that MTBE may be degraded by different mechanisms under oxic conditions. The strains PM1 and R8 are thought to cleave the C – H bond in the initial rate-limiting biochemical reaction step via a monooxygenase reaction, leading to a significant carbon and hydrogen isotope fractionation. In contrast, the isotope pattern found for strains L108 and IFP 2001 suggest a reaction type similar to an acidic hydrolysis reaction (S_N1), which may cleave the C – O bond of the ether linkage as the kinetic reaction step for MTBE degradation. However, other hypothesis such as the presence of non-fractionating rate-limiting processes associated with the uptake of the substrate (e.g. transport limitation or diffusion through the microbial cell membrane) [14, 67], different monooxygenase reaction mechanisms or enzymes that have reached catalytic perfection [10] should not be totally ruled out.

The ETBE degradation by L108 and IFP 2001 was also found to be associated with a low carbon (–0.68 to –0.8) and slight hydrogen isotope fractionation (–11 to –14) [40]. In summary, more studies on aerobic degradation of fuel oxygenates are needed.

Only limited information is available on isotope fractionation of TBA, which is a by-product of MTBE manufacturing or occurs as a major main metabolite of MTBE degradation. The carbon isotope fractionation of TBA ($\varepsilon C = -4.21 \pm 0.07$‰) was studied in cometabolic aerobic microcosms [41], however, hydrogen isotope enrichment factors are not available yet.

5.2 Anaerobic Biodegradation

The isotope fractionation of fuel oxygenates have been studied with microcosms and enrichment cultures since anaerobic isolates are yet not available. The anaerobic degradation of fuel oxygenates is a relatively slow process and requires long experimental times. Therefore only a few studies exist, of which the majority concern the carbon isotope fractionation.

Anaerobic biodegradation of MTBE appeared to cause a consistent but substantially higher carbon isotopic enrichment (εC from –8.1 to –16‰), whereas hydrogen fractionation seemed to be less pronounced (εH = –16‰)

as compared to aerobic conditions. The relatively low hydrogen fractionation in comparison to carbon fractionation has led to the hypothesis that the first reaction may be an enzymatic hydrolysis of the O – C-methyl bond [66], which requires further evaluation.

In an initial study, Kolhatkar et al. [23] demonstrated carbon isotope fractionation (εC = –9‰) during anaerobic MTBE degradation in a laboratory microcosm with material obtained from a gasoline station site in Parsippany, NJ. Although the electron-accepting processes were not clearly identified, methanogenic conditions have been sometimes assumed [10]. Lately, Kuder et al. [66] monitored MTBE carbon and hydrogen isotope fractionation using enrichment cultures derived from the same microcosms, which exhibited a slightly higher fractionation factor (εC = –13‰) although not statistically different from the previous one.

Somsamak et al. [38, 39] performed studies on MTBE degradation by different anaerobic cultures enriched from sediments from two locations, the Arthur Kill, an intertidal strait between New Jersey and Staten Island, NY and the Coronado Cays, an estuarine site within the vicinity of the San Diego Bay, CA. Batch degradation studies were carried out under sulfate-reducing and methanogenic conditions and reported almost identical carbon enrichment factors of between –13.4 and –14.6‰. The similar magnitude of carbon isotope fractionation in all enrichments, regardless of culture or electron-accepting condition, suggests that the terminal electron-accepting process may not significantly affect carbon isotope fractionation during anaerobic MTBE degradation in these enrichment cultures [39]. The carbon isotope fractionation upon degradation of TAME under similar conditions was found to be slightly lower (–11 to –14‰) than for MTBE. Since there is limited information on carbon isotope fractionation of fuel oxygenates other than MTBE and only a very few studies exist on hydrogen isotope fractionation, more studies are needed for a more complete view of the anaerobic degradation pathways. In particular, studies concerning the isotope fractionation of ETBE under anaerobic conditions are needed. However, microcosm studies [57, 68] indicate that this compound may not be biodegradable under anoxic conditions.

6 Abiotic Isotope Effects

The CSIA concept relies on the presumption that only biodegradation significantly alters the isotope composition of contaminants in the aquifer. Other processes such as dilution, evaporation and sorption–desorption that are also involved in the attenuation of contaminants are considered not to affect their isotope composition to a significant extent. Smallwood et al. [17] found that equilibrium partitioning of MTBE from an organic phase (e.g. spilt gasoline)

to water did not lead to a significant shift in isotopic composition. Hunkeler et al. [41] reported slight carbon isotope enrichment of MTBE during partitioning processes such as organic phase/gas phase (0.50 ± 0.15‰), aqueous phase/gas phase (0.17 ± 0.05‰), and organic phase/aqueous phase (0.18 ± 0.24‰). These εC values were found small in comparison to carbon isotope fractionation measured during biodegradation of MTBE in most microcosms. However, these abiotic isotope effects were in the same order as εC found recently for strains L108 and IFP 2001 [40] and thus caution is needed when interpreting small isotope enrichment patterns.

Recently, Kopinke et al. [69] hypothesised sorption may lead to isotope fractionation due to transport processes in contaminated aquifers. Although the extent of carbon and hydrogen isotope fractionation of fuel oxygenates upon sorption has not yet been studied, it is unlikely that sorption processes can cause an isotope effect in stationary plumes. No evidence has been obtained from field experiments to show that sorption causes a significant carbon and hydrogen isotope fractionation for BTEX compounds in real contaminated aquifers [70, 71]. In addition, due to its poor physical and chemical adsorption characteristics, MTBE did not significantly sorb to humic acids or other hydrophobic surfaces in aquifers and therefore it is unlikely that isotope composition of MTBE and other fuel oxygenates can be significantly affected by multiple sorption steps [69].

Non-peer-reviewed publications are still available on the isotopic effects related to the two main abiotic pathways for ethers, oxidation and hydrolysis under acidic conditions. However, preliminary results on isotopic fractionation associated with the reaction of potassium permanganate ($KMnO_4$) with MTBE showed a carbon enrichment factor between –4.2 and –4.9‰ [18]. These εC values seem higher than the ones reported upon aerobic biodegradation of MTBE (from –0.28 to –2.4‰) suggesting potentially different reaction pathways and opening new lines of research and discussion for the future.

7
Two-Dimensional Isotope Analysis for Identification of Degradation Pathways

The combined use of hydrogen and carbon isotope analysis was proposed as a tool for characterising the pathway of biodegradation in the field [64, 65]. This hypothesis presumes that degradation pathways can be clearly distinguished by the characteristic isotope fingerprint left by the isotope fractionation pattern in the residual substrate fraction.

Kuder et al. [66, 72] first observed a strong correlation between δ^{13}C and δ^{2}H values during MTBE degradation by anaerobic enrichment cultures. The regression slope (1.3) corresponded approximately to the ratio of εH/εC

(–16/–13=1.2) and was also very different from the relationship that would be expected from aerobic biodegradation of MTBE (e.g. PM1 had an average slope of $\varepsilon H/\varepsilon C$= –34.75/-2.20=15.8). Aerobic biodegradation has been shown to cause a relatively small carbon isotopic fractionation but a strong shift in hydrogen isotopic signatures; whereas anaerobic biodegradation results in strong isotopic enrichment for both elements. This fractionation pattern can be used as a strong indicator for the existence of different reaction mechanisms [65].

The main advantages for such plots (δ^2H versus δ^{13}C) are that they are intuitively accessible (can be easily constructed even from field data) and avoid the influence of non-isotope fractionating rate-limiting processes or other dilution effects because both elements may be affected in the same way [64].

However, an update of the available field data for carbon and hydrogen isotope fractionation fall in the category of anaerobic as well as aerobic degradation and may demonstrate the potential of this approach (Fig. 1). Nevertheless, the current complications when using the two-dimensional isotope

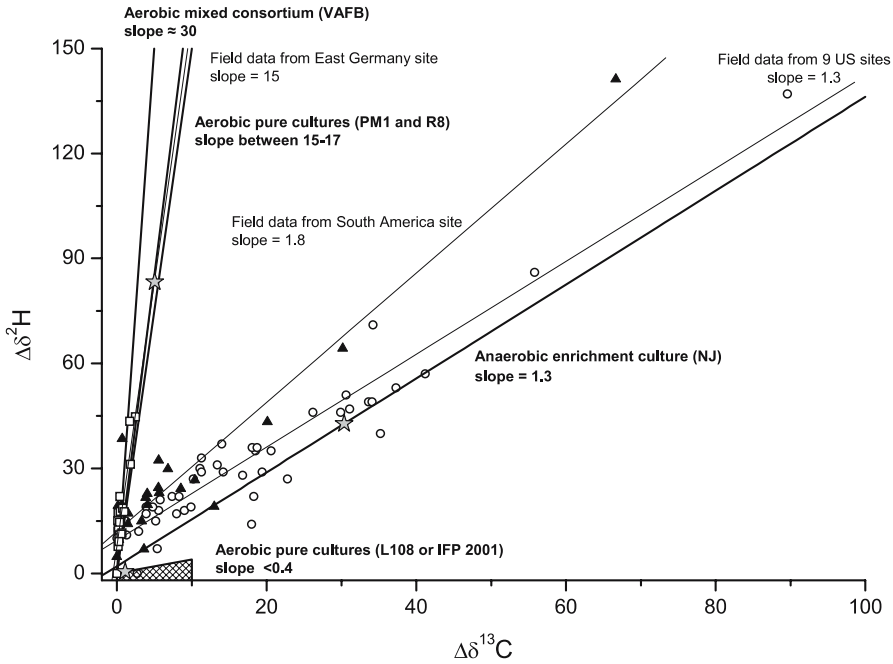

Fig. 1 Hydrogen versus carbon isotopic shifts for MTBE. The *solid lines* illustrate both aerobic and anaerobic MTBE laboratory degradation studies with pure strains or mixed cultures, to date. Three field data sets are plotted: *open circles* from nine different contaminated sites close to gasoline stations in the USA [66]; *solid triangles* from a former industrial landfill in South America [65]; and *open squares* from a refinery site in East Germany. The *grey stars* represent the 90% biodegradation point for each of the main MTBE fractionation patterns discovered so far

analysis to distinguish between anaerobic and aerobic MTBE degradation are also illustrated because the pattern observed for aerobic strains L108 and IFP 2001 is very similar to the pattern reported for anaerobic MTBE degradation. Due to this overlap, caution is needed for the interpretation of field data simply plotting hydrogen vs. carbon isotopes [40].

8 Experience from Field Sites

In field studies, a conventional approach for monitoring the microbial transformation of organic contaminants is to document the abundance of metabolites. However, the mere presence of the intermediate *tert*-butyl alcohol (TBA) has been considered inappropriate for providing evidence for natural attenuation of fuel oxygenates in many cases because it is a component of the original gasoline [73] or can originate from other industrial processes [74]. Another common indicator, the depletion of oxygen or other electron acceptors, as well as the production of methane, can be associated with the degradation of other gasoline components in general and not to MTBE in particular. Nevertheless, in a contaminated field site in Germany, TBA was found to be a useful intermediate for identifying MTBE degradation under possibly microaerophilic conditions [6]. In this case, the concentration of other pollutants at the fringes of the plume was very low and TBA accumulation was strongly correlated to MTBE degradation. However, the correlation between TBA and MTBE concentrations does not always provide direct proof of in-situ biodegradation.

Kolhatkar et al. [23] applied CSIA for the first time to characterise the MTBE degradation pathway in a retail gasoline station in Parsippany, NJ. The study followed two approaches: (i) the construction of anaerobic microcosms with the sediments of the contaminated aquifer and (ii) long-term monitoring data of the groundwater from several monitoring wells. In both systems the decrease of MTBE concentration was correlated with an increase of the $\delta^{13}C$ along the groundwater flow path in the field. Similar carbon enrichment factors, –8.1‰ in the field and –9.2‰ in the laboratory incubations, were reported to be higher than in previous aerobic studies. These results demonstrated that anaerobic biodegradation was the dominant natural attenuation mechanism for MTBE at this site. Additionally, negligible changes in $\delta^{13}C$ for TBA were observed in the field site, which did not correlate with the TBA concentrations. This fact was explained by the mixture of TBA present in the original gasoline as well as the TBA formation during MTBE biodegradation.

Later, Zwank et al. [65] proposed an average carbon isotopic enrichment factor for anaerobic biodegradation at –8.63‰, which served as indicator in opposition to the lower aerobic enrichment factor ($\varepsilon C = -1.82‰$). In their study, carbon as well as hydrogen isotopic analyses were applied to assess

the fate of MTBE and TBA in a groundwater plume at a former industrial landfill in South America where MTBE was disposed in open ponds [65]. MTBE isotope composition changed from the source regions along the major contaminant plume (–26.4‰ to + 40.0‰ for $\delta^{13}C$; –73.1‰ to + 60.3‰ for $\delta^{2}H$) indicating substantial biodegradation, whereas TBA isotope signatures remained constant suggesting the absence of TBA degradation. The proposed two-dimensional isotope analysis indicated anaerobic biodegradation of MTBE along the entire plume in a consistent way. The slope of the linear regression (1.8) was closer to the anaerobic isotopic pattern. The $\delta^{13}C$ and $\delta^{2}H$ values were strongly correlated ($R^2 = 0.91$). A similar result was obtained by Kuder et al. [66] when plotting field data from nine different contaminated sites close to gasoline stations in California and New Jersey. The changes in the $\delta^{13}C$ and $\delta^{2}H$ were comparable to those observed in anaerobic enrichment cultures (slope 1.3) and showed a strong correlation ($R^2 = 0.92$), even when coming from different locations. This may demonstrate that anaerobic biodegradation was the dominant process and that the aerobic degradation pathway was probably marginal. In fact, the use of the anaerobic carbon enrichment factor obtained by Kuder et al. [66] has been proposed as a conservative assumption for predicting the least extent of biodegradation of MTBE in field studies [19]. Once the biodegradation was calculated, Wilson et al. [19] evaluated the contribution of the TBA concentration in groundwater, which derived from the biodegradation of MTBE, and compared it with the TBA concentration measured at the site.

Preliminary results from a refinery site in East Germany show that sometimes aerobic MTBE degradation processes may be dominant. Although the extent of the biodegradation seems to be lower, the correlation of the isotopic composition was reasonable ($R^2 = 0.8$). The slope of the linear regression is similar to the aerobic isotopic pattern observed in degradation experiments with strains PM1 or R8. This fact may indicate that even when the aquifer is considered mainly anoxic, the microaerophilic conditions in the groundwater table can dominate the MTBE biodegradation, as observed in other contaminated field sites such as Leuna [6].

9 Uncertainty Related to the Quantification of In-Situ Biodegradation in Contaminated Field Sites

Although biodegradation of pollutants in contaminated aquifers has been demonstrated by CSIA, it is questioned whether the concept can be practically used to quantify microbial decomposition. The Rayleigh equation was originally developed for homogeneous systems [21] while in the subsurface, contaminants can migrate at different velocities due to physical heterogeneity. A recent analytical modelling approach [75] revealed a systematic under-

estimation of in-situ biodegradation when applying the Rayleigh equation for quantification purposes. However, this error was found to be a relatively small (< 5% underestimation). Therefore, the quantification of biodegradation by the CSIA approach can also give reliable results in aquifers with heterogeneities such as various longitudinal dispersion effects, degrees of biodegradation, plume geometries, and travel times. In addition, this was demonstrated in a multi-tracer test at a field site [71], showing the applicability of the CSIA concept for monitoring the biodegradation of BTEX compounds using three different independent methods.

To this end, for a proper assessment of biodegradation by the CSIA method, one important issue is to choose the right laboratory-derived α (or ε) for the initial step of biodegradation that is dominant in the investigated aquifer [9]. This is not such a simple task because the geochemical conditions are not always so well known and several electron-accepting processes can occur concomitantly in aquifer material. MTBE carbon enrichment values (εC) of mixed cultures were lower than those of pure cultures. On one hand this shows the variability. On the other hand, if organisms are present in a mixed culture that fractionates the substrate to a different extent (as shown in last experiments with several pure aerobic strains) the average isotope enrichment factor might be lower. This makes the selection of an appropriate ε for quantitative work difficult and can lead to strong variable fractionation in mixed cultures depending on the dominance of the organism actually

Table 2 Expected carbon and hydrogen isotopic shifts for increasing extents of biodegradation calculated by a modified Rayleigh equation (Eq. 3) and the different MTBE degradation patterns reported so far in the literature (carbon and hydrogen enrichment factors, εC and εH, respectively)

Extent of biodegradation	Anaerobic enrichment cultures [66]		Aerobic pure strain PM1 [37]		Aerobic pure strain L108 [40]	
	εC = – 13	εH = – 16	εC = – 2.2	εH = – 34.75	εC = – 0.48	εH= – 0.2
B [%]	$\Delta\delta^{13}$C [‰]	$\Delta\delta^{2}$H [‰]	$\Delta\delta^{13}$C [‰]	$\Delta\delta^{2}$H [‰]	$\Delta\delta^{13}$C [‰]	$\Delta\delta^{2}$H [‰]
1	0.1	0.2	0.02	0.3	0.005	0.002
10	1	2	0.2	4	0.1	0.02
20	3	4	0.5	8	0.1	0.04
30	5	6	0.8	12	0.2	0.1
40	7	8	1	18	0.2	0.1
50	9	11	2	24	0.3	0.1
75	18	22	3	49	0.7	0.3
90	30	38	5	83	1	0.5
99	62	76	10	174	2	0.9
99.9	94	117	15	271	3	1

growing best. Therefore, isotope enrichment factors from uncharacterised microcosm studies should always be used with caution and may not be representative for isotope fractionation in the field. Indeed, in a case study, fractionation may vary by more than an order of magnitude depending on the bacteria responsible for degradation (see Table 2). In addition, at field scale, the extent of biodegradation is also influenced by the relative contribution of oxic and anoxic conditions along the flow path. The use of two-dimensional isotope analysis can help to some extent in selecting a ε for a quantitative assessment of MTBE biodegradation, but it is not definitive. At least, selection of a larger ε in the case of aerobic biodegradation (e.g. $\varepsilon C = -1.82‰$ as suggested by Zwank et al. [65]) will give a conservative estimate and not result in an overestimation of in-situ biodegradation.

10 Future Needs

Investigation of the indigenous microbial consortium in the aquifer and the identification of the organisms by molecular biological methods in the environment might help to improve the selection of appropriate isotope enrichment factors suitable for the assessment of in-situ degradation at sites contaminated by fuel oxygenates.

To date, anaerobic MTBE-degrading strains have not been isolated and the mechanisms of anaerobic MTBE degradation have not yet been elucidated. Moreover, only a limited number of enrichment cultures have been used to study isotope fractionation and, in particular, data to evaluate the variability in isotope fractionation under anoxic conditions are missing. The isotope enrichment factors for more microbial strains as well as for electron-accepting conditions, in particular for other fuel oxygenates, is needed.

In addition to the measurement of the carbon and hydrogen isotopic compositions, compound-specific determination of $\delta^{18}O$ values has been proposed to be useful for further elucidation of degradation mechanisms of ethers and alcohols. Oxygen isotope fractionation may allow the unravelling of the degradation mechanism if the carbon–oxygen bond is affected in the rate-determining step of the degradation reaction [46].

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Spreading of MTBE and Chlorinated Hydrocarbons in Groundwater: Comparison of Groundwater Transport and Plume Dimensions

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Abstract Based on a physical-chemical-biological database, the behavior of MTBE and CAH (chlorinated aliphatic hydrocarbons) in the subsoil is described and compared. In contrast to MTBE, CAH can form independent phase bodies that can infiltrate deep into aquifers. Due to its striking higher solubility, MTBE spreads much faster in groundwater. The longest CAH plume recorded in literature so far amounts to 10 000 km. The longest reported MTBE plume reaches 1900 m. Interpreting the available worldwide data, spreading of MTBE groundwater contaminations leads plume lengths that fall rather into the category of the BTEX as into the class of CAH. A substantial reason for comparison with the lower CAH plume expansions might consist of the fact that MTBE plumes—due to high water solubility and thereby the connected fast development of the MTBE source transfer—progress comparatively fast into the stable and/or regressive status of the plume development. Beyond this, MTBE infiltrates as subordinated portion of gasolines (predominantly 1–3 wt % in regular grade fuel and/or premium fuel), in comparatively low quantities into the subsoil, so that these comparatively low quantities do not possess large source strengths over longer periods. Only spills with very large gasoline quantities may longer MTBE plumes develop under certain conditions.

Keywords MTBE · TBA · Plume length · Spreading velocity · Retardation

Abbreviations

LNAPL	Light non-aqueous phase liquids
BTEX	Benzene, toluene, ethylbenzene, xylene
CAH	Chlorinated aliphatic hydrocarbons

cis-DCE	*cis*-1.2-dichloroethene
1.2-DCA	1.2-dichloroethane
DNAPL	Dense non aqueous phase liquids
ETBE	Ethyl-*tertiary*-butyl-ether
hpa	Hecto pascal
MTBE	Methyl-*tertiary*-butyl-ether
PCE	Tetrachloroethene
TCA	1.1.1- trichloroethane
TCE	Trichloroethene
VC	Vinylchloride
TBA	*tertiary*-butyl-alcohol
vol%	Volume percent
wt%	Weight percent

1 Introduction

Since January 1, 2005, actualized guidelines concerning the oxygen contents in gasolines apply in Europe. Fuel oxygenates are oxygen-containing compounds that are added to automobile fuels in order to increase the anti-knock property and to improve burning behavior. As possible oxygenate compounds, alcohols and ethers come into question. The importance of these gasoline additives has increased strongly across Europe in the last years. Since the European Union decided to lower the aromatic contents to less than 35% as of January 1, 2005, this led to an increased use of oxygenates to guarantee fuel quality. Beyond this, the portion of oxygenates in gasolines in respect to directive 2003/30/EC (Bio Fuel Directive) will increase in the next years. Following this Europe-wide stipulation, the portion of bio components in fuels must amount to at least a weight percentage of 5.75 as from January 1, 2010.

In the past, the most important gasoline additive used in Germany was MTBE (methyl-*tertiary*-butyl-ether). Due to the Bio Fuel Directive, the share in bio-constituents is to be increased, therefore MTBE will only be used in larger quantities in gasolines until 2006 and after that will be replaced by ETBE (ethyl-*tertiary*-butyl-ether). This conversion is connected with the fact that ETBE, contrary to MTBE, can be produced more easily from bio components by means of ethanol as an intermediate product.

In the last years in North America, extensive MTBE groundwater contamination became known. The extent of this contamination in the US has led to the prohibition of MTBE as a gasoline component in numerous US states and its partial replacement by ethanol[1]. In the last 5 years, an increasing number of MTBE groundwater contaminations have also been detected in

[1] Also in Germany, in the meantime, there are discussions as to whether ethanol should also be used in the future in addition to ETBE.

Germany. MTBE possesses a high potential to form long plumes. Inspecting MTBE groundwater contamination *tertiary*-butyl-ether (TBA) has to be considered as an important metabolite of the MTBE degradation. Beyond this, TBA is an impurity in MTBE and was formerly blended to gasoline to improve solution properties of gasoline components.

In Germany, most groundwater contaminations are caused by chlorinated hydrocarbons[2] [1]. This is proven among other things by the fact that the majority of groundwater remediations demanded by state authorities concerns CAH. Of special importance among the CAH contaminations are the compounds PCE (tetrachloroethene), trichlorethene (TCE), 1.1.1-trichloroethane (1.1.1-Tri) and 1.2-dichloroethane (1.2-DCA). Beyond this, the apparent metabolites from TCE resulting from microbiological decay[3] in the order *cis*-1.2-dichloroethene (*cis*-DCE) and vinyl chloride (VC) display mentionable groundwater hazards. CAH were often used in the past as degreasing, solvent and detergent materials.

From the stated reasons, it is of interest to compare the spreading behavior of the historically most important oxygenate MTBE and of CAH. This evaluation will supply important information about probable transport mechanisms of MTBE in the groundwater and affect thus the risk management of MTBE groundwater contamination.

2 Chemical Physical Data and Biodegradation

To which degree harmful groundwater compounds can lead to an endangerment of the groundwater is to be justified primarily by their spreading potential. The spreading of the contaminants in groundwater is based on their chemical-physical-biological properties. These are submitted below from a comparative view.

MTBE and TBA with densities of $< 1\ g/cm^3$ belong to the so called LNAPL (light non aqueous phase liquids) whereas CAH with densities of $> 1\ g/cm^3$ belong to the DNAPL (dense non-aqueous phase liquids). While DNAPL (heavy phase) tend to migrate as an independent phase in greater depths, LNAPL (light phase) float on the groundwater table.

The most remarkable characteristic of MTBE and TBA is their high water solubility (Fig. 1). The water solubility of MTBE amounts to approx. $50\ g\,l^{-1}$ and TBA is totally mixable with water. In comparison, the solubilities of CAH are very much lower. PCE, with $160\ mg\,l^{-1}$, has a water solubility approx. 310 times lower than MTBE. CAH dechlorination tends to result in an increas-

[2] The term CAH is used in the following as a synonym for "chlorinated aliphatic hydrocarbons", e.g., tetrachloroethene, trichlorethene, trichloroethane

[3] TCE is also the first decay product of PCE

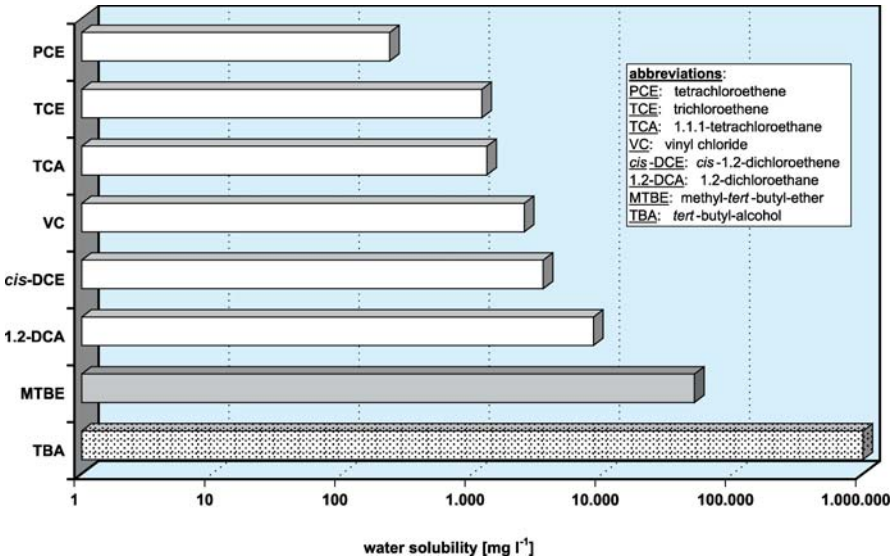

Fig. 1 Solubility of MTBE and TBA in comparison to chlorinated ethenes; data for water temperatures of 20 °C

ing water solubility. The best water-soluble CAH is 1.2-DCA with approx. 8.500 mg l^{-1}. Altogether it is important that the water solubilities of MTBE and TBA exceed the scale of the CAH.

The log K_{OC} value determines the distribution of a substance between water and soil, mainly dependent on the organic carbon content of the soil. A low K_{OC} value means that little absorption at the soil particles takes place. The K_{OC} values of MTBE and TBA are very low according to the polarity of these compounds and clearly lie below 4 mg l^{-1}. CAH possess a clearly higher tendency for adsorption to organic substances with values between 38 ml g^{-1} (1.2-DCA) and 300 ml g^{-1} (PCE).

The Henry constant determines the distribution of a substance between air and the water phase. The lower the value, the more the substance tends to accumulate in the water phase. The Henry constants of MTBE and TBA constitute 0.0017 Pa m^3/mol and 0.00038 Pa m^3/mol, respectively. On the other hand, the CAH with readings of 0.17 (*cis*-DCE) and 1.16 (VC) exhibit clearly higher values and are consequently well desorbable. Only 1.2-DCA possesses a clearly smaller value with 0.049 (Fig. 2). An important conclusion from this is that MTBE and even more extremely TBA are not effectively desorbable and thus not strippable in water treatment plants.

The vapor pressure describes the inclination of a compound to convert from its liquid phase into the gas phase. VC possesses with approx. 3500 hPa by far the highest vapor pressure of all considered substances. The vapor pressure of MTBE with approx. 330 hPa is roughly 3.5 higher than that of benzene.

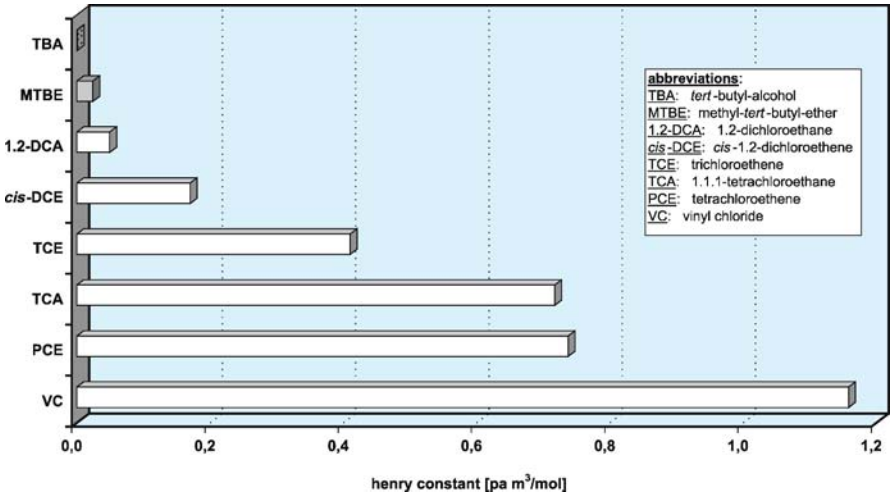

Fig. 2 Henry constants of MTBE and TBA in comparison with chlorinated ethenes; data for temperatures of 20 °C

Noticeable vapor pressures show *cis*-DCE, 1.1.1-TCA, TCE and 1.2-DCA. The least values exist for TBA and PCE (Fig. 3).

The biodegradation of CAH is strongly dependent on the environmental milieu characteristics of the aquifers. An important realization consists of the fact that the transformation from PCE to TCE only takes place under anaerobic conditions. On the other hand, the further disintegration of chlorethene

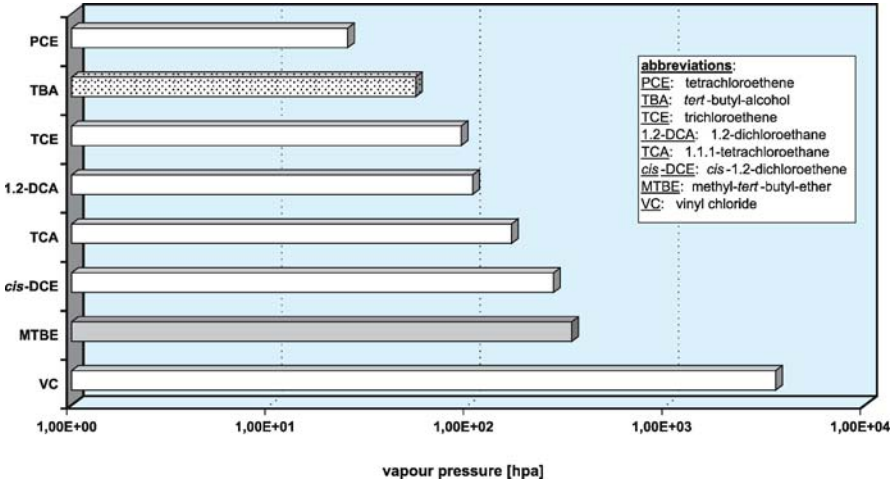

Fig. 3 Vapor pressure of MTBE and TBA in comparison with chlorinated ethenes; data for temperatures of 20 °C

from TCE on, is possible under anaerobic and aerobe conditions. The pollutants can directly be used as carbon or energy source of microorganisms, or, which is of greater practical importance, they are transformed cometabolic by enzymes, which are produced by bacteria consuming cosubstrates (organic substances like oil components, organic acids, lactates) [2].

MTBE is under natural conditions hardly degradable and is classified as persistent by the EPA. The increasing work with MTBE in the last few years has shown, however, that the MTBE degradation under special conditions can function nevertheless, since comprehensive literature on that topic has been made available. The present level of knowledge was recently described in a publication of Püttmann and Koenen [3].

Concluding overall statements on the decay of MTBE are not possible. Whether natural decay is possible depends on the respective site and in particular on the environmental conditions. Due to field observations at different MTBE groundwater plumes, it can be stated that an efficient decay of MTBE through pure natural processes can usually not be observed. The presence of other gasoline compounds, for example BTEX, seems to limit the MTBE decay at least so far as long as the BTEX single substances are diminished. In most cases, however, distinct TBA values—most frequently with concentrations of 10–50% of the MTBE concentrations—can be observed.

3 Transport in Groundwater

The spreading of contaminants in groundwater is a complex process and steered by a number of different factors. The substantial parameters that determine the spreading behavior are arranged in Table 1.

Comparing the spreading behavior of CAH and MTBE is to be considered that by the spill of the CAH into the subsoil usually a pure phase product of PCE, TCE or 1.1.1-TCA is infiltrated. Most CAH contaminations were released by degreasing plants in the metal manufacturing industry. Due to their noticeable vapor pressure, CAH develop a gas phase body in the unsaturated zone, which is characterized by high CAH concentrations in soil air.

In contrast to this, spills of pure MTBE are restricted to sites of the producing industry and by far in most spills MTBE is a component of gasolines with the consequence that it is a complex gasoline phase that infiltrates the subsoil. Due to its high vapor pressure, a first differention in terms of gas phase body having a high MTBE concentration can be built up in the unsaturated zone, however, it may also contain the other light volatile gasoline compounds (e.g., benzene). Although the vapor pressure of TBA is clearly lower than that of MTBE, TBA can also appear as a subordinated component in the gas phase body since it can also be a primary component in gasolines. According to their high solubilities, MTBE and TBA are preferentially solved in water.

Table 1 Influence parameters on the migration behavior of organic compounds in groundwater [4]

General parameters	Aquifer parameter	Material parameter
Amount	Permeability	Water solubility
Source strength	Flow speed	Sorption
Age	Advection	Retardation
Migration time	Vertical flow paths[a]	Diffusion
Co-contaminants	Dispersion	Microbiological decay/metabolism
	Dilution	Gas transfer in unsaturated zone
	Environment[b]	
	Chemical composition	
	Sorbents[c]	
	Microorganisms	
	Nutrient offer	

[a] If necessary, the influence of existing vertical flow paths is to be estimated, since otherwise incorrect evaluations can occur about the plume lengths [4]
[b] All general environment data such as temperature, Eh, pH etc.
[c] All relevant sorbents such as organic material, clay minerals

Therefore a second differentiation from the gasoline phase takes place in the seepage water, in which the concentrations at TBA and MTBE are a far higher than those of the remaining gasoline components.

As soon as the gasoline product reaches the groundwater a third differentiation develops in that way that the most soluble gas components—at first MTBE—are preferentially solved in groundwater and transported along the flow path. Since gasolines are LNAPL in contrast to CAH, they do not infiltrate deep into the aquifer. If TBA is present as a primary gas compound, the spreading behavior of TBA is steered by its unlimited water solubility. The very low Henry constants of MTBE and TBA lead to a preferred transfer into the water, where a significant concentration reduction from degassing into the unsaturated zone is not to be expected.

CAH reveal a completely different behavior in the groundwater. They can penetrate deeply into the aquifer and vertically spreading phase bodies can develop. From the study of numerous groundwater contaminations can be derived that CAH as DNAPL differ considerably from the LNAPL such as gasolines by the fact that they develop substantially larger source strength with the same entry quantities. This high source strength is a consequence of the penetration of CAH in different distribution forms into the saturated zone. Those CAH phase bodies usually reach some meters into the aquifer. They exhibit an extremely large surface, which offers the flowing groundwater ideal conditions for solution processes and loading with these substances in a solved form. As a consequence long CAH contaminant plumes can develop.

Since CAH are characterized by very much lower water solubilities in contrast to MTBE CAH remain existent as an own phase in the aquifer over very long periods, in extreme cases decades to centuries. The high Henry constants of CAH principally promote a transfer from the groundwater into the seepage zone. Concentration reduction can take place due to degassing into the unsaturated zone, a process which is supported by the high vapor pressures of most CAH.

The solution process of a mixed phase like gasoline is determined by Raoult's law, which states that the individual gasoline components go into solution according to their mole fractions in the product, so that the theoretical maximum solubilities of the single compounds cannot be achieved.

In Table 2 the concentrations of different compounds of gasolines depending on their volume share—based on Raoult's law—are arranged and it is calculated which groundwater concentrations can occur at maximum.

As seen from Table 2 even lower MTBE concentrations in gasolines can clearly generate higher MTBE concentrations in groundwater compared to BTEX. Due to the perfect mixing ability with water, the derivation of higher concentrations of TBA in groundwater is more strongly pronounced. Already low MTBE or TBA contents in products are sufficient in order to cause high concentrations in groundwater. It is even reported that if jet fuel is spilled, high MTBE concentrations in groundwater can occur. By such a jet fuel spill MTBE concentrations of about 609 μg/l were determined in groundwater, although the MTBE concentration in the nozzle fuel amounted to only 0.02% [5]. Thus despite the occurrence as a subordinated component of gasolines, MTBE can appear in groundwater in higher concentrations than the CAH PCE and TCE exhibiting solubilities of 160 and 1200 mg/l.

The relationship between the groundwater flow velocity and the transport speed of the compound is described by the retardation factor R. A compound which is not held back at all, moves with the same speed as water and there-

Table 2 Maximum MTBE and BTEX equilibrium concentrations in water in contact with gasoline (modelled on Weaver et al. (1999) [5])

Material	Water solubility (mg/l)	Volume %/ gasoline types	Maximum concentration (mg/l)
MTBE	50 000	11/Super Plus	5500
MTBE	50 000	2/Super	1000
TBA	1 000 000	0.1	1000
Benzol	1780	1/Super	18
Toluol	535	13/Super	70
Ethylbenzol	161	2/Super	3
o-Xylol	175	11/Super	19

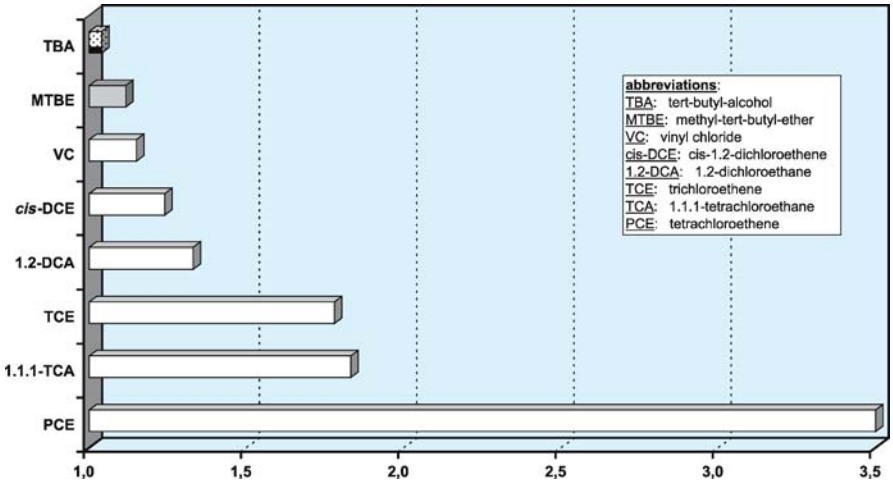

Fig. 4 Retardation values of organic compounds

fore possesses the value R = 1. A substance, which is transported with half of the speed of groundwater, possesses the value R=2. MTBE and TBA possess retardation factors around 1 at carbon contents around 0.01%, so that both substances are transported in the groundwater without delay and exhibit behaviors like ideal tracers. Higher carbon contents change these transport behaviors only insignificantly. On the other hand, the retardation factors for CAH are clearly higher with values between approx. 2 and 15 (Fig. 4). The consequence is that CAH are transported clearly more slowly than MTBE and TBA in the groundwater. For example, the retardation factors amount for *cis*-DCE to approx. 2, for 1.2-DCA to approx. 4 and for PCE and 1.1.1-TCA to approx. 14. An exception forms VC with similar retardation factors (around 1) like both oxygenates.

4
Plume Studies

4.1
Plume Types

With spills of MTBE containing gasoline, three plume types depending on the time of the spill event can be differentiated in groundwater (Fig. 5):

Type 1: Young gasoline spill: MTBE plume spreading corresponds to that of the BTEX

Type 2: Middle aged gasoline spill: MTBE spreading towards groundwater flow further advanced as that of the BTEX

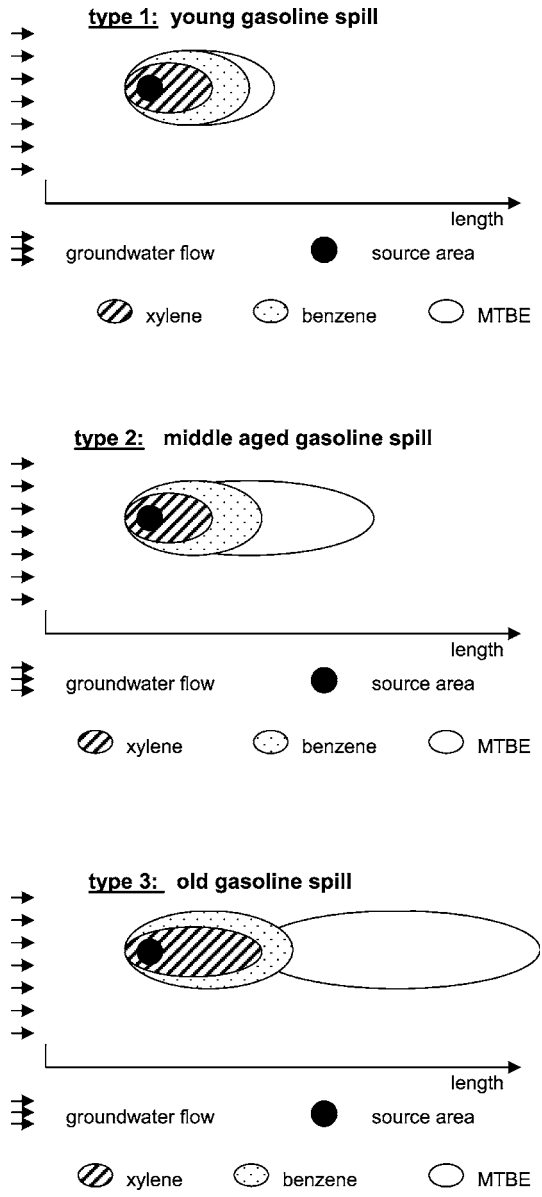

Fig. 5 Types of MTBE plumes

Type 3: Old gasoline spill: MTBE plume "torn off" from the source area and MTBE downstream spreading from the source whereas the BTEX plume is developed further upstream

The TBA behavior can be described as equivalent to the MTBE spreading. Since the abiotic features, like sorption, evaporation and chemical decay are

of subordinated importance, only biodegradation can be applicable as limiting factor for the spreading behavior of MTBE and TBA.

With CAH, groundwater contamination the dissolved CAH can spread horizontally over long distances without changing the vertical position, since the CAH solution possesses the same density as water. Depending on whether at the source of CAH contaminations CAH phase is present, two different types of CAH plumes develop (Fig. 6):

Type 1: CAH phases are present in the source area in the saturated zone, high CAH concentrations are measured in direct vicinity of the source area.

Type 2: The source of CAH is exhausted, the maximum concentrations are found downstream of the source area.

Irrespective of these two plume types—due to biological processes—the CAH plumes are often structured into different zones of metabolites. In the case of PCE as the original substance, a plume differentiation into plume sections develops, in which typical substances—such as PCE-TCE-*cis*-DCE and VC—predominate.

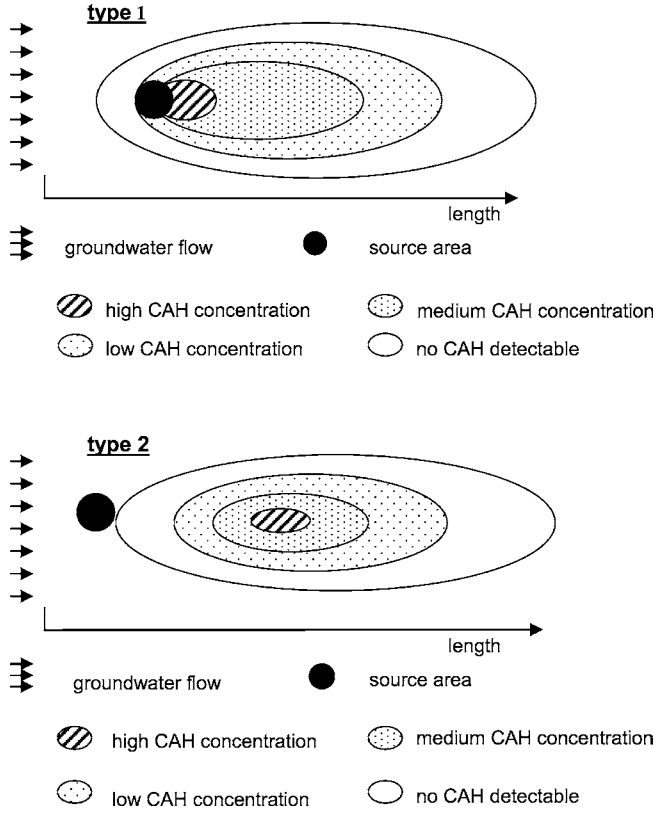

Fig. 6 Types of CAH plumes

4.2 Plume Dimensions

For the data evaluation upon the dimensions of CAH and MTBE groundwater plumes a literature study was carried out. On the basis of the evaluation of 40 CAH plumes in Germany, average plume lengths of approx. 1080 m and maximum of 8200 m were determined [4]. The longest CAH plume found in the literature exhibits a length of 10 000 m (CAH plume in Germany [6]).

From Fig. 7 it can be seen that the majority of the examined CAH plumes possess a length of less than 500 m. It is noticeable however that the very long plumes show a high portion of PCE (60–98%), what regarding the high retardation factor and the low water solubility of PCE was not inevitably to be expected. Site-specific views lead to the result that these long CAH plumes are characterized by the following specific features:

- large spills with registered spill amounts of usually more than 10 000 kg
- high permeability (k-values $> 1 \times 10^{-3}$ m/s) and partially steeper groundwater gradients (approx. > 0.001). The groundwater flow velocities are high;
- extensive oxidizing conditions in the downstream section of the plume and no and/or subordinated formation of degradation products of CAH;
- low concentrations of organic material in sediments and thus relatively "sterile" aquifers without high capacities for the adsorption of substances contained in water.

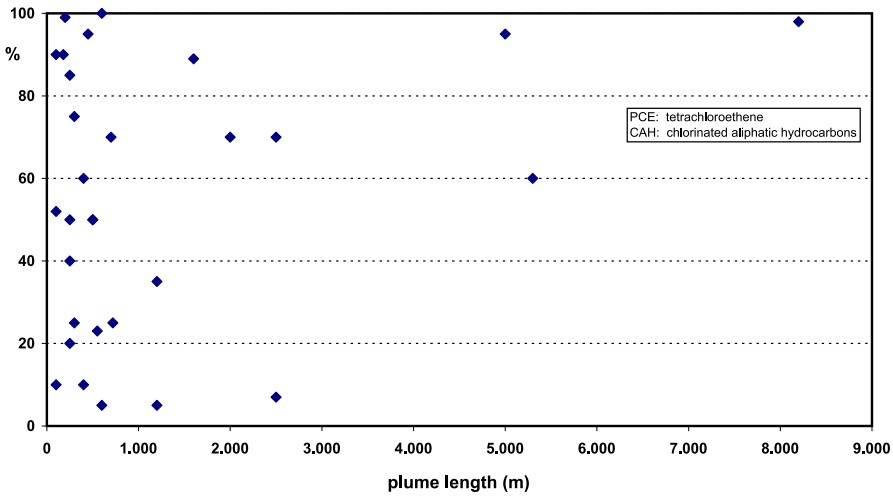

Fig. 7 PCE amount in % of CAH-Sum versus plume length

These observations are supported by statistic investigations from the US [6]. Here it was ascertained that the presence of VC and with that the dechlorination tends to result in the formation of shorter CAH plumes [7].

Compared to the CAH contaminations, not much is known so far about the dimensions of MTBE plumes in Germany. By far more extensive data are available from the US. The longest MTBE plume described in literature in the US so far has a length of approx. 1800 m [8].

The results of different studies accomplished in the US states of California, Florida, Texas and Arizona can be summarized as follows:

- MTBE plumes are similar in length to BTEX plumes. MTBE plumes reach rarely lengths of over 75 m [9]. The relative short MTBE plumes may be due to the fact that CAH have been used substantially longer than MTBE.
- MTBE plumes are predominantly stable and show a similar plume length as BTEX plumes [10]. Plumes with lengths of over 150 m and MTBE concentrations of > 100 μg/l in the more downstream plume sections are very rare [11].
- The average lengths of MTBE plumes lie at approx. 50 m and the majority of the MTBE plumes points to a regressive status [12]. The plume lengths fall below approx. 90 m.
- There are however also exceptions of the previously described plume lengths with clearly larger ranges. In 16 US states, MTBE plumes with lengths between 330 and 1600 m were determined [13].

The results for this data evaluation are not easily transferable to Central Europe. For example, the spreading of longer MTBE plumes in Florida is limited by climatic conditions (subtropical climate), flat ground-water levels, low groundwater gradients, and high rates of groundwater formation. These features, which strongly influence the plume dimensions, are regionally very specific and not representative for situations in Central Europe.

As a result of the literature study, the longest known MTBE plume in Germany so far is that at the site LEUNA with a length of approx. 1900 m [14]. Amongst the eight MTBE projects under work by the author at present, the maximum MTBE plume length amounts to 1200 m. As the microbiological decay of MTBE in most cases does not lead to a strong decrease of MTBE concentrations in all these plumes, it is probable that with the appropriate source strengths and high flow velocities longer MTBE plumes can also develop. It should be considered that plume length principally depends strongly on the time the detection of the MTBE plume took place; the later the plume is determined, the further the plume has moved from the source area.

Apart from the spreading of MTBE, the spreading of TBA is of interest. Field experiences show that near the source zones no or only an insignificant amount of decay of TBA takes place. This points to the fact that TBA under the here existing reducing conditions is, to a large extent, persistent.

According to Kolhatkar, TBA plumes in 75% of all cases are shorter than MTBE plumes, which suggests biological decay in further downstream sections under aerobic conditions [12]. There can sometimes be overlays of TBA decay and TBA formation and such interpretations are to be seen however with caution, since TBA was also added to gasolines in former times[4]. According to fuel-quality guidelines, up to 7 volume percentage TBA may be contained in gasolines.

5 Conclusions

In summary, the following differences in respect to the spreading behavior between CAH and MTBE could be noted:

- CAH are DNAPL and can penetrate deeply into the aquifer.
- MTBE and TBA are LNAPL and float on the groundwater only in cases of very large spill amounts. Both substances as independent phases do not deeply infiltrate into the aquifer.
- MTBE and TBA are much more water soluble than CAH and temporarily form independent phases on the groundwater surface in cases of very large spills.
- MTBE and TBA are transported in groundwater much faster than the other gasoline compounds. Both become hardly retarded and exhibit a very mobile behavior in groundwater.
- CAH are far less mobile; the retardation affects the spreading behavior of the different CAH.
- The most important CAH compounds (PCE and TCE) can be better diminished anaerobically, starting with the metabolite *cis*-DCE the aerobe decay is more effective.
- If possible at all under the respective site conditions, MTBE and TBA are better diminished aerobically than anaerobically.
- The CAH decay ends frequently with *cis*-DCE, a MTBE degradation often cannot be recognized in the field.
- Under specific site conditions, degradation products can accumulate, with the CAH *cis*-DCE and VC, with MTBE TBA.
- MTBE and TBA spread in the groundwater without limitations and are approximately just as fast transported as groundwater. In most cases, natural biological decay is subordinate.

The spreading of CAH is affected by more factors than for MTBE. To these parameters belong the infiltrations of striking phase bodies into larger aquifer depths, often associated with larger source strengths and persistence, the stronger adsorption and the delayed substance transfer (stronger retardation)

[4] At present, TBA concentrations are usually low in German gasolines (< 0.2%).

as well as site dependent stronger biological decay. On the other hand, MTBE spreading is affected almost exclusively by biological degradation and dilution effects.

As a consequence of the deeply infiltrated phase bodies, high CAH concentrations appear close to the source and in the downstream plume section. Pronounced CAH zoning in the way that higher CAH values arise in the upper groundwater zone is relatively rare. In contrast, it is often stated that within thicker aquifers, a pronounced zoning with higher MTBE contents in the upper groundwater levels is present.

During the evaluation of data from approx. 750 CAH groundwater contamination plumes it was found that approximately 75% of all considered CAH groundwater contaminations in principle did not develop longer contaminant plumes [4]. The CAH sources in these cases were predominately found on industrial sites, which lay very frequently at or in direct proximity to rivers. A hydraulic connection between rivers and groundwater usually exists, which makes a formation of CAH plumes not possible in these cases. Regarding potential MTBE sources, the portion of source areas which lie in the vicinity of rivers should be by far lower, since this situation for gas stations is of far less importance than for industrial sites. A consequence of this is that due to the location differences, a comparatively higher number of longer MTBE plumes could exist.

As described, the transport of MTBE compared to CAH is steered by the flow conditions themselves. The tracer behavior is expressed by the fact that the flow conditions considerably determine the MTBE spreading. Therefore, for an adequate treatment of MTBE groundwater contaminations, the flow conditions must be known as well as possible. It has to be noted that vertical flow paths within an aquifer or between aquifer systems are often underestimated. For example, contamination can dive by "windows" between aquifer systems into deeper groundwater systems and there continue further spreading. Figures 8a–d illustrate how MTBE transport follows the respective flow conditions. If such transport mechanisms are not recognized in field investigations, this can lead to a wrong conclusion about the apparent limitation of plumes in the aquifer.

Furthermore, field observations show that MTBE groundwater plumes become broader than comparable CAH plumes with varying groundwater flow directions, which can be explained by the fact that due to the high transport velocity of MTBE, the plumes are spread faster with changing flow directions. What at first sight appears to be a strong lateral dispersion, is in fact a consequence of longitudinal MTBE transport under different flow conditions.

With reference to the presented theoretical views it is to be assumed that MTBE possesses the potential for the formation of longer plumes. This theoretical finding however contradicts the available data, as the longest determined MTBE plume with maximally approx. 1900 m is clearly shorter than the longest CAH plume with plume lengths of approx. 10 000 m. In this con-

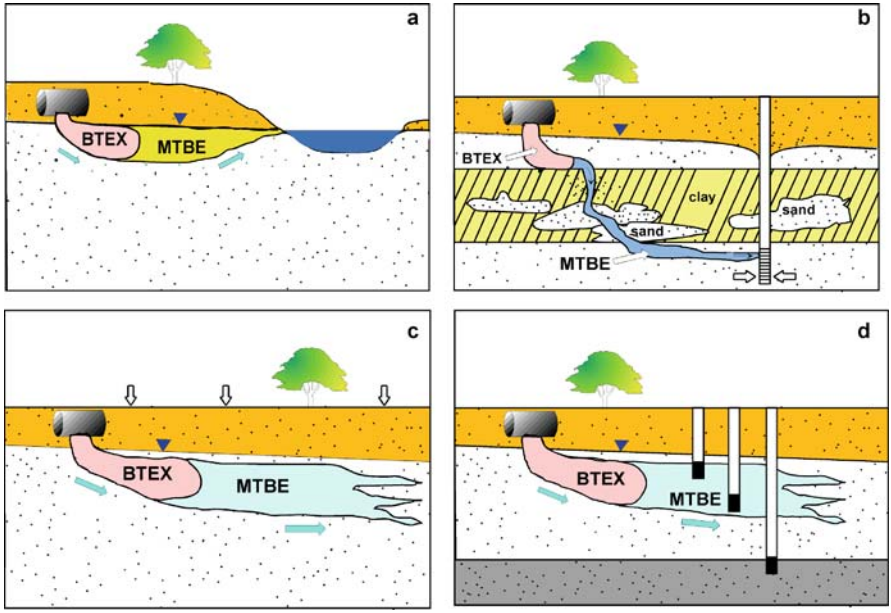

Fig. 8 Spreading of MTBE at different conditions of groundwater streams

text it is amazing that from North America (considering the high number of known MTBE groundwater contaminations) the maximum known plume length is only 1800 m. In comparison, the authors know of approx. 25 MTBE plumes in Germany where the longest plume already shows an expansion of approx. 1900 m.

In summary, the multitude of field findings from the US clearly indicate that MTBE plumes in most cases are comparatively short, and the determined plume lengths frequently amount to 50–400 m. These plume lengths concern predominantly groundwater contamination arising from gas stations, which are by far the most frequent cause of MTBE groundwater contamination.

Thus the available results point to the fact that MTBE in most cases does not form markedly long plumes, even though this can be seen in individual cases. This leads to the conclusion that the spreading potential of MTBE is to be classified between the BTEX and the CAH. Available information about the plume lengths of CAH, BTEX, and MTBE are graphically illustrated in Fig. 9.

Consequently, MTBE groundwater contaminations reach plume lengths that fall in the category of BTEX as well as in the class of CAH. MTBE plumes might nevertheless often exceed the lengths of BTEX plumes. A substantial reason for comparison with the lower CAH plume expansions might consist of the fact that MTBE plumes—due to high water solubility and thereby the connected fast development of the MTBE source transfer—progress com-

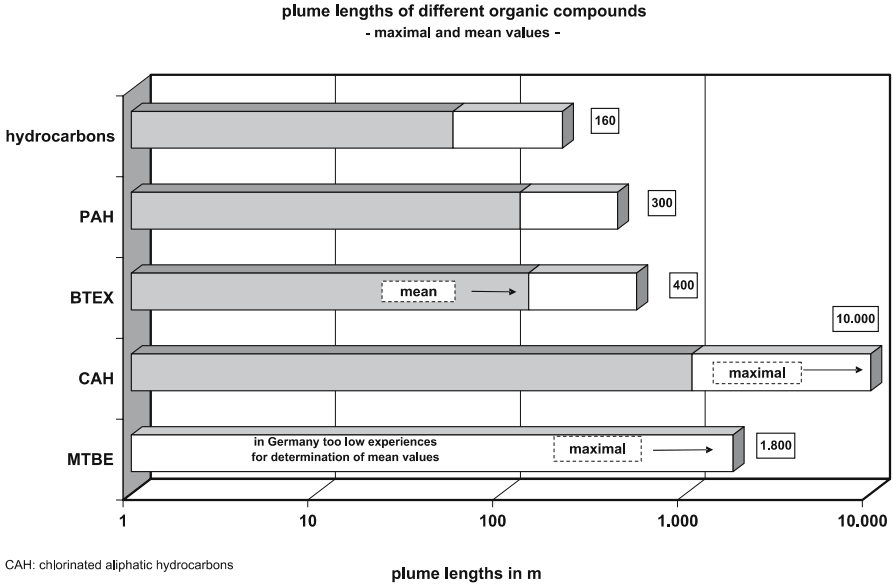

Fig. 9 Plume lengths of different organic compounds; maximal and mean values

paratively fast into the stable and/or regressive status of the plume development. Beyond this, MTBE infiltrates as subordinated portion of gasolines (predominantly 1–3 wt % in regular grade fuel and/or premium fuel), in comparatively low quantities into the subsoil, so that these comparatively low quantities do not possess large source strengths over longer periods. Only in the case of very large gasoline spills can longer MTBE plumes develop in aquifers with high flow velocities.

Regarding risk management, it is important to identify MTBE plumes having a high potential to develop long plumes in a very early stage of plume development. Only this strategy prevents contamination of extensive groundwater volumes and large funds for remediation can be saved. It is to be noted that under certain conditions TBA can enrich itself in the groundwater and in addition TBA is substantially more difficult to be remediated than MTBE. The possible remediation technologies for MTBE and TBA can be referred to in the literature [15, 16].

In 2005, Germany began to substitute MTBE with ethyl-*tertiary*-butylether (ETBE), so that MTBE will hardly be contained in any German gasolines at the start of 2007. Regarding the risk of ETBE, no data is yet available. However, ETBE has been used for several years in other European countries, but still no comprehensive knowledge exists on ETBE spreading into groundwater. Because the characteristics of ETBE are very similar to those of MTBE, it is highly probable that the spreading behavior of ETBE will not differ substantially from MTBE groundwater spreading.

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Enhanced Natural Attenuation of MTBE

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Abstract MTBE contamination in groundwater is an increasing environmental problem and treatment costs using conventional remediation technologies will increase if water is contaminated by MTBE. Generally, natural attenuation (NA) and enhanced natural attenuation (ENA) are possible low-cost alternatives to conventional techniques. Since biodegradation of MTBE is comparably slow under field conditions and often limited by the environmental conditions, optimizing these conditions within the framework of an ENA approach can be a useful means to enhance the natural degradation process.

One potential limitation of the ENA approach is that MTBE is mineralized by only a few specialized bacteria and mainly under aerobic conditions. Co-metabolic biotransformation of MTBE by aerobic, alkane-degrading bacteria has also been reported. Although several studies have demonstrated anaerobic biodegradation, anaerobic MTBE degradation rates are very low compared to aerobic rates.

Introducing a source of pure oxygen into a MTBE-contaminated aquifer has been shown to be a successful means to enhance biodegradation efficiency. At higher organic loadings, H_2O_2 can be used as an additional oxygen source. There is also some evidence that nitrate can be used as an alternative electron acceptor. Recent investigations have also demonstrated enhanced MTBE degradation under methanogenic conditions generated by the dosing of electron donors such as alcohols.

For the field application of ENA measures, different technological solutions such as direct gas, slurry or liquid injections have been developed during the past few years.

Keywords Biodegradation · Ground water · Enhanced natural attenuation · MTBE

Abbreviations

CFU	colony forming unit
EPA	US Environmental Protection Agency
ENA	enhanced natural attenuation
K_{OC}	carbon-based partitioning coefficient
K_m	substrate half-saturation constant
K_S	oxygen half-saturation constant
MTBE	methyl *tert*-butyl ether
NA	natural attenuation
ORC	oxygen-releasing compounds.

1 Introduction

The gasoline additive methyl *tert*-butyl ether (MTBE) has been used in large quantities both in the US and Europe only within the past twenty years. In 1999, about 21 million tons were produced globally, with 3.3 million tons in the European Union [1].

Over only a few years of intense use, MTBE has become one of the most frequently detected ground water pollutants in the US. More than 400 000 leaking underground storage tank sites have been identified by the US Environmental Protection Agency (EPA) since 1988 [2]. In Europe, although field data are currently rare, there are also a growing number of reports about releases of MTBE containing gasoline [3]. A recent study from Germany, including measurements from gasoline spill sites, found severe MTBE contamination at five out of ten sites selected with peak concentrations of up to 87 mg L^{-1} [3, 4].

MTBE is highly soluble in water (43–50 g/l), and the organic carbon-based partitioning coefficient (K_{OC}) of 11 cm^3/g is low [5]. This results in minimal sorption and retardation in natural aquifers (retardation factor 1.1 [6]). Moreover, natural MTBE biodegradation rates are commonly low. The half-life of MTBE in ground water has been estimated at 2–3 years [6]. As a consequence of its low adsorption, its high solubility, and its recalcitrance to biodegradation, MTBE can be transported in ground water over long distances. Several waterworks, e.g. in the USA, Denmark [7] and Germany, are experiencing MTBE contamination within their water supply wells. Since MTBE has very low taste and odor threshold concentrations, even low MTBE concentrations between 20–40 μg/l can significantly impair drinking water quality.

Because of its physicochemical properties and its environmental behavior, MTBE contamination is an increasing problem and treatment costs using

conventional remediation technologies will increase if water is contaminated by MTBE. Therefore, new cost-efficient remediation technologies are needed.

Natural attenuation (NA) and enhanced natural attenuation (ENA) are possible low-cost alternatives to conventional techniques. During the NA process, pollutants are removed from ground water by different processes including volatilization, adsorption, dispersion, hydrolysis and biodegradation.

But unlike other gasoline components, MTBE is known to be only slowly removed from ground water under natural conditions. Because of its physicochemical properties, the reduction of MTBE mass by physical and chemical processes is relatively limited. Volatilization is not very efficient and hydrolysis at neutral pH is slow. The low K_{OC} implies that retardation and adsorption to soil particles are also low. Therefore, bioremediation may play the main role in mass reduction of MTBE during the NA process. However, the structure of the MTBE molecule (tribasic chain branching, 1-carbon chain lengths and ether bonding) indicates that the substance is comparably resistant to microbial degradation. Numerous early reports have confirmed this persistence. On the other hand, there are a large number of studies demonstrating MTBE degradation at the lab-scale [8–10].

In the field, degradation of MTBE is slow and often limited by the environmental conditions. Optimizing these conditions within the framework of an ENA approach can therefore be a useful means to enhance the natural degradation process.

2 MTBE Biodegradation under Natural Conditions

In contrast to former reports, recent investigations have demonstrated significant MTBE degradation not only in lab-scale experiments but also under natural conditions. However, there seems to be a wide variation in the intrinsic potential for MTBE biodegradation depending on residence time and the degree of MTBE contamination, availability of electron acceptors, the type and concentrations of co-contaminants as well as the geological and hydrogeological site conditions [11–14]. As described by Salanitro et al. [15], native microorganisms from a contaminated site in Port Hueneme degraded 10 mg L^{-1} within 10 days. In contrast, only two out of ten microcosms constructed with material from different sites in California, Louisiana, Illinois, Nevada, Ohio, Texas, Michigan and New Jersey were able to degrade MTBE [16]. Kane et al. [11] measured significant MTBE degradation in two out of four samples from MTBE spills in California. In contrast, material from a site in Sacramento showed no significant degradation in 75 days [11]. However, an absence of MTBE degradation in microcosm studies over weeks or even months does not imply that there are no microorganisms able to degrade MTBE. Long lag times have been documented in several studies. In microcosm studies

with aquifer material from the Leuna site in Germany, for example, all six microcosms degraded MTBE but only after a lag time of about 70 days [17]. This was much faster than measured in earlier experiments with material from a field experiment in Borden (Ontario), where lag times reached 18 months [13]. These difficulties in measuring MTBE degradation have created the perception that MTBE-degrading bacteria are rare in natural environments [5].

However, compared to the degradation of hydrocarbons or even BTEX compounds, the most soluble compounds in gasoline [9], natural MTBE degradation rates documented to date are relatively low. Under field conditions, first-order degradation rates for MTBE between 0.04 [18] and 2.7 per year have been described [19]. Summarizing ten different field studies, Wilson et al. [6] estimated a median MTBE degradation rate of 0.41 per year. This is about one-third to one-fourth of the rates described for benzene [6]. As a result, the half-life for MTBE has been found to be at least 2 years in most natural ground water systems, compared to 2–3 months for BTEX; the contaminant plumes for MTBE are therefore normally much longer than those of BTEX. Whereas BTEX compounds are transported in ground water seldom farther than 100 m [20], MTBE plumes can reach more than one thousand meters [21].

However, compared to the natural degradation rates, specific degradation rates of MTBE-degrading bacteria are not low. The maximum specific degradation rates for MTBE mineralizing strains have been estimated to be above 1 g MTBE g cells^{-1} day^{-1} (3 g MTBE g cells^{-1} day^{-1} for the strain ENV425—*Hydrogenophagaflava*) [22], between 0.9 and 1.9 g MTBE g cells^{-1} day^{-1} for strain L108 [23], 1.2 g MTBE g cells^{-1} day^{-1} for Rubrivirex spec. PM1 [24] or up to 1200 mg L^{-1} day^{-1} in a lab-scale column experiment [25]. These rates are comparable to the lower range of rates described for BTEX compounds [26]. One explanation for the contradiction between lab-scale results with pure strains and field degradation rates can be given by the growth properties of the MTBE-degrading strains. Since microbial growth using MTBE as the energy and carbon source is energetically ineffective, the growth rates of the specialized bacteria are very low. The generation times described for MTBE-mineralizing bacteria are between several days to several weeks [6]. This is much longer than for most other bacteria that have typical generation times of several hours. Thus, if ground water becomes contaminated with MTBE, a long time may pass before an effective population of MTBE-degrading bacteria is established. Starting at a density of about 1 colony forming unit (CFU) ml^{-1} of MTBE-degrading bacteria, with a generation time of two days, it will take about 40 days to reach the concentration of approximately 10^6 bacteria per ml necessary to degrade 1 mg L^{-1} MTBE. With a generation time of two weeks, 280 days are required. Moreover, environmental conditions are often limited and optimal cell numbers for the degradation of pollutants cannot be reached. These limitations in cell numbers and growth

rates may be overcome by bioaugmentation within the framework of an ENA approach.

An additional way to overcome the limited availability of specialized bacteria is the utilization of cometabolic pathways for the degradation of MTBE. It has been shown that at least the aerobic MTBE degradation starts with a monooxygenase reaction. Monooxygenases are common among ground water microbial populations and are involved, for example, in the degradation of hydrocarbons. There are a growing number of strains that have been found to be able to degrade not only aliphatic hydrocarbons but also MTBE. However, the monooxygenase enzymes of most hydrocarbon-degrading bacteria are obviously not induced by MTBE themselves. Therefore, different co-substrates such as n- and iso-alkanes, cycloalkanes and even alcohols have been used as effective co-substrates for MTBE remediation [27–31].

With respect to the cleanup goals, the kinetic properties of MTBE degradation have to be taken into account as well. The half-saturation constants for MTBE, as described so far, are comparably high. The Km values of different MTBE-degrading cultures have been estimated between 0.33 (enriched culture from refinery activated sludge [32]) and more than 50 mg/l (strain PM1 [33]). As a consequence, the degradation of MTBE at concentrations below 1 mg L^{-1} is relatively slow, and times necessary to reach the remediation goals (commonly between 0.2 and 0.005 mg L^{-1}) are much longer than those estimated from a zero- or first-order degradation model. These kinetic limitations cannot be overcome even if environmental conditions are optimized by ENA measures.

2.1 Electron Acceptors

Biodegradation of MTBE has been shown at a variety of locations. In most cases, it has been found to be an aerobic or microaerobic process starting with a monooxygenase reaction [8, 9, 11, 12, 24, 34]. Since monooxygenases are the key enzymes for the degradation of a variety of natural substances such as hydrocarbons and lignins, the phylogenetic potential for the degradation of MTBE should be available at most contaminated sites. However, even this reaction may be the critical step limiting the efficiency of MTBE degradation in natural environments. The most critical feature of the monooxygenase reaction is its special oxygen demand. As shown in several studies, MTBE-degrading bacteria require higher oxygen concentrations than many other aerobic bacteria which use oxygen as the only terminal electron acceptor. The half-saturation constant K_S for oxygen in the respiratory chain is usually less than 0.1 mg L^{-1}. Thus, aerobic metabolism normally occurs at maximum rates above 0.5 mg L^{-1} oxygen [6]. Bacteria which degrade MTBE via the monooxygenase enzymes have a second requirement for oxygen as a substrate for the monooxygenase. Unfortunately, the K_S values of the monooxy-

genases are often much higher than those estimated for the enzymes in the respiration chain. K_S values described so far varied between 0.9 mg L^{-1} and 3 mg L^{-1} [6, 21]. As a result, optimal MTBE degradation requires exceptionally high oxygen concentrations. Salanitro et al. [16] reported significant inhibition with the BC-1 culture at oxygen concentrations below 1 mg L^{-1}. Under natural conditions, oxygen concentrations are commonly low and thus MTBE degradation is limited by the oxygen availability. Moreover, potential co-substrates such as hydrocarbons are often more readily degradable than MTBE. The degradation of these compounds can consume a significant portion of the dissolved oxygen making the initial steps of the MTBE biodegradation very slow or even impossible. On the other hand, MTBE by itself has been found to be unable to induce the initial monooxygenase in most bacteria and co-substrates could significantly enhance the potential for MTBE degradation (see Sect. 2). However, the benefits of co-substrates such as hydrocarbons, for example, are often overcome by the limited availability of oxygen. As a result of the different and conflicting properties of the monooxygenase enzymes, aerobic degradation seems likely to occur only at the fringes of the plume or at least outside of the BTEX and hydrocarbon plumes [6, 21]. Optimizing the oxygen supply can therefore be a very effective ENA measure to enhance the degradation of MTBE.

Besides aerobic MTBE degradation, anoxic or even anaerobic degradation has also been described in the literature. Bradley et al. [35] described MTBE degradation under denitrifying conditions. There was also some evidence for MTBE degradation under denitrifying conditions at a test site in Leuna (Germany) [17].

MTBE degradation under Fe(III) reducing conditions has been described as well [36, 37] but the rates were very low. Landmeyer et al. [36] calculated a first-order degradation rate of 0.06 per year or a half-life of 12 years. Data on natural MTBE degradation under sulfate reducing conditions are rare and indicate that degradation is either very slow or non-existent. Amerson and Johnson [38] used MTBE labelled with the stable carbon isotope ^{13}C to follow MTBE degradation at Port Hueneme, California. They found no evidence for MTBE degradation over one year. More recently, Somsamak et al. [39, 40] demonstrated MTBE degradation in microcosm and isotope fractionation studies.

MTBE degradation under methanogenic conditions seems to be more promising. However, results from field studies are somewhat conflicting. However, where MTBE degradation did occur, it was comparably rapid. At a site in Elizabeth City (North Carolina), Wilson et al. [19] calculated degradation rates between 2.2 and 5 per year. These findings have been verified in lab-scale experiments. The average degradation rate was 3 $year^{-1}$ corresponding to a half-life of three months. At a contaminated site at Long Island, a degradation rate of 5.2 $year^{-1}$ was calculated. But at three other sites, the rates were about one order of magnitude slower [6].

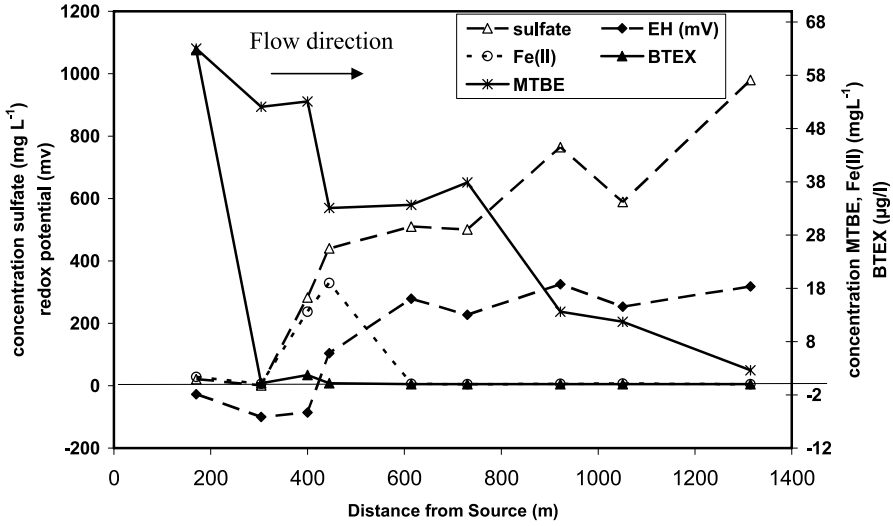

Fig. 1 Concentrations of redox potential and electron acceptors along the center line of the MTBE plume at the UFZ test site in Leuna (Germany)

Summarizing the field results, the degradation of MTBE under methanogenic conditions seems to be an effective alternative to the aerobic process and degradation rates calculated so far are at least comparable to aerobic rates. However, suitable environmental conditions for methanogenesis, especially the redox state, are often limited to the region in the immediate vicinity of the source. As shown at the UFZ test site in Leuna (Germany), MTBE degradation in the MTBE-only area of the plume is obviously not efficient enough to reduce the redox potential to Fe(III) reducing, sulfate reducing or even methanogenic conditions [21]. On the contrary, the redox potential in this region increased and Fe(II) became re-oxidized after the BTEX compounds had been completely degraded (Fig. 1). These findings confirmed former results by Salanitro et al. [15]. The MTBE plume at the USN Hydrocarbon National Environmental Test Site at Port Hueneme was characterized by microaerobic rather than anaerobic conditions. The dissolved oxygen concentrations in the ground water were usually in a range between 0.2 and 1.5 mg L^{-1} and the redox potential measured in the field varied from + 30 mV to + 200 mV [15].

At these sites, environmental conditions suitable for supporting anaerobic or even methanogenic bacterial populations do not exist.

2.2 Availability of Native Microorganisms

In principle, bacteria potentially able to degrade MTBE should be available at most contaminated sites. This can be concluded from our knowledge about

the degradation pathways. The key enzymes are monooxygenases that are involved in a variety of natural degradation processes (see Sect. 2.1). Most of the reactions in the subsequent degradation of metabolites are also involved in basal metabolism and are therefore ubiquitous in the microbial environment. In fact, at many sites only very low cell numbers of MTBE-degrading bacteria are found. This can be deduced from long lag phases in microcosm studies [11, 13, 17] and may be the result of the long generation times of the bacteria as well as inadequate environmental conditions. As a result of these unfavorable conditions, it takes months or even years after an MTBE spill to establish a significant MTBE-degrading microbial population. In a large controlled-release experiment at the Canadian Forces Base Borden (Ontario) starting in 1988, Hubbard et al. found no evidence for natural MTBE degradation up to 476 days after the MTBE release [41]. However, in 1995 the concentrations were significantly lower than expected based on dilution and dispersion and in 1996 after 3000 days, only 3% of the injected MTBE mass remained [12, 13].

In summary, there seems to be a wide variation in the intrinsic potential for MTBE biodegradation depending on time and degree of the MTBE contamination, availability of electron acceptors, concentrations and types of co-contaminants as well on the geological and hydrogeological site conditions [11–14]. These very different natural conditions have to be taken into account if ENA measures are being considered.

3
Methods to Enhance the Natural Biodegradation Process

Compared to lab-scale experiments and engineered treatment systems, natural degradation of MTBE is commonly slow. As shown above, there are significant limitations for MTBE-degrading bacteria in the aquifer. Slow growth rates and low yields of MTBE-mineralizing bacteria prevent bacteria from maintaining significant rates of contaminant degradation. The low natural oxygen transfer rates in ground water also limit the growth of the MTBE-degrading bacteria. Therefore, a variety of different measures have been tested to enhance the natural degradation within the framework of an ENA approach. Several of these measures have also been verified and applied in pilot- and full-scale treatments. These measures include technologies to increase the oxygen supply by air and oxygen sparging [42–45], hydrogen peroxide dosing [17] or the use of oxygen-release compounds (ORC) [18]. Other measures have stimulated the in situ biodegradation rate by bioaugmentation [15, 44] or by the addition of co-substrates such as aliphatic and cyclic hydrocarbons (methane [31], propane [45], butane [29], iso-butane [46], pentane [47], cyclohexane [30]) and alcohols [31, 45].

3.1 Enhanced Oxygen Supply

The recent practice of increasing the oxygen supply is, without doubt, the most effective way to enhance degradation efficiency in an aquifer. There are many technologies currently available to increase the oxygen concentration of contaminated ground water, and there have been many attempts to increase the rate of aerobic in situ degradation of MTBE.

At several sites, adding dissolved oxygen to the aquifer was the only action that was necessary to stimulate the biodegradation of MTBE. Javanmardian and Glasser [42] reduced the MTBE concentration in ground water, by one order of magnitude, by simply sparging with air. However, the injection of air into the aquifer may have an unintended side effect, a reduction in the permeability of the aquifer in the intended zone. Therefore, in most treatment systems, oxygen injection is preferred to air sparging. Smith et al. [44] tested pure oxygen sparging in an in situ field study at Port Hueneme, California. Two test plots (A and B) were supplied with oxygen. Plot B was also inoculated with strain PM1, which is able to degrade MTBE as a sole source of carbon and energy. The MTBE concentrations up-gradient from the test plots varied between 1.5 mg L^{-1} and 6 mg L^{-1}. Six months after the start of the oxygen injection, MTBE decreased substantially in the shallow zone in both plots. In the deeper zone, downstream concentrations also decreased in plot A and to a lesser extent in plot B which was inoculated with PM1. In this study, the degradation of MTBE was obviously limited by the availability of oxygen rather than by low numbers of specialized bacteria. Addition of oxygen to the indigenous microbial population was sufficient to stimulate the MTBE biodegradation and there was no need for bioaugmentation.

The same was true at the UFZ test site in Leuna (Germany). In a so-called conditioning unit (for a full description see Sect. 4.2), the aquifer was divided into five separate strips (each 9.5 m wide and 100 m long). Three out of the five plots (plots 2, 3 and 4) were amended with oxygen by means of pure oxygen or addition of H_2O_2. Additionally, plot 3 was inoculated with strain L108. This strain had been previously isolated at the UFZ and was able to degrade MTBE as the sole source of carbon and energy [23]. Plot 4 was supplied with either 1- or 2-propanol. Significant MTBE biodegradation was measured two months after starting the oxygen addition. With H_2O_2 injection (corresponding to 520 mg O_2 L^{-1} ground water) the MTBE concentration in the ground water decreased from 30–35 mg L^{-1} to about 10 mg L^{-1}. As shown in Fig. 2, there was no effect of either bioaugmentation or the addition of co-metabolites even at the highest H_2O_2 injection rate. The biodegradation rate was clearly limited by the oxygen availability and there was no need for further measures such as bioaugmentation or co-metabolite addition.

At many sites, especially the Leuna site discussed here, the added oxygen has to meet not only the oxygen demand of MTBE degradation but

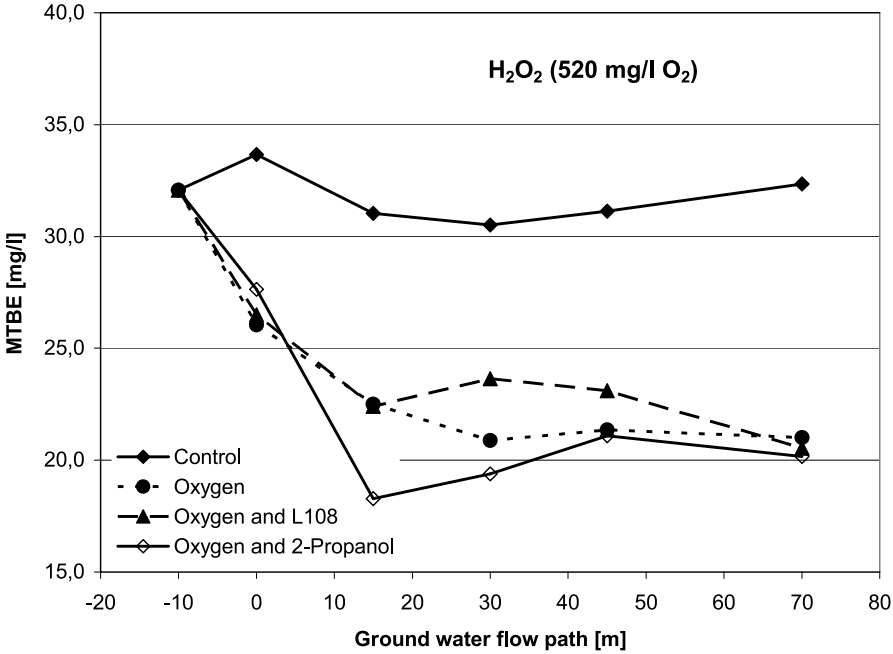

Fig. 2 Effect of oxygen supply, bioaugmentation and co-substrate addition to the biodegradation of MTBE at a test site in Leuna (Germany)

also the biochemical demand of other organic substances in the ground water or adsorbed to the aquifer matrix, as well as the non-biological oxygen demand associated with reduced minerals in the aquifer. These side reactions may exceed the oxygen consumed by the degradation of the pollutant itself. At the Leuna test site, the oxygen concentrations in the ground water decreased to less than 0.1 mg L^{-1} within the first 15 m of the 100 m long strips, which was mainly caused by the action of different site reactions, e.g. nitrification [17].

There are several further studies at different sites that confirm the efficiency of oxygen addition in stimulating the natural MTBE degradation; these studies have been summarized by Wilson et al. [6].

As demonstrated by Wilson et al. [43] in their field experiments at the Vandenberg Air Force Base (California), oxygen addition can induce high degradation rates by the autochthonous microbial populations. After a lag period of only two months, MTBE was degraded in the aerated zone from several hundred mg L^{-1} to less than 10 mg L^{-1}. The apparent pseudo-first-order degradation rate was estimated to be 5.3 day^{-1}. With excess dissolved oxygen and additional injection of MTBE, this degradation rate was enhanced up to 8.6 day^{-1}.

However, there are also a large number of studies where oxygen addition alone was insufficient or additional measures such as bioaugmentation were advantageous [15, 45, 48] (compare Sects. 3.3 and 3.4).

3.2 Alternative Electron Acceptors and Anaerobic Processes

Adding alternative electron acceptors may be a useful tool in the context of ENA. Nitrate, for example, has been used as an especially cost efficient and easy to handle alternative to oxygen. Although MTBE degradation with alternative electron acceptors has been measured in lab-scale experiments, the degradation rates are commonly low and there exists to date no field study using alternative electron acceptors for an ENA approach.

However, Bradley et al. reported a rapid mineralization of MTBE labelled with [^{14}C] in denitrifying surface water [35]. At the UFZ test site in Leuna (Germany), nitrate has also been tested for its suitability in ENA and there is some evidence for nitrate utilization [17]. However, these studies are continuing and conclusive results are not yet available.

Several investigators have also described efficient MTBE degradation under methanogenic conditions [19]. Several promising field studies are under investigation.

3.3 Cometabolic Biodegradation

Bacteria growing on a co-substrate and co-metabolizing MTBE can grow much more rapidly than those utilizing MTBE as the growth substrate. For one particular strain (*Pseudomonas aeroginosa*) growing on pentane, Garnier et al. determined a doubling time of 0.15 days [47]. Moreover, there are many more bacteria that can degrade MTBE cometabolically than those that can mineralize MTBE as the sole source of carbon and energy [27–31] (see Sect. 2. Several attempts have therefore been undertaken to utilize these advantages for increasing the MTBE degradation efficiency at contaminated sites. However, to date there have been many lab-scale studies, but only one investigation that was successful in increasing MTBE biodegradation under field conditions.

Steffan et al. [45] isolated a propane-oxidizing strain ENV 425 which was able to convert MTBE to CO_2. This strain has been used to establish a biobarrier in an MTBE plume in New Jersey [49]. An air-sparging system at the site was tested, but was not successful in degrading MTBE. Therefore, propane was added to the sparge air for 10-minute periods at intervals of three hours each. Since natural microbial populations were low at the site (perhaps because of an unfavorable environment of about pH 3), one month later a culture of the propane-oxidizing strain ENV 425 was injected directly

to the sparge wells and the pH was adjusted to pH 7. Within three months after inoculation, the MTBE concentration decreased by about 90% from its initial range of 90 mg L^{-1} to 320 mg L^{-1}. The maximum removal was 97% from 87 mg L^{-1} to 2.5 mg L^{-1}. When the sparge system was turned off, the MTBE concentrations increased again.

However, at the test site in Leuna (Germany), co-metabolic degradation was successful at the lab scale [31] but did not increase the MTBE degradation under field conditions (Fig. 2). At this site, MTBE degradation was limited by the oxygen supply. At low oxygen supply, co-substrate degradation inhibited MTBE degradation by its own oxygen consumption (see Sects. 3.1 and 4.2).

In summary, co-metabolic MTBE degradation can be a useful means to enhance the natural degradation of MTBE. However, suitable environmental conditions such as an adequate oxygen supply, sufficient numbers of MTBE-degrading bacteria and favorable geochemical conditions must also be naturally present or established using enhancement methods such as are described above.

3.4 Bioaugmentation

There are several field applications which indicate that efficient MTBE degradation can be established only by the optimization of environmental conditions. There are also a variety of studies, however, where additional measures such as bioaugmentation were necessary or advantageous to achieve acceptable degradation [15, 45, 48]. In most of these cases, only those bacterial strains that utilized MTBE as the sole source of carbon and energy were used for bioaugmentation. Only Steffan et al. [49] preferred bioaugmentation with propane-degrading bacteria to achieve an optimal performance of MTBE degradation.

Since natural bacterial populations of MTBE-degrading bacteria are low at many sites, bioaugmentation can help to shorten the lag phase before significant MTBE degradation becomes detectable. Salanitro et al. [15] compared in situ biostimulation (oxygen supply only) and bioaugmentation at the USN Hydrocarbon National Environmental Test Site at Port Hueneme, California. Three test plots were located in the MTBE-only portion of the plume and included control (not treatment), O_2-only, and O_2 +(bioaugmentation) zones. Before treatment, the MTBE concentrations varied from 2.7 mg L^{-1} to 6.7 mg L^{-1} in the shallow monitoring wells and from 5.7 mg L^{-1} to 8.9 mg L^{-1} in the deep wells. After starting the aeration, MTBE decreased in the O_2-only plot to 0.01 to 0.1 mg L^{-1} after a lag period of 186–261 days, indicating the stimulation of natural MTBE biodegradation. In contrast, in the O_2 + (bioaugmentation) plot, MTBE concentrations began decreasing after only 30 days and concentrations decreased to between < 0.001 and 0.01 mg L^{-1}. These results clearly illustrate the beneficial effects of the bioaugmentation measures.

Spinnler et al. [48] applied similar technology to an MTBE plume in Connecticut and obtained comparable results. However, the initial concentrations in the most highly contaminated well were higher than 100 mg L^{-1} and, thus, much higher than described for the site in Port Hueneme. The reduction in MTBE concentrations at the site in Connecticut varied between one and four orders of magnitude.

The efficiency of bioaugmentation has also been confirmed by other investigators and in additional studies [8, 50, 51]. However, bioaugmentation may be inefficient at sites containing an efficient autochthonous population or even at sites where natural conditions are unsuitable for microbial growth. Therefore, the individual situation at the site should be proven before starting bioaugmentation.

4
Technical Solutions for the ENA Approach

ENA measures have been shown to be an efficient and low cost tool to eliminate MTBE from contaminated ground water. However, for optimum effect, efficient technologies are needed. These technologies have to meet a variety of different requirements, including: (1) Create and maintain suitable conditions for microbial growth and MTBE degradation; (2) sustain existing microbial biomass, or generate new biomass to attain efficient MTBE degradation rates; and (3) ensure a continuous flow of contaminated ground water through the treatment zone.

For this purpose, a variety of different technologies have been developed for the technical application of ENA measures, including technologies for the addition of oxygen such as air and oxygen sparging [42–45], hydrogen peroxide dosing [17] and the use of oxygen-releasing compounds (ORC) [18]. Other measures have stimulated in situ biodegradation by bioaugmentation [15, 44] or by the addition of co-substrates [45].

4.1
Direct Gas Injection

One of the simplest methods for adding oxygen to ground water is sparging with air. The main advantages of air sparging technologies are the relatively simple technical equipment and low costs.

Air sparging technologies have been described, for example, by Javanmardian and Glasser [42] and by Leeson et al. [52] and have been used for the elimination of MTBE concentrations up to 40 mg L^{-1}.

A serious disadvantage of air sparging is the so-called gas clogging effect. Nitrogen bubbles remaining after oxygen consumption are known to reduce the water filled porosity thus reducing the permeability of the aquifer in the

intended treatment zone. This reduced permeability may result in a partial bypass of contaminated ground water around the treatment zone. Physical model studies at the test site in Port Hueneme demonstrated that there appears to be an optimal air injection rate, above which the aquifer permeability decreases [52].

However, the degree of the gas clogging effect depends not only on the gas injection rates but also on the geological and hydrogeological properties of the aquifer which differs from site to site. To overcome this "trial and error" nature of the air sparging practice, recent treatments prefer the injection of pure oxygen instead of air. Oxygen sparging has been successfully applied by different investigators and at several different sites [15, 43, 44]. The operation of the oxygen injection systems was either continuous [43, 45] or intermittent [15, 44]. Continuous oxygen injection commonly provides higher dissolved oxygen concentrations than intermittent injection. On the other hand, increased gas saturation in the aquifer can significantly reduce the hydraulic conductivity. This gas clogging is not only the result of excess oxygen supply but also results from the increased accumulation of dissolved nitrogen and other permanent gases in the gas bubbles [53]. Therefore, periodic O_2 gas injection has been preferred in most applications. Trapped gas bubbles remaining in the aquifer have been shown to provide the ground water with oxygen for extended periods between the gas injection cycles. Optimized intervals between subsequent gas injections allow the remaining nitrogen and other permanent gases to re-dissolve, thus preventing significant gas clogging.

The optimal conditions depend on the concentrations of pollutants, and also on the oxygen demand of the different site reactions and on the geological and hydrogeological setting. For optimal performance, Salanitro et al. [15] selected an intermittent oxygen injection of 1700 L delivered 4–8 times per day to a plot that was 6 m long and 15 m wide and which contained MTBE concentrations up to 8.9 mg L^{-1}.

As shown in several studies, optimal performance of the ENA approach requires not only an optimal timing of the gas injection system but also an optimal arrangement of the injection wells in the aquifer. For this purpose, Salanitro et al. [15] as well as Smith et al. [44] used an array of injection wells arranged in two to four rows. The injection wells were installed at two different depths depending on the geological structure of the aquifer. In addition, Smith et al. [44] optimized the horizontal and vertical positions of the gas injection points according to the MTBE concentrations in the aquifer.

A very interesting alternative to direct gas injection by means of gas injection wells is the diffuse release of oxygen via semi-permeable tubing placed in the aquifer. The gas diffusion technique overcomes all the disadvantages associated with direct gas injection such as gas clogging. Wilson et al. [43] demonstrated very high performance with a silicon tubing system applied in a pilot plant at the Vandenberg Air Force Base, California.

Besides its use for adding oxygen, direct gas injection has also been used for supplying the aquifer with gaseous co-substrates. Steffan et al. [49] used direct gas injection for the combined addition of oxygen and propane. The addition of propane used existing air sparging equipment. Application of propane in the field, however, may raise concerns about creating explosive in situ mixtures of propane and air. To meet these requirements, propane was injected in pulses and the concentrations in the mixture did not exceed 0.2% propane in air.

4.2 The Conditioning Unit

A conditioning unit has been developed and constructed by the UFZ—Helmhotz Center for Environmental Research (Germany, patent applications: DE 199 48 828, WO 01/24952). This treatment facility allows a controlled flow of contaminated ground water and the addition of a variety of components including nutrients, electron acceptors, catalysts and microorganisms. The degradation of the pollutants can be increased by means of microbial communities established in the conditioning chamber and/or by means of reactive substances. In the conditioning unit, the following treatments are applicable:

- Oxygen supply by oxygen gas or hydrogen peroxide;
- Addition of electron acceptors such as nitrate or Fe(III);
- Addition of pre-adapted bacteria;
- Addition of different gaseous or liquid cometabolites.

The upstream ground water is captured by sheet piles followed by hydraulic passive flow through the conditioning unit. After passing the conditioning

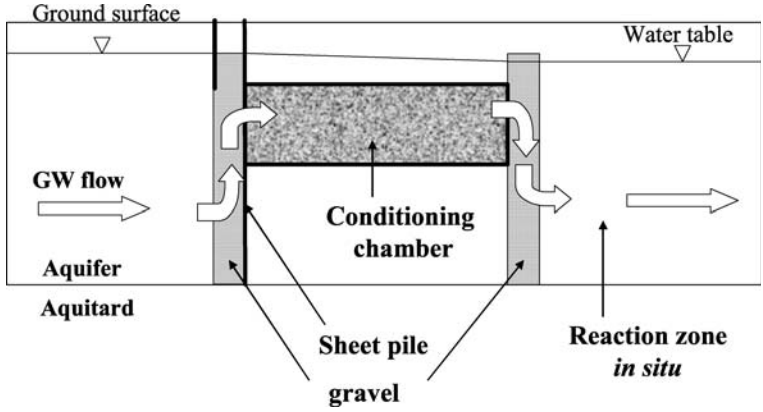

Fig. 3 Schematic diagram of the conditioning unit (cross section)

chamber, the conditioned ground water is subsequently re-infiltrated into the down-gradient aquifer, which is used as the reaction area. The schematic construction of the treatment facilities is shown in Fig. 3. The performance of the conditioning unit has been studied at the UFZ test site in Leuna (Germany) (see also Sect. 3.1) [17].

A comparable treatment system has been described by Wilson et al. [43]. A pilot scale test facility was filled with non-native highly permeable pea gravel. However, the 4–8 day ground water residence time in the treatment facility classifies this technology as a funnel-and-gate system rather than an ENA measure.

4.3 Liquid and Slurry Injections

Liquid and slurry injections are commonly employed for the addition of various ENA components to ground water such as microorganisms, nutrients or dissolved co-substrates. Injections of H_2O_2 or metal peroxides have also been used for increasing the oxygen supply within the framework of ENA.

Microorganisms and nutrients are commonly injected via common monitoring wells. In more recent applications, special injection wells for bioaugmentation were installed by the Geoprobe® technique [16, 43, 44, 48]. In all studies, oxygenation began several weeks prior to the injection of bacterial cultures. Salanitro et al. injected a total of 6000 L at several points along a 6.1 m transect. Individual injections of 20 L each were performed, spaced roughly 0.3-m vertically and horizontally [15]. Smith et al. installed nine individual injection points for biomass injection in two rows interspaced between the oxygen sparging wells. [44].

The techniques for liquid and slurry injections are comparable to the microbial injections.

The use of oxygen release compounds has been described in several studies to be an efficient means to enhance the oxygen concentration in aquifers. In this approach, metal peroxides (MgO_2 or CaO_2) are introduced as solids or slurries into the aquifer. The metal peroxides slowly release dissolved oxygen by hydrolysis and thus act as a long-term oxygen source. The injection of MgO_2 slurries as an ENA measure for MTBE remediation has been described, for example, by Landmeyer et al. [54]. They injected a slurry consisting of 30 kg MgO_2 in 30 L of uncontaminated water into each of 18 boreholes located along a single 29 m wide transect across the plume. The slurry was injected at 1.7 m centers. The injection occurred vertically from the bottom of the contaminated aquifer to about 0.6 m above the average water table surface. At a monitoring well two meters downgradient of the injection wells, the concentration of dissolved oxygen reached 12 mg L^{-1} and remained at or above 2 mg L^{-1} for at least one year.

5 Comparative Consideration of Different Technologies

The efficiency of the ENA approach for the remediation of MTBE has been demonstrated by a variety of lab-scale and field studies. Several different technologies such as air and oxygen sparging, hydrogen peroxide dosing or the use of oxygen release compounds have been developed and applied. Other measures have stimulated in situ biodegradation by bioaugmentation or by the addition of co-substrates.

Pure oxygen sparging has been shown to be a successful means to enhance biodegradation efficiency. In several applications, initial MTBE concentrations up to 10 mg L^{-1} have been reduced to below 0.01 mg L^{-1}. Concentrations above 100 mg L^{-1} have been reduced at least tenfold by means of biobarriers containing injection wells that have been arranged in two or more rows across the plume. At higher organic loadings, H_2O_2 or metal peroxides can be used as additional oxygen sources.

One of the main advantages of oxygen injection technologies is that the degree of oxygen supply can be regulated by the number and arrangement of the injection wells. However, spatial heterogeneity of the aquifer hydraulic conductivity may restrict the ability to provide oxygen to all regions of an MTBE plume. To overcome this problem, comprehensive geological and hydrogeological information is necessary.

A conditioning unit may be a useful alternative, especially at sites with heterogeneous aquifers. Since all the ground water is captured upstream and transported through the conditioning chambers, the demand for geological and hydrogeological information is relatively low. However, the conditioning unit allows only single-point additions of, for example, oxygen or nutrients. Therefore, the efficiency of the conditioning unit has been found to be limited to contaminated sites with an oxygen demand of less than 300 to 400 mg L^{-1}.

Bioaugmentation and cometabolic biodegradation may be a useful supplementary means at those sites with limited indigenous MTBE degradation potentials. They could also be useful as tools for reducing the often long lag times which occur before MTBE degradation can begin. However, at several test sites, there was no demand for additional measures after oxygen was supplied.

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Bioremediation of groundwater contaminated with MTBE/TBA

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Abstract Because of organoleptic issues and potential health risks, groundwater containing methyl *tert*-butyl ether (MTBE) and *tert*-butyl alcohol (TBA) is of concern. Regulatory limits exist in several countries and remediation of MTBE/TBA is needed. Although an in situ MTBE/TBA-biodegradation capacity is not omnipresent, an increasing number of MTBE/TBA-degrading axenic strains and consortia are being isolated. Bioremediation, in situ or ex-situ in bioreactors, is considered an interesting and cost-effective option. Degradation may occur under in situ conditions (natural attenuation), in other cases additives may be required to increase the activity of naturally present MTBE/TBA-

degraders (biostimulation). At contaminated sites where an indigenous MTBE/TBA-degradation potential is lacking, bioremediation is feasible upon addition of ex situ cultivated MTBE/TBA-degraders (bioaugmentation).

Keywords Aerobic biodegradation · Bacteria · Biostimulation · Bioaugmentation · Degradation pathways

Abbreviations

BCR	Biomass concentrator reactor
BTEX	Benzene, toluene, ethyl benzene and xylenes
BTF	Biotrickling filter
CO_2	Carbon dioxide
DIEE	Di-iso ethyl ether
DIPE	Di-isopropyl ether
dw	Dry weight of cells
ETBE	Ethyl *tert*-butyl ether
FBR	Up-flow fluidized bed reactor
GAC	Granular activated carbon
h	Hour
HIBA	2-Hydroxyisobutyric acid
HIBAL	2-Hydroxy-2-methylpropanal
HRT	Hydraulic retention time
LUST	Leaking underground storage tank
MBR	Membrane bioreactor
MTBE	Methyl *tert*-butyl ether
MPD	2-methyl 1,2-propanediol
N	Nitrate
NA	Natural attenuation
ND	Not determined
ns	Not specified
P	Phosphate
POB	Propane oxidizing bacterium
PPR	Porous pot reactor
SBF	Submerged biofilter
SBR	Sequencing batch reactor
TAA	*tert*-amyl alcohol
TAME	*tert*-amyl methyl ether
TBA	*tert*-butyl alcohol
TBF	*tert*-butyl formate
TBM	*tert*-butoxy-methanol

1 Introduction

MTBE is an organic compound, mainly used for replacing lead as a car fuel octane enhancer. Additionally, MTBE is an oxygenate added to car fuel to improve the combustion efficiency of gasoline. Other oxygenates are ethers

such as ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME), diisopropyl ether (DIPE) and alcohols as *tert*-amyl alcohol (TAA) and *tert*-butyl alcohol (TBA). MTBE has become a widespread pollutant of ground- and surface waters, mainly as a result of leaking underground gasoline storage tanks or from accidental spills. In groundwater, MTBE is often accompanied by other gasoline constituents, mainly benzene, toluene, ethyl benzene and xylenes (BTEX) and TBA. Due to low sorption on soil particles and resistance to in situ degradation, MTBE contamination plumes migrate rapidly through aquifers, posing a threat to uncontaminated water resources. Mainly because of its low odor and taste thresholds, MTBE in groundwater is of concern, whereas potential health risks remain a matter of debate.

TBA is currently widely accepted as stable metabolic intermediate or dead-end product of MTBE. In itself, TBA in groundwater is of concern as potential health risks have been reported [1].

Regulatory limits exist or are being composed in a growing number of countries for MTBE, and in a lesser extent for TBA [2]. As such, there is an increasing need for remediation technologies dealing with MTBE/TBA-contaminated groundwater. Conventional remediation technologies for gasoline-contaminated groundwater, including chemical oxidation, air stripping, and adsorption onto activated carbon as reviewed by Fayolle et al. [3], have proven to be inefficient and impractical when MTBE and/or TBA are among the contaminants [4–6]. Bioremediation of contaminated groundwater is considered a cost-effective and energy-efficient alternative for the treatment of MTBE/TBA-polluted groundwater [7, 8]. The major advantage of bioremediation is the potential complete mineralization of the hazardous compounds MTBE and TBA to harmless products such as carbon dioxide (CO_2), biomass, and water.

This chapter focuses on MTBE/TBA-degrading microorganisms and their application for remediation of groundwater contaminated with MTBE and/or TBA.

2 MTBE/TBA-Degrading Microorganisms

More than a decade ago, MTBE was considered recalcitrant to biological degradation under both aerobic and anaerobic conditions [9–12]. More recently, an increasing number of studies describe bioconversion of MTBE in contaminated groundwater, under anaerobic but primarily under aerobic conditions [13–15]. In 1994, Salanitro et al. [14] reported the first isolation of an aerobic mixed bacterial culture that was capable of degrading MTBE. Three years later, Mo et al. [13] reported the first axenic cultures able to degrade MTBE as the sole carbon source. Since that time, several MTBE/TBA-degrading cultures have been enriched and isolated from con-

taminated aquifer material or from contaminated groundwater treatment systems as activated sludge from municipal water treatment plants. During the enrichment process, MTBE/TBA-degrading microorganisms are preferentially proliferated in the microbial community by adding MTBE or TBA to the system as carbon source while reducing other carbon sources to a minimum. Enrichments in laboratory-scale systems [16, 17] as well as in bioreactors treating MTBE-contaminated groundwater have been reported [18–20]. Generally, it requires months to years to enrich a stable culture that is dominated by MTBE and/or TBA-degraders [17, 18].

2.1 Aerobic Degradation

2.1.1 Axenic and Mixed Bacterial Cultures Capable of Growth on MTBE and/or TBA

An overview of bacterial cultures with the capacity to grow on MTBE and/or TBA as the sole source of carbon and energy is given in Tables 1 and 2. In regard to axenic cultures, (Table 1), MTBE degradation is not limited to a single genus or group of bacteria. Reported genera include *Rhodococcus*, *Arthrobacter* and *Mycobacterium*, all high GC and Gram-positive bacteria [13, 21]. Further, many genera belong to a certain subdivision of the phylum Proteobacteria: examples include *Methylobacterium* (α subdivision) [13] and many β-Proteobacteria, e.g., *Methylibium petroleiphilum* PM1, *Hydrogenophaga flava* ENV735, *Ideonella*-like bacteria and *Burkholderia cepacia* CIP I-2052 [22–26]. The latter is only capable of degrading TBA, not MTBE. Bacterial isolates UC1 and UC2 are closely related to *M. petroleiphilum* PM1 (99% and 98%, resp.) and UC3 is related to the genus *Mycobacterium* (97%) [27].

In addition to axenic bacterial strains, mixed bacterial cultures capable of MTBE and/or TBA-degradation have been reported (Table 2). Bacteria closely related to some of the above-mentioned axenic cultures where found present in these mixed bacterial cultures, i.e., *M. petroleiphilum* [40, 41], *Hydrogenophaga* [40, 42] and *Methylobactium* [42]. For example, *M. petroleiphilum* PM1 was isolated from the mixed bacterial culture enriched by Eweis et al. [28].

Also, other bacteria than reported axenic MTBE-degrading cultures have been associated with MTBE or TBA-degradation in the mixed cultures in Table 2. Salanitro et al. [14] reported that bacterial culture BC-1 contained at least four or five organisms, including coryneforms, pseudomonads, and achromobacters. None of the obtained axenic isolates was able to grow on MTBE as the sole carbon source. Other reported bacteria include many uncultured members of the Cytophaga-Flexibacter-Bacteroides group [20, 43] and some α-Proteobacteria: *Hyphomicrobium vulgare* [42, 43] and some *Sphin-*

Table 1 Selection of axenic bacterial cultures with the capacity to grow on MTBE and/or TBA as sole source of carbon and energy

Bacterial culture	Source of enrichment	Growth substrates	Refs.
Methylobacterium mesophilicum	Activated sludge, contaminated soil and soil near a Gingko tree	Incomplete MTBE degradation to TBA	[13]
Rhodococcus sp.	Activated sludge, contaminated soil and soil near a Gingko tree	Incomplete MTBE degradation to TBA	[13]
Arthrobacter ilicis	Activated sludge, contaminated soil and soil near a Gingko tree	Incomplete MTBE degradation to TBA	[13]
Methylibium petroleiphilum PM1	Compost biofilter [28]	MTBE, TAME, ETBE, DIPE, TAA, TBA, Benzene, Toluene, Xylene	[22, 29–31]
Hydrogenophaga flava ENV735	Contaminated ground water and activated carbon filter treating MTBE	MTBE, no BTEX	[23, 26, 32]
Mycobacterium austroafricanum IFP 2012	Activated sludge from urban wastewater treament plant	MTBE, TAME, TAA, (ETBE)	[21, 33–35]
Mycobacterium austroafricanum IFP 2015	Drail water from MTBE-supplemented gasoline storage tank	MTBE, ETBE, TBF, TAME, TBA	[36]
UC1, UC2	MTBE-degrading porous pot reactor [37]	MTBE	[27]
UC3	MTBE-degrading fluidized bed reactor [38]	MTBE	[27]
Ideonella sp. L108	MTBE-contaminated aquifer Leuna, Germany	MTBE, (TAME), ETBE	[25, 39]
Ideonella sp. L10	MTBE-contaminated aquifer Leuna, Germany	TBA, HIBA	[25, 39]
Burkholderia cepacia CIP I-2052	Activated sludge of a wastewater treatment plant	TBA, TAA	[24]

Table 2 Selection of mixed bacterial cultures with the capacity to grow on MTBE and/or TBA as sole source of carbon and energy

Bacterial culture	Source of enrichment	Growth substrates	Refs.
BC-1	Chemical plant biotreater sludge	MTBE, DIPE, ETBE and TAME, no benzene or toluene	[14]
MTBE degrading culture	MTBE-degrading enrichment in biofilter	MTBE, TAME	[28]
F-consortium	Groundwater and aquifer material from two MTBE-contaminated sites	MTBE, TBA, TAME, BTEX	[18, 46, 50]
Enriched mixed culture	Gasoline-contaminated soil	ETBE, MTBE, TAME	[51, 52]
Consortium	Activated sludge treating petrochemical waste waters	MTBE	[45]
Consortium	Enrichment from activated sludge, mixed culture provided by J. Salanitro, DIEE-degrading biofilter enrichment and contaminated aquifer bacteria	MTBE, BTEX	[43]
RS24	Gasoline-contaminated aquifer in Ronan, Montana	MTBE	[48]
Consortium	MTBE-degrading consortium [18] enrichment in biotrickling filter	MTBE, TBA, BTEX	[41, 53]
Enrichment culture	5 environmental samples including MTBE-contaminated soil	MTBE	[44]
Consortium	Enrichment in porous pot reactor	Incomplete MTBE degradation to TBA	[54]
Enrichment culture	MTBE-degrading enrichment from membrane bioreactor [55] and from two porous pot reactors [43] plus activated sludge	MTBE, TBA, BTEX, TAME, DIPE, TAA	[42, 56]
Consortium	Activated sludge enriched in sequencing batch reactor	TBA	[20]
Enrichment	Contaminated aquifer bacteria enrichment in upflow submerged biofilter	MTBE	[40]
VITO-consortium	Contaminated soil enrichment	MTBE, TBA, BTEX	[17]

gomonas sp. [20, 40, 42–44]; *Nitrospina* sp. (δ-Proteobacteria) [43] and *Streptomyces* [44]. Acuna-Askar et al. [45] associate *Micrococcus*, *Acinetobacter lwoffii* and *Bacillus* sp. with MTBE-degradation in the enriched consortium.

Isolation of stable axenic strains from mixed bacterial cultures is not always feasible [17, 46]. The following reasons have been proposed to explain the observed difficulties in isolating axenic MTBE-degrading microorganisms:

- Microorganisms growing on MTBE/TBA produce low biomass even after long incubation times making it difficult to specifically enrich MTBE-utilizing strains.
- Platings on solid agar growth media are classically used to isolate axenic strains. But MTBE/TBA-degrading bacteria may not all be culturable on solid media. Moreels et al. [17] reported a bacterial consortium able to grow relatively fast in liquid medium, without showing growth on solid agar plates with the same growth medium.
- MTBE/TBA degradation in enrichments from contaminated sites can be co-metabolic, requiring an alternative growth substrate for MTBE/TBA-degradation [47]. For example, isolates from the consortium enriched by Kern et al. [48] are closely related to *Pseudomonas* Ant9 and *Rhodococcus koreensis*, but were not able to grow on MTBE nor TBA as the sole source of carbon. Isolations using platings on selective growth media with MTBE/TBA as sole carbon source would have missed these strains.
- Platings on unselective rich growth medium are therefore frequently used. The presence of fast-growing bacteria, however, may mask slow-growing bacteria present in the same consortium. The selection of colonies at different times over longer incubation periods of plates might provide a solution [36].
- Several genes reported to be responsible for MTBE-degradation potential are found to be highly unstable [25, 49]. MTBE-degradation potential could therefore be lost by isolation in non-selective media. ETBE and MTBE-degrading *R. ruber* IFP 2001 has been reported to spontaneously undergo chromosomal deletion, which resulted in the loss of the ability to degrade ETBE [49]. Similarly, MTBE-degradation by *Ideonella*-like strain L108 was easily lost by incubation on non-selective media [25].

Reported specific MTBE degradation rates for both axenic and mixed aerobic bacterial cultures are in the range of 8.5–52 mg MTBE g dry weight^{-1} h^{-1} (Table 3), with the exception of 250.2 mg MTBE g dw^{-1} h^{-1}, reported for axenic culture *Hydrogenophaga* ENV735 [23, 26]. However, this bacterial species requires yeast extract (0.01%) in order to efficiently degrade MTBE.

Reported aerobic TBA-degradation rates are typically higher than MTBE-degradation rates, as reported for two well-documented axenic bacterial cultures *M. austroafricanum* IFP 2012 [21] and recently isolated *Ideonella* sp. L108 and L10 [25] (Table 3). The specific TBA degradation rates in Table 3 for *M. austroafricanum* IFP 2012 and for *Ideonella* sp. L108 and L10 were calculated with resting cells grown on TBA and with MTBE-grown late log phase

Table 3 Specific degradation rates for a selection of MTBE and/or TBA-degrading cultures

Bacterial culture	Specific degradation rate mg g dry weight^{-1} hour^{-1} (initial concentration)			Yield coefficient mg dw mg MTBE^{-1}	Refs.
	MTBE	TBA	HIBA		
Methylibium petroleiphilum PM1	50	ND	ND	0.18	[22, 29–31]
Hydrogenophaga flava ENV735	133.8–250.2 (25 mg l^{-1} MTBE, 0.01% YE, 25–30 °C)	ND	ND	0.2–0.4 (0.01% YE)	[23, 26, 32]
M austroafricanum IFP 2012	23.2 ± 1.9 (30 mg l^{-1} MTBE)	23.3 ± 0.8–39 ± 2 (34.8–200 mg l^{-1} TBA)	51.8 ± 2.3 (35.4 mg l^{-1} HIBA)	0.44 ± 0.01 (200 mg l^{-1} MTBE)	[21, 33–35]
M austroafricanum IFP 2015	ND	59.9	ND	ND	[36]
Ideonella sp. L108	15.9–31.7	66.7–93.4	62.5–249.9	0.3–0.4	[25, 39]
Ideonella sp. L10	Not degraded	ND	62.5–249.9	Not degraded	[25, 39]

ND: not determined

cells, respectively. Reported degradation IFP2012 rates of HIBA are even higher (Table 3) for resting cells of *M. austroafricanum* grown on MTBE and cells of *Ideonella* sp. L108 and L10 grown on TBA, 2-HIBA and isobutyrate, respectively. These high rates might explain why HIBA, currently widely accepted as a stable metabolic intermediate of MTBE degradation (see Sect. 3.1), is not always detected in batch experiments during degradation of MTBE and/or TBA. Most bacteria able to metabolize TBA will also degrade MTBE, however, a few exceptions have been reported [24, 25].

2.1.2
Co-metabolic Degradation of MTBE and TBA by Bacteria

Especially 5 to 10 years ago, most of the reported axenic bacterial cultures that degrade MTBE co-metabolically, have been discovered due to several studies that pointed out the ability of (soluble) alkane monooxygenases to convert MTBE to TBA [57, 58]. Consequently, many alkane-utilizing bacterial axenic cultures and consortia have been reported that co-metabolize MTBE (Tables 4 and 5). Growth substrates supporting co-metabolic aerobic MTBE-degradation are diverse, comprising the following carbon sources:

n-alkanes: methane, propane, butane, pentane, hexane, isooctane, 1- and 2-propanol
Alcohols: methanol, butanol
(co)pollutants: camphor, trichloroethylene, cyclohexane, tetrahydrofuran and benzene

It can be noted that several of these *n*-alkanes are also present in gasoline, i.e., hexane, pentane, isooctane, thus providing alternative carbon sources in gasoline-polluted MTBE source zones. Reported specific co-metabolic, aerobic MTBE-degradation rates are comparable with the MTBE-degradation rates reported for axenic and mixed cultures that are capable of growth on MTBE and/or TBA. Specific degradation rates range from 0.33 mg MTBE g dw^{-1} h^{-1} for PEL-B201 with growth on benzene [59] to 43.6 mg MTBE g dw^{-1} h^{-1} for *Rhodococcus ruber* IFP 2007 for MTBE-conversion to TBA with growth on ethanol, as cited by Fayolle et al. [60]. One exceptionally high calculated maximum co-metabolic degradation rate was reported, 102.7 ± 6.6 and 177.7 ± 1.9 mg MTBE g dw^{-1} h^{-1}, with *n*-pentane-grown and *n*-alkane-grown cells, respectively, reported by Smith et al. [61] for *Pseudomonas mendocina* KR-1 for relatively high initial MTBE concentrations in batch experiments (3.7 mg l^{-1} MTBE).

2.1.3
Fungi

Other organisms than bacteria are reported to degrade MTBE and/or TBA. Kharoune et al. [51, 52] reported a mixed culture consisting of a wide variety

Table 4 Selection of axenic bacterial cultures with the capacity to co-metabolize MTBE and/or TBA

Bacterial culture	Isolated from	Carbon source	Co-metabolism substrates	Refs.
Rhodococcus ruber IFP 2001, IFP 2007	Activated sludge	Incomplete ETBE degradation to TBA	Incomplete MTBE degradation to TBA Incomplete TAME degradation to TAA	[49, 62, 63]
Hydrogenophaga ENV421	POB	propane	MTBE, ETBE and TAME	[58]
Propane oxidizing bacterium ENV425	POB	propane	MTBE, ETBE and TAME	[58]
Pseudomonas putida CAM	–	camphor	ETBE, TAME Incomplete MTBE degradation to TBA	[58]
Pseudomonas putida GPo1	–	*n*-octane	Incomplete MTBE degradation to TBA	[64]
Mycobacterium vaccae Job5	POB	propane	MTBE	[58, 65–67]
Xanthobacter	POB	propane	MTBE	[57]
Pseudomonas	–	*n*-pentane,	Incomplete MTBE degradation to TBA	[58, 61]
mendocina KR-1		trichloroethylene, 2-methyl pentane		
PEL-B201	MTBE-contaminated aquifer	benzene	MTBE	[59]
Arthrobacter	Natural- and domestic-gas-contaminated site	butane and butanol	MTBE	[68]
RS24	Gasoline-contaminated aquifer	2-propanol	MTBE	[48]
Pseudonocardia sp. ENV478	Membrane bioreactor treating industrial wastewater	tetrahydrofuran	Incomplete MTBE degradation to TBA	[69]

POB Propane oxidizing bacterium

Table 5 Selection of mixed bacterial cultures with the capacity to co-metabolize MTBE and/or TBA

Bacterial culture	Isolated from	Growth substrates	Co-metabolism substrates	Refs.
Consortium Borden	Gasoline-contaminated soil, Borden, Canada	2-pentane, *n*-pentane, hexane, 3-methylpentane, cyclohexane (slow)	MTBE	[70, 71]
Three *Pseudomonas* sp.	Contaminated soil	*n*-pentane (> 1 mg pentane)	MTBE	[72]
Consortium	Cyclohexane bioscrubber	cyclohexane	MTBE, ETBE, TAME and TBA, benzene, toluene	[73]
Consortium M1 and M2, Enriched on different alkanes	Samples of bioremediated and polluted LUST sites in Mexico	pentane, hexane and isooctane	MTBE (pentane), incomplete MTBE degradation to TBA	[16]
Several enrichment cultures	Activated sludge mixture and contaminated aquifer	methane, propane, butane, 1-propanol	MTBE	[74]

of microorganisms, including bacteria, protozoa, and fungi. Fortin et al. [18] reported that the enrichment from contaminated groundwater consisted of at least six Gram-positive and negative bacteria, bacilli and cocci, fungi, protozoa, and rotifers.

Apart from being present in mixed bacterial cultures, several axenic fungal cultures have been associated with MTBE-degradation [75, 76]. Hardison et al. [75] reported an axenic fungal culture, *Graphium* sp., capable of incomplete MTBE-degradation to TBA after growth on n-butane and propane. The reported co-metabolic specific degradation rate is 0.93 mg MTBE g dry weight of mycelia^{-1} h^{-1} (initial concentration about 18 mg l^{-1} MTBE). More recently, Magaña-Reyes et al. [76] described a hexane-degrading axenic fungal culture consisting of a *Fusarium* sp. enriched in a biofilter removing hexane [77] and capable of growth on TBA, TAME, hexane and MTBE when induced with TBA. The authors further report degradation rates of 2 mg MTBE g dry weight^{-1} h^{-1} ($\sim$ 50 mg l^{-1} MTBE, 30 °C) and 5.6–8.7 mg MTBE g dry weight^{-1} h^{-1} ($\sim$ 80 mg l^{-1} MTBE, 30 °C) after growth on hexane and after induction with TBA, respectively. The calculated specific TBA degradation rate is 25.4 mg TBA g dry weight^{-1} h^{-1} ($\sim$ 220 mg l^{-1} TBA). In addition, yield coefficients of 0.18 mg dry weight mg MTBE^{-1} and 0.36 mg dry weight mg TBA^{-1} have been reported. These specific degradation rates are generally lower than those reported for bacterial cultures (Sect. 2.1.1).

2.2 Anaerobic Degradation

Initially, MTBE was considered recalcitrant under anaerobic conditions. However, a few studies have indicated MTBE-degradation under methanogenic [15, 78], sulfate-reducing [79] and iron-reducing conditions [54, 80]. In general, results on anaerobic conditions that support degradation of MTBE or TBA are controversial, as reviewed by Fischer et al. [81] and Pruden et al. [54]. In addition, in most experiments, the bacterial cultures capable of anaerobic MTBE-degradation have not been identified. Fischer et al. [81] report anaerobic degradation in 4 out of 30 batch microcosms under sulphate- and nitrate-reducing conditions, inoculated with aquifer material from three MTBE-contaminated groundwater wells at Leuna, a former refinery site in Germany. However, the lag time was 3 months and reported degradation rates of MTBE were very low. Moreover, benzene (260 μg l^{-1}), a common co-contaminant of MTBE, was observed to inhibit anaerobic MTBE-degradation. The first enrichment of a mixed bacterial consortium capable of converting MTBE to TBA under iron reducing conditions was recently reported by Pruden et al. [54]. The authors report several δ-Proteobacteria (uncultered strains) to be present in the bacterial consortium. Anaerobic specific degradation rates were significantly lower than rates obtained in aerobic studies, i.e., 1.25×10^{-3} mg MTBE g VSS^{-1} h^{-1}.

3
Aerobic MTBE/TBA Degradation Pathways

Many MTBE-degrading bacterial cultures listed in Tables 1 and 2 have been used to elucidate the degradation pathway of MTBE. An overview of results obtained in selected literature is schematically summarized in Figs 1 and 2. A distinction has been made between the upper pathway (MTBE to HIBA) and lower pathway (HIBA to CO_2). Recently, the enzymes and genes that are responsible for MTBE-degradation have been reviewed by Müller et al. [82] and Ferreira et al. [83], respectively.

3.1
Degradation Pathway from MTBE to HIBA (Upper Pathway)

In brief, MTBE is degraded to HIBA (2-hydroxy-isobutyric acid), through TBA (*tert*-butyl alcohol), with production of formaldehyde. On the other hand, formation of *tert*-butyl formate (TBF) and formate from MTBE has been reported as well [57, 75]. The formation of TBF has been mainly associated with bacterial cultures as *M. austroafricanum* en *M. vaccae* who seem to use the same monooxygenase for degradation of MTBE and TBA [84]. Further intermediates of MTBE degradation to HIBA are depicted in Fig. 1, accompanied with selected literature sources that have reported these intermediates. Formaldehyde production during MTBE oxidation might contribute to the slow and inefficient growth of MTBE-utilizing organisms on MTBE, due to the toxic effect of formaldehyde on bacterial growth [84]. Most of the genes responsible for MTBE degradation to TBA have been found in co-metabolic organisms, as for Chauvaux et al. [49] (gene cluster) and Hernandez-Perez et al. [63] (cytochrome P450) for *R. ruber* IFP 2001. Recently, Ferreira et al. [34] reported the first cloning and characterization of genes involved in growth on MTBE, in *M. austroafricanum* IFP 2012. The gene cluster identified was involved in degradation of MPD (2-methyl 1,2-propanediol) to HIBAL (2-hydroxy-2-methylpropanal) and HIBAL to HIBA (Fig. 1).

3.2
Degradation Pathway from HIBA to CO_2 (Lower Pathway)

For further degradation of HIBA to CO_2 and bacterial biomass, three different pathways have been described by Steffan et al. [58]. Intermediates involved in all three suggested degradation pathways are given in Fig. 2. Recently, the enzyme and coding gene responsible for degradation of HIBA by *Ideonella* strains L108 and L10 as well as by the closely related TBA-degrading *B. cepacia* CIP I-2052 [24] have been identified [25]. The enzyme identified, a cobalt-dependent isobutyryl-coenzyme A mutase (ICM), converted 2-hydroxyisobutyryl-CoA to 3-hydroxyisobutyryl-CoA, as depicted in Fig. 2.

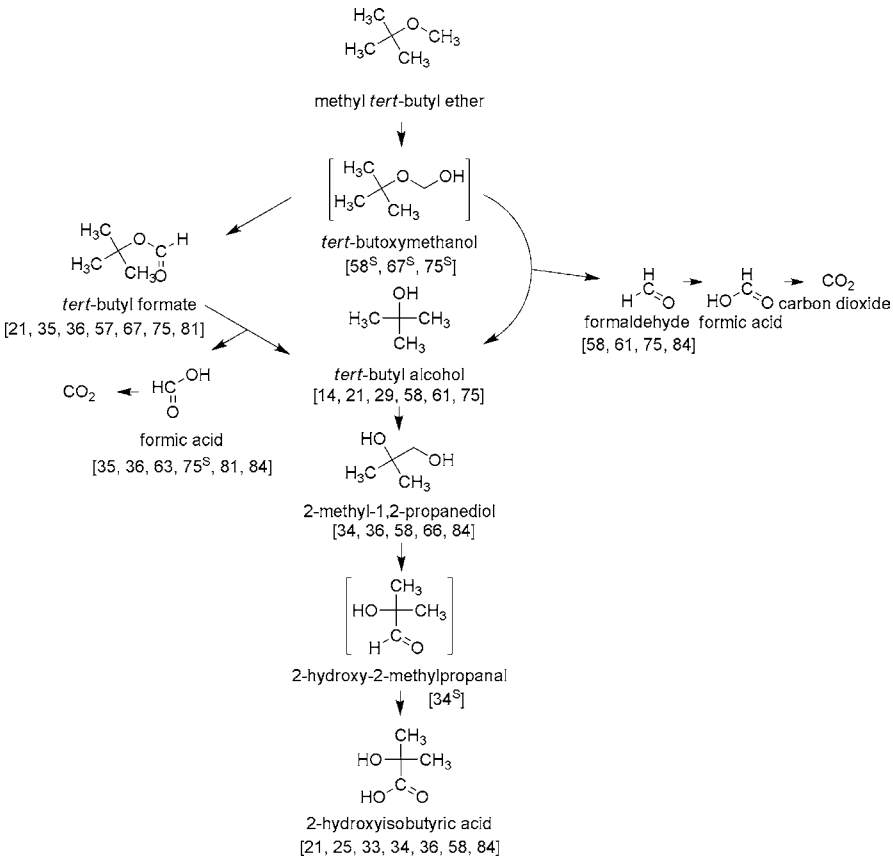

Fig. 1 Reported aerobic biodegradation pathways to transform MTBE/TBA into HIBA (upper pathway) ^{S}: Suggested but not detected

The authors pointed out that the cobalt-dependency of this enzyme may explain why several axenic bacterial cultures require Co for degradation of TBA and HIBA [21, 24, 25, 60]. Most remarkably, the same gene (nearly 100% identity) was found in the finished genome of *M. petroleiphilum* PM1 [22], suggesting a horizontal gene transfer between both cultures.

4 Biostimulation versus Bioaugmentation

The existence of MTBE/TBA-degrading microorganisms has been described earlier in this chapter. However, in order to be of help for bioremediation purposes, the microorganisms need to be active under specific environmental conditions. When considering contaminated groundwater, the ideal situation

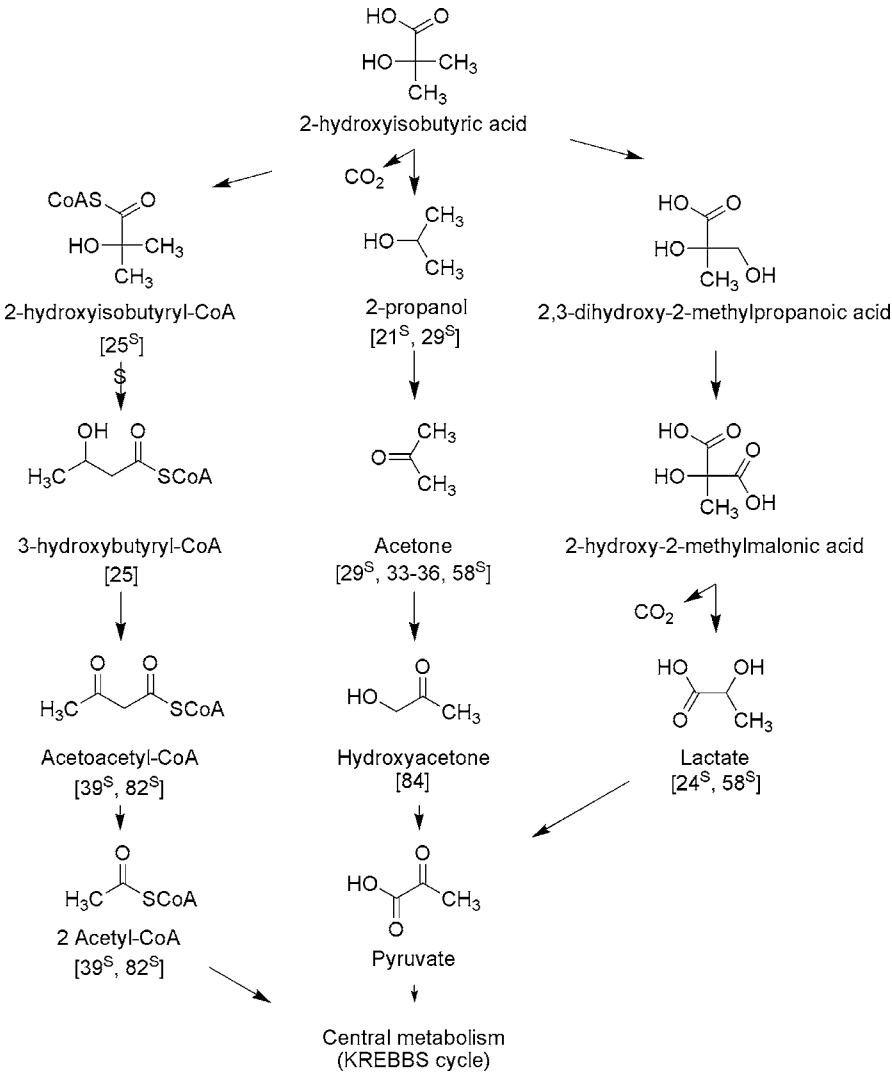

Fig. 2 Reported aerobic biodegradation pathways to convert HIBA to CO_2 (lower pathway) ^{S}: Suggested but not detected, S: Carbon-skeleton rearranging reaction

would be biodegradation of MTBE/TBA under in situ conditions by microorganisms present is the aquifer. This refers to natural attenuation processes, where contaminants released in the environment are degraded by naturally occurring processes without any active human interference. Generally, the in situ dissolved oxygen concentration is limited, leaving anaerobic processes as the only possibility for bioremediation. Indications for anaerobic degradation have been reported, but are associated with very low degradation rates

(see Sect. 2.2). As such, natural attenuation is often insufficient to control rapidly migrating MTBE/TBA groundwater contamination plumes.

Human intervention to stimulate MTBE/TBA-degradation is therefore required at many contaminated sites for a faster remediation (in situ or ex situ). Biostimulation of the indigenous microbiota may include addition of oxygen, nutrients (N, P) and/or co-substrates. The determining factor for success of biostimulation is the presence of indigenous MTBE/TBA-degrading bacteria at the site. However, although many MTBE-degrading bacterial cultures have been reported and enriched from MTBE-contaminated soil, not all soils contain indigenous bacteria that are capable of MTBE-degradation. For example, Moreels et al. [85] examined the biological MTBE-degradation potential at different sites in Belgium. Based on long-term lab-scale microcosm tests, in only one out of five MTBE-contaminated soil samples was the indigenous microbial population found to be able to biodegrade MTBE. Also, two uncontaminated soils did not show a MTBE-degradation capacity. From these results it was concluded that biostimulation is only feasible at a limited number of sites. The soil samples used in this study were collected in 2001. In 2006, soil samples of three additional MTBE-contaminated locations in Belgium were collected and examined (unpublished data). Two sites showed a potential to degrade MTBE indicating that an intrinsic aerobic MTBE biodegradation potential is more often present at Belgian gasoline/MTBE-contaminated sites than estimated based on the results of the first experiments. This could be due to longer exposure, with possible acclimation, of indigenous microbial communities to MTBE. A better characterization of MTBE-contamination these days also allows to sample soil in hot-spots of MTBE and older MTBE-pollution. But still, for the third site no convincing MTBE-biodegradation potential was found.

The absence of a MTBE/TBA-biodegradation potential at some contaminated sites implies that injection of oxygen and substrates to stimulate the present bacteria will not result in a decrease of the MTBE-concentration at all locations. This provides opportunities for bioaugmentation, where bacteria, specially selected for their MTBE-degradation capacities, are grown in the lab and added to soils together with oxygen and the necessary substrates/nutrients.

In addition, in situ biodegradation rates can be much slower than rates in soils bioaugmented with special MTBE-acclimated bacterial cultures. Salanitro et al. [86] report both biostimulation by pure oxygen sparging and bioaugmentation with axenic culture MC-100 at the USN National Test Site at Port Hueneme, in Oxnard, California. MTBE biodegradation was observed even in the absence of bioaugmentation, but bioaugmented plots showed higher MTBE-removal rates and a greater extent of TBA degradation.

In all cases, the success of bioaugmentation relies on the survival of augmented biomass. However, oxygen supply is also important. At the U.S. National Environmental Technology Test Site, at Port Hueneme, California,

biostimulation by sparging with oxygen or air and bioaugmentation with MTBE-degrading *M. petroleiphilum* PM1 indicated that rates of MTBE removal were similar in both inoculated and uninoculated plots amended with oxygen [87]. The limiting factor to in situ MTBE-degradation seemed to be oxygen delivery, mainly to the deeper zones.

5
Possibilities of Bioremediation for MTBE/TBA Contaminated Groundwater

For remediation of contaminated groundwater, biological processes can be applied in both in situ as well as ex situ remediation techniques. In both cases, the microorganisms demand control of various parameters in order to degrade MTBE/TBA. Most importantly, pH, dissolved oxygen and temperature dictate bacterial growth and thus bioremediation success, beside the presence and survival of MTBE-acclimated MTBE/TBA-degraders.

5.1
Ex situ Remediation in Bioreactors

A commonly used ex situ bioremediation technology is referred to as pump-and-treat, where groundwater is extracted from the soil and treated in a controlled environment. Ex situ treatment is a commonly used technology to contain and treat BTEX-polluted groundwater at gas stations via air stripping and activated carbon. Although not optimal for MTBE/TBA, as mentioned before, this technique is often used when MTBE/TBA is present. Pumping of groundwater followed by ex situ MTBE treatment can be used for example for source zone clean-up and for sites where in situ treatment is not possible or is inefficient. Also, when urgent measures are required to prevent contamination plumes from further expansion, pump-and-treat can be used while working out other remediation options [88]. The treatment of the pumped groundwater can be optimized by including a bioreactor in the treatment chain. This reactor may be colonized by microorganisms present in the groundwater, or may be inoculated by specialized MTBE/TBA-degraders cultivated in laboratory conditions.

Ex situ bioremediation relies on management of groundwater flow, with optimization of retention times in the bioreactor, biomass retention in bioreactors, temperature and pH control and most importantly, maintaining aerobic conditions. In addition, co-contaminants as BTEX compounds or iron, Fe(III), can jeopardize aerobic degradation of MTBE, both in and ex situ.

5.1.1 Bioreactor Types

Two fundamentally different types of bioreactor setups can be distinguished. In the first type of reactors, MTBE-degradation occurs by bacteria in suspension in continuously stirred tank reactors (CSTR) (Table 6). An obvious advantage of this setup is the optimal mixing of MTBE-degrading biomass, contaminants and oxygen, reducing transport limitations to a minimum. However, specialized adaptations are required to prevent washout of biomass from the reactor. Three different methods exist.

- In a membrane bioreactor (MBR), biomass is separated from cleaned groundwater by special membranes [26, 55, 89]. In general, high pressure is used for effluent filtration.
  - A special type of membrane bioreactor is called a porous pot reactor (PPR) [19, 37, 90, 91]. A 0.48-cm-thick filter grade porous polyethylene membrane is used in all applications to separate biomass from effluent. The same format is used to enrich bacterial cultures in continuous suspension systems, both under aerobic and anaerobic conditions [43, 54].
  - Another special type of membrane bioreactor, a biomass concentrator reactor (BCR) [42, 56] uses gravity to filtrate the suspension containing bacterial mass and groundwater.
- A second example of suspension reactors is a sequencing batch reactor (SBR). This reactor is operated in different sequential stages to optimize MTBE-removal and biomass retention [92]. The same setup was used to enrich TBA-degrading bacterial consortia [20, 93].

The second reactor setup type is commonly referred to as a biofilm bioreactor. This reactor is filled with packing material onto which a microbial biofilm is allowed to develop and biomass retention is achieved without the need for special membranes. Selected examples of this second type of reactor are provided in Table 7.

Again, different types of biofilm bioreactors can be distinguished. The differences here are the direction of contaminated groundwater flow in the reactor and the influent flow rate which can cause expansion of the support material:

- Upflow fixed bed reactors or submerged biofilters (SBF) have upward groundwater flow, the packing material is submerged under water and no bed expansion is obtained [45, 94–97].
- Up-flow fluidized bed reactors (FBR) use increased groundwater inflow to achieve support material expansion, typically 125–150% of packed bed volume [26, 38, 98–101].
- Biotrickling filters (BTF) have a complete different setup, as contaminated groundwater is trickled down through the reactor [53]. An immediate advantage is that extra oxygen supply is not necessary.

Numerous examples of possible packing materials that allow bacterial immobilization exist, examples include both natural materials as soil [105], lava rock [18, 50], expanded clay [94, 97], sand [95, 98], natural fibers [102] and aquifer material [106] and synthetic materials as perlite [53, 107, 108], granular activated carbon (GAC) [26, 38, 100, 101], glass beads or rings [96] and polypropylene [18, 107], apart from many commercial applications.

Biomass retention in immobilized systems relies on a balance between attrition of bacteria because of high shear forces due to influent flow and the growth of new biomass. Therefore, the main difficulty in treating MTBE-contaminated groundwater with fixed film bioreactors is the low reported biomass yield with growth on MTBE in continuous systems, typically 0.08–0.26 mg TSS mg $MTBE^{-1}$ [26, 42, 55, 56].

The biotrickling filter setup is also used to bioremediate MTBE in the gaseous phase [18, 50, 103, 104]. This setup is often used for the off-gas treatment of air stripping systems for remediation of groundwater contaminated with MTBE [109]. Lindberg et al. [92] report that the SBR setup is a good pretreatment reactor especially at fluctuating influent conditions, but not good at treating low MTBE concentrations due to a possible net biomass washout.

5.1.2 Lab-Scale Simulations of Bioreactors

The performance of bioreactors can in a first stage be simulated in continuous up-flow columns. Most of the biofilm bioreactors in Table 7 are lab-scale simulations in a column setup [26, 38, 45, 53, 94–97, 100–102]. A column experiment that was set up to evaluate the performance of a bioreactor inoculated with the VITO consortium [85] will be described as an example [95]. Here, two Plexiglas columns (diameter 4 cm, length 50 cm) were filled with filter sand. One of these columns was inoculated with the VITO consortium ($\pm 6 \times 10^{10}$ cfu/column). Artificial contaminated groundwater, comprising diluted minimal mineral medium polluted with MTBE (10–40 mg l^{-1}) only or in combination with BTEX-compounds (5 mg l^{-1}), was pumped through the columns. The hydraulic retention time (HRT) in the columns was initially 1 day. Along the columns, sampling points were present at different distances from the entrance (bottom of the columns), which allowed the determination of pollutant concentration profiles as well as the evolution of pH en dissolved oxygen concentration (DO) along the columns. Extra oxygen was added to the aerobic column systems via a diluted solution of hydrogen peroxide using a syringe pump (final concentration < 0.01%). The columns were operated for more than 9 months, with continuous MTBE inflow.

During the first test period of the column experiment, high concentrations of MTBE (40 mg l^{-1}) without BTEX were used in the influent of the columns. Within 1 week after the inoculation, MTBE-degrading activity was

Table 6 Selection of bioreactors with MTBE-degradation in suspension: MBR, BCR, and SBR

Reactor type	Scale	Inoculum	Remarks	Refs.
MBR	Lab	*H. flava* ENV735		[26]
MBR	Lab	*H. flava* ENV735		[89]
PPR	Lab	MTBE-degrading mixed culture [43]		[19, 37]
MBR	Pilot	MTBE-degrading consortium [43] and activated sludge		[55]
SBR	Lab	Consortium		[92]
PPR	Lab	MTBE-, BTEX-degrading consortium Pruden 2001		[90]
BCR	Field	MTBE-degrading enrichment from membrane bioreactor [55] and from two porous pot reactors [43] plus activated sludge	1 m^3 pilot model, 8 m^3 field scale model	[42, 56]
PPR	Lab	MTBE-, BTEX- and ethanol degrading consortium contaminated groundwater in continuous	Removal MTBE, TBA, BTEX, PAH from culture and crude-oil degrading culture	[91]

SBR: Sequencing batch reactor,
MBR: Membrane bioreactor,
BCR: biomass concentrator reactor,
PPR: Porous pot reactor

Table 7 Selection of bioreactors with MTBE-degradation in biofilm: FBR, PPR, SBF, and BF

Reactor type	Scale	Inoculum	Remarks	Refs.
SBF	Lab	Activated sludge enrichment	Groundwater treatment	[45]
FBR	Lab	*Hydrogenophaga flava* ENV735	Groundwater treatment	[26]
FBR	Field	Consortium	Groundwater treatment	[98, 99]
SBF	Lab	MTBE/ETBE/TAME-degrading consortium enriched from gasoline- contaminated soil	Groundwater treatment	[96]
FBR	Lab- and field scale (comparison)	Various sources including gasoline-contaminated soil and water samples	Groundwater treatment	[100]
FBR	Lab	Consortium operating MBR and consortium University of Cincinnati	Groundwater treatment	[101]
SBF	lab- and 20 times up-scaled lab-scale	MTBE-degrading consortium	Groundwater treatment	[94, 97]
FBR	Lab	MTBE-degrading consortium [43]	Groundwater treatment	[38]
SBF	Lab	Consortium [45]	Groundwater treatment	[102]
SBF	Lab	VITO consortium	Groundwater treatment	[95]
BTF	Lab	Consortium biotrickling filter [18]	Groundwater treatment	[53]
BTF	Pilot	Self-seed	MTBE removal from gas phase	[28, 103]
BTF	Lab	Ground water and aquifer material from two field sites	MTBE removal from gas phase	[18, 50]
BTF	Lab	MTBE-degrading enrichment from wastewater treatment plant	MTBE removal from gas phase	[104]

SBF: Submerged biofilter or up-flow fixed/packed bed bioreactor; FBR: up-flow fluidized bed reactor; BTF: biotrickling filter; ns: Not specified

observed in the inoculated column, where the concentration of MTBE was reduced from 40 mg l^{-1} to less than 20 mg l^{-1}. Further reduction of the MTBE-concentration was inhibited by depletion of the dissolved oxygen. During the second test period, the MTBE-concentration in the influent was lowered to 10 mg l^{-1}, which resulted in a more complete degradation of MTBE, as is shown in Fig. 3. In a third test period, some BTEX-compounds (mainly benzene) were added to the influent. A delay of the MTBE-degradation was seen, during which the BTEX-compounds were degraded, consuming the available oxygen. Once the BTEX-compounds were degraded and sufficient oxygen remained or was additionally provided, the MTBE-degradation started.

The results of the column tests clearly show that the inoculated consortium has potential for enhancing MTBE-biodegradation in continuous flow systems such as bioreactors. Within a week after the inoculation, MTBE-degradation was observed, and a single inoculation event led to a MTBE-degradation over more than 9 months.

Biofilm bioreactors (attached growth) are relatively inexpensive, but biomass clogging and support media acidification (nitrification and others) may result in the deterioration of their performance and might limit their applicability. Another basic limitation lies in the availability of enough surface area on the support for microbial attachment and, the difficulties associated with enriching bacterial strains acclimated to attached growth and high shear stress. The ability of MTBE/TBA-degraders to attach to the support medium is an advantage when used in a biofilm bioreactor. Not all MTBE-degrading bacterial cultures are suitable for inoculation in biofilm bioreactor systems. Vainberg et al. [101] report that *H. flava* ENV735 did not readily attach to the GAC support medium to be used in the intended FBR, so other mixed and pure cultures had to be found. A number of bacterial cultures were screened for their ability to attach to the side of glass shake flasks during growth. On the other hand, Pruden et al. [38] report that although the seed culture of

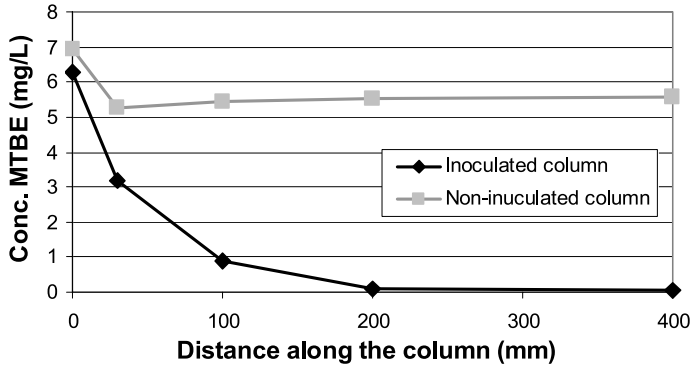

Fig. 3 Performance of a lab-scale fixed-bed bioreactor simulation with and without an MTBE-degrading inoculum (VITO consortium)

a FBR reactor was enriched under suspended growth conditions in a porous pot reactor, it was able to adapt to attached growth conditions without any notable delay. In order to select microorganisms capable of attachment to support materials, the consortium [18] used in several column applications packed with various support systems as soil [105] and expanded perlite [53], was grown attached to the exterior surface of a silicon tubing while a small air stream was passed through the tubing.

Startup times ranging from 1 week [95] up to 6 months, have been reported for biofilm reactors before low and stable effluent concentrations are achieved [26, 37, 38, 42, 94, 100, 101]. Based on lab-scale biofilm bioreactor studies problems associated with aeration [96], temperature, loading rates, biomass control [101] and pH have been reported and require special attention during the design of full-scale FBR systems. Significant pH decreases, mainly due to the nitrification of ammonia, have been observed in column systems [97, 105]. In addition, pH increases can significantly impair reactor performance [37].

Relatively long reaction times may be required in biofilm bioreactors to obtain the required MTBE-effluent concentrations. For example, Kharoune et al. [96] report a 98% removal of MTBE with a 24 h HRT, while the performance declined significantly with a HRT of 13 h. However, Zein et al. [42] reported that for the BCR, the important variables affecting performance where sludge age and high biomass solids, not HRT. In general, lower effluent MTBE concentrations can be achieved with membrane bioreactors, as transport limitations of contaminants in bacterial biofilms are circumvented [42, 55, 56]. On the other hand, Pruden et al. [38] report lower TBA effluent concentrations obtained with the fluidized bed bioreactor setup.

5.1.3 Field Applications

Not many field applications using the above-mentioned lab-scale reactors have been reported to date. Zein et al. [56] report the application of their biomass concentrator reactor, derived from the porous pot design of Wilson et al. [19], at the former gas station site at Pascoag, RI, USA. The reactor was capable of remediation of various contaminants, including MTBE, TBA, TBF, BTEX, TAME, DIPE, TAA, methanol and acetone, by pump-and-treat. Significantly lower effluent concentrations than the effluent of an air stripper that shared the contaminated water feed were achieved. However, fouling of the polyethylene membranes due to biological biofilms and iron precipitation required extra maintenance of the bioremediation technology. Zein et al. [56] pointed out that ground water temperature to as low as 13 °C did not decrease reactor performance whereas O'Connell et al. [99] reported that temperatures below 16 °C compromised reactor performance. Stringfellow et al. [100] found that in both field and laboratory studies, one reactor grew

MTBE-degrading bacteria spontaneously, while another reactor did not. The authors concluded that the success of MTBE treatment is dependent on more than the introduction of a specific MTBE-degrading culture.

5.2 In situ Remediation

In situ remediation technologies for groundwater refer to techniques where contaminated groundwater is treated in the subsurface by biological and/or physicochemical processes (including chemical oxidation, photocatalysis, stripping and soil vapor extraction). Since no pumping of groundwater to the surface is required, in situ remediation technologies are more passive than ex-situ techniques. This chapter focuses only on bioremediation techniques, which can be applied separately or as "polishing steps" in combination with other in situ and ex situ remediation actions.

5.2.1 Types of in situ Bioremediation

Bioremediation strategies include natural attenuation, biostimulation as well bioaugmentation. The latter two can be applied in different ways, as shown in Fig. 4.

A first choice may be to remove all the pollutants from the groundwater, which is feasible and preferred for small contaminant plumes. Another approach is to control the pollution by preventing further migration of the con-

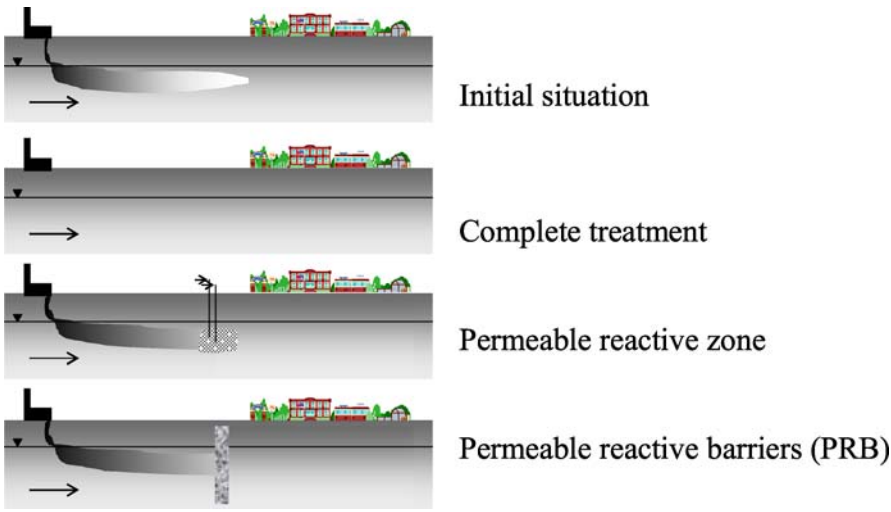

Fig. 4 Different approaches for in situ biostimulation/bioaugmentation

taminated water to uncontaminated aquifers, where they might pose a threat to for instance drinking water reserves or extraction points. This can be realized by local stimulation of MTBE/TBA-biodegradation in an area perpendicular to the direction of groundwater flow [86, 110]. When aquifer material is replaced by other filling material, to increase for instance the permeability, this area is called a permeable reactive barrier. Without replacement of aquifer material, the term permeable reactive zone is used. Both are passive remediation applications as MTBE/TBA is removed from the groundwater while it flows through the reactive zone/barrier under influence of the naturally present hydraulic gradient.

In situ bioremediation relies on the presence (or successful addition) and survival of suitable microorganisms able to degrade MTBE and/or TBA. As biodegradation rates for efficient bioremediation are mainly observed under aerobic conditions, the addition of a sufficient amount of oxygen to the subsurface is crucial. The following techniques have been applied for introducing oxygen in the subsurface to enhance MTBE/TBA-biodegradation:

- Airsparging [7].
- Slow oxygen releasing compounds such as MgO_2 [111, 112] and CaO_2 [107].
- Oxygen diffusers [113, 114].
- Recirculation of groundwater [112, 115] which is especially interesting when in situ bioremediation is combined with pump-and-treat.
- Addition of hydrogen peroxide (H_2O_2) [115]. However, with addition of higher concentrations (2%), chemical oxidation can not be excluded.
- In situ electrolysis [116].

5.2.2 Lab-scale Simulations of in situ Remediation Techniques

Lab-scale simulations to verify the presence of an indigenous MTBE/TBA biodegradation potential at test sites and to determine the influence of additives on the degradation, are typically performed via batch degradation experiments (microcosm tests). The microcosms described by Moreels [17] consisted of 120-ml glass vials containing 6 g aquifer material and 54 ml of a defined simulated groundwater medium. The systems were incubated statically at 20 °C. In time, aqueous sub-samples were taken from the incubated microcosms for chemical analyses, and for pH en dissolved oxygen concentration determinations. Mineral nutrients, nitrogen (N) and phosphor (P), were added as NH_4NO_3 and $Na_2HPO_4 \cdot 2H_2O$, respectively, until a C/N/P ratio of 100/10/1 was reached. From time to time, oxygen was injected in the headspace to prevent the dissolved oxygen concentration to decrease below 5 mg l^{-1}. This test procedure was applied to determine the MTBE-biodegradation potential at a specific Flemish contaminated site where the groundwater contained up to 24 000 μg l^{-1} MTBE [88]. Different aerobic and anaerobic test conditions were set up: an abiotic control, a condition without

amendments, a condition with nutrients and several conditions with addition of nutrients and a carbon source (benzene, TBA, 2-propanol, propane, *n*-heptane, MSBE, pyruvate or yeast extract). No degradation of MTBE was obtained during an experimental time of more than 2 years. These results suggested that (I) no MTBE-biodegrading bacteria were present among the indigenous microorganisms at the site, or (II) that certain factors where inhibiting the MTBE-degradation by these microorganisms.

The same microcosm was subsequently used to simulate in situ bioaugmentation. After about 1.5 years of incubation, an aliquot of the MTBE-degrading VITO consortium [17], containing 1.0×10^8 cells, was added. The MTBE-concentration subsequently decreased within a few weeks from 15 mg l^{-1} to less than 10 μg l^{-1} (Fig. 5). Re-addition of MTBE resulted again in MTBE biodegradation. No decreases in the MTBE-concentration were observed in the non-bioaugmented control or the abiotic control. The inoculated batch tests continued degrading MTBE during more than 1 year. It was concluded that no inhibiting factors were preventing MTBE-degradation at the site, but rather the lack of suitable microorganisms.

As for biofilm bioreactors, lab-scale simulations of reactive zones for bioremediation of groundwater contaminated with MTBE and TBA can be simulated in continuous up-flow columns [107, 108]. Liu et al. [107] report a lab-scale simulation of two reactive barriers for bioremediation of MTBE using two upflow fixed-bed columns. The first column, simulating an oxygen-releasing biobarrier layer, contained oxygen-releasing calcium peroxide and the inorganic salts potassium dihydrogen phosphate and ammonium sulphate to provide nutrients for the immobilized microorganisms in the second column, and at the same time to act as a pH buffer. The second column, representing a biodegradation layer, contained an immobilized MTBE-degrading bacterial consortium. With a high influent concentration of MTBE (160 mg l^{-1}) and a high HRT of 80 h, the authors reported a relatively low MTBE-removal efficiency of 50% and TBA effluent concentration

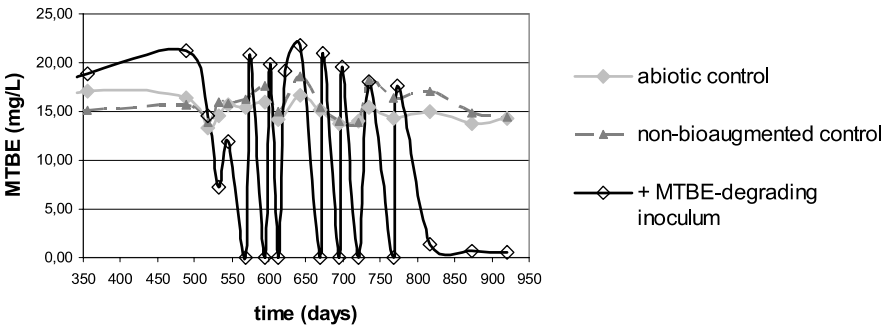

Fig. 5 Bioaugmentation of an aquifer without an indigenous MTBE-biodegrading potential—lab-scale simulation (VITO consortium was added at day 500)

Table 8 Selection of field application of in situ bioremediation techniques to treat MTBE/TBA-contaminated groundwater

Remediation type	MTBE/TBA-degraders involved	Additives (others than microorganisms)	Refs.
Biostimulation combined with dual phase extraction	Laboratory-cultured indigenous bacteria	Nutrients, oxygen (vacuum extraction, recirculation, MgO_2)	[112]
Biostimulation	Ex-situ enriched indigenous microorganisms	Nutrients, Oxygen (recirculation of groundwater, H_2O_2)	[115]
Biostimulation	Indigenous population	Oxygen (oxygen diffusers)	[113]
Biostimulation & bioaugmentation	*M. petroleiphilum* PM1	Oxygen (air sparging oxygen gas)	[87]
Biostimulation & bioaugmentation	Propane oxidizing bacterium ENV425	Oxygen and propane	[118]
Biostimulation & bioaugmentation in a reactive zone	Indigenous population Consortium MC-100	oxygen (oxygen gas or air injection)	[7]
Biostimulation	Indigenous population	Oxygen (in situ electrolysis)	[116]
Biostimulation in a reactive zone	Indigenous population	Oxygen (oxygen diffusers)	[114]
Biostimulation & Bioaugmentation	Bacterial consortium MC-100	Oxygen (O_2 gas injection)	[86]
Biostimulation	Indigenous population	Oxygen release compounds application (ORC), MgO_2	[111]

of 10 mg l^{-1}. Suggested measures to improve the performance of the proposed barrier are the use of other acclimated microorganisms or increased reaction times (HRT).

5.2.3 Field Applications

In 2004, the U.S. Environmental Protection Agency reported more than 50 pilot and full-scale in situ bioremediation projects (EPA report). In Europe, the number of reported field applications is limited. In general, biostimulation and bioaugmentation are complementary, but reported lag times are shorter, and MTBE-degradation rates can be higher with bioaugmentation [86], although exceptions are reported as well [87]. Some characteristics of a selection of field implementations of in situ bioremediation projects is given in Table 8. As reported above for lab-scale bioreactor applications, oxygen supply is considered a determining factor for bioremediation success [117]. In regard to co-contaminants, Königsberg et al. [111] report that MTBE degradation only occurred after BTEX concentrations were significantly reduced. In a bioaugmentation project using propane-oxidizing bacterium strain ENV425 [58], indications were reported that native microorganisms increased in abundance or became dominant over injected ENV425 [118].

6 Conclusions

Biological processes offer interesting opportunities for remediation of groundwater contaminated with MTBE/TBA. Up to now, mainly lab-scale simulation of ex situ and in situ remediation technologies have been described, while reports of applications in the field are still limited, especially in Europe. A growing number of field data are expected the coming years.

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Adsorption and Abiotic Degradation of Methyl *tert*-Butyl Ether (MTBE)

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Abstract This chapter explores the role of abiotic reactions such as acid catalysis (hydrolysis) as well as the adsorption of methyl *tert*-butyl ether (MTBE) and other fuel oxygenates in environmental issues as the remediation of these substances is notoriously difficult. First of all, these methods are briefly classified with other abiotic technologies. The suitability of hydrolysis and adsorption for the remediation of water contaminated by fuel oxygenates is then discussed in detail, with information being provided about the principle of the reactions, potential catalysts and sorbents, limitations of the reactions, and practical implications. To conclude, the possible application of hydrolysis and adsorption in combination with other remediation techniques is also examined.

Keywords Adsorption · Acid catalysis · Hydrolysis · Abiotic remediation

Abbreviations

BTEX	Benzene, toluene, ethylbenzene, xylene
DIPE	Diisopropyl ether
ETBE	Ethyl *tert*-butyl ether
GAC	Granular activated carbon
MTBE	Methyl *tert*-butyl ether
NOM	Natural organic matter
PRB	Permeable reactive barrier
TAA	*tert*-Amyl alcohol
TAME	*tert*-Amyl methyl ether
TBA	*tert*-Butyl alcohol
UV	Ultraviolet radiation

Symbols

A	Adsorption potential [$J\,mol^{-1}$]
b	Empirical exponent (dimensionless)
β	Heterogeneity coefficient (dimensionless)
c	Aqueous phase concentration [$mg\,L^{-1}$]
c_e	Equilibrium concentration [$mg\,L^{-1}$]
c_i	Initial concentration [$mg\,L^{-1}$]
E	Adsorption potential at which the sorbed-phase concentration is $1/e(= 36.8\%)$ of the maximum capacity [$J\,mol^{-1}$]
H	Air–water partition coefficient (Henry's Law coefficient) (dimensionless)
k	Affinity coefficient (dimensions depending on the equation)
K_{OC}	Organic carbon coefficient [$L\,kg^{-1}$]
K_{OW}	Octanol/water partition coefficient (dimensionless)
n	Exponent (dimensionless)
q	Solid phase concentration [$mg\,g^{-1}$]
q_m	Maximum adsorption capacity [$mg\,g^{-1}$]
R	Ideal gas constant ($8.314\,J\,mol^{-1}\,K^{-1}$)
ρ	Density of the sorbate [$kg\,L^{-1}$]
S	Aqueous solubility of the sorbate [$mg\,L^{-1}$]
T	Temperature [K]
V_m	Micropore volume of the adsorbent [$L\,kg^{-1}$]

1 Introduction

Ever since oxygenates began to be substituted for leaded fuel additives, their positive environmental effects (i.e. less toxic exhaust) have been offset by an increasing number of contaminated sites. Due to the physical and chemical properties of methyl *tert*-butyl ether (MTBE) associated with its low biodegradability, removing the most common fuel oxygenate from contaminated water is still a major challenge. This chapter summarizes abiotic remediation technologies and focuses on acid catalysis and the adsorption of MTBE and other fuel oxygenates as alternative strategies for the clean-up of contaminated water.

2 Critical Properties Influencing the Environmental Behaviour and Remediation of MTBE and Other Fuel Oxygenates

The physicochemical properties of fuel oxygenates closely affect not only their environmental behaviour but also the remediation technology to be used. In contaminated groundwater, the ratio between the rates of water and oxygenate movement is described by the retardation factor. This provides a rough indication of the contaminant distribution in the environment and also helps in choosing the most suitable remediation strategy. The retardation may be influenced by several soil and contaminant properties; those relevant for fuel oxygenates are listed in Table 1 [1–17]. By way of clarification, these critical properties of oxygenates are compared with those of benzene, a harmful gasoline compound frequently present in MTBE-contaminated groundwater.

Water solubility (the measure of a compound's ability to dissolve in water) has a strong influence on the transport capacity of oxygenates underground. Compounds with very high water solubility such as MTBE, ethyl *tert*-butyl ether (ETBE) and *tert*-amyl methyl ether (TAME) (20–62 $g\,L^{-1}$) or which are infinitely soluble in water (e.g. *tert*-butyl alcohol, TBA) are much more widely distributed in the environment than BTEX compounds.

Adsorption onto soil particles is characterized by the organic carbon coefficient (K_{OC}). This parameter varies greatly depending on the carbon content of the soil. Nevertheless, MTBE shows a lower tendency to adhere to soil compared to benzene. These characteristics of MTBE are discussed in detail in Sect. 5 in connection with the suitability of adsorption-based techniques.

The octanol/water partition coefficient ($\log K_{OW}$) is an indication of accumulation in adipose tissue. Compared to benzene, fuel oxygenates tend to accumulate less, as their octanol/water partition coefficient is up to six times lower than that of benzene.

The at least 50% higher vapour pressure of MTBE, ETBE and diisopropyl ether (DIPE) compared to benzene describes the stronger volatilization tendency of these fuel oxygenates. Therefore these substances are amenable to soil vapour extraction.

Another important parameter for choosing a proper remediation method is the Henry's law coefficient (H). This parameter characterizes the partitioning of a substance between the water phase and the gas phase. Due to the lower value of this coefficient for MTBE (Table 1), remediation using technologies involving contaminant transfer from water to the gas phase (e.g. air stripping) is less efficient in comparison to benzene.

Biodegradability is also a crucial aspect regarding the fate and behaviour of fuel oxygenates in the environment. Ethers such as MTBE are characterized by stable ether bonding. In addition, access to the ether linkage is much harder because of the *tert*-butyl group [18]. Therefore the biodegradation

Table 1 Key physicochemical properties of MTBE and other fuel oxygenates compared with benzene [1–17]

	MTBE	ETBE	TAME	DIPE	TBA	Benzene
Sum formula	$C_5H_{12}O$	$C_6H_{14}O$	$C_6H_{14}O$	$C_6H_{14}O$	C_4H_9OH	C_6H_6
Density at 25 °C [g cm^{-3}]	0.744	0.73	0.77	0.736–0.7491	0.791	0.88
Water solubility at 25 °C [g L^{-1}]	43–54.3 35.5 (at 20 °C) 62.1 (at 5 °C)	~ 26	~ 20	2.04 (at 20 °C)	Infinitely soluble	1.78
log K_{OW} (–)	1.20	1.74	No data	1.52	0.35	2.13 1.56–2.15
log K_{OC} (–)	1.091 1.035 1.049	2.2 0.95	2.2 1.27	1.82 1.46	1.57	1.8–1.99 1.50–2.16
Henry's Law coefficient at 25 °C (–)	0.0292 0.030/0.03 0.022 0.0555 0.0117 (at 10 °C)	0.1087	0.05191	0.4075 0.195 0.2399	4.803×10^{-4} 4.864×10^{-4} 4.251×10^{-4} 5.927×10^{-4} 4.8×10^{-4}	0.2219
Vapour pressure at 25 °C [mm Hg]	245–256	152	68.3	149–151 (at 20 °C)	40–42	76 95.19

of these substances is less efficient than for other components (e.g. BTEX) present at MTBE-contaminated sites.

3 A Brief Overview: Abiotic Remediation Techniques

Apart from biodegradation, abiotic technologies are important alternatives for the remediation of water contaminated with MTBE and other fuel oxygenates. Various reports describe in detail the methods used for the remediation of groundwater, soil and drinking water, focusing on their performance, costs and effectiveness under site-specific conditions [19, 20]. A brief summary of abiotic remediation technologies is given in Table 2, with technologies grouped into accumulative and destructive. They include gas-based technologies (air sparging, air stripping), oxidation (using hydrogen peroxide, ozone, ultraviolet radiation, permanganate), adsorption (on activated carbon, zeolites and resins) and other strategies (soil vapour extraction, membrane separation and acid hydrolysis). The comments refer to their performance at the contaminated sites as well as to possible improvements.

Remediation strategies may involve the use of one or more technologies with an increase in overall efficiency (e.g. multi-phase extraction – a combination of soil vapour extraction and groundwater pump-and-treat). From those presented above, an overview of hydrolysis in acidic conditions and adsorption on different media is presented in the following sections.

4 Acid Catalysis of MTBE in Aqueous Solution

The acid catalysis of MTBE plays an important role in many different areas of application. The key aspect is the synthesis of MTBE using solid acid catalysts since it has become the most important fuel oxygenate in the world. As this reaction is reversible, the cleavage of MTBE is used to gain pure isobutene, a basic chemical required for various products. Furthermore, the hydrolysis of MTBE has been investigated regarding its role in environmental chemistry. Besides its prominence in analysis, the use of this reaction in the treatment of contaminated water is discussed.

Synthesis. During the production process, MTBE is formed by the reaction of isobutene and methanol via acidic catalysts (Eq. 1):

$$(CH_3)_2C = CH_2 + CH_3OH \rightleftarrows (CH_3)_3C - O - CH_3\,. \qquad (1)$$

These materials can be ion exchange resins, heteropoly acids, acidic zeolites as well as chemically modified zeolites [33]. The reversibility of this reaction leads to the conclusion that the cleavage of ethers is also possible

Table 2 Abiotic remediation technologies for MTBE

Process		Technology/ method	Comments
Destructive	Chemical oxidation	Fenton's reagent	Sensitive to pH variations [21] Significant improvement in MTBE degradation when combined with sonication [22] Cyclical regeneration of Fe^{2+} from Fe^{3+} and H_2O_2 [21]
		O_3/H_2O_2	Insignificant degradation only by O_3 oxidation, promising results with O_3/H_2O_2 combination [23, 24]
		UV/H_2O_2	Low degradation under UV irradiation (without H_2O_2), formation of hazardous by-products (e.g. formaldehyde) [25, 26] Degradation highly influenced by H_2O_2 and MTBE initial concentrations [27, 28]
		Permanganate	Causes slower oxidation than using O_3 or H_2O_2, but has a longer half-life in the environment [19] Possible precipitation of manganese dioxide [19]
Accumulative	Cross-media distribution (solid phase)	Adsorption	Insignificant on soil particles Can be affected by the competition from other compounds Preferentially on certain materials
	Cross-media distribution (gas phase)	Air sparging	Low efficiency due to low Henry's Law constant, increased air flow required [19] Off-gas treatment required
		Air stripping	Low efficiency due to low Henry's Law constant [18] High removal percentage only by high air/water ratio (high packed towers required) [29] Off-gas treatment required

Table 2 (continued)

Others	Acid hydrolysis	Use of solid acid catalysts positively tested at laboratory scale
	Soil vapour extraction	Amenable for ethers used as fuel oxygenates Off-gas treatment required
	Membrane separation	Satisfactory results at 30 °C [30] and good at 80 °C [31] but lower than BTEX (e.g. toluene) Efficiency strongly dependent on membrane characteristics but independent of gas-phase parameters [32] High efficiency (but very costly!) obtained in modular steps [18]

using acidic catalysts. This explains why besides the synthesis of MTBE the cleavage of this substance is of high importance in the production of pure isobutene (> 99.5%). A side reaction of isobutene with water leads to TBA formation [33].

Hydrolysis. The role of the hydrolysis of several fuel oxygenates during common static headspace analysis was investigated by Lin et al [34]. Samples with an initial concentration of 500 $\mu g\,L^{-1}$ of ether and a pH of 2 were heated for up to 150 min at 80 °C before analysis. After 30 min no degradation was observed, while after 150 min only DIPE showed no hydrolysis. The detection of the reaction products TBA, ethanol and *tert*-amyl alcohol (TAA) demonstrates the hydrolysis of TAME, ETBE and MTBE. The reactivity of the fuel oxygenates was TAME > ETBE > MTBE > DIPE. Moreover, it should be noted that preserved samples at pH 2 (with hydrochloric acid) that were adjusted to pH 7.0 before analysis yielded excellent recoveries [34]. Information about the influence of acid preservation during the analysis of MTBE is also given by Schumacher et al [35]. Preservation of the samples with sulfuric acid yielded recoveries of at least 94% after storage at 6 °C for four months. Other methods that do not include a heating step have not been sensitive to hydrolysis during analysis when samples have been preserved with acid. Consequently, preserving and preparing the sample prior to analysis is of major importance to prevent the ether used as fuel oxygenate being underestimated and the related alcohol, which is a reaction product of hydrolysis and biodegradation, being overestimated.

The next sections summarize several investigations into the role of hydrolysis in remediation.

4.1 Principle and Definitions

Typically, ethers react with molecular oxygen to produce peroxides [36]. However, those containing a *tert*-butyl group (e.g. MTBE) do not form peroxides because the first reaction step – the insertion of an oxygen molecule into the α carbon–hydrogen bond – is not possible at the *tert*-alkyl group.

Nevertheless, all ethers can be protonated to a certain extent but strong acids are necessary due to the low pK of the ether bond (mostly between – 2.0 and – 4.0). The protonation leads often to a further reaction of the molecule. The "Zeisel ether cleavage" using hydroiodic acid is a well known chemical reaction in organic chemistry [36]. Depending on the acid and solvent used, the reaction products are halogenated compounds or the related alcohols. MTBE can be hydrolysed with strong acids in aqueous solutions (Eqs. 2 and 3), leading to the reaction products TBA and methanol [37]:

$$(CH_3)_3C - O - CH_3 + H^+ \rightleftarrows (CH_3)_3C - OH^+ - CH_3\,, \tag{2}$$

$$(CH_3)_3C - OH^+ - CH_3 + H_2O \rightleftarrows (CH_3)_3C - OH + CH_3 - OH + H^+\,. \tag{3}$$

These reaction products have also been observed in laboratory experiments with solid acid catalysts [37–44]. However, since a coherent mass balance has not been provided, other reaction products as well as other elimination reactions (e.g. adsorption) may well be possible. Recently, other possible reaction products such as isobutene have been identified [38].

As the acid in this hydrolysis functions as a catalyst, the use of more environmentally friendly solid acid catalysts instead of acidic solutions is discussed in the next section.

4.2 Use of Catalysts for the Hydrolysis of MTBE

The use of solid acid catalysts has several advantages over acidic solutions. On the one hand, using solid materials reduces the risk of toxicity and corrosion, and on the other it improves the separation and the regeneration of the catalyst. They could feasibly be used in reactors of pump-and-treat systems and in permeable reactive barriers (PRBs). Table 3 provides an overview of the ma-

Table 3 Hydrolysis of MTBE on selected catalytic materials

Catalyst	Reaction products	Solid:water ratio [g : mL]	c_i [mg L^{-1}]	c_e [mg L^{-1}]	Refs.
Resins					
Amberlite IR-120$^+$	TBA and methanol	Not available exactly	1000	190	[37]
Zeolites					
H-ZSM-5 (80)	TBA, methanol	0.5:50	2000	350	[41, 42]
H-ZSM-5 (25)	TBA, methanol	0.5:50	2000	1420	[41, 42]
H-ZSM-5 (30)	TBA	0.25:18	100	< 1	[40]
H-BEA (25)	TBA	0.5:50	2000	440	[41, 42]
H-BEA (75)	TBA in traces	0.25:18	100	< 5	[40]
H-BEA (150)	TBA in traces	0.25:18	100	< 5	[40]
H-BEA (300)	TBA in traces	0.25:18	100	< 5	[40]
H-MOR (15)	No products	0.5:50	2000	1970	[41, 42]
H-MOR (25)	TBA	0.25:18	100	61	[40]
HY (30)	No products	0.5:50	2000	1930	[41, 42]
Others					
Nafion	TBA, methanol, isobutene, acetone	4:50	50	15	[38]

terials investigated in laboratory experiments and includes information about the reaction products detected.

Zeolites in acidic form, strongly acidic ion exchange resins and other catalysts have been used to observe reaction pathways of MTBE transformation as well as to determine the efficiency of this method in terms of MTBE remediation. Theoretically, materials with acidic centres and a minimum pore size similar to the molecule size of the ether to be treated can be used for the hydrolysis of ethers. As mentioned above, zeolites in acid form can be used as catalysts in the synthesis of MTBE. The reversibility of this reaction suggests the use of this kind of catalyst during hydrolysis as well. Additionally, it should be mentioned that zeolites have a regular structure with defined pore and channel size. This means that using these materials rules out competition with larger molecules. The structure of the zeolite defines the accessibility for reactant and product molecules whereas the silica/aluminium ratio is of major importance in the number of acidic sites. Then again, other catalysts may provide a higher acidity on their surface, e.g. "superacidic" catalysts. The acidic strengths of various zeolites and other materials are given in Table 4. Furthermore, the accessibility of the acidic sites within the material has to be taken into consideration, as investigated by Centi et al. [41, 42].

The extent to which the processes of hydrolysis and adsorption contribute to the elimination of ether from contaminated water has still to be elucidated. To this end, detailed desorption experiments need to be performed and all the reaction products precisely analysed.

Centi et al. [41, 42] also found the formation of the reaction products during hydrolysis to depend on the initial MTBE concentration, a higher initial MTBE concentration leading to a higher TBA/methanol ratio. Although information on this aspect is limited, this is an interesting point regarding the further treatment of the compounds formed. Laboratory experiments were mainly accomplished starting with high MTBE initial con-

Table 4 Acidic strength of selected acid catalysts

Catalyst	Hammett acidity	Refs.
Zeolites		
Zeolite H-ZSM-5	– 5.6 to – 3.0	[45]
Zeolite H-MOR	– 5.6 to 0.8	[45]
Zeolite H-Y	– 5.6 to 1.5	[45]
Others		
Nafion	Approx. – 12	[38]

centrations ($\geq 1\,g\,L^{-1}$) typical of areas around the source of contamination. Oehm et al. [40] carried out batch tests with initial MTBE concentrations of $100\,mg\,L^{-1}$ also resulting in the formation of TBA as a reaction product. Furthermore, the possible use of zeolite H-ZSM-5 to treat MTBE has been consistently suggested [40–42]. At any rate, more information is needed on whether acid catalysts can be used to remediate contaminated water with lower concentrations.

4.3 Limitations of the Reaction

Acid hydrolysis is dependent on the concentration and strength of acidic centres provided by the catalyst. In the case of zeolites, these centres are formed by exchangeable hydrogen ions that can be replaced by other cations present in water. When catalytic materials are used to treat MTBE-contaminated water, the accessibility of the acidic centres is crucial. Detailed investigations of the blockage of pores by co-contaminants and dissolved matter are essential and therefore experiments with groundwater are required. The impact of temperature on the rate of hydrolysis also needs to be investigated. Although most of the experiments in the literature were performed at ambient temperature, more investigations at groundwater temperature are required to assess the feasibility of using acidic catalysts.

Another important factor is the salt concentration of the contaminated water. In the presence of high salt concentrations, the rate of hydrolysis is reduced [40, 44]. Nevertheless, even at high salt concentrations zeolites might still be usable as sorbents [40].

4.4 Practical Implications

Materials with acidic sites have to replace strong acids in the hydrolysis of MTBE and other fuel oxygenates, as using acids would cause strongly acidic effluents and corrosion. Catalytic materials used in MTBE remediation need to have the following properties: insolubility in water and therefore easy separation of the catalyst from the treated water, chemical stability, and feasibility for regeneration. Possible applications of these materials include PRBs as well as packed-bed reactors in pump-and-treat procedures.

In the case of hydrolysis, the occurrence of reaction products entails the further treatment of the contaminated water. It is conceivable that biological processes may make an essential contribution to the mineralization of the readily biodegradable reaction products [40].

Another advantage of using catalytic materials is that they also function as an adsorbent for MTBE and/or the reaction products. This is evident from several laboratory studies [39–42].

To our knowledge, no field studies have been conducted using acid hydrolysis for the remediation of MTBE-contaminated water. Therefore, details for practical implications can only include knowledge gained at laboratory scale.

Prior to deciding whether hydrolysis can be included in treatment, investigations should involve characterization of the contaminated water especially regarding its salt concentration: the higher the salt content, the more inefficient the use of catalysts with acidic centres. Furthermore, combining methods might increase the efficiency of the remediation strategy, although this is another area where no field studies have been conducted yet. In particular, the use of zeolites might be a promising method as these materials are able to act simultaneously as catalysts with acidic centres and as adsorbents.

One disadvantage that has to be resolved is the relatively high costs for synthetically produced zeolites. This may become less important if combined methods (e.g. involving biodegradation) with high remediation efficiency can be developed.

5 Adsorption of MTBE

Adsorption on granular activated carbon (GAC) is widely used to remove organic compounds from contaminated water as it is relatively inexpensive. In the case of gasoline spills, the set-up selected for the remediation of certain groups of compounds (e.g. BTEX) may not be equally effective for all the compounds present. The efficiency of the elimination of fuel oxygenates was considered unsatisfactory and, thus, the need for new adsorbents has become increasingly urgent in recent years. Adsorption was mainly attained by using materials with well-defined porous structures like zeolites and synthetic resins. Below, an introduction to theoretical adsorption mechanisms is given along with selected findings on the adsorption-based removal of MTBE by zeolites and resins in comparison with the use of GAC.

5.1 Mechanisms and Definitions

According to the definition given by IUPAC [46], “adsorption is the enrichment [...] of one or more components in an interfacial layer” and can be subdivided into chemical adsorption (chemisorption) and physical adsorption (physisorption). In chemisorption, the adsorbate becomes bound to the solid surface by a chemical bond, forming a monolayer. In physisorption, which is a reversible process, adsorption takes place mainly by van der Waals and electrostatic forces between adsorbate molecules and the atoms composing the adsorbent surface.

5.1.1 Monolayer Theory

In monolayer adsorption, all the adsorbed molecules are in contact with the surface layer of the adsorbent [46]. The adsorbate molecules are thus adsorbed on a fixed number of localized sites, each of which can only hold one adsorbate molecule (the molecules of the adsorbate are not deposited on others already adsorbed, only on the free surface of the adsorbent). The most suitable models describing the monolayer theory are the Langmuir-type ones. They presume that all adsorption sites are energetically equivalent and that there is no interaction between the adsorbed molecules [47].

5.1.2 Multilayer Theory

Depending on the adsorption conditions (e.g. temperature), the adsorbent molecules can adhere in excess to the molecules already in contact with the adsorbent surface and form more than one layer [46]. Braunauer, Emmet and Teller (BET) proposed in 1938 the first model for the adsorption of gases on multilayers, which hypothesizes in addition to the Langmuir assumptions that each molecule in the first layer serves as an adsorption place for molecules in the second layer and so on [48].

5.1.3 Pore-Filling Mechanism

Both monolayer and multilayer mechanisms apply to a single flat surface or a porous surface with very large pore radii. However, they do not apply to adsorbents with pores comparable in size to the molecule of the adsorbate, due to the strong influence of the pore walls. In this case, the adsorption follows a pore-filling mechanism in which the molecules are adsorbed in the available spaces between macropores [49]. This is well described by the pore-filling model developed by Dubinin–Polanyi, originally used for vapour phase adsorption [50]. The model was extended to aqueous solutions and applied to the adsorption of organic compounds on highly microporous materials [51].

5.1.4 Adsorption Isotherms

According to the IUPAC nomenclature [46], an adsorption isotherm represents the ratio between the quantity adsorbed and the composition of the bulk phase under equilibrium conditions at constant temperature. Depicted in a graph, the adsorption isotherm is a plot of the amount adsorbed as a function of the contaminant's concentration in the fluid phase. The shape

of the adsorption isotherm is also important and IUPAC identified six types of adsorption isotherms. Based on their shape, the plots provide information about the adsorption mechanisms, porosity and surface area of the adsorbent [46].

In practice, the adsorption isotherms are described by different equations, the most common of which are listed in Table 5 (classification by the number of fitting parameters).

Regarding the linearity or non-linearity character of the adsorption of fuel oxygenates on porous media, non-linear behaviour was ascertained by most authors. This confirms the pore-filling nature of adsorption. Hardly any linear fitting has been reported in the literature: solely the adsorption of MTBE on mordenite [58] and on all-silica zeolites [59]. Although most of the authors identified a kind of plateau appearing at high equilibrium concentrations due to the filling of micropores (and thus suggesting a better fitting of Langmuir-type isotherms), many authors have used Freundlich isotherms to characterize their experimental data. Only some recent work reported by Bi et al. [60] and Stefan [61] have taken into consideration a more detailed description of MTBE adsorption on synthetic resins and zeolites by fitting several models to experimental data and comparing the results. It has also been concluded that the Freundlich isotherm is not suitable for describing the adsorption data, a conclusion previously reached by Lin et al [62].

Table 5 Adsorption isotherms by the number of fitting parameters

Sorbent	Equation	Refs.
Two fitting parameters		
Langmuir	$q = q_{\mathrm{m}} \frac{kc}{1+kc}$	[47]
Freundlich	$q = kc^{1/n}$	[52]
Temkin	$q = \frac{RT}{b} \ln(Ac)$	[53]
Three fitting parameters		
Tóth	$q = q_{\mathrm{m}} \frac{kc}{\left(1+(kc)^{\beta}\right)^{1/\beta}}$	[54]
Polanyi–Manes	$q = V_m \rho \exp\left[-\left(\frac{RT \ln \frac{S}{c}}{E}\right)^{b}\right]$	[51]
Redlich–Peterson	$q = q_{\mathrm{m}} \frac{kc}{1+kc^{n}}$	[55]
Langmuir–Freundlich	$q = q_{\mathrm{m}} \frac{kc^{n}}{1+kc^{n}}$	[56]
Four fitting parameters		
Generalized Langmuir	$q = q_{\mathrm{m}} \left[\frac{(kc)^{n}}{1+(kc)^{n}}\right]^{m/n}$	[57]

5.2 Sorbents for the Removal of MTBE

Various laboratory-scale projects have been carried out to estimate the sorption affinity and adsorption mechanisms of MTBE on different porous materials. Most of the results have been obtained from batch tests, the adsorbents used varying from natural soils [63] to natural and synthetic resins [60, 62, 64] and zeolites [58, 59, 65–69]. Parallel studies were performed by most authors to compare the adsorption potential of the above-mentioned adsorbents with GAC. Table 6 shows the adsorption of MTBE on zeolites and resins, where q_m represents the maximum adsorption capacity at equilibrium, c_i is the initial concentration and c_e the equilibrium concentration [61].

The uptake of MTBE by resins and zeolites was also studied by Davis et al. [64] and Hung et al. [69] but the maximum adsorption capacity was not reached. Instead, the loading of adsorbents with MTBE (q^*) was given for two equilibrium concentrations: 100 and 1000 $\mu g\,L^{-1}$ (Table 7) [61].

In addition to the data presented above, some other authors present the efficiency of MTBE adsorption as a percentage (Table 8) with values ranging from 5% (adsorption on zeolite Y) to 96% (adsorption on mordenite). Nevertheless, the results published may not be relevant as they closely depend on the set-up of the experiment (mass of adsorbent and volume of MTBE solution) [61].

Table 6 Adsorption of MTBE on zeolites and resins (I)

Sorbent	q_m [$mg\,g^{-1}$]	c_i [$mg\,L^{-1}$]	c_e [$mg\,L^{-1}$]	Refs.
Resins				
Ambersorb 563 (untreated)	76.6	0.57–177	0.11–97.3	[60]
Ambersorb 563	75.0	0.57–177	0.112–103	[60]
Ambersorb 563	44.9	10–50	1.0–50.0	[62]
Amberlite XAD4	58.6	0.57–177	0.155–125	[60]
Amberlite XAD7	8.50	0.57–177	0.306–139	[60]
Dow Optipore L493	74.7	0.57–177	0.109–105	[60]
Zeolites				
Zeolite ZSM-5 powder	73.5	91.8	0.06–25.6	[61]
Zeolite ZSM-5 granules	47.9	81.1	0.09–72.1	[61]
Zeocarb Na	0.31	50.8	19.2	[65]

Table 7 Adsorption of MTBE on zeolites and resins (II)

Sorbent	q^* [mg g^{-1}]	c_i [mg L^{-1}]	c_e [mg L^{-1}]	Refs.
Equilibrium concentration = 1000 μg L^{-1}				
Ambersorb 563	16.2	5–2500	0.6–2500	[64]
Ambersorb 572	13.8	5–2500	1.0–2000	[64]
Polysorb MP-1	0.80	5–2500	No data	[64]
Equilibrium concentration = 100 μg L^{-1}				
Mordenite	2.94	0.50–0.60	0.02–0.60	[69]
HiSiv 1000	0.07	0.50–0.60	0.09–0.32	[69]

Table 8 Removal percentage of zeolites for MTBE

Sorbent	Sorbent [g]	Volume [mL]	c_i [mg L^{-1}]	c_e [mg L^{-1}]	Removal [%]	Refs.
Mordenite	0.005	25	0.100	0.004	96	[58]
Zeolite ZSM-5	0.005	25	0.100	0.037	63	[58]
Zeolite Y	0.005	25	0.100	0.949	5	[58]
H-β zeolite	0.005	25	11	7.700	30	[59]
Deal. β zeolite	0.005	25	11	1.200	89	[59]
All-silica β zeolite	0.005	25	11	0.590	95	[59]

Compared to the results obtained using resins and zeolites, the uptake of MTBE by GAC shows similar values, corresponding to a wide range of influent concentrations (from 50 μg L^{-1} to 434 mg L^{-1}). The maximum adsorption capacities vary from below 1 mg g^{-1} to 122 mg g^{-1} (Table 9) and in cases where this is not known, the loading of GAC with MTBE (q^*) is given for equilibrium concentrations of 100 and 1000 μg L^{-1} (Table 10) [61].

5.3 Limitations of Adsorption

The most commonly used adsorbent to remove organic compounds from contaminated water, GAC, is not very effective for MTBE or other fuel oxygenates due to their high water solubilities and low partition coefficients. Moreover, the reasons for contamination with fuel oxygenates are often gaso-

Table 9 Adsorption of MTBE on GAC (I)

Sorbent	q_m [mg g^{-1}]	c_i [mg L^{-1}]	c_e [mg L^{-1}]	Refs.
F300	122	434	0.05–117	[70]
PAC 200	62.5	171	0.10–2.10	[70]
F300	58.0	48	No data	[65]
HD-4000	42.0	183	No data	[70]
TL 830	37.9	98	0.617–50.7	[61]
Hydrodarco B	25.7	164	No data	[70]
Picazine	20.2	172	No data	[70]
F600	19.9	5	No data	[28]
Darco KB	19.5	171	No data	[70]
GRC-22	10.5	1.03	0.0005–0.64	[71]
F400	9.30	5	No data	[28]
F400-HO	4.30	1.03	0.0009–0.82	[71]
F400	3.20	1.03	0.0009–0.89	[71]
F600	1.43	0.05	0.00065–0.025	[72]
F400	0.69	0.05	0.002–0.035	[72]

Table 10 Adsorption of MTBE on GAC (II)

Sorbent	q^* [mg g^{-1}]	c_i [mg L^{-1}]	c_e [mg L^{-1}]	Refs.
Equilibrium concentration = 1000 μg L^{-1}				
Hypercarb	6.50	5–2500	No data	[64]
F400	3.10	5–2500	No data	[64]
Equilibrium concentration = 100 μg L^{-1}				
F300	1.94	0.05–0.06	0.012–0.400	[69]
Unicarb	1.60	0.05–0.06	0.017–0.680	[69]
F400	1.51	0.05–0.06	0.033–0.430	[69]
WPH	0.75	0.05–0.06	0.060–0.700	[69]

line spills and therefore a number of groups of compounds may be found at the same site. The preferential adsorption of these competing compounds (e.g. BTEX) in mixed solutions reduces the affinity of the adsorbents for MTBE and the other fuel oxygenates. In most cases, the efficiency of GAC adsorption is diminished by the obstruction of water flow through the GAC pores caused by precipitation of iron and manganese, hard water compounds

(e.g. calcium carbonate), various coagulants and additives used in removal of turbidity, as well as biological growth [73]. The effect of obstruction is enhanced in waters with high content of natural organic matters (NOM), since the big NOM molecules frequently block the pores of GAC, making it difficult for the much smaller molecules of the fuel oxygenate to penetrate [68]. Moreover, the more easily adsorbable compounds may even displace the oxygenates already adsorbed and thus the overall efficiency of the adsorbents could be decreased [19].

5.4 Practical Implications

Adsorption on GAC is a common technology for the above-ground remediation of MTBE contaminations, proven also on full-scale applications [19]. Despite this, the negative effects of pore blocking and competition suggest the replacement of GAC with alternative adsorbents. Using zeolites, there is a higher probability to avoid inhibition and competition for the adsorption sites [74]. Nevertheless, most of the published results are from laboratory experiments. However, one pilot-scale and one full-scale application using zeolites have been published recently [75]. The process for the treatment of water contaminated by apolar compounds, based on the use of apolar zeolites in PRBs has been patented [74]. Adsorption also plays an important role in the natural attenuation of contaminated sites as well as in some in situ and ex situ remediation technologies like pump-and-treat (above-ground treatment of extracted water), PRBs or soil vapour extraction (adsorption of vapour phase contaminants).

6 Summary and Conclusions

The abiotic processes based on acid hydrolysis and adsorption have been evaluated for their applicability in the remediation of contaminated water containing fuel oxygenates.

Adsorption can be classified as an accumulative process involving the transition of the contaminant from the water phase (or gas phase) to the solid phase. Due to their log K_{OC} values, other hazardous substances (e.g. benzene) present at MTBE-contaminated sites show better adsorption on activated carbon. Alternatively, other adsorbents like zeolites have been widely investigated in laboratory studies as well as in one pilot-scale and one full-scale application, showing promising results. Nevertheless, it should be mentioned that the availability of data dealing with the adsorption behaviour of fuel oxygenates other than MTBE is limited. Further studies are needed to identify the most suitable adsorbent for each contaminant as well as for mixed contam-

inations in groundwater. Hydrolysis plays an important role in the analysis of ethers used as fuel oxygenates and can potentially be used in the remediation of contaminated sites. At present, acid hydrolysis is being investigated at the laboratory scale using solid acid catalysts (e.g. zeolites). Basically, this reaction has been proven to efficiently break down the stable ether bonding of MTBE. At any rate, TBA and other reaction products require further treatment. Additionally, the effectiveness of hydrolysis has to be investigated under groundwater conditions to estimate the influence of factors such as temperature and salt concentration.

Generally speaking, the applicability of abiotic processes such as acid hydrolysis and adsorption for the remediation of contaminated waters with fuel oxygenates is limited by the physicochemical properties of the ethers and the groundwater conditions. Consequently, combining a number of remediation techniques, including hydrolysis and/or adsorption, might be a promising way to meet site-specific requirements.

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Microbial Degradation of MTBE in Reactors

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Abstract The use of methyl *tert*-butyl ether (MTBE) has resulted in serious contamination of many groundwater supplies worldwide. Literature investigations were performed with the aim of improving knowledge on the use of bioreactors for removal of MTBE from con-

taminated groundwater. Among the important findings were: membrane bioreactors and fluidized bed reactors had the highest volumetric removal rates of all reactors studied, in the order of 1000 mg/(l d); competition for oxygen, nutrients and occupancy between MTBE degraders and oxidisers of co-contaminants such as, ammonium and the group of benzene, toluene, ethylbenzene and xylenes, may reduce the removal rates of MTBE, or prevent its removal in reactors. With mathematical modelling, the long startup time required for some MTBE degrading reactors could be predicted. Long startup times of up to 200 days were due to the low maximum growth rate of the MTBE degraders, in the order of 0.1 d^{-1} or less, at 25 °C. However, despite this, high volumetric MTBE removal rates were found to be possible after the startup period when the biomass concentration reached a steady state.

Keywords Biodegradation · Co-contaminants · Modelling · MTBE · Reactors

Abbreviations

AUSB	Aerobic upflow sludge bed reactor
b	Decay constant
BTEX	Benzene, toluene, ethylbenzene and xylenes
COD	Chemical oxygen demand
Con.	Concentration
DEE	Diethyl ether
DIPE	Diisopropyl ether
D_{MTBE}	MTBE diffusion coefficient
D_{O_2}	Oxygen diffusion coefficient
Eff.	Effluent
ETBE	Ethyl *tert*-butyl ether
FBR	Fluidized bed reactor
GAC	Granular activated carbon
HRT	Hydraulic retention time
K_s	Half saturation constant
MBR	Membrane bioreactor
MTBE	Methyl *tert*-butyl ether
O_{min}	Minimum concentration before oxygen limitation
PBR	Packed bed reactor
RBC	Rotating biological contactor
Recirc.	Recirculation
Rem.	Removal
S_{MTBE}	Dissolved bulk MTBE reactor concentration
S_{O_2}	Dissolved bulk oxygen reactor concentration
TAME	*Tert*-amyl methyl ether
TBA	*Tert*-butyl alcohol
TCE	Trichloroethylene
Temp.	Temperature
TSS	Total suspended solids
μ_{max}	Maximum growth rate
Vol.	Volume
$v_{O_2,MTBE}$	Stoichiometric coefficient for oxygen and MTBE
VSS	Volatile suspended solids
Y	Yield coefficient

1 Introduction

Methyl *tert*-butyl ether (MTBE) has been used since the 1970s as a fuel oxygenate in order to reduce smog and emissions from internal combustion engines. MTBE also has octane enhancing properties, which help prevent knocking inside engines. It is produced with light ends from the crude oil distillation process, which might have otherwise been unusable, and is favourable from the point of view of refiners. It is less expensive and can be produced more readily compared to other compounds such as ethanol, which can also act as oxygenates [1–4].

However, despite its positives MTBE has a bad reputation of causing pollution of water supplies when accidentally released in the environment. Studies from the United States (US) found that as many as 250 000 sites may have been polluted from leaking underground fuel tanks [5, 6].

The main problem associated with MTBE in drinking water is its low odour and taste threshold. It is said to impart a turpentine-like flavour to drinking water. It is likely to be detected at concentrations from 10–40 ppb [7, 8]. However, the threshold value does vary a lot, for instance a value of 2–2.5 ppb was reported by Fiorenza and Rifai [9]. MTBE is not retarded by aquifer material, and in addition, it has a high solubility of approximately 50 g/l at room temperature [7]. It can, therefore, quickly dissolve in groundwater and pollute it.

Currently, we have no reports of MTBE drinking water guidelines set by the European Union (EU) or the US regulators. However, the state of California has set a limit of 5 μg/l [10, 11]. In Denmark, the limit value is also set at 5 μg/l, but preferable below 2 μg/l [12].

The tertiary structure of MTBE leads to a steric hindrance to an enzymatic attack on the molecule [13]. Compounds with ether bonds are also generally relatively stable [14]. For these reasons, MTBE is a rather difficult compound to degrade by naturally occurring microorganisms in groundwater. MTBE does not sustain microbial growth well, and its degradation is associated with low biomass yields [15, 16]. MTBE which has volatilised to the atmosphere will decomposed readily, there by the action of free radicals [17, 18]. The problems associated with MTBE in the environment are therefore mainly associated with groundwater.

Physical processes such as air sparging and sorption unto granular activated carbon (GAC) can be used for removing MTBE from groundwater. However, these processes typically do not work very well due to its physical properties [7]. When air sparging is applied for remediation of groundwater, a much longer time is needed for removing a contaminant plume when MTBE is present, compared to plumes with only the mix of benzene, toluene, ethylbenzene and xylenes (BTEX) [19]. MTBE has a low affinity for sorption to the

organic phase. Application of GAC sorption processes also works much better for BTEX compounds than for MTBE.

The metabolic product *tert*-butyl alcohol (TBA), which is often present with MTBE, is also considered a groundwater contaminant. Due to its physical properties, it is much more difficult to remove from groundwater than MTBE through the physical processes mentioned [20]. Air sparging and sorption unto GAC cannot be considered as viable options for TBA removal from groundwater.

Bioremediation in engineered systems can be used for removal of MTBE from groundwater. There are also naturally occurring microorganisms which have been shown to completely mineralise MTBE under aerobic conditions. Several pure strains that have been isolated and studied can mineralise MTBE. They do so by direct metabolism, whereby, MTBE is used as the sole carbon and energy source [15, 21–25].

Many other aerobic strains are also able to use MTBE in cometabolic reactions with other substrates [26–30]. Cometabolism is the fortuitous transformation of a compound by enzymes which were produced for degradation of another substrate. The compound which is being incidentally transformed is not used either for growth or to provide energy for the microorganism [31]. There is a strong correlation between organisms which can degrade and grow on branched alkanes, and their ability to cometabolise structurally analogous compounds such as MTBE. Simple branched alkanes are abundant in gasoline; therefore, the application of cometabolic cultures for remediation of gasoline impacted MTBE plumes in reactors is an interesting prospect [32].

Degradation under methanogenic conditions and the use of nitrate, sulphate and ferric iron has been shown to a limited extent [33–38]. Compared to aerobic MTBE degradation, removal rates under anaerobic conditions are extremely slow and long acclimatisation periods are required. It cannot be considered as a feasible remediation option until further research is carried out.

Remediation of MTBE using aerobic biologically engineered systems has the potential to be successfully used as an option for removing MTBE from drinking water. One of the most crucial aspects of reactor design and control is the challenge to operate a reactor with a high concentration of MTBE degrading bacteria and the ability to remove MTBE down to the prevailing drinking water standards or lower. Reactors which utilise biofilms have good applicability in this regard. Biofilms have the ability to maintain very high biomass concentrations, and are considered to be very robust and stable in terms of their ability to resist changing and different kinds of environmental conditions [39]. Several studies using biofilm reactors in experimental systems have shown that MTBE can be removed down to less than 1 μg/l [40–42].

MTBE removal has also been documented in sand filters of drinking water works in Denmark. MTBE was removed from concentrations of about 10–65 μg/l down to concentrations below 5 μg/l [43]. One key observation in this study was that the MTBE degrading organisms seemed quite robust. When the filter was left standing for 4 weeks, the MTBE removal capacity could be re-established within this time. Optimisation of biological filters in drinking water works for MTBE removal should be considered in remediation options for MTBE removal.

In order to fully utilise the potential of bioremediation for MTBE removal in reactors, several areas are of considerable challenge and interest:

1 Understanding the characteristics and behaviour of biofilms vs. suspended biomass reactor systems. What is the role of biofilms or suspended biomass in the bioremediation of MTBE?
2 Understanding the characteristics of the different reactor types suitable for MTBE bioremediation. What are the properties of these reactors that make them suitable for bioremediation of MTBE, and what are their operational characteristics?
3 Understanding of the most important process parameters which affects the degradation of MTBE in reactors. What are the effects of for e.g., oxygen, nutrients, toxicants, co-contaminants, temperature and pH on the degradation of MTBE in bioreactors?
4 Understanding of the factors influencing the time required for startup of MTBE degrading bioreactors. This time can vary from about 20 to over 200 days. How do we predict the startup time of MTBE degrading reactors? Can we reduce the time required for startup?
5 Understanding of the role and potential of cometabolism. How can cometabolic MTBE degrading cultures be exploited with a view to improve bioremediation of MTBE in reactors?
6 Application of mathematical models as a tool for approaching the previously mentioned challenges. What can mathematical models tell us about the degradation of MTBE in bioreactors? How can they be used to increase understanding of the factors which are most important for bioremediation of MTBE?

Literature investigations were used in order to address the six listed challenges; these are considered to be some of the most important aspects related to the bioremoval of MTBE in reactors. The focus is on the use of aerobic bioreactors for aqueous phase MTBE removal by direct metabolism. The discussions on cometabolism are confined to its own section. The concepts and information provided are mainly applicable to the *ex situ* remediation of MTBE contaminated groundwater. The ideas presented, however, can also be applied to MTBE removal in drinking water treatment or industrial applications. Most of the discussions are equally valuable to TBA and other ethers used as fuel oxygenates. These are for example, ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME) and diisopropyl ether (DIPE).

2 Biofilms versus Suspended Growth

Biofilm reactors are ideally suitable for their ability to remove MTBE from contaminated water, one of the advantages been derived from the growth of biofilms in these reactors. A biofilm can simply be regarded as "microorganisms immobilized at a substratum (i.e., the support surface) generally in association with an organic polymer" [44]. A more general description may also include microorganisms in flocs and pellets. The growth stages of a biofilm can be typically divided into three phases: 1) lag; 2) exponential; and 3) stationary. Figure 1 shows the growth phases which are important to understand when dealing with biofilm reactor systems [45, 46].

Microbial reactor systems utilizing biofilms for degradation of organic compounds such as MTBE have several advantages compared to systems using suspended or planktonic biomass. Microorganisms growing on MTBE as sole carbon and energy source are some of the slowest set of aerobic heterotrophic bacteria currently known. Their doubling time is greater than 10 days at 20 °C [47, 48] compared to a few hours for general heterotrophs growing on easily degradable compounds [49]. With such long doubling times necessary for growth of the MTBE degrading microorganisms, the choice of a reactor system which can retain the microorganisms in a biofilm becomes logical. Bacteria attached to a surface inside a biofilm are protected from washout with the stream of flowing water, and, therefore, short retention times can be used. Generally, a high biomass concentration can be reached inside biofilms, up to 100 kg/m^3 VSS. This is more than an order of magnitude higher than the biomass concentration typically present in suspended biomass systems [49, 50]. The higher biomass concentration that can

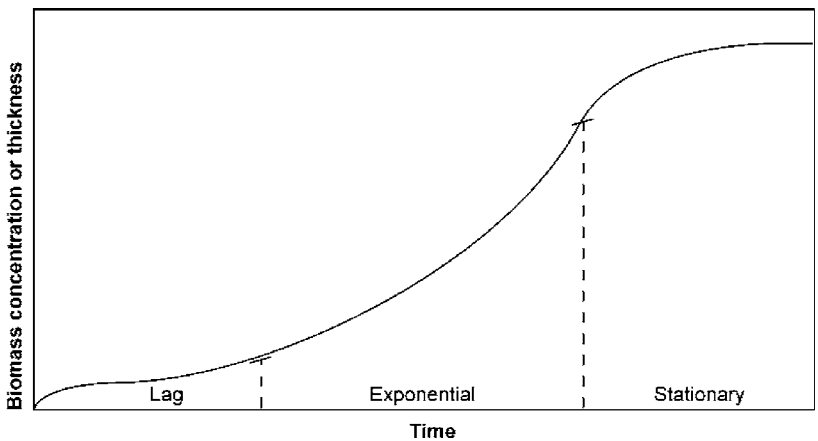

Fig. 1 The growth stages of a biofilm. The plot can be divided into three phases: (1) lag, (2) exponential and (3) stationary or plateau (modified from [45])

be achieved in biofilm reactors compared to suspended systems results in increased volumetric removal rates in the reactors.

Biofilms are more specifically groups of cells embedded in an organic matrix [44]. Therefore, the cells which are actually participating in the removal processes are often protected from undesirable conditions in the bulk phase of a reactor. Biofilms can suitably adjust their internal environmental conditions (e.g., pH, temperature, oxygen or toxicants) to make their removal processes favourable [39].

Biofilms are able to maintain both fast and slow growing organisms within close proximity inside the matrix. Many different types of organisms can be involved in the removal processes. A potentially faster and more thorough conversion of substrates can be obtained compared to systems employing suspended biomass. Microorganisms inside a biofilm have the ability to optimally arrange themselves spatially, both within the biofilm and inside a reactor. This may be advantageous in terms of the rates at which substrate conversions can occur. Several different compounds may be simultaneously converted within the biofilm, which may not have been as efficient otherwise. The volumetric removal efficiency of suspended growth systems, however, may approach that of biofilm systems, if the biomass is prevented from washing out from the system. This may be accomplished by incorporating a biomass clarifier and a recycle loop to the reactor or incorporating a membrane which prevents the biomass from leaving the system [1, 51, 52]. The sludge age within the system will be greatly increased, while still maintaining a relatively short hydraulic retention time (HRT).

2.1 Oxygen or MTBE Limitation in a Biofilm?

Biofilms that become too thick, however, may prevent full penetration of substrates; the reaction rates become more dependent on the diffusion of substrates inside the film. Therefore, procedures for controlling the biofilm thickness may be necessary in some reactor systems [53]. Oxygen may also become limited within such biofilms, reducing transformation rates of MTBE. It is possible to estimate whether oxygen or MTBE is limited inside a biofilm by the following expression [49]:

$$\frac{S_{O_2}}{S_{MTBE}} = \frac{D_{MTBE}}{D_{O_2}\, v_{O_2,MTBE}} \tag{1}$$

where S_{O_2} and S_{MTBE} are the dissolved bulk oxygen and MTBE reactor concentrations, respectively, in chemical oxygen demand (COD) units; $v_{O_2,MTBE}$ is the stoichiometric coefficient for oxygen and MTBE; D_{O_2} and D_{MTBE} are the oxygen and MTBE diffusion coefficient, respectively. D_{O_2} and D_{MTBE} are estimated as 1.7×10^{-4} and 0.6×10^{-4} m^2/d respectively [49]. While $v_{O_2,MTBE}$

is equal to 1.07 g COD_{MTBE}/gO_2 and was deduced from the stoichiometric expression for mineralization of MTBE.

Solving expression 1, one obtains the following: $S_{O_2} = 0.33S_{MTBE}$. Therefore to prevent oxygen limitation in the biofilm of a reactor in which the oxidation of MTBE is controlled by diffusion inside the film then the following must hold: $S_{O_2} > 0.33S_{MTBE}$, on a COD basis.

3 Reactor Types for MTBE Removal

Figure 2 shows the bioreactor types which are most suitable for MTBE removal. The packed bed reactor (PBR), fluidized bed reactor (FBR) and the membrane bioreactor (MBR) are widely applied. Both the PBR and the FBR are often categorised as fixed film reactors in the literature.

The main reason for the popularity of the three widely used reactors lies in their ability to effectively retain a very high biomass concentration in their biofilms with a high sludge age and short hydraulic retention time.

The other biofilm systems such as the rotating biological contactor (RBC) and the aerobic upflow sludge bed reactor (AUSB) shown in Fig. 2 could possibly be applied for MTBE removal, and may posses some unique advantages. However, to our knowledge these reactors have never been applied for MTBE removal so far.

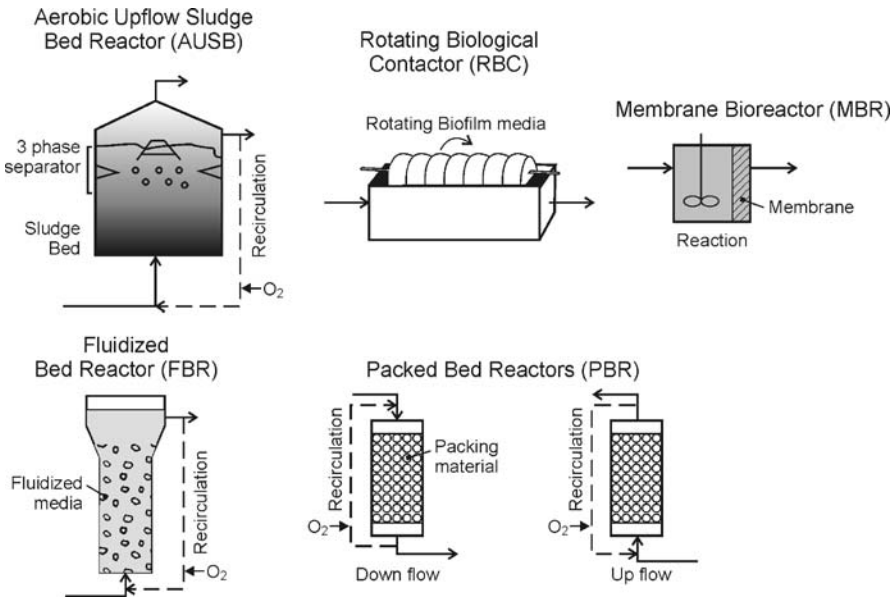

Fig. 2 Reactor types suitable for MTBE biodegradation

RBCs have been used widely used for wastewater treatment in the past and are well understood; furthermore, abundant information is available in the literature on their operation and design [54]. The upflow sludge bed reactor has also been applied successfully in the past. However, experience is only widely available on its application to anaerobic wastewater treatment [55, 56]. One of the key requirements for application of the AUSB for MTBE bioremediation, however, depends on the ability of the MTBE bacteria to agglomerate and form dense granules [57]. The granules also need to attain a settling velocity in the range of 40–100 m/h to function properly inside a reactor [58].

It has also been reported that many facilities manufacturing MTBE successfully use the activated sludge process for treating MTBE and TBA at high concentrations [20]. Applicability of the system may be limited at field sites were MTBE concentrations are often much less than 100 mg/l. The organic carbon loading rate to the system may be too low to sustain the activated sludge biomass.

3.1 Packed Bed Reactors

PBRs can be divided into two sub categories: upflow and downflow. The downflow type can be either operated with saturated media or unsaturated media, the upflow type is operated with saturated media. The downflow unsaturated media PBR is typically referred to as a trickling filter. Trickling filters have a long history and have been widely applied to wastewater treatment for more than a century. All PBRs primarily consist of a support media for biomass attachment and development, an influent distribution system and an effluent draw-off system (if recycling is used). These reactor types have advantages in their simplicity of design and construction. The hydraulics of the system is mainly plug flow, but approaches the behaviour of a completely mixed reactor, if a high recycle is incorporated. PBRs can be operated at high hydraulic loading rates since biomass washout is eliminated. They also have a good resistance to shock and toxic loads. Proper selection of the filter media is critical in order to ensure a high as possible biofilm liquid contact area inside the reactor and for prevention of clogging problems. Filter media have traditionally been a random packing of stones. More advanced plastic type media are, however, now available; they are much lighter and have higher specific surface areas. The filter media can be made of polypropylene lattice, wire, fritted glass particles and of varied sizes and shapes [39]. When PBRs are applied for wastewater treatment the fluid flow used is generally 1–2 m/h with a height to diameter ratio of 1–2. Furthermore, a sufficient amount of inlets should be present to ensure uniform distribution of the influent [59].

The biomass yield coefficient (Y) for MTBE is very low, only about 0.1–0.2 g VSS/g MTBE [16, 47]. Therefore, the rate of biomass accumulation

between the pores of the filter material is slow. If other compounds are present in the influent which can be utilized as substrate for bacteria a faster accumulation of biomass may occur. Clogging localized at the influent section may also be a problem since the microbial growth rates there are higher than at other sections of the reactor. Clogging may also occur from precipitation of iron or calcium ions. In order to prevent clogging in PBRs backwashing installations may be necessary.

3.2 Fluidized Bed Reactors

The FBR uses essentially the same basic design as the PBR. The main difference is that the liquid or liquid gas mix applied to the influent has a sufficiently high upflow velocity which results in fluidisation of the filter media particles. The created high upflow velocity of the FBR is normally provided by recycled effluent. Oxygenation of the system can be incorporated in the recycle loop (Fig. 2). FBR hydraulics is somewhere between a plug flow and a completely mixed system. The upflow velocities applied may vary from 2–30 m/h depending on the density of the support material. FBRs have similar properties compared to PBRs in terms of their ability to handle high hydraulic loads and resistance to toxicants. They, however, have advantages in that clogging will not be a problem, since the void spaces between particles in the reactor will be larger. The fluidisation process constantly allows for shearing off excess biomass from the particles, which enables control of the biofilm's thickness. Particle sizes reported in the literature applied to wastewater systems are in the range 0.2–2 mm [39, 59]. Fluidisation increases the effective surface area available for biomass growth. Typically, significantly higher loading rates can be applied when compared to PBRs. Hydraulic residence times are normally less than 1 h. The support material typically used for MTBE removal is GAC [60, 61], however, sand may also be used. GAC is able to provide MTBE removal prior to the startup of the biological process and during shock loadings through sorption. Expansion of the bed height may occur over time as the biofilm grows inside the reactor, leading to bed loss. The particles may have to be removed dislodged of biomass and returned to the reactor to prevent this. The reactor can be shaped either cylindrically or tapered-like with a height to diameter ratio in the range of 2–5. FBRs are often said to have high running costs due to the high energy consumption, operator maintenance and process control [20].

3.3 Membrane Bioreactors

In a MBR, biomass is separated from the treated effluent by membranes inside a completely mixed system which only allows the clear water to pass

(Fig. 2). The biomass is suspended within the system, and it has to be designed to maximise the permeable barrier surface area. The MBR has the obvious advantage of complete control over the sludge retention. Biomass concentrations as high as 12 g/l total suspended solids (TSS) have been reported for MBRs treating MTBE. The biomass in these systems has also been found to have a high enzyme activity [62]. These properties are advantageous for obtaining high volumetric removal efficiencies. Furthermore, the high biomass concentration attainable allows the system to treat polluted streams with very high influent concentrations. The system can also be started up in a very short time if seeding with an acclimatised biomass is done.

Three types of membranes have been applied so far for MTBE degrading reactors: 1) A ceramic cross-flow ultrafiltration membrane with a molecular cut-off of 300 kDaltons and pore size 0.02 μm [40]; 2) an internal hollow fibre membrane [62]; and 3) a porous polyethylene, 0.48 cm thick membrane with pore size of 18–28 μm [42, 52, 63, 64]. Interestingly, it was reported by the authors who used this latter polyethylene membrane mentioned that there was no need to apply a pressure across the membrane for operation in their reactor.

Biomass growth on the surface of the membrane is often a problem in these systems. This biomass growth is normally difficult to avoid and leads to fouling of the membranes which reduces its permeability. Membrane systems may have disadvantages in terms of the high capital costs for membranes, operational costs related to the need for a high transmembrane pressure and for fouling control. Pre-treatment of the influent to membrane systems may also be necessary in the case when dissolved ions such as iron may precipitate on membrane surfaces, which increases fouling problems. Precipitation of iron at a concentration of 5 mg/l leading to fouling was reported on membrane surfaces in a MTBE degrading reactor [64]. However, membranes are becoming less expensive and more functional with time; hence, their application in reactor system may have a bright future.

Membrane Systems may also be alternatively configured such that it is desired to have biofilm growth on the membrane surface and oxygen diffusing through the other side of the membrane. This system has an obvious advantage, in that, stripping of MTBE is most likely reduced compared to the configuration shown in Fig. 2. The alternative configuration, however, is generally less common and has never been applied to MTBE removal to our knowledge.

3.4
Reactor Applications from the Literature

Tables 1–3 show a comprehensive analysis of past reports of MTBE removal in the literature. The tables summarise relevant information on different studies conducted in PBRs, FBRs and MBRs.

Table 1 Packed bed reactor applications

Reactor description	Influent characteristics	Operational data	Treatment efficiency	Startup time (d)	Comments	Refs.
Type: Upflow packed bed *Vol.:* 2 l *Bed:* sintered glass rings	*Inlet:* MTBE, ETBE, TAME *Con.:* 10–100 mg/l each *Recirc.:* 650 l/d	*HRT:* 13 h *VSS:* ~ 1 g/l *Temp.:* 28 ± 1 °C O_2*:* > 2 mg/l *Recirc.:* yes	*Rem.:* > 99% for MTBE, TAME and ETBE at 135–140 mg/(l d) loads *Eff.:* 1–2.2 μg/l	40	Reactor seeded with ether degrading biomass; at 13 h HRT removal rate was 133–170 mg/(l d) for all ethers; ETBE removed the fastest	[65]
Type: Upflow packed bed *Vol.:* 0.5 l *Bed:* Filtralite®	*Inlet:* MTBE, TBA *Con.:* 3.2 mg/l MTBE *Load:* 258 mg/(l d)	*HRT:* 9.8 min *Temp.:* 19 ± 1 °C O_2*:* > 2 mg/l (outlet)	*Eff.:* 30 μg/l	~ 120	Maximum MTBE removal rate after 3 months was 19 mg/(l h)	[66]
Type: Upflow packed bed *Vol.:* 1.2 l *Bed:* glass beads	*Inlet:* MTBE *Con.:* 150 mg/l	*HRT:* 1 day O_2*:* 14.5 mg/l *Recirc.:* non	*Rem.:* 70%		Reactor seeded with petrochemical plant activated sludge; dominant species are *Micrococcus*; MTBE removal is comparatively low	[67, 68]
Type: Upflow packed bed *Dim:* 100× 5 cm *Packing:* quartz	*Inlet:* MTBE *Con.:* ~ 160 mg/l *Flow:* 500 ml/d O_2*:* > 4 mg/l	*HRT:* 80 h *Temp.:* ~ 25 °C *Recirc.:* no	*Rem.:* 50%		Removal is low compared to similar systems, however, the operational time was only 33 days	[69]
Type: Down flow packed bed *Bed:* anthracite and sand *Area:* 80 m^2	*Inlet:* MTBE *Con.:* 10–55 μg/l *Flow:* 4–28 m^3/h	*HRT:* 10–72 min *Temp.:* > 10 °C *Recirc.:* non	*Rem.:* 95–100% *Eff.:* < 5 μg/l		Studies conducted on a drinking water filter	[43, 70]

Table 1 (continued)

Reactor description	Influent characteristics	Operational data	Treatment efficiency	Startup time (d)	Comments	Refs.
Type: Trickling Filter *Vol.:* 0.7 l *Bed:* soil	*Inlet:* MTBE *Con.:* 13 mg/l *Load:* 0.1–2.5 mg/(l h)	*HRT:* 4.8–84 h *Recirc.:* non	*Rem.:* 100% upto loads 2.5 mg/(l h)		Simultaneous nitrification	[71]
Type: Trickling Filter	*Inlet:* MTBE *Con.:* 0.1–25 mg/l *Flow:* 1–35 m^3/h *Load:* 3–5 g/(m^3 h)	*HRT:* 0.1 h *Temp.:* > 14 °C	*Rem.:* > 90% *Eff.:* 10 µg/l		Studies conducted at 15 field sites; treatment costs about \$0.3/m^3 groundwater	[72]

Table 2 Fluidized bed reactor applications

Reactor description	Influent characteristics	Operational data	Treatment efficiency	Startup time (d)	Comments	Refs.
Type: Fluidized bed *Vol.:* ~ 900 l *Bed:* GAC	*Inlet:* MTBE, BTEX *Con.:* ~ 9.6 mg/l MTBE *Flow:* 15 l/min *Recirc.:* 121 l/min	*Temp.:* 10.6–23.8 °C O_2*:* 2.5 mg/l (outlet) *Recirc.:* yes	*Rem.:* 96% MTBE	30–40	Reactor seeded with bio-active GAC; a longer time for start up was required in another similar reactor	[61]
Type: Fluidized bed *Vol.:* 1.56 l *Bed:* GAC	*Inlet:* MTBE *Con.:* 10–50 mg/l *Recirc.:* 840 l/d *Flow:* 5–20 l/d	*HRT:* 1.7–10.8 h *Temp.:* 27–29 °C O_2*:* 4 mg/l *Recirc.:* yes	*Rem.:* > 98% upto 700 mg/(l d) loads	30–50	*Iso*-pentane may have initiated startup in a similarly operated reactor through cometabolism	[61]
Type: Fluidized bed *Vol.:* 7.88 l *Bed:* GAC	*Inlet:* MTBE, BTEX *Con.:* 7.8–8.8 mg/l MTBE *Con.:* 2 mg/l BTEX *Recirc.:* 150% (bed vol.) *Flow:* 22.7–36.4 l/d	*HRT:* 1 h (empty bed) *Temp.:* 20 °C O_2*:* > 2 mg/l *Recirc.:* yes	*Rem.:* 99.9% MTBE and BTEX *Eff.:* 18–20 μg/l MTBE *Eff.:* 1–2.2 μg/l BTEX	30	BTEX added to influent after 225 days; instantaneous removal of BTEX. Reactor seeded with PM1 type culture from membrane reactor	[60]
Type: Fluidized bed *Vol.:* 4.5 l *Bed:* GAC	*Inlet:* MTBE *Con.:* 10 mg/l *Flow:* 0.1 and 0.34 l/h	*HRT:* 3 and 1 h *Expansion:* 125% *Recirc.:* yes O_2*:* 2 mg/l	*Rem.:* 90 and 99% at 1 and 3 h HRT respectively *Eff.:* 100 μg/l at 3 h HRT	~ 30	Reactor seeded with the MTBE degrading strain ENV735 taken from a membrane bioreactor	[62]

Table 2 (continued)

Reactor description	Influent characteristics	Operational data	Treatment efficiency	Startup time (d)	Comments	Refs.
Type: Fluidized bed *Bed:* Sand	*Inlet:* MTBE *Con.:* 1.7 mg/l (max) *Flow:* 40 l/min	*Recirc.:* yes O_2*:* ~ 8 mg/l	*Eff.:* < 1 μg/l	~ 150	Reactor seeded with PM1 cultures	[73]
Type: Fluidized bed *Vol.:* 3.53 m^3 *Bed:* Sand	*Inlet:* MTBE, TBA *Flow:* 60 l/min *Con.:* 12 mg/l MTBE *Con.:* 300 μg/l TBA *Recirc.:* 180 l/min	*Recirc.:* yes *HRT:* 1 h	*Eff.:* < 1 μg/l MTBE and TBA		Reactor seeded with PM1 cultures; higher levels of dissolved oxygen greatly increased MTBE's removal rate	[73]
Type: Fluidized bed *Vol.:* 4.5 l *Bed:* GAC	*Inlet:* MTBE, TBA *Con.:* 350 mg/l MTBE *Con.:* 170 mg/l TBA *Recirc.:* ~ 20 l/h	*HRT:* 7.5 h *Temp.:* 25–30 °C *TSS:* > 10 g/l *Expansion:* ~ 127% *Recirc.:* yes O_2*:* > 1 mg/l	*Eff.:* 1 ± 15 μg/l MTBE *Eff.:* 3 ± 3 μg/l TBA	~ 20	Reactor seeded bio-active GAC; summary given here applicable to phase 5 of the reactor operation; BTEX removed without effects on MTBE removal	[41]

Table 3 Membrane bioreactor applications

Reactor description	Influent characteristics	Operational data	Treatment efficiency	Startup time (d)	Comments	Refs.
Type: Membrane *Vol.:* 9.95 l *Membrane:* polyethylene	*Inlet:* MTBE *Load:* 370 mg/(l d) *Flow:* 2.37 l/d	*HRT:* 4.2 days *Temp.:* 20 °C	*Rem.:* 99.9% *Eff.:* ~ 1 μg/l	100–200	BTEX, DIPE, DEE and ethanol were also degraded in similar reactors with no effect on MTBE's removal	[74]
Type: Membrane *Membrane:* polyethylene	*Inlet:* MTBE *Con.:* 150 mg/l *Flow:* 2.37 l/d	*HRT:* 4.2 days *SRT:* > 20 days *VSS:* ~ 1 g/l (max) *Temp.:* 20 °C O_2*:* > 3 mg/l	*Rem.:* > 99.99% *Eff.:* < 1 μg/l	100–200	Reactor seeded with MTBE acclimatized biomass; max VSS concentration reached was 2.5 g/l	[42]
Type: Membrane *Vol.:* 5.9 l *Membrane:* ceramic ultrafiltration	*Inlet:* MTBE *Con.:* 5 mg/l *Flow:* 142 l/d	*HRT:* 1 h *SRT:* 150–400 days *VSS:* ~ 3.5 g/l (max) *Temp.:* 18–20 °C O_2*:* 3 mg/l	*Rem.:* 99.99% *Eff.:* 0.32 ± 39 μg/l	~ 150	Membrane fouling resulted in the need for increasing transmembrane pressure over time	[40]
Type: Membrane *Vol.:* 6 m^3 *Membrane:* polyethylene	*Inlet:* MTBE, BTEX *Con.:* 2.9 mg/l MTBE *Flow:* 19 l/h O_2*:* > 8 mg/l	*HRT:* 6 h *VSS:* 2.5 g/l *Temp.:* 13–26 °C	*Rem.:* 99.91% MTBE *Rem.:* 99.98% BTEX *Eff.:* 2.62 μg/l MTBE	70–90	Reactor seeded with MTBE and BTEX enriched cultures; no pressure was required for water flow through the membrane	[64]

Table 3 (continued)

Reactor description	Influent characteristics	Operational data	Treatment efficiency	Startup time (d)	Comments	Refs.
Type: Membrane *Vol.:* 85 l *Membrane:* microporous hollow fiber	*Inlet:* MTBE *Con.:* 1 g/l *Flow:* 1.2 l/h	*HRT:* 3 days *TSS:* 12 g/l O_2*:* 2 mg/l	*Rem.:* 99.99% *Eff.:* 0.1 mg/l	10–20	Reactor started with an MTB degrading culture; infinite SRT first 160 days; MTBE removal rate was 1008 mg/(l d) at 1 day HRT	[62]
Type: Membrane *Vol.:* 1 m^3 *Membrane:* polyethylene	*Inlet:* MTBE *Con.:* 5 mg/l *Flow:* 104.17 l/h O_2*:* > 3 mg/l	*HRT:* 4 h *SRT:* > 100 days *VSS:* ~ 1 g/l *Temp.:* 10–25 °C *Recirc.:* yes	*Rem.:* 97.93% *Eff.:* < 1 μg/l	20–50	Reactor seeded with MTBE and BTEX enriched cultures; no pressure was required for flow of water through the membrane	[63]

From the tables it can be concluded that generally MTBE can be removed in excess of 99% in the investigated reactors. Many of these reactors removed MTBE down to very low effluent concentrations in the ppb range. In some of the reports the concentration was even below the Danish and Californian drinking water limit of 5 μg/l of MTBE. It is also clear that high inlet concentrations of MTBE can be treated in these reactors; some of the studies have even reported concentrations greater than 1 g/l MTBE. High volatile suspended solids (VSS) concentrations can also be achieved inside the reactors; some of the concentrations have been greater than 1 g/l. The volumetric degradation rates estimated from the tables have shown that FBRs and MBRs generally have the highest volumetric removal rates followed by PBRs. The maximum removal rates reported for both FBRs and MBRs were about 1000 mg/(L d) and approximately 450 mg/(L d) for PBRs. It is also evident that both MTBE and BTEX present in a contaminated groundwater plume can be biologically degraded simultaneously.

3.5 Process Comparison and Summary

Table 4 shows a ranking of the different systems based on some typical process characteristics. It may be considered subjective; however, it gives a good overview of the properties of the different systems. The ranking given to each reactor for each characteristic should be considered more from a general perspective than specifically related to MTBE. All the reactor systems ranked can be regarded as being excellent overall in terms of their MTBE removal ability.

Table 4 Ranking of different reactor types suitable for MTBE biodegradation in terms of typical process characteristics. The reactors shown are the fluidized bed reactor (FBR), packed bed reactor (PBR), rotating biological contactor (RBC), membrane bioreactor and the aerobic upflow sludge bed reactor (AUSB)

Reactor → Characteristics ↓	FBR	PBR	RBC	MBR	AUSB
Loading rates	4	3	3–4	3–4	3
Biofilm control	4	2	4	4	3
Biomass retention	3	3–4	3–4	4	3–4
Startup capability	2	2	2	4	4
Operation/control ease	2	4	3	2–3	2
Handling of inlet fluctuations	2	3	4	4	4
Handling of clogging	4	2	4	2	3
Documentation	3	4	3	2	1–2

Notes: A ranking from 1–4 is given to each reactor, where 4 is the best and 1 is the worst

4
Process Parameters Affecting the Degradation of MTBE

Microbial processes have several parameters which affect their rates and general applicability. Since the MTBE bacteria are rather slow growers, it is very important to carefully consider these factors in order to fully exploit the potential that bioremediation offers. The following variables are considered important for the MTBE degradation process:

- Oxygen and nutrients
- Co-contaminants
- Potential toxicants
- Temperature and pH

4.1
Oxygen and Nutrients

Both oxygen and nutrients are required by the MTBE degrading organisms. The oxygen requirements for the degradation process can be deduced by writing a stoichiometric expression for the mineralization of MTBE with the production of biomass:

$$C_5H_{12}O + 6.961O_2 + 0.078H^+ + 0.078NO_3^- \rightarrow 0.078C_5H_7NO_2 + 4.61CO_2 + 5.78H_2O$$

or

$$C_5H_{12}O + 7.11O_2 + 0.078OH^- + 0.078NH_4^+ \rightarrow 0.078C_5H_7NO_2 + 4.61CO_2 + 5.922H_2O$$

The biomass composition is $C_5H_7NO_2$, taken from McCarty [75], and the Y is taken as 0.1 g VSS/g MTBE or 0.078 mol VSS/mol MTBE [47, 60]. The COD equivalent of 1 g MTBE is 2.73 g COD. The oxygen requirement is approximately 2.5 g O_2/g MTBE degraded based on the stoichiometry for the mineralization of MTBE. There is some extra oxygen consumption arising from the endogenous decay of the microorganisms.

The dissolved oxygen half saturation constant (K_s) for microbial respiration has been reported to be less than 0.1 mg/l. It was found to be related to cell size for many organisms tested. Aerobic metabolic activities should therefore proceed at maximum rates when the dissolved oxygen concentration is 0.4 mg/l or higher [76]. However, according to more recent studies done on the aerobic degradation of MTBE, the K_s value for dissolved oxygen has been mainly higher. Table 5 shows some reported values for the K_s of dissolved oxygen during MTBE degradation.

Table 5 The dissolved oxygen half saturation constant (K_s) or the minimum concentration before oxygen limitation (O_{min}) occurred measured during the degradation of MTBE for different cultures

Culture	O_{min} (mg/l)	K_s (mg/l)	Refs.
Mixed culture		0.9	[77]
Vapour phase biofilter consortium		3	[78]
Vapour phase biofilter consortium		0.16	[79]
BC-1	1		[80]

Dissolved oxygen concentrations are generally kept at about 2 mg/l for aerobic bioreactors using suspended biomass. However, for attached growth biofilm processes 2 mg/l oxygen may be insufficient to ensure that no limitation occurs within the biofilm [54]. Estimates done in the first section of this chapter have shown that the bulk oxygen concentration should be greater than $0.33S_{MTBE}$ on a COD basis to avoid its limitation.

Elements such as nitrogen, phosphorous, sulphur, iron and trace components are also necessary for the microbial process. The nitrogen source for degradation of MTBE can come from either nitrates or ammonium. No significant difference was observed in the biodegradation rates of MTBE when either nitrates or ammonium was used as the nitrogen source [79, 81]. Most trace elements are only needed at concentrations well below 1 mg/l. For remediation of contaminated groundwater, trace elements are most likely present. Two strains of microorganisms have been reported to have a special requirement for cobalt ions during degradation of TBA [22, 82].

4.2 Co-contaminants

Co-contaminants, including BTEXs and inorganic compounds such as ammonium or iron may influence the degradation rates of MTBE in reactors due to different mechanisms. This may be due to three factors: 1) competitive or non-competitive inhibition by BTEX compounds; 2) microbial competition in reactors for occupancy, oxygen and nutrients; and 3) fouling of reactor and biological flocs due to iron precipitation.

4.2.1 Inhibition by BTEX Competition

Competitive inhibition occurs when two or more different substrates compete for access to the same microbial enzyme system. Both competitive and non-competitive inhibition may result in the degradation of one substrate being repressed in the presence of another.

It has been shown in both field and batch experiments that BTEX compounds may partially or totally inhibit the degradation of MTBE. In field experiments it was shown that MTBE degradation only occurred after the BTEX concentration had been reduced. Using batch experiments, it was shown that the presence of xylenes together with MTBE resulted in a 43% inhibition of MTBE degradation. In these reports, the authors stated that competitive inhibition by the BTEX compounds was responsible for inhibiting the degradation of MTBE [78].

Batch studies showed that benzene inhibited the degradation of MTBE by the pure culture PM1. MTBE was not degraded until benzene was depleted. The study confirmed that PM1 was capable of also degrading benzene. This study, overall, was very detailed giving rise to many questions. However, the authors stated that MTBE and benzene degradation in PM1 may have been induced by two different pathways [83].

When the biodegradation of MTBE was investigated in laboratory columns packed with aquifer sediments it was shown to degrade only in the absence of BTEX. In this study, it was concluded that MTBE would not degrade in the presence of significant concentrations of more readily degradable contaminants such as BTEX compounds [84].

Both trichloroethylene (TCE) and toluene were found to have inhibitory effects on the degradation of MTBE in FBRs due to a form of competition. The authors further stated that the high loading rates of TCE and toluene may not have been the only factor leading to inhibition [85]. Inhibition of MTBE degradation by BTEX was observed in a trickling filter reactor which had a MTBE degrading strain involved in direct metabolism. It was not identified what mechanism was responsible for the inhibition [79].

4.2.2 Competition for Reactor Occupancy, Oxygen and Nutrients

The maximum growth rate (μ_{max}) reported for aerobic BTEX degrading and nitrifying organisms at 25 °C lies in the range 3–9 d^{-1} [86] and about 0.6–1 d^{-1} [49] respectively. These μ_{max}s are over an order of magnitude higher than that reported for MTBE [47]. BTEX degraders and nitrifiers, therefore, have a competitive advantage in growth over MTBE degraders. Their faster growth rates can result in them becoming more dominant in a reactor, out-competing the MTBE degraders for occupancy, oxygen and nutrients. The presence of these co-contaminants could, therefore, have the effect of lowering MTBE removal rates, when compared to the situation where MTBE is the only contaminant being removed [48].

A study involving the oxidation of MTBE and ammonium in a PBR showed that ammonium oxidation occurred at a faster rate than that of MTBE. It was also found that the ammonium oxidisers were more dominant than the MTBE degraders at the inlet of the reactor. Model results showed that if the supply

of oxygen was insufficient for the complete oxidation of both MTBE and ammonium the removal of MTBE could either be prevented or reduced, while that of ammonium remained unchanged. The generally faster removal rates of ammonium compared to MTBE is attributed to their ability to effectively out-compete the MTBE degraders for oxygen and occupancy in some sections of the reactor [48]. The competition for oxygen can also become a problem for onsite remediation of MTBE polluted groundwater. In these situations the dissolved oxygen concentration typically is less than 10 mg/l. BTEX or ammonium concentrations even as low as 1–2 mg/l may prevent the degradation of MTBE.

Chemical oxidants, such as hydrogen peroxide, can be used to supply addition oxygen. However, it has been shown to reduce the degradation rates of MTBE due to inhibition, even at a concentration less than 1 mmol/L [87].

4.2.3 Precipitation of Iron

Some co-contaminant ions which are typically present in groundwater such as iron may precipitate in reactors and coat the biofilm. The coating of biological flocs or carrier material in a reactor may potentially interfere with the biofilm formation. Furthermore, clogging of reactors by iron precipitation creates the need for backwashing of PBRs or cleaning of membranes, which can result in biomass loss. Early loss of biomass from a reactor system may have a more pronounced effect on the MTBE degraders than other microbes since their growth rates are the slowest.

4.2.4 Summary of the Effects of Co-contaminants

Co-contaminants, however, do not always result in an effective lowering of MTBE degradation rates. Both BTEX and MTBE were degraded in bioreactors and all compounds were successfully removed down to low ppb ranges without accumulation of metabolic intermediates or inhibitory effects. There was also no indication that BTEX may have lowered the MTBE degradation rates [41, 60, 64, 88, 89]. In some studies BTEX was shown to have an enhancing effect on the degradation of MTBE [83, 88, 90]. In another study, it was also pointed out that an MTBE degrading culture could be maintained on toluene, which is a more favourable substrate. Growth on toluene did not affect the MTBE degrading capacity [91]. In all of these studies it appears that degradation of MTBE occurred as a result of direct metabolism.

It is not straightforward to predict how co-contaminants affect MTBE degradation rates in reactors. It is complex and depends on the relative substrate concentrations of the different compounds, the nature of the biological reactions and the reactor configuration, transformation capacity and adap-

tation. If, for example, the concentration of a co-contaminant is much lower compared to that of MTBE, it can hardly be expected that it will result in a lowering of MTBE degradation rates. Likewise, if MTBE is being metabolised in a reactor operated well below its maximum possible loading rates, then a small addition of co-contaminants should not be expected to affect the degradation of MTBE. It is also interesting that some MTBE degrading cultures will degrade some BTEX compounds by direct metabolism. BTEX present together with MTBE in gasoline plumes may reduce startup time reactors used for plume remediation, and increase the stability of the biomass.

4.3 Potential Toxicants

In addition to completion for microbial enzyme systems by co-contaminants, such as BTEX compounds, which can result in inhibition of MTBE degradation, MTBE's degradation may also be affected by the toxicity effects of these compounds. The accumulation of toxic intermediates formed in the degradation process can also lead to inhibition.

Compared to ethers for example, BTEX compounds are potentially more toxic to unacclimatised microorganisms due to their relatively high organic carbon partition coefficient [1]. Therefore, they are expected to bind readily to biological membranes, resulting in possible negative effects on their functionality. However, based on the literature, BTEX compounds can apparently be fed to continuous fixed film reactors at concentrations even in the range close to their water solubility limit without any inhibitory effects [92]. In this study, *o*-xylene was found to be most inhibitory among the BTEX compounds and only at a concentration over 100 mg/l (water solubility 175 mg/l). Benzene was the least inhibitory; concentrations even at 1 g/l did not show any inhibitory effects. In this study, it was concluded that their fibrous bed reactor with its immobilised biomass had an inherent ability to resist the effects of toxicity and adapt to the BTEX compounds. Studies on the degradation kinetics of toluene (nitrate as electron acceptor) in a biofilm reactor showed that toluene in the presence of benzene, ethylbenzene and xylenes could be degraded at concentrations greater than 10 mg/l without indication of inhibition [93]. Toluene concentrations at 6 mg/l showed no inhibitory effects on its own degradation in a fixed film aerobic reactor in another study [94].

Formaldehyde, a well known microbial inhibitor, is produced as an intermediate in the microbial degradation of MTBE [1]. However, no reports so far have shown that this intermediate may accumulate to toxic levels.

Both MTBE and TBA can be considered to have little or no inhibitory effects at the concentrations within the range of a few ppm normally encountered in groundwater plumes. At a concentration less than 1 g/l, the presence of MTBE, DIPE, ETBE or TBA alone was shown to have no inhibitory effects to microorganisms degrading acetate under anaerobic conditions [95]. Inlet

concentrations of 350 and 170 mg/l for MTBE and TBA, respectively, fed simultaneously to a FBR were successfully removed down to a few micrograms per litre without inhibitory effects [41].

4.4 Temperature and pH

Temperature affects all microbial processes; a higher temperature generally means higher microbial growth rates. In general, metabolic rates double for every 10 °C rise in temperature [31]. Biodegradation of MTBE in reactors will generally take place at the prevailing ambient conditions, this temperature may vary from about 5–25 °C in the northern hemisphere. Based on all the reports studied so far, MTBE degrading organisms operate well within these temperature ranges. The MTBE degradation rates in batch cultures were much slower at 10 °C when compared to 25 °C [96, 97].

The pH of a biological system also has an impact on the process rates; normally the pH should be maintained within a narrow range. It was reported that the optimal pH range of an MTBE degrading culture in a biofilter was 6.5–7.8 [89, 97–99]. Operation of MTBE degrading bioreactors way outside of the normal optimal range of pH is likely to affect the process [81]. The degradation of MTBE does not consume or release a net amount of protons. Therefore, in most cases pH control is not necessary. However, in the case of acidic groundwater, high dissolved carbon dioxide concentration, or significant nitrification activity, addition of alkalinity is necessary to maintain an optimal reactor pH.

5 Reactor Startup

The startup time or the time taken for reaching the maximum removal potential (or a steady state) of a reactor designed for MTBE removal has been shown to vary from a few days to over 200 days [41, 42]. This means that it is critical to predict the startup time before a bioremediation strategy for MTBE removal can be implemented. Alternative treatment options must be implemented until the full remediation capacity of the biological treatment process can be reached. Physical treatment methods such as chemical oxidation, stripping or activated carbon sorption are good options to be added down stream of the biological system. With the use of simple models representing our system we are able to predict for example the startup time or dynamic removal of the interested components in the reactor's inlet stream. If our reactor is operated optimally in terms of nutrients and correct pH, then, the initial biomass concentration and the presence of co-contaminants can be considered to be two of the most important factors in determining the time for startup [48].

Other factors such as temperature and the presence of toxins, which affect the growth rate of the MTBE degrading organism, are also expected to have an influence on reactor startup time.

5.1 Initial Biomass Concentration

A high initial seed of microorganisms previously acclimatised to similar conditions as the new reactor system will reduce the startup time. The startup time of MBRs for MTBE removal was shown to be approximately 20 days when seeded with 5 g/l TSS of an MTBE degrading culture (ENV735) [62]. Two other MBRs operated under similar conditions for MTBE removal, but seeded with a much lower initial biomass concentration took approximately 150 and 200 days for startup [40, 88]. Other studies with FBRs used for MTBE removal showed that the startup process could be only 20–30 days if the reactor was seeded with cultures already adapted to MTBE degradation [41, 60].

Model simulations showed that by increasing the initial seed concentration of MTBE degrading biomass in bioreactors by 10 times reduced the startup time by 50–100 days [48].

5.2 Co-contaminants

Previously in this chapter, it was shown that co-contaminants present in MTBE degrading reactors may have possible effects on the degradation of MTBE. Co-contaminants may either increase or reduce the time required for startup of a bioreactor. Co-contaminants such as ammonium or BTEX can result in an out-competing of MTBE degraders by nitrifiers and BTEX oxidisers. Co-contaminants with higher growth rate oxidisers and/or in higher concentrations than that of MTBE may reduce the growth of the MTBE biomass compared to a situation where MTBE were present alone. A lowering of the growth rate of the MTBE biomass effectively increases the startup time for a reactor. The co-contaminant oxidisers can also occupy more favourable positions inside biofilms enabling them to out-compete the MTBE degraders for access to oxygen and nutrients.

Model simulations showed that startup time for degradation of MTBE in a mixed reactor would be increased by increasing concentrations of co-contaminants [48].

It has been reported that some BTEX compounds may stimulate the growth of MTBE degraders [83, 88, 90]. Growth on BTEX for microorganisms is expected to be much more favourable than with MTBE. The presence of BTEX in reactors may reduce reactor startup times for MTBE degradation if these compounds increase the quantity of the MTBE degrading biomass.

The strain *M austroafricanum* IFP 2012 degrades MTBE as sole carbon and energy source, and it has been shown to grow on ethanol, *iso*-propanol, toluene and xylenes. The cell yield of this strain when grown on TBA was 0.6 g VSS/g TBA, the TBA grown cells have also been shown to degrade MTBE [22]. The MTBE degrading strain PM1 isolated by Hanson et al. [15] is reported to be capable of rapid growth on ethanol and TBA [96]. The feeding of these compounds to reactors seeded with PM1 may offer possibilities for quick startup of the MTBE degrading activity.

6 Cometabolism

Degradation of MTBE by cometabolism is probably more widespread in the environment than direct metabolism if the number of strains that have been identified so far performing each type of metabolism is used as the judging criteria. Cometabolic degradation occurs when the organism degrades MTBE incidentally using the enzymes that were produced from growth on a primary substrate. MTBE does not provide either energy or electrons for biomass production during its degradation.

Some hydrocarbon components which are typically present together with MTBE in a gasoline plume, such as simple branched alkanes (e.g., *iso*-butane), have been shown to act as primary substrates for degradation of MTBE and other ethers by cometabolism. The general view is that organisms which can degrade these alkanes will likely be able to degrade MTBE through cometabolism. This ability is related to analogous properties of the molecules of MTBE and the branched alkanes [29, 32, 100].

Several propane oxidising strains have been shown to cometabolically degrade MTBE. The strains were able to grow on several other organic compounds including ethanol and 2-propanol [30, 62, 101]. The strain *G. terrae* isolated from an urban wastewater treatment plant was also able to degrade both MTBE and TAME by cometabolism using ethanol as the carbon source [102]. In another study, cometabolism of MTBE by a benzene-grown culture called PEL-B201 was also shown. Preliminary results had suggested that cometabolism of MTBE could also occur by cultures grown on cyclohexanone, *o*-xylene or camphor [78].

It was reported that *iso*-pentane initiated the biodegradation of MTBE in a FBR through cometabolism. Interestingly, after *iso*-pentane reportedly initiated the degradation of MTBE, MTBE removal continued for more than 60 days without the need for its re-addition. Degradation of a MTBE and BTEX mix in the reactor resulted in a 96% removal of the MTBE [61].

In cometabolism, both the primary and the cometabolic substrates are competing for the same enzyme system. The presence of the primary sub-

strate is necessary to induce the enzyme system of the cell, but it does not need to be present at all times during degradation of the cometabolic substrate. Due to competitive inhibition, the degradation rate of the cometabolic substrate is often slower in the presence the primary substrate than when degraded alone [26, 29]. Transformation of the cometabolic substrate has also been shown to be mostly partial and many cometabolic MTBE degrading strains tend to accumulate TBA in batch studies [29, 30, 103–105]. This aspect may be a problem which has to be addressed for the applicability of these strains in bioreactors.

The affinity of cometabolic strains for MTBE will be generally lower than that of direct metabolising strains. For this reason, the K_s values for MTBE in cometabolic strains is often much higher compared to strains which transform MTBE by direct metabolism. The K_s values reported for cometabolic MTBE degrading strains were mostly high. Hyman et al. [32] reported values ranging from 10.56–44 mg/l for nine different strains, while Smith et al. [104] reported a value of 1140 ± 180 mg/l. The K_s values reported for MTBE degrading strains which use direct metabolism have typically not exceeded 10 mg/l [47, 106].

Cometabolic strains grow much faster on simple organic compounds compared to strains which degrade MTBE by direct metabolism. Cometabolic strains could be used in bioreactors to achieve a fast startup of MTBE degradation by supplying the primary substrate to the reactor. They could also be grown separately either on support material or in membrane systems to high concentrations. This would enable almost immediate startup of fixed film reactors or MBRs. Operational strategies which can reduce the effect of competitive inhibition in reactors should be considered. Since cometabolic strains tend to have high K_s values this is a disadvantage when trying to achieve very low effluent concentrations. The optimal dose (frequency and quantity) of a primary substrate that is required to operate MTBE degrading reactors should be investigated. There is also a need to verify if MTBE is fully mineralised in reactors when cometabolism is used, it is undesirable to have TBA in the effluents.

7 Modelling MTBE Degradation

Models are now an indispensable part of all aspects of biological reactor design, operation and control. Models can be used to gain *a priori* information for bioremediation systems that are being planned. They can be used as a testing platform for our hypothesis of the biological and physical processes occurring inside in a reactor, and they can be used to predict the dynamic changes of the substrate and biomass profiles during the startup phase of reactor operation. However, before the model is made the objectives must be defined in order that (only) the relevant concepts and processes are incorporated.

Models can be represented in the form of a process matrix; the matrix shows all the components of the models, processes, rate kinetics, mass balances and stoichiometry. A thorough outline on the use of process matrix is described in the activated sludge model [107]. All growth processes are based on Monod kinetics and switching functions to literally turn on or off different biological processes.

7.1 Model Application

The model and examples used in this section are centred on the modelling of a 1 m long laboratory PBR. The model describes the growth and decay of MTBE degraders, nitrifiers and other general heterotrophs. The influent to the PBR contains ammonium (1 mg N/l) and MTBE (10 mg/l or 27.3 mgCOD/l). Ammonium is fully nitrified while MTBE is mineralised by oxygen. For a full description of the model and its implementation see Waul [48].

Figure 3 shows the modelled dynamics of both the MTBE's biomass and substrate profiles in a PBR as a function of the reactor's depth. The figure shows that the biomass concentration increases uniformly over the column's depth within the first 150 days of reactor operation. There is also a corresponding increase in the substrate removal rate within this period, which is evident from the increased steepness of the substrate profiles (Fig. 3b). Full steady state of the biomass is reached between 200–300 days and there is no further improvement in reactor performance.

The biomass concentration at the base of the reactor (0 m) is over 10 times greater than at the top (1 m) at 500 days. The biomass at the base of the reactor has a faster growth rate than at the top.

The time required for the reactor to reach its full removal potential is in approximate agreement with some experimental studies which have reported the startup time of their reactors [40, 42].

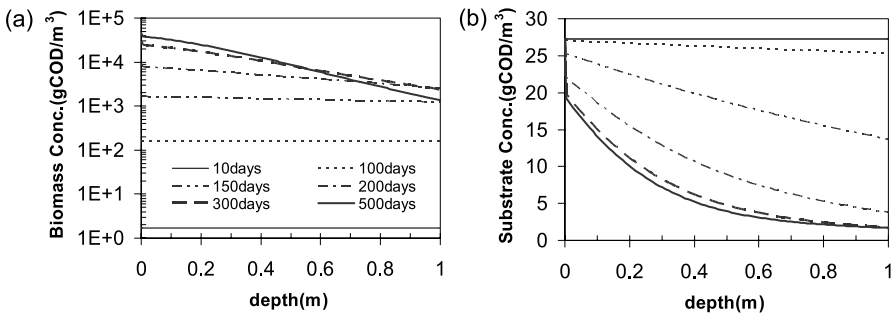

Fig. 3 The modelled dynamics of **a** biomass concentration plotted on a log scale and **b** substrate concentration for MTBE as a function of reactor depth in a packed bed reactor

7.2 Model Parameters

The outcome of a model is closely linked to the values of the model parameters. Parameters are the values that cannot be measured directly and thus must be estimated. Model parameters are normally only valid for one set of environmental conditions. There will be changes in both μ_{max} and the decay constant (b) when there is a change of reactor temperature. Normally, parameters will differ depending on the source to some degree, so careful thought must be given to the use of parameters for the modelling process. Table 6 shows a set of parameters for the modelling of MTBE degradation, with MTBE being used as the sole substrate for growth and energy of the biomass.

The range of the reported measurements available for the μ_{max} of MTBE is quite large. However, there is enough evidence based on Waul [48] that it is much less than the μ_{max} for nitrifiers at 30 °C (0.16 d^{-1}). A good starting value for modelling should be about 0.1 d^{-1}. The value for b reported by Fortin et al. [47] is considered very low, in well tested models such as the activated sludge model 1 (ASM1), the b is about 10% of the μ_{max} values [107]. Based on the data of Hanson et al. [15], a K_s of approximately 136 mg COD/l was estimated for the PM1 culture, however, this is considered very large and most likely out of range. It is more consistent with the K_s values for cometabolic MTBE degrading strains. A good starting value for modelling should be less than 20 mg COD/l. All evidence so far suggests that the Y for MTBE is typically much lower than for other heterotrophic bacteria. The generally accepted range of values for Y is 0.1–0.2 g VSS/g MTBE.

There is a large uncertainty in the μ_{max} and K_s values. Only a few authors have studied the kinetics of MTBE so far. It is suggested that experiments are

Table 6 Model parameters for MTBE degradation

Parameter	Symbol	Units	Value	Refs.
Maximum growth rate	μ_{max}	d^{-1}	0.1 ($T = 30$ °C) 0.86 ($T = 30$ °C) 0.07–0.5	[47, 106, 108]
Half saturation constant	K_s	mgCOD/l	4.1 15.63	[47, 106]
Decay constant	b	d^{-1}	0.001 ($T = 30$) 0.12 ($T = 30$)	[47, 106]
Yield coefficient	Y	g VSS/g MTBE	0.11 0.21–0.28 0.18 0.1 –0.14	[15, 16, 47, 60]

performed if a full scale reactor is to be implemented, so that these parameters can be estimated for the particular system.

8 Conclusions

Based on the literature investigations conducted, it was found that reactors which utilise biofilms are all capable of achieving high biomass concentrations, values even greater than 10 g/l TSS have been reported [41]. These high concentrations are critical for the high rate removal of MTBE in reactors since the MTBE degraders are some of the slowest growing organisms known, their growth rates are in the order of 0.1 d^{-1} or less, at 25 °C. Too high biomass concentrations, however, may lead to thicker than necessary biofilms, causing efficiency problems due to diffusion limitations of substrates or clogging in packed bed systems. Therefore, it is necessary to control the thickness of the biofilms in fixed film processes in an optimal range. To prevent oxygen limitation inside the biofilms the bulk oxygen concentration must be as follows: $S_{O_2} > 0.33S_{MTBE}$, on a COD basis.

The reactor types applied for MTBE removal have been identified as being the packed bed reactor (PBR), fluidized bed reactor (FBR) and the membrane bioreactor (MBR). The aerobic upflow sludge bed reactor and the rotating biological contactor have been identified as two possible candidate reactors which do posses some advantages and can be applied for MTBE removal. More research is, however, required to further exploit the advantages these reactors may posses. The maximum removal rates reported for both FBRs and MBRs were about 1000 mg/(l d) and about 450 mg/(l d) for PBRs. Both MTBE and BTEX present in a contaminated groundwater plume can be biologically degraded simultaneously.

The typical co-contaminants present in MTBE polluted groundwater are usually BTEXs, ammonium and iron. These co-contaminants will affect the degradation of MTBE in reactors, due to the presence of their oxidisers. The growth rate of both BTEX degraders and nitrifiers are higher than that of MTBE degraders. Therefore, competition for access to oxygen, nutrients and reactor occupancy will mostly favour the organisms which oxidise the co-contaminants. In a reactor system where the oxygen supply is limited, oxidation of the co-contaminants will take precedence over that of MTBE degradation. It does not appear that toxicity of BTEXs will inhibit MTBE degradation over the long term. However, the presence of BTEX compounds in MTBE degrading reactors may interfere with the MTBE degradation enzyme system through competitive or non-competitive inhibition. This will have the effect of reducing MTBE degradation rates. However, if the MTBE biomass can grow on for example, co-contaminants such as BTEXs, this is important in terms of having high MTBE removal rates. The presence of iron in

groundwater will lead to fouling of MBRs and clogging of PBRs, which affects their performance.

The initial biomass concentration and the presence of co-contaminants have been found to influence the startup of MTBE reactors. Higher initial seed concentrations generally lead to a faster reactor startup. MBRs or FBRs seeded with a high biomass concentration can be started within 10–30 days. Reactors seeded with only a low biomass concentration will generally take about 150–200 days to achieve startup. The organisms which oxidise co-contaminants will compete with the MTBE degrading biomass for dominance and occupation in reactors. Therefore, high concentrations of co-contaminants can increase the time required for reactor startup in some systems.

Cometabolic cultures in MTBE degrading reactors may have some positives. The cometabolic strains normally grow much faster than strains which utilise direct metabolism. Furthermore, the simple branched chain alkanes used as energy source during cometabolism reactions are normally present in MTBE plumes caused by gasoline leaks. The use of cometabolic strains can result in faster reactor startup. Knowledge of the applicability and limitations of cometabolic strains in bioreactors is limited and needs further research.

Adequate understanding of biological reactions would be incomplete without using mathematical models for further analysis. Models increase our knowledge of the biological processes. Some results from using models for MTBE have been shown; the model have predicted startup times and evaluated the dynamic performance of a MTBE degrading PBR.

9 Future Outlook

There is evidence that MTBE is being phased out in many places, especially in parts of the US because of the widespread contamination it has caused. So far, ethanol seems to be the replacement. Ethanol can be degraded fairly rapidly, so long as the concentrations are not toxic. Therefore, from the point of view of bioremediation of contaminated groundwater, ethanol is a suitable replacement. Other ethers such as: ETBE, TAME and DIPE can also be used as substitutes for MTBE. Indications so far suggest that the same principles apply for their bioremediation. The ease at which biodegradation will occur are as follows: ETBE > TAME, MTBE > DIPE [48, 65].

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Remediation Technologies and Costs for Cleaning MTBE Contaminated Groundwater

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Abstract MTBE (methyl-*tert*-butyl-ether) is the most important fuel additive to have been used in the USA and Germany within the last 20 years. In the last 3 years, California and some other American states have substituted MTBE by ethanol. In Germany MTBE was replaced by ETBE (ethyl-*tert*-butyl-ether). Due to widespread MTBE use, spills from underground fuel tanks locally has caused intensive groundwater contamination, which is favoured by the tracer-like behaviour of the compound. In cases of remediation needs, MTBE is difficult to clean due to its physical-chemical-biological characteristics. The possible remediation technologies are classified and described. These technologies can be differentiated into "pump-and-treat measures" and "alternative technologies". For groundwater cleaning applying pump-and-treat, several procedures can be chosen. How-

ever, in most cases stripping is used, which results in high costs for cleaning of the stripped air. Regarding alternative technologies, a lot of lessons have been learnt in optimizing the biological technologies over the last 5 years. Since MTBE-degrading bacteria at most sites have developed some years after the spill event, biostimulation by biobarrier techniques have a good chance of cleaning MTBE-contaminated groundwater by an in-situ method. In the case of successful remediation, large amounts of costs can be saved compared to pump-and-treat applications.

Keywords Biobarrier · MTBE · Remediation costs · Remediation technologies · TBA

Abbreviations

AOP	Advanced oxidation process
BTEX	Benzene, toluene, ethylbenzene, xylene
CAH	Chlorinated aliphatic hydrocarbons
cis-DCE	*cis*-1.2-Dichloroethene
CT	Carbon tetrachloride
1.2-DCA	1.2-Dichloroethane
DNAPL	Dense non-aqueous phase liquids
DVGW	Deutsche Vereinigung des Gas- und Wasserfaches ("German Technical and Scientific Association for Gas and Water")
ETBE	Ethyl-*tert*-butyl-ether
hPa	Hectopascal (100 Pa)
LNAPL	Light non-aqueous phase liquids
MTBE	Methyl-*tert*-butyl-ether
MPPE	Macroporous polymer extraction
NOx	Nitrogen oxide
PAH	Polycyclic aromatic hydrocarbons
PCE	Tetrachloroethene
TA-Luft	Technische Anleitung zur Reinhaltung der Luft ("Technical Instructions on Air Quality Control")
TBA	*tert*-Butyl-alcohol
TBF	*tert*-Butyl formate
TCA	1.1.1-Trichloroethane
1.1.2.2-TCA	1.1.1.2-Tetrachloroethane
TCE	Trichloroethene
VC	Vinyl chloride
vol%	Volume percent
wt%	Weight percent

1 Introduction

Methyl-*tert*-butyl-ether (MTBE; synonymous with *tert*-butyl methyl ether and methyl *tert* butylether) has been used as an additive for car fuels in Europe and the USA since the 1970s. Since the beginning of the 1980s MTBE has gained in importance as an additive in petrol in Germany. In 2001 the added MTBE quantity in gasolines in Germany totalled 680 000 t [1].

The use of MTBE as a fuel additive leads to improvements in the gasoline quality (so-called oxyfuels: oxygen-containing gasolines). First of all, burning of the fuels is optimized as the content of harmful components in exhaust gases is reduced (benzene, ozone, NOx and CO). A second positive effect is the improvement in the anti-knock property of the fuel (increase in the octane rating).

For the improvement of air quality in California at the beginning of the 1990s, the Clean Air Act prescribed that gasolines must contain a season-dependent minimum content of 2.7 wt % oxygen, in order to obtain a more efficient burn. This was reached by adding high MTBE portions to the gasoline (up to 15 vol %). Consequently, in the 1990s MTBE production and consumption rose strongly in the USA. In 1998 MTBE (with an output of 9.3 E6 t) quantitatively ranked fourth of the chemicals manufactured in the USA.

In contrast to the development in the USA, in Germany MTBE was added with the objective of replacing the lead compound (lead-tetra-ethyl) in gasolines in order to guarantee the anti-knock property of the gasolines (increase in the octane rating).

Meaningful for the intensified use of MTBE as fuel compound in Europe was the guideline 85/535/EWG of 5 Dec 1985 for the saving of crude oil by the use of replacement material components, which was partly replaced by the fuel quality guideline dated 13 Oct 1998. After that, mixing of MTBE up to 15 vol % was permitted.

In 2001 the MTBE content in all gasoline types averaged 2.1% in Europe. In Germany the average MTBE content in the year 2001 amounted to 0.43 vol % in regular grade fuel (market share 32.1%), 3.0 vol % in the euro-super (market share 64.1%) and 10.2 vol % in the super-plus fuel (market share 3.8%). In the super-plus, peak values up to 15% by volume were measured [1].

In Germany in the late 1990s, possible environmental pollution caused by MTBE was under consideration, due to reports about the contamination of drinking water wells in the USA. In most cases gasoline stations were identified as the origin of the groundwater impurities. Some German Federal States reacted to these references. Nowadays, the analysis for MTBE is mandatory for soil and groundwater investigations carried out within the range of gasoline stations in the Federal States of Bavaria and Rheinland Pfalz. In Baden-Württemberg an appropriate guideline for the monitoring obligation is in preparation.

Applying state-wide sampling programs at groundwater wells in Bavaria, Brandenburg and Baden-Württemberg it was determined that the MTBE concentrations in the groundwater were low (low: $< 1\ \mu g\ L^{-1}$). One of the DVGW research projects showed that the number of MTBE concentrations lying above the detection limit was clearly higher in the groundwater in urban areas than in rural regions [2].

Within the framework of a diploma thesis carried out at the UFZ (Umweltforschungszentrum Leipzig Halle) ten cases of gasoline damage were exam-

ined for MTBE. The examined locations concerned eight gasoline stations and two fuel depots in Sachsen, Baden-Württemberg and Mecklenburg-Vorpommern. Groundwater sampling measured MTBE concentrations between 29 and 87 800 mg L^{-1}. Since a pollution of the groundwater with MTBE in at least three of the examined cases was certainly caused by a defect at a newer gasoline station (established or restructured after 1990) these investigations are of special interest [3].

MTBE is currently replaced by ethyl-*tert*-butyl-ether (ETBE) in Germany. ETBE is produced from bioethanol, which is a product of alcohol production from plants like sugar beet and wheat. Since the chemical-physical-biological characteristics of ETBE are very similar to those of MTBE it is highly probable that both substances in groundwater exhibit a similar behaviour with respect to plume development and remediation technologies. In addition, the possibility of using ethanol as a gasoline component at present is being discussed in Germany.

2 Material Properties and Behaviour of MTBE in Groundwater

For the evaluation of technologies that might be suitable for remediation of MTBE-contaminated groundwater, knowledge about the chemical-physical properties of the substance is of great importance. The most important physical-chemical data of MTBE are arranged in Table 1. The liquid belonging to the group of volatile hydrocarbons, is colourless and has a boiling point of 55.3 °C. The vapour pressure is about three times higher than that of benzene and amounts to 270 hPa at 20 °C. The Henry constant shows a low value of approximately 0.02. The log K_{OW} value (octanol–water distribution coefficient) is

Table 1 Chemical-physical and environmental relevant data of MTBE

Melting point (°C)	– 108.6
Boiling point (°C) (1013 hPa)	55.3
Flash point (°C)	– 28
Density (g cm^{-3}) (25 °C)	0.74
Water solubility (mg L^{-1} at 25 °C)	50 000
Vapour pressure (Pa at 20 °C)	27 000
Dynamic viscosity (mPa s at 20 °C)	0.36
Surface tension (mN m^{-1} at 20 °C)	20
Henry constant (20 °C)	0.017
K_{OC}	10
log K_{OW}	1
Taste threshold value water (µg L^{-1})	2.5–600
Water endangerment class (WGK)	1

approximately 1.0. From this it can be concluded that MTBE has a small tendency to be enriched in non-polar media. The data for the K_{OC} value (organic carbon–water distribution coefficient) lie in the region of 9–12 [4]. The most noticeable characteristics of MTBE for humans are the intense smell and taste.

The solubility value most frequently quoted in the German literature is 50 mg L^{-1} at 25 °C. For an environmentally relevant substance MTBE exhibits a remarkably high solubility. At 20 °C this is about 24 times higher than that of the most soluble BTEX component (benzene) and approximately 360 times higher than that of ethylbenzene (benzene and ethylbenzene are components of gasoline).

In the atmosphere, MTBE is subject to degradation by OH radicals; photolysis (direct decomposition by sunlight) hardly plays a role. The calculated half-life amounts to 3–6 days, depending upon OH radical concentration [5, 6].

According to field observations in groundwater, MTBE is hardly microbiologically degradable by pure natural processes alone. The half-life of MTBE in the groundwater is estimated at approximately 2 years [6]. In laboratory studies a microbiological degradation could be proven; however, decay took place more slowly than the degradation of BTEX aromatics. MTBE reduction runs preferentially in aerobic environments and is most effective if no other carbon source is available and the oxygen content is at the milligram per litre level. However, in the last few years at a lot of sites MTBE-degrading cultures could be identified in the field, which shows that at least there exists a chance of biological decay by natural processes.

As reduction products in laboratory tests, *tert*-butyl-ether (TBA) and *tert* butyl formate (TBF) could be proven, whereby TBF is converted rapidly to TBA. CO_2 remains at the end of the TBA decay [7]. The fact that TBA can also be a primary component of gasolines (within the %-range) makes it often difficult to interpret the degradation path under field conditions.

3 Types of MTBE Contamination and Plumes

In Europe approximately 98% of today's MTBE output is used for the formation of approximately 3 E6 t of gasoline. Subordinated quantities are needed as solvents in the pharmaceutical industry.

In principle, the following types of MTBE contamination can be differentiated regarding production, transport and use of MTBE as a gasoline component:

Type 1 Production of MTBE. In Europe approximately 25 companies produce MTBE at about 35 different locations.

Type 2 Transport of MTBE from the manufacturers for further usage. MTBE is either added directly to the gasoline in MTBE-producing refineries

(on-site) or transported by the producers (refineries as well as chemical and petrochemical plants) to the non-manufacturing MTBE refineries (off site, transport over inland waterways and in railway tankers).

Type 3 Formulation in the refineries (mixing of MTBE in the gasoline, on-site and off-site).

Type 4 Storage of formulated gasoline in fuel depots.

Type 5 Transport from formulated gasoline depots to temporary storage facilities/consumers (gasoline stations).

Type 6 Storage and handling by consumers (gasoline stations).

Type 7 Losses from automobile tanks and emissions into the soil, e.g. through traffic accidents (these emissions are of minor importance due to the small release rates in single cases).

While with the types 1 and 2 pure MTBE contamination can develop, types 3 to 7 are always associated with gasolines and the substances contained in them (alcanes, cycloalcanes, BTEX, further additives etc.). Thus, differences result in the required treatment of MTBE-contaminated soil and groundwater according to types of contamination (types 1–2 or 3–7). Due to the very large number (approximately 15 000 gasoline stations as compared to about seven manufacturers in Germany), gasoline station contaminations are of much greater importance. Further, the sites of MTBE manufacturers usually have suitable monitoring systems, by which MTBE pollution of the groundwater may be determined. The most frequent causes of contamination can be stated as follows:

- Leakages at service stations and piping systems (fuel depots and gasoline stations)
- Incorrect filling at fuel depots and gasoline stations
- Transportation accidents
- Averages by fire events and explosions (fuel depot, gasoline stations)
- Inappropriate cleaning of transport containers (inland waterway crafts, railway tankers)
- Pipelines

On the basis of the chemical-physical characteristics and the experiences available today concerning the treatment of MTBE contaminations, three different types of MTBE plumes can be differentiated:

Type 1 MTBE plume development corresponds almost to that of the BTEX

Type 2 MTBE plume development towards flow path is further advanced than those of the BTEX

Type 3 Highest MTBE concentrations have already left the source area while the BTEX plume centre is still present in the source area or very close to it

The described types are schematically represented in Fig. 1. The three different spreading scenarios can be explained by different source strengths in

connection with different time scales. While type 1 is concerned with recent damage, the plume development of type 2 can be traced back over a longer time to when the spill took place. With type 3 the source strength is relatively low and the plume (due to the high solubility) is already separated from the source area.

Since the spreading behaviour of organic substances in subsoil is very complicated depending on the site (subsoil structure) and material characteristics, at this point only a simplified description is given. For detailed information refer to the available technical literature [8, 9].

The behaviour of MTBE in aquifers is described below. It is fundamental to differentiate between spills of MTBE-containing gasoline and pure MTBE spills.

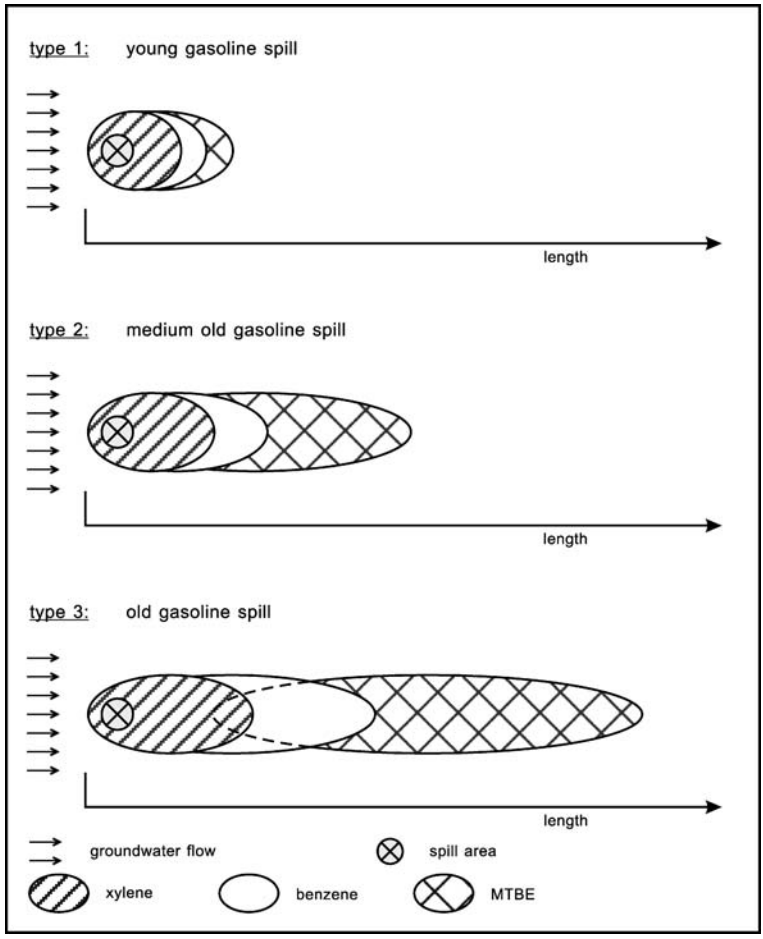

Fig. 1 MTBE plume types

3.1
Spills of Gasoline Containing MTBE

Upon infiltration of gasoline containing MTBE into the soil, MTBE is initially mixed with the other gasoline components, representing a gasoline–MTBE mixed phase. A first differentiation of the mixed phase occurs in the unsaturated zone. As MTBE has an approximately three times higher vapour pressure than benzene, it forms a vapour phase body in the unsaturated zone. The vapour pressures of different organics are shown in Fig. 2. As soon as MTBE infiltrates as gasoline–MTBE mixed phase into the groundwater, a second differentiation takes place, since MTBE is substantially more soluble than the BTEX. A third differentiation occurs in the seepage path, in which the percolation water contains higher MTBE than BTEX contents. Thus, at sufficient MTBE supply in the seepage zone, MTBE preferentially enters the groundwater as dissolved phase.

Due to its high solubility and its strikingly low adsorption behaviour, MTBE migrates in the groundwater significantly faster than BTEX. MTBE behaves almost like an ideal tracer. The K_{OC} values, as meaningful parameters for the evaluation of the adsorption behaviour for some relevant organic substances, are represented in Fig. 3.

In groundwater MTBE is subject to no or minor microbiological decay, thus leading to the formation of the aforementioned contamination plumes (plumes type 1–3).

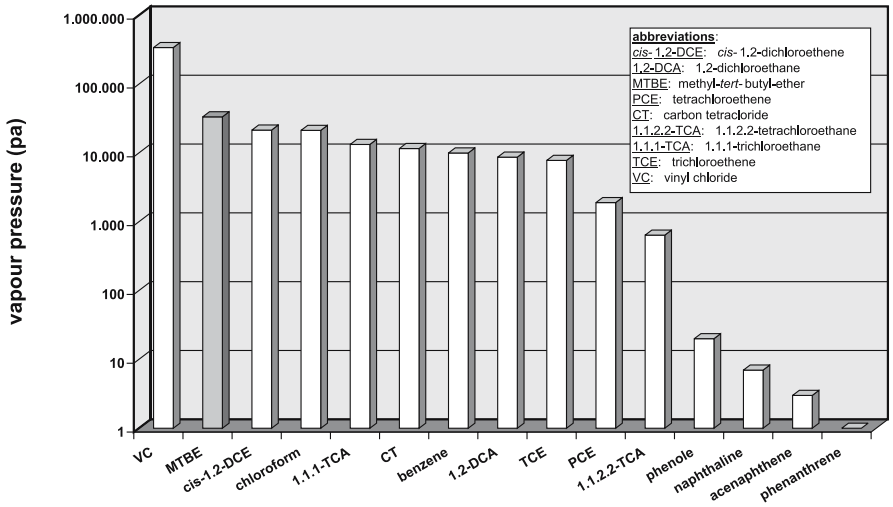

Fig. 2 Vapour pressure of organic compounds at 20 °C, in pascals

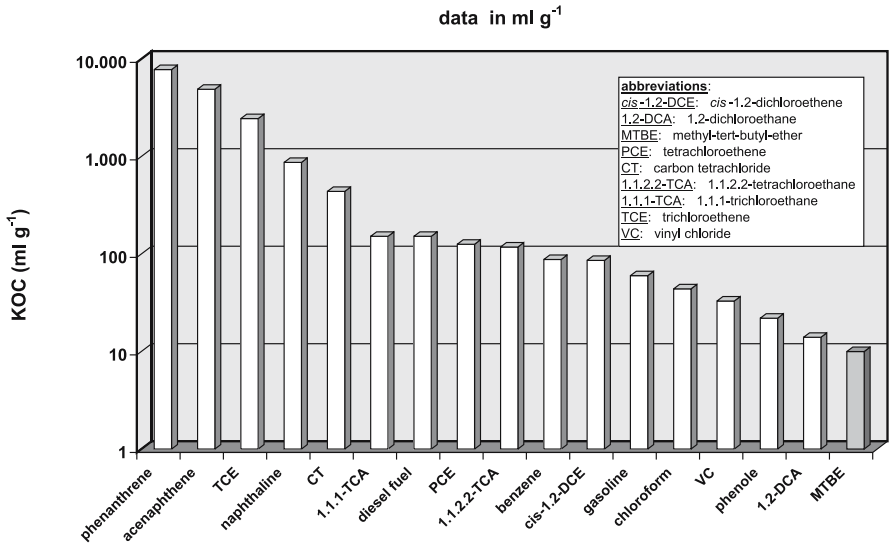

Fig. 3 KOC values of organic compounds

3.2 Pure MTBE Spills

- At adequate quantities MTBE easily infiltrates into the soil due to its low viscosity and low surface tension.
- The high vapour pressure of MTBE leads to the development of a distinct vapour phase body (compare Fig. 2).
- Due to its low density of 0.74, MTBE does not penetrate deeply into the groundwater in the source area. In Fig. 4 the densities for groundwater impurities of relevant organic contaminants are comparatively represented. MTBE has the lowest density of all specified compounds.
- Floating of MTBE as an independent phase may occur in the case of infiltration of large amounts of MTBE in a short time. The very high solubility of MTBE (approximately $50\,g\,L^{-1}$ at 25 °C) leads to preferential dissolution of MTBE in the groundwater. In most cases MTBE is subject to no or minor microbiological degradation and a contaminant plume develops (compare Fig. 5).

With larger MTBE spills it is to be noted that MTBE affects the dissolution of other organic compounds, i.e. the dissolved quantity of other organic substances is increased. At MTBE contents of more than 1% ($> 10\,000\,mg\,L^{-1}$) this effect is to be expected for BTEX.

Due to its high mobility on the one hand and its normally minor degradation in the aquifer on the other hand, MTBE can lead to an endangerment of

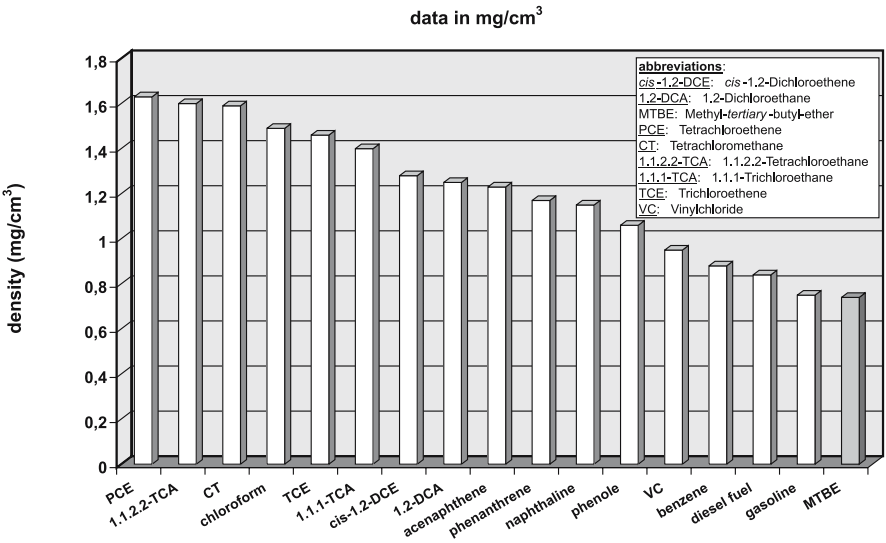

Fig. 4 Density of organic compounds

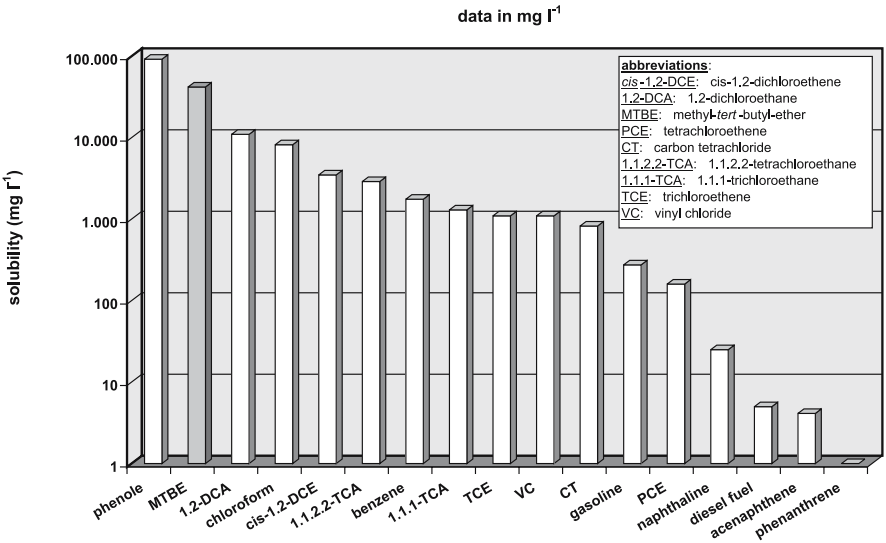

Fig. 5 Water solubility of organic compounds

subjects of protection (e.g. water supply plants) to a far greater extent than BTEX. According to estimations from insurance companies the number of possible MTBE groundwater contaminations in Germany is estimated at approximately 1500.

For the detection of groundwater contamination it is important that MTBE is organoleptically perceptible by humans at low concentrations, so that in many cases a chemical analysis is initially not necessary for further investigations. The odour threshold of humans varies between 2.5 and 190 $\mu g\,L^{-1}$ and the taste threshold between 2.5 and 690 $\mu g\,L^{-1}$ [10].

4 Remediation Techniques

Around the middle of the 1990s a series of larger groundwater contaminations caused by MTBE were determined in the USA. These arose primarily from the underground fuel tanks of the gasoline stations and partly led to contamination of drinking water wells. In Germany the first MTBE groundwater impact was detected some years later. Due to the lower safety standards of retail stations, the extent of groundwater contamination in the USA (especially in California) is to be classified as substantially larger than in Germany.

North America is leading in the development of remediation technologies because of the earlier detection of MTBE groundwater contamination. For the

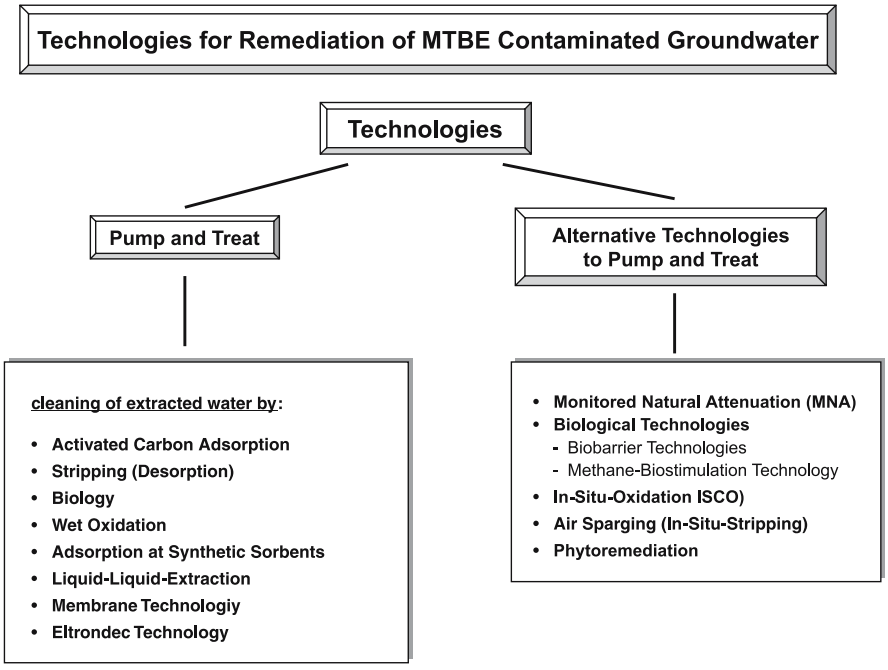

Fig. 6 Technologies for remediation of MTBE contaminated groundwater

groundwater remediation of MTBE spills, a large spectrum of techniques is theoretically possible.

Structuring of the technologies is shown in Fig. 6. In principle one can differentiate between pump-and-treat and other technologies (here, alternative technologies). With pump-and-treat there exist different techniques that can be used for groundwater remediation. Since some of the remediation techniques for pump-and-treat and the alternative procedures are still at the stage of research/development, in practice only few have been used so far.

An important decision parameter for the definition of the remediation technology is the MTBE threshold value that needs to be achieved for the treated water. This depends on the kind of discharge (waste water channel, rain water channel, re-injection into the groundwater) and the general site situation. At present, for discharge into sewers MTBE concentrations of about 50 $\mu g\,L^{-1}$ are commonly specified.

4.1 Cleaning Technologies for Pump-and-Treat

4.1.1 Activated Carbon Adsorption

According to Fig. 3, MTBE possesses a low K_{OC} value, so that activated carbon has a low capacity for the adsorption of MTBE. According to the available isotherms, the loading capacity for water activated carbon for an MTBE infed concentration of 100 $\mu g\,L^{-1}$ lies at approximately 0.1 wt % (fresh activated carbon). Somewhat higher loadings can be obtained with fresh activated carbon from coconut shell (approximately 0.15–0.2 wt%). For treating MTBE-contaminated air, the loading capacity under favourable conditions is up to approximately 4% (no competition adsorption and air flow with low dampness due to a previous drying process).

The activated carbon adsorption of MTBE is determined in strong measure by competition adsorption with other organic substances contained in water. Included in these are organic compounds (general gasoline components, e.g. aliphates, cycloaliphates and BTEX) and also naturally occurring substances (NOM, natural organic matter). The consumption of activated carbon for MTBE adsorption can rise due to a high content of NOM, for example. Also displacement of MTBE by other gasoline components, primarily BTEX, can occur and thus lower MTBE adsorption drastically. Since MTBE is transported in groundwater much faster than BTEX, in the case of plume treatment a prognostic approach has to be considered in respect to other substances entering the capture zone of the remediation well after some delay. In the preliminary investigations possible disturbing effects should be considered (e.g. from iron, manganese, carbonate).

Applications for activated carbon adsorption predominantly exist in treating low water volumes ($< 1\ m^3\ h^{-1}$), very low MTBE concentrations, and for cleaning natural water for drinking water supply.

4.1.2 Stripping

As shown in Fig. 7, MTBE possesses a comparatively low Henry constant of approximately 0.02. In practice it has been clearly confirmed that MTBE is comparatively less strippable than other frequently occurring groundwater contaminants, such as tetrachloroethene, trichloroethene, *cis*-1.2-dichloroethene, 1.1.1-trichloroethane, benzene and toluene.

Clearly, higher elimination rates for MTBE can be achieved by increasing the air : water ratios for stripping. For desorption of the above-mentioned well-strippable contaminants, generally air : water ratios of 1 : 50 are applied. For MTBE remediation much higher air : water ratios of at least 1 : 200 are necessary. With these increased volumes of air, cleaning efficiencies of over 95% can be obtained by one packed column. With the use of two stripping columns the cleaning efficiencies rise to over 99%.

Due to increased air : water ratios disturbances can occur with low iron and manganese concentrations, leading to mineral precipitations. It is advisable to clarify these possible processes by thorough preliminary investigations.

A further possibility for the improvement of the desorption effect exists in heating up the groundwater. With the rise in temperature of the raw waters a clear increase of the Henry constant for MTBE is obtained. It should

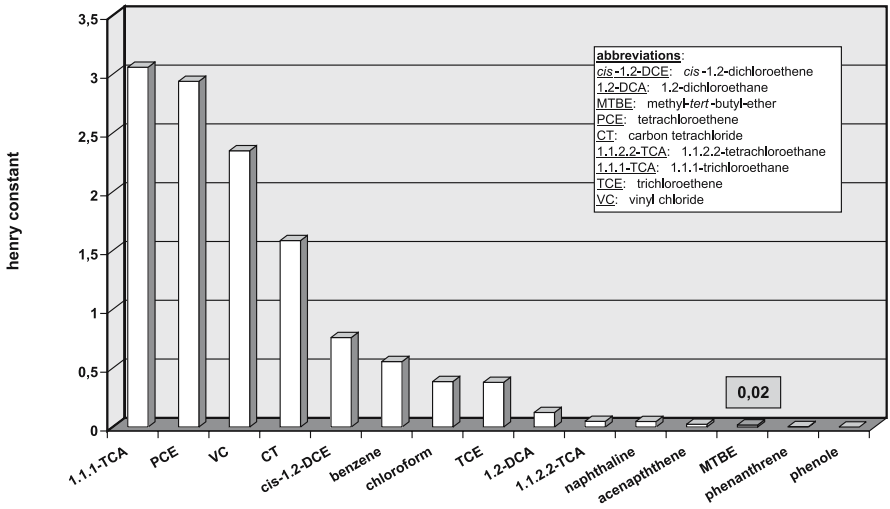

Fig. 7 Henry constants of organic compounds

be taken into consideration, however, that MTBE forms an azeotrope with water.

As Fig. 8 shows, the Henry constant of 0.008 at 3 °C increases to 0.027 by heating the water to 25 °C [11]. Therefore, the higher stripping efficiencies of some MTBE remediation projects observed in the summer are reasonable.

Since the heating of water is comparatively expensive, an increase of water temperature is economically meaningful only if a heat source is available at the site that can be integrated by heat exchange processes, without the large expense of heating the groundwater to be cleaned.

With the use of stripping plants it is cost-relevant whether cleaning of the stripping exhaust air has to be carried out. MTBE concentrations in groundwater are often approximately 2000 $\mu g\,L^{-1}$ with a water pumping rate in the order of 5 $m^3\,h^{-1}$. Setting up an air : water ratio of 300 : 1 and a stripping efficiency of 95% results in MTBE levels of approximately 6 $mg\,m^{-3}$ in the exhaust air and MTBE stripping air fluxes of approximately 9.5 $g\,h^{-1}$. These MTBE concentrations and MTBE fluxes clearly lie below the threshold values of the German "TA-Luft" (50 $mg\,m^{-3}$ or 0.5 $kg\,h^{-1}$), so that no necessity exists for exhaust air purification. On the other hand, the principle is to be noted that, at the time of the execution of remediation projects, if possible, no contaminant transfers should take place into other environmental compartments. In this respect it is an individual case decision, in coordination with the responsible regulators, whether exhaust air purification is necessary and which exhaust air levels are to be set up. In this context it is of importance that MTBE in air is diminished relatively rapidly. The half-life of MTBE in the

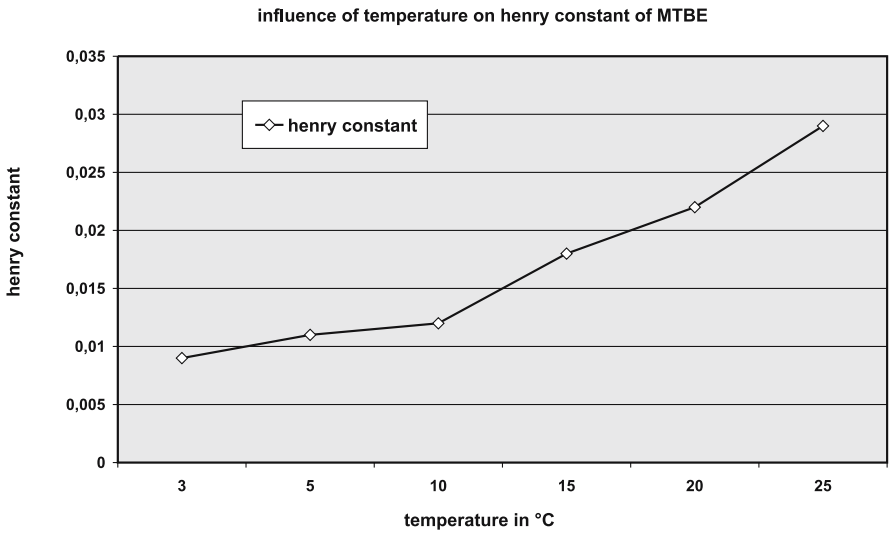

Fig. 8 Influence of temperature on Henry constant of MTBE

atmosphere lies at 2–6 days, in contrast to the half-life of several years in the groundwater.

For the cleaning of stripping exhaust air, activated carbon filters are usually used. In the case of previously dried stripping exhaust air, loading capacities can be obtained of up to 4% with high-quality activated carbon (fresh coal from coconut shell; regenerated activated carbon has a much lower loading capacity). The competition adsorption, in particular through BTEX, has already been referred to.

Catalysts are offered as further cleaning technologies. These are particularly of interest if higher organic levels are present in the exhaust air of the stripping plants. At high concentrations an autotherm operation of the catalyst is possible. There are, however, very high air : water ratios and thus strong dilutions have to be taken into account. Hence catalysts are only an advantage in the case of extremely high MTBE concentrations. Biofilters are another alternative, but so far hardly used due to the varying operating conditions (seasonal influences).

4.1.3 Biology (Reactors)

In remediation projects there always exists a strong interest in the use of biological techniques. The following reactor techniques are nowadays available for the cleaning of contaminated groundwater [12]:

- Activated sludge process (ASP)
- Sequencing batch reactor (SBR)
- Membrane bioreactor (MBR)
- Fluidized bed reactor (FBF)
- Submerged biofilter (SBF)
- Trickling biofilter

Laboratory tests succeeded in proving MTBE biodegradation in most of these listed techniques. For field tests, however, too little data is available for well-founded evaluation. A problem with the field tests often exists in too-low MTBE concentrations of around 10–100 $\mu g\,L^{-1}$. These concentrations are not high enough to form sufficient biomass. In most laboratory tests a lag phase of several weeks precedes the beginning of MTBE decay. The actual MTBE decay starts only after the BTEX decay. After the lag phase, a decay of up to 99.95% could be obtained; however, the flow rates were only small (laboratory conditions). According to the recent available level of knowledge, aerobic decay is more effective than anaerobic decay. However, anaerobic decay is also found (iron-reducing, subordinated sulfate-reducing) under special microcosm conditions [12].

4.1.4 Wet Oxidation (Advanced Oxidation)

Wet oxidative procedures for cleaning groundwater were known in Germany towards the end of the 1980s and were mainly used for the cleaning of groundwater containing chlorinated aliphatic hydrocarbons (CAH; light volatile substances like tetrachloroethene, trichloroethene, 1.1.1-trichloroethane, BTEX and PAH). In the USA these procedures are included in "advanced oxidation processes" (AOP). Primarily, the oxidizing agents ozone and hydrogen peroxide are used. In addition, the groundwater to be treated is illuminated with UV lamps. Good cleaning results were obtained with the combined use of ozone and hydrogen peroxide. The possible formation of decay products and their toxicological relevance have to be considered. According to the present state of information, these attempts have been essentially accomplished in the USA. So far no practical application for MTBE cleaning under field conditions is known from Germany.

In the context of laboratory tests accomplished in the USA the following further oxidation and/or catalytic methods were tested:

- Potassium permanganate
- Special aluminium compounds
- Titanium dioxide
- Fentons reagent (mixture of iron and hydrogen peroxide)

From the literature it is to be emphasized that oxidative destruction of MTBE consumes about 2–5 times more energy than is needed for the elimination of BTEX. The most important problems of this technology are connected with the fact that wet oxidation is not, in principle, a process particularly suitable for the destruction of MTBE. All organic components suitable for oxidation are oxidized by that process and thus consume the oxidizing agents and UV energy. Therefore, the oxidative effort strongly depends on the composition of the raw waters. Further, limitations arise with the operation of the oxidation plants when there are high iron and manganese concentrations in the water. Also, corrosion of plant parts should be taken into account.

An interesting option is the combination of wet chemical and biological treatment techniques. Here, the decay products that develop with the wet oxidation are biologically diminished. However, the flow rates obtained so far are only very low.

4.1.5 Adsorption to Synthetic Sorbents

This adsorption technology uses polymer resins as sorbents (Amberlite, Ambersorb, XUS, Reillex). The polymers consist of polystyrene, polyvinylepyridine and polymethylacrylats. In addition, carbonate resins and zeolithes (sili-

cic acid-rich mordenite) are of importance. Laboratory tests clearly showed that with special sorbents (e.g. carbonatic resins) higher MTBE loadings could be obtained than with activated carbon. The sorption capacity is, in sequence, Ambersorb 563 > L493 > XAD4 > XAD7. Furthermore, it was found that xylene can be substantially better sorbed than MTBE, and that MTBE is significantly better sorbed than *tert*-butyl-ether (TBA) [13].

An advantage of most synthetic sorbents over activated carbon is the better regeneration ability, for which hot steam is usually used. With the application of these procedures in remediation projects, a good infrastructure and supplying logistics (electricity, hot steam) are clearly an advantage. Since the sorbents materials react sensitively to fine particles (e.g. clay, fine silt), iron and manganese, the possible occurrence of these substances should be clarified before hand.

4.1.6
Liquid–Liquid Extraction (MPPE Technology)

The MPPE technique has so far been used mainly for cleaning of water containing CAH, BTEX and PAH. The company Akzo Nobel as patentees developed a special macroporous polymer extraction (MPPE) material for the treatment of groundwater containing MTBE. The fundamental suitability of the new material was proved in first field tests. The suitability under longer pilot operation has not yet been investigated. The previous remarks concerning possible process disturbances also apply to this procedure.

4.1.7
Membrane Technology

A new membrane procedure was tested with field tests in Port Hueneme (California). By means of special hollow fibre membranes (HFM) and the additional creation of a vacuum on the exterior of the membranes MTBE can be transferred into the gas phase and destroyed by thermal procedures, for example. The efficiency of the technology strongly depends on the vacuum applied, the water temperature and the retention time. Very good results were obtained with low flow rates of 4 L min^{-1} [14].

4.1.8
Electron Beam Technology (Eltrondec)

The irradiation of water with electrons leads to the formation of radicals, which destroy the organic contaminants. The effect of the process is thus similar to wet oxidation, so that this technology in the USA also falls into the category of “advanced oxidation process”. The result of laboratory tests can be summarized as follows: MTBE was decomposed with low

irradiation doses; however, the MTBE decay products TBA and TBF were formed [15].

In Germany no field tests with groundwater containing MTBE have yet been accomplished.

Summarizing, it can be stated that a wide range of techniques for cleaning of pumped groundwater is available. Except for the activated carbon adsorption and desorption, the other procedures are still in development regarding their utilization in remediation projects.

The stripping technology constitutes an approved technology, which has to be adapted to the chemical-physical data of MTBE to achieve the necessary cleaning goals. Therefore, MTBE pump-and-treat projects so far have almost exclusively used stripping technologies. The cleaning efficiency of desorption reaches 95% without difficulties and if necessary more than 99% by applying a two-column plant. The other gasoline components, in particular BTEX, can be removed from groundwater with an efficiency of more than 99.5%.

4.2 In-Situ Technologies

4.2.1 Biological In-Situ Remediation (Enhanced Natural Attenuation)

Based on available literature, the in-situ decay of MTBE is in principle difficult and occurs under natural conditions very slowly or not at all. A substantial reason for this is the structure of the MTBE molecule in the form of a tertiary chain and an ether bond. Only very few microorganisms are able to consume MTBE as an exclusive carbon source for mineralization [6]. Further, in spill areas the microbial activities create an oxygen-depleted zone and under such anaerobic conditions MTBE is difficult to degrade by natural processes alone. A better chance for MTBE decay occurs in the downstream area after MTBE is separated from the other gasoline components due to its higher transport velocity.

Applying biological techniques, unwanted decay products of MTBE can develop. In laboratory tests the decay products *tert*-butyl-ether (TBA) and *tert*-butyl-formate (TBF) were proven, though TBF is converted rapidly to TBA again.

On the basis of experiences in North America, increasing interest in the possible application of biological techniques for the remediation of MTBE groundwater contamination is also developing in Germany. At the location of the Canadian armed forces in Borden (Ontario) ongoing field experiments point to the fact that biological processes have led to a strong decay of MTBE over several years. Since general agreement exists on the fact that MTBE decay by biological processes is not easy to achieve, these results point out that under special environmental conditions and/or by special bacteria such decay

is possible. Similar to the decay of CAH, co-metabolitic processes could lead to an effective decay of MTBE. Alcanes (pentane, hexane, heptane) are to be regarded as co-metabolitic effective components [6].

A large-scale research project at the German petrochemical site Leuna is examining whether biological techniques are applicable by stimulating the biological decay processes (enhanced natural attenuation, ENA). Boundary conditions for the optimization of the natural decay potential are to be determined by examination of different procedures [6]. According to present planning, the investigations will initially run to 2007.

4.2.1.1 Biobarrier

The biobarrier procedure has been used for approximately 9 years in large-scale field experiments at Port Hueneme in California and at various other sites. The goal of this technique is to develop an in-situ reactive zone in the groundwater. The decay of MTBE is performed under aerobic conditions in the biobarrier. In test fields the different technologies of biostimulation (stimulation of autochthone bacteria populations) and bioaugmentation (injection of allochthone bacteria) were tested. To favour decay conditions, oxygen gas (or alternatively air) is injected into the groundwater. The groundwater containing MTBE flows through the biobarrier (oxygen curtain) and is destroyed by biological decay. According to reports, groundwater cleaning of over 99.9% has been achieved [16]. In the last few years a lot of lessons have been learnt about optimizing the decay conditions. One of the key prerequisites for achieving an effective MTBE decay is the optimal distribution of oxygen in the aquifer, combined with generation of an appropriately sized reactive aerobic zone. In Germany at present similar projects are being planned.

4.2.1.2 Methane Biostimulation

The company Biopract, as licensees of the procedure in Germany, carried out laboratory tests with the goal of stimulating the autochthonous organisms by injection of air with 4% methane [17]. As further substances, gaseous sources of nitrogen and phosphorus were added. It was ascertained that MTBE is well degradable through methanotrophic bacteria, whereby the decay rate depends on the oxygen content. For further development of the technology, field tests would be helpful.

4.2.2 In-Situ Oxidation

According to laboratory tests, destruction of MTBE is possible; however, TBA is formed as a decay product. There is still insufficient experience for an application in the field.

4.2.3 Air Sparging

Air sparging has been applied to remediation of CAH (especially tetrachloroethene and trichloroethene) and BTEX for many years, but it is only conditionally suitable for the treatment of MTBE-contaminated groundwater. The low Henry constant of MTBE has an unfavourable effect since under in-situ conditions the substance can hardly be transferred from the water into the gas phase. To avoid cross-contamination of the unsaturated zone, air sparging is usually combined with a soil air vapour extraction. However, air sparging can stimulate biological processes, which may lead to a significant MTBE decay.

An application of the procedure is meaningful only in homogeneous and well permeable subsoil, because otherwise "air bags" form in the groundwater. Further, in iron and manganiferous groundwater mineral precipitation has to be expected with the effect of blocking aquifer zones.

As a special technique "bio-sparging" is mentioned. Stimulation of biological decay is accomplished by air injection into the unsaturated zone.

4.2.4 Phytoremediation

Phytoremediation uses the water requirement of deep-rooted plants. Thus hydraulic control of contaminated groundwater can be achieved ("hydraulic containment"). Further, certain plants are able to transform the organic groundwater contaminants.

On the basis of laboratory and field tests accomplished in the USA an in-situ cleaning of groundwater containing MTBE can be achieved by cultivating plants. In a field study, poplars were planted downstream of a MTBE plume. The calculated reduction of MTBE concentration in the groundwater amounted to approximately 37–67% within 10 days [18]. However, the examination of applicability of phytoremediation requires field tests. It is conceivable that phytoremediation could be used if high order subjects of protection are not directly concerned and if the necessary reaction area and time can be accepted.

4.2.5 Monitored Natural Attenuation

Due to the often observable limited natural decay of MTBE in the field the use of "monitored natural attenuation" (MNA) has to be seen with caution. At sites that are characterized by a higher groundwater velocity, the application of MNA is hardly possible. A meaningful use of natural attenuation is limited to the employment of ENA (enhanced natural attenuation).

5 Costs

Due to the longer and more extensive experiences in North America it is still meaningful to rely on cost specifications for remediation from the USA. Meanwhile, cost data for the stripping technology is also available in Germany; however, it is difficult to receive precise and reliable data about further alternative procedures with the exception of activated carbon adsorption and stripping.

As already described, in Germany pump-and-treat is used almost exclusively for the remediation of MTBE groundwater contamination. Hence, the costs of the following cleaning methods are compared with each other on the basis of data collected in the USA [4]:

- Stripping technology
- Wet oxidation
- Activated carbon adsorption
- Resin adsorption

To be able to compare the costs of the different technologies with each other, the investment and operating costs were calculated over the redemption and for each cubic metre of groundwater ("totally amortized costs"). For cost calculation a pumped water rate of $14\,m^3\,h^{-1}$ was used. The MTBE raw water concentration amounts to $2000\,\mu g\,L^{-1}$ and the demanded plant discharge value is set to $20\,\mu g\,L^{-3}$. A two-stage design is intended for the stripping plant and an activated carbon plant is considered for stripping air cleaning. The efficiency of the stripping plant is about 99%. The costs of sampling, analysis and consultants are not included in the cost calculations.

The results of cost considerations are shown in Fig. 9. The treatment costs for the stripping technology are $0.81\,€\,m^{-3}$ groundwater and increase for wet oxidation ($0.87\,€\,m^{-3}$), activated carbon adsorption ($1.17\,€\,m^{-3}$) and resin adsorption ($1.21\,€\,m^{-3}$). Pretreatments for iron, manganese or carbonate elimination have been disregarded in the calculations. Including pretreatment, costs would rise by a factor of 0.5–1 approximately. With the costs of the stripping technology the largest portion is allotted to the energy costs.

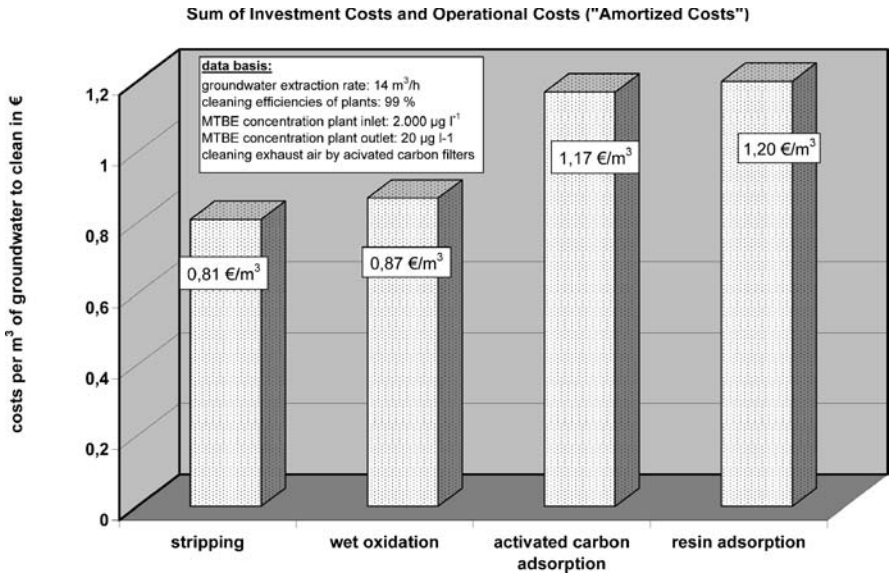

Fig. 9 Comparison of treatment costs of different technologies for cleaning of MTBE-containing groundwater

It is important to note that the costs actually arising in the projects are always to be seen as individual site-specific cases. A flat-rate view is not advisable. The costs determined for the stripping technology were compared with the expenditures that would develop with an operation in Germany, and have been confirmed.

According to cost calculations accomplished by the author, the expenditures for combined remediation of MTBE and BTEX contaminated groundwater are usually around 20–100% higher than the costs of pure BTEX cases. Pump-and-treat groundwater remediation with stripping technology is used with exhaust air purification only behind the first stripping tower (values to be maintained: exhaust air-laterally 5 mg m^{-3}, water-laterally 20 µg L^{-3}). Both the capital outlays for the plants and the operating costs for the plant are included in the cost comparison calculations. A financing of the investment and of the operating costs as well as the consultant and discharge costs of the groundwater after cleaning were not considered. This concerns medium-sized MTBE contaminations (quantity of water to be treated approximately 5 m^3 h^{-1}, MTBE content in the groundwater to be cleaned approximately 2000 µg L^{-3}).

The variation of the remediation costs depends considerably on a whole set of conditions. The essential are compiled in the following:

- Is exhaust air purification necessary or not?
- If exhaust air purification is demanded by regulators, is it necessary for the first and the second stripping columns or only for the first one?

- Which MTBE concentrations were fixed for water discharge of the cleaning plant and exhaust air behind the activated carbon filters?
- How high is the energy price? (in practice this varies between 0.05 and 0.15 € kWh^{-1}.)
- Do fees for water discharge of the cleaned water arise and, if so, how high are these?
- Will there be mineral precipitation due to the composition of the raw waters in the treatment plant? Due to higher air : water ratios for MTBE elimination the risk for such mineral precipitation increases (in particular iron and manganese compounds).
- Which costs are necessary for plant monitoring?
- Does the cost calculation include the capital costs or not?

6 Future Considerations

At present in California and Denmark a MTBE threshold value of 5 $\mu g\,L^{-1}$ for drinking water is used. The American Environmental Protection Agency (EPA) has recommended a sensory justified intervention value of 20–40 $\mu g\,L^{-1}$ for drinking water [4]. Drinking water with MTBE contents of about 30 $\mu g\,L^{-1}$ are 20 000 to 100 000 times lower than the MTBE exposition margin, with which carcinogenic or non-carcinogenic effects with rodents were observed. A study group of the LAWA (German working group on water issues of the Federal States) suggested a concentration of 15 $\mu g\,L^{-1}$ as a threshold value above which a groundwater contamination demands remediation (minimum concentration limit according to LAWA definition). All the previous mentioned values are not toxicologically justified and are exclusively based on the noticeable organoleptical characteristics of MTBE (smell and taste).

A proven technique for remediating MTBE-contaminated groundwater is available with pump-and-treat and application of the stripping technology for cleaning the extracted groundwater. To remediate the unsaturated zone, in most cases soil air extraction can be used, which can be carried out at acceptable costs. However, groundwater remediation by pump-and-treat means remediation activities over longer periods and at high costs.

In the last few years, knowledge of the biological behaviour of MTBE in the subsoil has increased considerably. Field investigations have found out that MTBE-degrading cultures are present at many MTBE-contaminated sites. Thus the potential to degrade MTBE by natural processes is proven. On the other hand, it was learnt that natural attenuation processes in most cases are not effective enough to degrade MTBE below certain threshold values.

The logical consequence is to improve the biological decay of MTBE by applying enhanced natural attenuation to such an extent that this technol-

ogy can be applied to clean MTBE-contaminated groundwater below special threshold concentrations. Through many projects carried out over the last few years, especially in the USA, it was discovered that MTBE and TBA can be remediated at many sites by application of biological in-situ technologies. Great success could be achieved especially by applying the oxygen curtain technology [19].

At present in Germany projects have started to use the capacity of biological processes for remediation of MTBE-contaminated groundwater. Cost comparisons between pump-and-treat and biological technologies so far show that the costs for biological remediation are generally considerably lower than for pump-and-treat. For most projects, costs range in the order of 25–60% of the costs for pump-and-treat. Since the chances for successful biological remediation are highly dependent on the site conditions it is highly probable that biological in-situ options cannot be applied at each site. Thus, a very thorough site investigation and a fundamental up-scaling in remediation planning is of great importance. If the work is professionally carried out, the chances of biological remediation of MTBE groundwater contamination should be considerably increased.

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Removal of MTBE and Other Fuel Oxygenates During Drinking Water Treatment

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Abstract MTBE and other fuel oxygenates threaten the water sources for drinking water production. Due to their persistence in the environment, these substances pass through the subsoil unchanged and thus are not reliably retained by riverbank filtration. Conventional drinking water treatment technology—i.e., aeration and adsorption on activated carbon—are not able to remove them in a feasible manner. New adsorption materials show better performance but high costs and low availability prevent their use in drinking water production. Chemical oxidation by advanced oxidation technology (i.e., ozone/H_2O_2 or UV-induced advanced oxidation processes) is most likely able to eliminate MTBE and other ethers, however, only with high expenses. Nanofiltration might be an option since the retention by nanofiltration membranes is quite high. However, for the production of drinking water, the resulting water has to be further conditioned in order to meet drinking water standards. In this book chapter the treatment technologies currently available for water treatment are illuminated in detail on their potential for removing MTBE and other fuel oxygenates from water.

Keywords Adsorption · Aeration · AOP · Bank filtration · Membrane filtration · Oxidation · Ozonation

1 Introduction

The ongoing discussion about the usage of MTBE was initiated in the late 1980s and 1990s by the detection of high concentrations of MTBE in drinking water wells in several parts of the United States [1, 2]. From this time on, many surveys and studies showed the almost ubiquitous appearance of MTBE in the aquatic environment not only in the U.S. but all over the world [3–13]. Therefore MTBE and its substitutes, mostly related ethers such as ETBE, TAME, or DIPE, came into the focus of water suppliers. The presence of MTBE in high concentrations spoils the taste and smell of drinking water, but even in concentrations below the taste and odor threshold MTBE is objectionable in drinking water, simply because it is a xenobiotic compound. Therefore the elimination potential of different drinking water treatment technologies had to be investigated. This chapter will present an overview over literature dealing with MTBE or related fuel oxygenates removal during drinking water treatment. The questions to be answered include: What can be done to remove MTBE? Which technology can be evaluated as economically feasible? and Which treatment process is already implemented?

2 Riverbank Filtration

Riverbank filtration as a treatment step during drinking water production is a commonly known and widely used technology in Europe [14–16].

The possibility of the elimination of organic substances in the subsoil by either sorption or biodegradation is common knowledge and has been the subject of many investigations. Schmidt et al. [17] for example published a comprehensive overview of the behavior of organic substances during riverbank filtration.

In the case of MTBE, sorption onto soil is very low, as expressed by its low soil adsorption coefficient ($\log k_{OC}$ around 1). It readily partitions into the water. Any elimination achieved during subsoil passage has therefore to be attributed to microbial degradation.

Degradation of MTBE by microbes has been reported in literature, however, the presence of the *t*-butyl group in the structure makes the ethers strongly unsusceptible for microbial attack and constrains biodegradation under normal conditions [18–20]. A wide variety of special MTBE degrading consortia or pure cultures have been found in lab-scale studies either degrading MTBE as sole carbon or energy source or via complex cometabolisms [21]. MTBE can be degraded under both aerobic and anaerobic conditions, however, the degradation takes place slowly. Aquifer sediment and groundwater microcosms from MTBE contaminated sites were shown to be able to degrade MTBE with and without nutrient addition under aerobic conditions [22, 23]. Concurrent presence of other carbon sources (e.g., BTEX), however, may inhibit MTBE degradation. Pilot- and full-scale bioreactors reached efficiencies of 75 to > 95%, but only at the expense of high residence times [20].

The summary of these facts clearly explains the positive findings of MTBE in riverbank filtrated water in Germany [16, 24, 25]. Baus et al. [25] found 40% of the MTBE passing unchanged through the riverbank of the river Rhine, whereby shock loads of MTBE (i.e., peak concentrations) in the river were smoothened out. The concentrations in the river Rhine fluctuated between 0.1 and 0.5 μg/L (annual average 0.23 and 0.26 μg/L in 2000/2001) whereas the riverbank filtrated water showed average concentrations of 0.10 and 0.09 μg/L, respectively. Similar values were observed by Achten et al. [16] who observed a 35% reduction of MTBE concentration. The residence time of the riverbank filtration site is between 15 and 70 days under aerobic conditions. The transformation of organic pollutants is, however, most effective in the first decimeters of river sediment.

Different observations were made at a riverbank filtration site at the river Main in Germany [16]. At this site, nitrate-, iron- and manganese-reducing conditions prevail and the residence time at two sampling points varies between 45–85 and 310–570 days, respectively. No elimination was observed after 60 m subsoil passage, which corresponds to a residence time of 45–85 days. MTBE was found to be as persistent as other poorly attenuable compounds in the Main water.

In respect to the behavior of alternative ethers in the underground there is not as much literature available as for MTBE. McKinnon et al. [1] noticed

that DIPE appears to travel even faster in the ground than MTBE. Schmidt et al. [26] observed *t*BA degradation under oxic conditions but not any evidence of substantial degradation in the absence of oxygen.

Ongoing measurements at the above-mentioned riverbank filtration sites leads one to suspect that ETBE, which is increasingly found in the river water in detectable concentrations since 2005, is also not eliminated by riverbank filtration (Sacher F (2007) Personal communication and unpublished data, DVGW-Technologiezentrum Wasser (TZW), Germany, www.tzw.de)

3 Aeration / Air stripping

The removal of MTBE by air stripping was investigated in various studies. Due to its high vapor pressure and low boiling temperature, it could be expected that MTBE is rather easily eliminated by air stripping. However, MTBE exhibits a good solubility in water and a low Henry coefficient, making the removal of MTBE from water rather difficult, especially at low concentrations (cp. Table 1).

For the elimination of MTBE from water by air stripping, high air-to-water ratios are required [19, 25, 27, 28]. This is made clear in several studies in liter-

Table 1 Physico-chemical parameters for MTBE, ETBE, and TAME

	MTBE	Refs.	TAME	Refs.	ETBE	Refs.
Water solubility [g/L]	43.0–54.3	[39, 40]	12	[9]	12	[9]
	48	[41]				
	50	[42]				
Vapor pressure [mbar]	326–334	[43]	91	[9]	203	[9]
Henry-constant [(atm m^3)/mol]	5.87×10^{-4}	[43]	1.3×10^{-3}	[9]	2.7×10^{-3}	[9]
	14×10^{-4}	[43]				
	30×10^{-4}	[43]				
	5.28×10^{-4}	[44]				
	5.28×10^{-4}–30×10^{-4}	[41]				
Henry-constant [dimensionless]	2.399×10^{-2}	[39, 40]				
	5.722×10^{-2}	[39, 40]				
	2.16×10^{-2}	[44]				
	1.8×10^{-2} at 20 °C	[44]				
	2.2×10^{-2}–12×10^{-2}	[41]				
	1.7×10^{-2} at 20 °C (temp. independent calculation)	[45]				
	$(5.55 \pm 1.22)\ 10^{-2}$	[46]				

ature concerning ozonation. In preliminary experiments all studies show that the loss of MTBE by sparging gaseous ozone into the reaction solution is less than 10% [27, 29–32]. Wagler et al. [33] claimed already in 1994 an air-to-water ratio of 100 : 1 to be necessary for an effective removal of MTBE. Other studies found the higher the air-to-water ratio the higher the removal percentages [1, 27, 34]. This is due to an increasing mass transfer, and thus the efficiency of the process mounts as well. With higher air-to-water ratios the required packing heights decrease. Sutherland et al. [27] showed that a decrease in air-to-water ratio from 150 to 75 : 1 leads to 1.5 to 3.0 times higher packing heights. Similar observations were made by Ramakrishnan et al. [35].

Compared to benzene, much higher air-to-water ratios are required to achieve the same elimination efficiency [20]. Therefore, if the treatment of water contaminated with BTEX and MTBE is designed for removal of MTBE, BTEX will be eliminated to a much larger extent [36].

At lower temperatures the efficiency of the process decreases due to the temperature influence on the Henry coefficient [36]. The required tower height will increase with decreasing temperature. On the other hand an increase in efficiency can be achieved by heating of the air stream [20, 28]. However, not only the behavior of the organic pollutant will be influenced by temperature but also the behavior of other water ingredients. The precipitation of iron, manganese, or carbonate might be enhanced at higher temperatures and lead to fouling and scaling. Moreover, bio-fouling is a problem occurring with air stripping especially at elevated temperatures.

McKinnon et al. [1] observed a higher removal efficiency for DIPE compared to MTBE. In a study by Sutherland et al. [37], DIPE ranged as being easiest eliminable among the ethers DIPE, ETBE, TAME, and MTBE and *t*BA and ethanol. The last two substances showed mass transfer coefficients two orders of magnitude lower than those of the ethers. This means that substances with lower Henry's law constants are much less effectively treated, but compounds with higher Henry's constants are more effectively treated with smaller columns and lower operating costs than MTBE.

Air stripping shows some advantages. It is a proven and reliable technology that is already widely used in drinking water applications [28, 36]. Moreover, the presence of other water ingredients is in a wide range not affecting the performance of the process, though experiments in demineralized water showed a slightly enhanced removal efficiency compared to natural water [34].

However, off-gas treatment is required in most cases [19, 20, 27, 28, 36]. Another disadvantage of this treatment process is the susceptibility towards scaling and fouling as well as corrosion.

Despite these disadvantages, air stripping has been applied as treatment technology for contaminated sites with higher MTBE contamination. Air stripping was shown to be the most economic option—at least at high flow rates; at low flow rates chemical oxidation processes showed comparable unit

treatment costs [37]. In Rockaway Township, NJ, USA, a groundwater system was installed in the early 1980s consisting of an air stripping tower with subsequent GAC adsorption [1]. The air stripping unit removed MTBE in concentrations around 40 μg/L to 95%; concurrently present DIPE was eliminated to 99%. In Kansas, a full-scale installation of packed tower air stripping system was implemented for treating drinking water containing concentrations of MTBE of up to 1 mg/L [38]. MTBE was eliminated to 94%. In Leuna, Sachsen-Anhalt, Germany, air stripping technology was used to remediate groundwater contaminated with PAC, BTEX, MTBE and other organic compounds [20] showing 90–95% efficiency. Similar results were obtained at a gasoline station in Germany, where MTBE concentrations of 210 μg/L were treated with an efficiency greater than 95% by an air stripping tower. However, since this installation was originally not designed for MTBE removal, the resulting costs for the remediation were 30–80% higher [20].

Stocking et al. [28] mention two identified water supplies in the U.S. that use air stripping as treatment technology, however, no further information on the identity are given. The MTBE concentrations were 96 μg/L and 900 μg/L and the achieved removal percentages were above 95% in both cases. In the second case, two packed towers were operating in series with an air-to-water ratio of 175.

4 Adsorption

4.1 General Remarks on Adsorption

The most common type of adsorbent in water treatment application is activated carbon because of its high availability and relatively low capital and installation costs. Many practical experiences are available concerning operation of activated carbon filters on full-scale, since it is a widely used treatment option in waterworks.

It provides a simple technology that is operating very stable and is relatively easy to implement. The equipment and the methods needed are well established and commercially available [28].

The activated carbons available nowadays can be distinguished by the origin of the carbon source. Bituminous/lignite coal, wood, or coconut shell are some of the main carbon sources used for purification of water.

When MTBE was first detected in groundwater, studies were undertaken shortly afterwards investigating the possibility of eliminating MTBE by adsorption on activated carbon [1, 33]. One of the first large-scale installations showed, however, that the tested bituminous-based carbon F-300 was not efficient in reliably removing MTBE and DIPE from contaminated well water.

Short breakthrough times required a frequent replacement of the coal, which proved to be too expensive.

From that time on, many studies have been undertaken illuminating the adsorption mechanisms and behavior of MTBE.

Adsorption Isotherms

For the evaluation of different adsorbents, the first measure is to determine adsorption isotherms that show the capacity of the coal for the target compound versus equilibrium concentration. These adsorption isotherms can be characterized by different adsorption models.

One of the most common models for the description of adsorption isotherms is the evaluation of the data according to Freundlich, as expressed in Eq. 1.

$$q = K\, c^{n} \tag{1}$$

q denotes the capacity in $[m_{\text{target compound}}/m_{\text{carbon}}]$ at equilibrium and c is the corresponding equilibrium concentration of the target compound in the liquid phase $[m/V]$. K and n are the so-called Freundlich parameters, which are used for fitting the experimental data.

When alternative sorbents were investigated, it was noticed that the Freundlich isotherm did not always show good congruence with the experimental data. The Dubinin–Astakov isotherm, which was originally developed for the sorption of gases, proved to be a more suitable isotherm equation for the description of the sorption process. The DA isotherm is given by

$$q = q_{\max} \exp\left[-\left(\frac{A}{E}\right)^{\eta}\right], \tag{2}$$

$$A = RT \ln\left(\frac{C_s}{C}\right) \tag{3}$$

with R = ideal gas constant (8314 kJ/mol K); T = temperature in K; and C_s = aqueous solubility (mg/L).

The parameters $q_{\max}$, E and η are typically used as fitting parameters, however, they represent physical characteristics of the solute and sorbent. E stands for the adsorption potential at which the capacity is 36.8% of the maximum capacity, whereas η is a measure for the heterogeneity of the micropores. The maximum adsorption capacity $q_{\max}$ denotes the filling of the micropores [47].

Tables 2 to 6 comprise data from literature dealing with the determination of isotherm parameters for different coal types and other possible adsorbents for MTBE.

Table 2 Freundlich parameters for the adsorption of MTBE on different activated carbons

Carbon	Temperature [°C]	Concentration MTBE	Matrix	Freundlich parameters K [(mg/g)(L/mg)^n]	n	Refs.
CC 602 (coconut shell-based)	20	0.001–10 mg/L	Organic free water unbuffered pH 7	13.804	0.46	[28]
CC-602 (coconut shell-based)	20 °C	1–1 000 000 µg/L	Organic-free water	13.9	0.46	[28]
GRC-22 (coconut shell-based)	Ambient	1 mg/L	n.g.	11.7	0.7101	[53]
GRC-22 (coconut shell-based)	n.g.	n.g.	Dist. water	11.7	0.714	[36]
PC (coconut shell)	25 °C	6000–100 000 µg/L	Dist. water	13.2	0.29	[28]
Unicarb (coconut shell based)	20±2	45–700 µg/L	Deionised water	5.89	0.59	[52]
207A (coal-based)	25 °C	6000–100 000 µg/L	Dist. water	12.9	0.26	[28]
F-300	Ambient	100 µg/L	Deionised water	7.6	0.64	[25]
F-300	Ambient	160–430 mg/L	Deionised dist. water	7.19/7.47	0.437/0.444	[34]
F-300	20±2	45–700 µg/L	Deionised water	9.55	0.66	[52]

Table 2 (continued)

Carbon	Temperature [°C]	Concentration MTBE	Matrix	Freundlich parameters K [(mg/g)(L/mg)^n]	n	Refs.
F-300 preloaded with 35 m^3/kg	Ambient	100 μg/L	Deionised water	> 1 000 000	12.7	[25]
F-400	22.4 ± 1.2 °C	50 μg/L	Distilled/deionised water, pH buffer phosphate 7.5	4.70	0.676	[51]
F-400	22.4 ± 1.2 °C	20 μg/L	Distilled/deionised water, pH buffer phosphate 7.5	7.15	0.692	[51]
F-400	10	0.06–1.5 mg/L	Simulated groundwater (MilliQ + $NaHCO_3$ HCl/NaOH/NaCl) pH 7	6.607	0.63	[28]
F-400	20	0.4–3000 mg/L	Simulated groundwater (deionised water, $NaHCO_3$ + NaCl) pH 7.2	3.090	0.59	[28]
F-400	n.g.	5–2500 mg/L	Synthetic groundwater: distilled deionised water, buffered 7.2 by $NaHCO_3$, NaCl	3.090±1.175	0.59±0.04	[47]
F-400	Ambient	1 mg/L	n.g.	4.48	0.5996	[53]
F-400	24 °C	102–628 μg/L	Dist./deionised water	6.0	0.48	[28]
F-400	20±2	45–700 μg/L	Deionised water	2.55	0.42	[52]

Table 2 (continued)

Carbon	Temperature [°C]	Concentration MTBE	Matrix	Freundlich parameters K [(mg/g)(L/mg)^n]	n	Refs.
F-400-HO	Ambient	1 mg/L	n.g.	7.67	0.6103	[53]
F-600	22.4 ± 1.2 °C	50 µg/L	Distilled/deionised water, pH buffer phosphate 7.5	15.72	0.707	[51]
F-600	22.4 ± 1.2 °C	50 µg/L	Distilled/deionised water, pH buffer phosphate 7.5	10.91	0.708	[51]
F-600	25 °C	10–100 µg/L	Dist. water	7.8	0.54	[28]
WPH (coal based)	20±2	45–700 µg/L	Deionised water	2.09	0.44	[52]
Hydrodarco-4000 (lignite based)	room temp.	180 mg/L	River water	3.96/4.03	0.450/0.453	[34]
Barnebey-Cheney	n.g.	10–270 µg/L	n.g.	3.65	0.75	[57]
Fischer	n.g.	10–270 µg/L	n.g.	3.07	0.48	[57]

n.g.: not given

Table 3 Freundlich parameters for the adsorption of other fuel oxygenates on activated carbon

Carbon	Temperature [°C]	Concentration MTBE	Matrix	Freundlich parameters K [(mg/g)(L/mg)^n]	n	Refs.
F-400	22.4 ± 1.2 °C	DIPE 50 µg/L	Distilled/deionised water, pH buffer phosphate 7.5	47.22	0.489	[51]
F-600	22.4 ± 1.2 °C	DIPE 50 µg/L	Distilled/deionised water, pH buffer phosphate 7.5	224.34	0.734	[51]
F-400	22.4 ± 1.2 °C	TAME 50 µg/L	Distilled/deionised water, pH buffer phosphate 7.5	14.01	0.452	[51]
F-600	22.4 ± 1.2 °C	TAME 50 µg/L	Distilled/deionised water, pH buffer phosphate 7.5	25.9	0.500	[51]
F-400	22.4 ± 1.2 °C	ETBE 50 µg/L	Distilled/deionised water, pH buffer phosphate 7.5	21.23	0.622	[51]
F-600	22.4 ± 1.2 °C	ETBE 50 µg/L	Distilled/deionised water, pH buffer phosphate 7.5	18.23	0.493	[51]
F-400	n.g.	*t*BA n.g.	Buffered, organic-free, spiked with salts (synthetic groundwater)	0.035	0.31	[28]

n.g.: not given

Table 4 Freundlich parameters for the adsorption of MTBE on different adsorbents

Carbon	Temperature [°C]	Concentration MTBE	Matrix	Freundlich parameters K [(mg/g)(L/mg)^n]	n	Refs.
Acrylic resin Polysorb MP-1	n.g.	5–2500 mg/L	Synthetic groundwater: distilled deionised water, buffered 7.2 by $NaHCO_3$, NaCl	0,759±3,388	1.15±0.55	[47]
Ambersorb 563	20±2	45–700 µg/L	Deionised water	20.50	0.65	[52]
Ambersorb 563	20 °C	0.57 to 177 mg/L	Millipore water	19.6/12.3	0.367/0.461	[56]
Ambersorb 563	10	0.07–0.6 mg/L	Simulated groundwater (MilliQ + $NaHCO_3$ HCl/NaOH/NaCl) pH 7	18.197	0.74	[28]
Ambersorb 563	20	0.6–2500 mg/L	Simulated groundwater (deionised water, $NaHCO_3$ + NaCl) pH 7.2	16.218	0.35	[28]
Ambersorb 563	25	0.001–0.380 mg/L	Synthetic groundwater	19.055	0.73	[28]
Ambersorb 563	25	0.006–73 mg/L	Organic free water buffered pH 7	4.365	0.36	[28]
Ambersorb 563	n.g.	5–2500 mg/L	Synthetic groundwater: distilled deionised water, buffered 7.2 by $NaHCO_3$, NaCl	16.218±1.096	0.35±0.02	[47]
Ambersorb 563	25 °C	1–50 mg/L	Deionised water	1.652	1.281	[59]

Table 4 (continued)

Carbon	Temperature [°C]	Concentration MTBE	Matrix	Freundlich parameters K [(mg/g)(L/mg)^n]	n	Refs.
Ambersorb 572	20±2	45–700 μg/L	Deionised water	20.28	0.61	[52]
Ambersorb 572	10	0.07–0.6 mg/L	Simulated groundwater (MilliQ + $NaHCO_3$ HCl/NaOH/NaCl) pH 7	18.197	0.67	[28]
Ambersorb 572	20	1–2000 mg/L	Simulated groundwater (deionised water, $NaHCO_3$ + NaCl) pH 7.2	13.804	0.46	[28]
Ambersorb 572	22.5–23	0.005–0.6 mg/L	Organic free water pH 6.8–7.0	18.197	0.68	[28]
Ambersorb 572	n.g.	5–2500 mg/L	Synthetic groundwater: distilled deionised water, buffered 7.2 by $NaHCO_3$, NaCl	13.804±1.148	0.46±0.03	[47]
C 18 bonded silica Hypersil	n.g.	5–2500 mg/L	Synthetic groundwater: distilled deionised water, buffered 7.2 by $NaHCO_3$, NaCl	No statistically significant decrease in MTBE concentration found		[47]
Graphitic carbon Hypercarb	n.g.	5–2500 mg/L	Synthetic groundwater: distilled deionised water, buffered 7.2 by $NaHCO_3$, NaCl	6.457±1,476	0.96±0.31	[47]
HiSiv 1000 zeolite	20±2	45–700 μg/L	Deionised water	0.19	0.43	[52]

Table 4 (continued)

Carbon	Temperature [°C]	Concentration MTBE	Matrix	Freundlich parameters K [(mg/g)(L/mg)^n]	n	Refs.
L493	20 °C	0.57 to 177 mg/L	Millipore water	3.42	0.710	[56]
Mordenite zeolite SiO_2/Al_2O_3 ratio = 90	20±2	45–700 µg/L	Deionised water	12.48	0.65	[52]
Optipore L-493	25	0.04–94 mg/L	Organic free, buffered pH 7	2.089	0.68	[28]
Optipore L-493	25	0.003–0.991 mg/L	Synthetic groundwater	0.955	0.49	[28]
XAD4	20 °C	0.57 to 177 mg/L	Millipore water	1.99	0.725	[56]
XAD7	20 °C	0.57 to 177 mg/L	Millipore water	0.138	0.842	[56]

n.g.: not given

Table 5 Dustinin–Anakov Parameters for the adsorption of MTBE on different resins

Resin type	Concentration range	Matrix	q_{max} [mg/g]	log E [kJ/mol]	η	Refs.
Carbonaceous resin Ambersorb 563	5–2500 mg/L	Synthetic groundwater: distilled deionised water, buffered 7.2 by $NaHCO_3$, NaCl	170	1.23±0.09	2.00±0.14	[47]
Carbonaceous resin Ambersorb 572	5–2500 mg/L	Synthetic groundwater: distilled deionised water, buffered 7.2 by $NaHCO_3$, NaCl	304	1.21±0.04	2.58±0.09	[47]
Ambersorb 563 untreated	0.57 to 177 mg/L	Millipore water	88.6	4.39	4.178	[56]
Ambersorb 563	0.57 to 177 mg/L	Millipore water	89.7	4.36	3.901	[56]
L-493	0.57 to 177 mg/L	Millipore water	89.5	4.29	9.950	[56]
XAD4	0.57 to 177 mg/L	Millipore water	72.3	4.26	3.724	[56]
XAD7	0.57 to 177 mg/L	Millipore water	8.8	4.25	4.422	[56]

Table 6 Freundlich parameters for the adsorption of *t*BA on synthetic resins

Carbon	Temperature [°C]	Concentration MTBE	Matrix	Freundlich parameters K [(mg/g)(L/mg)^n]	n	Refs.
Ambersorb 563	20 °C	0.154–86.9 mg/L	Millipore water	2.38/1.77	0.810/0.827	[56]
L493	20 °C	0.154–86.9 mg/L	Millipore water	0.171	1.038	[56]
XAD4	20 °C	0.154–86.9 mg/L	Millipore water	0.023	1.01	[56]
XAD7	20 °C	0.154–86.9 mg/L	Millipore water	0.0078	1.10	[56]
Ambersorb 563	n.g.	n.g.	Charnock well sample	1.778	0.85	[28]
Ambersorb 563	n.g.	n.g.	Buffered, organic-free, spiked with salts (synthetic groundwater)	8.128	1.20	[28]
Optipore L-493	n.g.	n.g.	Buffered, organic-free, spiked with salts (synthetic groundwater)	0.074	0.71	[28]

n.g.: not given

Column Tests

The determination of isotherm data gives a first impression of the adsorbability of a compound onto a specific adsorbent. However, since it is an equilibrium method, kinetic aspects of the adsorption are not accounted for. These influences can only be determined by column tests, where a specific amount of adsorbent is contacted with a steady influent concentration of the substance under investigation. In pilot- and full-scale applications, the impact of kinetics become more important and are often limiting for the removal efficiency of a given adsorber. Therefore, column tests have to be performed in order to predict the removal potential by adsorption.

Crittenden et al. [48, 49] developed a standardized test scenario based on down-scaling a full-scale adsorption filter, called the "rapid small-scale column test" (RSSCT). By down-scaling the process, it could be ensured that the kinetics in the small-scale tests are equal to those in a large-scale filter.

But other column installations not strictly following the above criteria are also able to give hints about the removal efficiency for MTBE by comparing the breakthrough curves with those from known substances [50]. The order of breakthrough was shown to be the same in the lab-scale installation as in implemented full-scale activated carbon filters from waterworks.

One criteria for the performance of an adsorbent in a column is the so called "adsorbent usage rate". It denotes the amount of adsorbent added per volume of water treated. The lower the adsorbent usage rate the more efficient the process. The parameter "capacity" describes the amount of substance adsorbed per amount of adsorbent added. Both values are subject to many variables such as kinetic conditions in the column, EBCT (empty bed contact time), water composition, etc.

In Table 7 the adsorbent usage rates, capacities and experimental conditions from several studies in literature are on display.

4.2 Impact of Adsorbent Type

In literature, numerous studies were undertaken regarding the removal of MTBE by means of adsorption. In Tables 2 to 6 the Freundlich and Dubini–Astakov parameters for various activated carbons and other adsorbents are given.

The activated carbons that were most widely investigated included bituminous coal based carbons such as F-300, F-400, F-600, or PAC 200, lignite-based (Hydrodarco-4000), wood-based (Picazine) and coconut shell-based carbons (GRC-22, PCB, Unicarb).

For the adsorption of MTBE high-energy pores are required whose energy is high enough to overcome the solubility factor—which is quite high for MTBE [19].

Table 7 Column tests

Carbon used	Type of column	Water matrix	Co-solutes of interest	MTBE inlet concentration	Carbon usage rate CUR	Capacity	Refs.
F-400	RSSCT	Groundwater	None	5.03–5.31 mg/L	0.44 g/L	9.3 mg/g	[27]
			None	0.963–1.26 mg/L	0.31 g/L	2.52 mg/g	[27]
			BTEX 1.1–3.6 mg/L	0.023–0.029 mg/L	0.26 g/L	0.11 mg/g	[27]
			BTEX 0.052–0.17	0.198–0.224 mg/L	0.16 g/L	0.86 mg/g	[27]
			BTEX	0.011–0.018 mg/L	0.11 g/L	0.25 mg/g	[27]
F-600	RSSCT	groundwater	None	5.03–5.31 mg/L	0.26 g/L	19.94 mg/g	[27]
			None	0.963–1.26 mg/L	0.15 g/L	5.52 mg/g	[27]
			BTEX 1.1–3.6 mg/L	0.023–0.029 mg/L	0.24 g/L	0.17 mg/g	[27]
			BTEX 0.052–0.17	0.198–0.224 mg/L	0.08 g/L	1.9 mg/g	[27]
			BTEX	0.011–0.018 mg/L	0.05 g/L	0.46 mg/g	[27]
GRC-22 (coconut-shell based)	n.g.	n.g.	n.g.	100–1000 μg/L	0.098 kg/m^3	10.5 mg/g (1000 μg/L)	[36]

Table 7 (continued)

Carbon used	Type of column	Water matrix	Co-solutes of interest	MTBE inlet concentration	Carbon usage rate CUR	Capacity	Refs.
CC-602 (coconut-shell based)	RSSCT EBCT 10 min 18±1°	Lake Perris water	None	20 μg/L	0.214 kg/m^3 (50% breakthrough)	72 μg/g	[55]
PCB (coconut-shell based)	RSSCT EBCT 10 min 18±1°	Lake Perris water	None	20 μg/L	0.137 kg/m^3 (50% breakthrough)	114 μg/g	[55]
CC-602 (coconut-shell based)	RSSCT EBCT 20 min 18±1°	Lake Perris water	None	20 μg/L	0.119 kg/m^3 (50% breakthrough)	136 μg/g	[55]
PCB (coconut-shell based)	RSSCT EBCT 20 min 18±1°	Lake Perris water	None	20 μg/L	0.096 kg/m^3 (50% breakthrough)	168 μg/g	[55]
n.g.	Groundwater remediation sites	Contaminated groundwater	BTEX present (1–23 mg/L)	> 270 μg/L	0.24–2.76 kg/m^3	n.g.	[28]
PCB (coconut-shell based)	RSSCT EBCT 20 min	Lake Perris water	303 μg/L BTEX	20 μg/L	233.66 g/m^3 (breakthrough 5 μg/L)	n.g.	[58]
Ambersorb 563	RSSCT EBCT 2.5 min	Lake Perris water	303 μg/L BTEX	20 μg/L	44.34 g/m^3 (breakthrough 5 μg/L)	n.g.	[58]
PCB (coconut-shell based)	RSSCT EBCT 20 min	Arcadia Well Field Water	100 μg/L *t*BA	1000 μg/L	296.56 g/m^3	n.g.	[58]
Ambersorb 563	RSSCT EBCT 2.5 min	Arcadia Well Field Water	100 μg/L *t*BA	1000 μg/L	45.54 g/m^3	n.g.	[58]

n.g.: not given

Lignite and wood-based coal show poor adsorption performance, whereas bituminous coal-based carbons exhibit low to moderate percentages of MTBE removal [19]. The higher the activation factor, the better the capacities of the coal types (F-600 better than F-400) [19, 51, 52]. F-300 proved to be the most efficient activated carbon in terms of carbon loading and carbon usage rates in a study of Wilhelm et al. [34] who compared various bituminous coal-, lignite- and wood-based carbon types.

Hung et al. [52] observed no significant difference in the adsorption performance between F-300, F-400 (bituminous coal-based) and Unicarb (coconut shell-based), but considerably poorer performance of WPH (bituminous coal-based).

Suffet et al. [53] showed superior adsorption properties for GRC-22 (coconut shell-based) over SA-30 and CA-30 (wood-based). Bituminous coal-based carbon types (F-200, Centaur), lignite-based Morit HD 3000 and wood based Picabiol had all lower capacities for MTBE.

In general, the comparison of different carbon types in literature yields higher capacity and better performance for MTBE adsorption for coconut shell-based carbons than for coal based carbon types [19, 20, 28, 53, 54].

A comparison of different coconut shell-based carbons (CC-602 and PCB) showed similar absolute capacities but a better performance and better adsorption kinetics of PCB during life time of the coal [55]. However, variations in coconut shell-based coal are unpredictable due to their production process. Other carbon types might be more predictable in the adsorption performance, but their adsorption characteristics are not good enough. Therefore new adsorption materials have to be developed [19].

Other adsorbent materials investigated in literature include carbonaceous resins such as Ambersorb 563, 572, 575, synthetic resins (Amberlite XAD4, XAD7), porous graphitic resins (Hypercarb), and zeolites (mordenite, ZSM-5, Beta, Y) with different SiO_2/Al_2O_3 ratios or pore sizes. Results are shown in Tables 4 to 6.

The carbonaceous resins and certain zeolites proved to be very promising in terms of MTBE removal efficiency, whereas the synthetic resins always showed poorer capacities.

Ambersorb 563 (carbonaceous resin) showed in all studies best performance [28, 47, 53, 54, 56], sometimes limited to MTBE concentrations below 5–10 mg/L [47, 53, 54]. Above that value the related carbonaceous resin Ambersorb 572 showed slightly superior removal efficiencies. In any case, Ambersorb resins had considerably higher capacities than activated carbon; e.g. at 500 µg/L MTBE initial concentration capacities of around 10–16 mg/g (Ambersorb 563 and 572) vs. 3–4 mg/g with F-400 [28, 47].

ZSM-5 and mordenite (both zeolites) showed adsorption capacities for MTBE similar to the carbonaceous resins [54]. Mordenite's superiority over activated carbon was documented by various studies [20, 28, 47, 52, 54, 57]. The aperture size of the zeolites is of significant influence for the adsorp-

tion performance. While mordenite and ZSM-5 have pore sizes similar to the kinetic diameter of MTBE (6.5 × 7.0 Å for mordenite, 5.3 × 5.6 Å for ZSM-5 compared to 6.2 Å kinetic diameter), HiSiv 1000, Beta-zeolite, and Y-zeolites exhibit larger pore sizes preventing MTBE from adsorbing efficiently [52]. Beta and Y-zeolites showed a very poor removal of MTBE [54, 57]. Besides the pore size difference, the high aluminum content of Y-zeolite (SiO_2/Al_2O_2 75) compared to mordenite (SiO_2/Al_2O_2 200) and ZSM-5 (SiO_2/Al_2O_2 1000) results in a higher charge and thus in a lower hydrophobicity which again lowers the MTBE adsorption [57]. Knappe and Rossner [54] observed no effect of exchangeable cations (H^+, Na^+, NH_4^+) on MTBE adsorption; the hydrolysis of MTBE to *t*BA is principally possible but was not observed under the study's conditions.

The synthetic resins Amberlite XAD4 or XAD7 showed only a rather poor potential for removing MTBE, the adsorption could not match the zeolites [56] or even the activated carbon F-400 [28, 47, 53]. Other resins such as porous graphitic Hypercarb exhibited a capacity twice as high as for activated carbon F-400 [47]; however, this special resin has to be activated prior to use with methanol, which limits its application in the field.

A major advantage of the use of synthetic resins is the possibility of designing the resin according to the sorbate and the specific conditions with regard to functional groups or pore size ranges [28]. Furthermore, they can be regenerated on-site by steam stripping, solvent extraction, or microwave irradiation, which lowers the regeneration costs [28, 58]. The regeneration with e.g., methanol results in minimal loss (max. 2%), however, a rather long regeneration time is needed [59].

Cost calculations showed that the expenses for zeolite application are in the same range as for coconut shell-based activated carbon and carbonaceous resin (Ambersorb) since material costs are similar. However, the estimated bed life for zeolites is up to six times longer than for activated carbon and the estimated adsorbent usage rate is only 25% [54]. Moreover, zeolites are thermally stable which implies an easier and faster regeneration [57].

4.3 Impact of NOM and Other Water Quality Parameters

Natural organic matter (NOM) might lower adsorption performance of adsorbents. It acts as a competitor for adsorption sites, may block the adsorption sites irreversibly, preloads the adsorbent, or clogs the pore space [28, 36, 55]. By a preferential sorption of NOM to the adsorbent already adsorbed substances may be released and a chromatographic effect occurs. Therefore the presence of NOM has to be considered for a precise evaluation of adsorption as a treatment process.

Sutherland et al. [27] observed no correlation between the capacity of F-400 and F-600 and COD in five different groundwaters at high MTBE

concentrations. Similar results were found by Wilhelm et al. [34] who compared different types of activated carbon. Initial concentrations of MTBE were as high as 200 mg/L, approximately 100 times higher than the NOM content. However, not only the concentration of NOM is decisive for the influence on the adsorption but also the constitution of different natural waters.

Hung et al. [52] noticed a decrease in adsorption capacity of F-300, F-400, and Unicarb due to the presence of NOM in river water and groundwater compared to demineralized water. The competition effect was stronger at lower initial concentrations of MTBE.

Coconut shell-based carbon seems to be less susceptible to fouling by NOM and other background water quality parameters, e.g., the precipitation of manganese, iron, or calcium carbonate [28]; however, a decrease in adsorption performance correlating with the TOC content of three different natural waters was observed as well [55]. Knappe and Rossner [54] found a reduction of adsorption capacity of up to 60% for the coal-based activated carbons, for the coconut shell-based carbons of around 20%. The presence of NOM in values of 0.5 mg/L TOC resulted in a decrease in adsorption capacity of coconut-based carbons [28, 53].

Zeolites were found to be almost unaffected by the presence of NOM [52, 54]. A maximum reduction of adsorption capacities of 0–23% for ZSM-5 was observed by Knappe et al. [54].

The carbonaceous resin Ambersorb 572 remains unaffected by the presence of NOM [28]. A study conducted in Santa Monica water showed no decline in capacity compared to demineralized water [53]. Moreover, Ambersorb is not prone to biofouling inside the pores.

The influence of physical water parameters such as pH or temperature on the performance of the adsorption process is quite small in the range relevant for drinking water production. Activated carbon (F-400) shows a slight decline in capacity with raising temperature whereas on Ambersorb 572 no effect was observed [28]. In column tests, the temperature influence observed was only small [59].

In the pH range between 6.5 and 8.5 no influence on Ambersorb performance was detected.

4.4 Impact of Concentration

The influence of inlet concentration of MTBE on the performance of an activated carbon adsorption column was studied in three references [27, 34, 55]. The authors found that the influent concentration of MTBE shows a strong correlation with capacity, a higher concentration resulting in a higher carbon loading [27, 34]. The carbon usage rate was found to increase with the inlet concentration [34, 55].

4.5 Impact of Co-Solutes

MTBE often occurs in conjunction with other gasoline components in the aquifer, therefore, competitive adsorption and the impact of co-solutes such as BTEX are of major interest for a full-scale application.

Various studies with all types of activated carbon found that MTBE is displaced from the carbon if other compounds are present [27]. It could be shown that BTEX compounds are preferentially adsorbed over MTBE [20, 27, 36]. Shih et al. [55, 58] observed a decisive reduction of adsorption of MTBE in the presence of 1950 μg/L BTEX resulting in a competitive displacement of MTBE and an increase in the carbon usage rate of 30%. The observed chromatographic effect is caused by the desorption of MTBE through displacement by better adsorbing compounds and the re-equilibration effect, if the inlet concentration of MTBE is decreasing [28].

But not only the activated carbons are subject to the impact of co-solutes. Bi et al. [56] showed that in a binary solution of MTBE and *o*-xylene the latter is preferentially adsorbed on carbonaceous resin leading to a reduction in adsorption capacity for MTBE. Similar results are found for *m*-xylene [28, 47] and other BTEX [58]. Davis and Powers [47] showed, however, that the influence is more pronounced on activated carbon (F-400) than on the carbonaceous resin Ambersorb (35% reduction vs. 11% reduction).

The competition for adsorption sites of MTBE with *t*BA is not as significantly pronounced as with other BTEX. Nevertheless, the capacities of activated carbons for MTBE (1000 μg/L) decreased in the presence of 100 μg/L *t*BA whereas the capacity of Ambersorb 572 remained unaffected [53].

4.6 Other Fuel Oxygenates

Only a few studies deal with the adsorption of alternative fuel oxygenates. The most common substitutes for MTBE are related ethers such as ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME) and di-*iso*propyl ether (DIPE). However, also ethanol (EthOH) or *t*BA are possible alternatives for MTBE.

The preferential selectivity of activated carbon for alternative oxygenates can be arranged according to the following rule: the less soluble the contaminant in water, the greater the preferential selectivity on the carbons [37], and the more difficult it is to treat with activated carbon. The order of relative selectivity is EthOH > *t*BA > MTBE > ETBE > TAME > DIPE [37, 51].

As with the case with MTBE, the activated carbon F-600 exhibits a higher capacity than the F-400.

The removal of *t*BA by adsorption is of special interest since *t*BA is a degradation product of MTBE not only during biodegradation but also during

chemical oxidation. The adsorption capacity of *t*BA on the carbonaceous resin Ambersorb 563 is significantly higher than on activated carbon [28]. However, the sorption potential is decisively lower than for MTBE [56]. The influence of co-solutes (*m*-xylene in binary solution) is negligible [56].

4.7 Practical Implications / Full-Scale Installations

Full-scale applications of the adsorption process for MTBE removal are so far only realized on remediation sites [20, 28]. Drinking water installations for the sole use of MTBE elimination are so far not implemented [28]. Nevertheless, the remediation of contaminated groundwater sites might be the first step towards drinking water treatment.

Only a few large-scale installations of GAC adsorption filters for the remediation of contaminated groundwater are reported in literature. The most known example is the implementation of a GAC F-300 adsorption filter for the purification of a contaminated groundwater in Rockaway Township, NJ, USA [1]. This installation showed, however, a very fast breakthrough of MTBE and DIPE, which imposed the necessity of frequent carbon replacements. The operators realized the high expenses and changed from carbon adsorption to a combined air stripping followed by carbon adsorption.

In Table 8 an overview over different pilot-scale field studies based on a report by Stocking et al. [28] is given.

However, for an industrial-scale installation, an economic evaluation has to be made not only considering the water quality but also taking into account regeneration costs. The performance of an adsorption process is dependent on NOM and other surrounding conditions. Synthetic resins have to be considered since their overall performance is comparable to GAC; if regeneration requirements are strict, they might even be the better choice.

5 Chlorination

The behavior of MTBE during chlorination has not been studied in literature so far. However, the detection of MTBE in finished drinking water implies that no elimination occurs during conventional disinfection with chlorine [25, 60–62]. The only reference of MTBE in combination with chlorine observed a possible interference of residual free chlorine with the analysis of MTBE with SPME and GC/MS detection [63].

Table 8 Pilot-scale/field studies [28]

Influent concentration of MTBE	Resin used	Flow rate	Effluent conc.	Capacity	Location
140–160 μ/L	L-493 followed by Ambersorb 563	114 L/h	n.d.		Oil refinery Bakersfield, California
200 μg/L	L-493 followed by Ambersorb 563	227 L/h	1–3 μg/L (L-493) n.d. after Ambersorb 563		World Oil Service Station
49 000–110 000 μg/L	Ambersorb 563	114 L/h	n.d. to break through of MTBE observed	50 mg/g	BP Oil Company
125 mg/L test (BTEX 75 mg/L) decreasing during test	PolyGuard	680 L/h		150 mg/g	Gas station Bellingham, MA

n.d.: not detectable

6
Chemical Oxidation

6.1
General Remarks

Chemical oxidation shows several potential benefits compared to other treatment options. The main advantage is the possible mineralization of organic substances to carbon dioxide and water. The substance can be completely destructed and is not only simply enriched or shifted into another phase [33]. Furthermore, there is also a disinfecting effect if ozone is used. Ozonation is the oxidative treatment process most widely spread in drinking water treatment—though it is mainly implemented for disinfection and the oxidation is only considered a beneficial side effect [28].

However, during chemical oxidation complete mineralization is only achieved with major expenses, i.e., high oxidant doses and ideal reaction conditions. Most often, the substances are only partially oxidized and side products or stable intermediates are formed. These by-products may be better biodegradable promoting bacterial growth. In some cases they might even be more toxic than the original substance resulting in even bigger problems for the drinking water supplier. Therefore, for a complete evaluation, possible by-product formation has to be taken into account [20, 28].

6.2
Ozonation

The impact of ozone on MTBE has been studied intensively in literature. For the reaction of ozone with MTBE two major reaction mechanisms have to be considered: firstly the direct reaction of ozone with MTBE and secondly the elimination via so-called AOP (advanced oxidation processes), where the reaction is induced by OH radicals as oxidants. The mechanism is strongly dependent on the pH. At low pH values, direct reaction with ozone prevails, especially if functional groups with high electron density are present (e.g., olefinic double bounds). As the milieu is getting more alkaline, radical mechanisms gain importance since ozone is decomposing into OH radicals in the presence of hydroxyl ions. From pH 11 only radical reactions are taking place [64].

The reaction of MTBE with ozone and OH radicals is following second order kinetics [18, 31, 65]. However, the reaction of MTBE with ozone alone is very slow as indicated by the reaction rate constants as given in Table 9. This observation is verified by various authors.

Baus et al. [25] found a maximum elimination efficiency of 40% in demineralized water at pH 6–7 with ozone doses varying between 0.5 and 5 mg/L (initial MTBE concentration 10 μg/L). At this pH the direct reaction of ozone

Table 9 Reaction rate constants for reactions with ozone and H_2O_2

	Ozone [L/mol sec]		OH radicals [L/mol sec]	
MTBE	< 1	[73]	1.6×10^9	[74, 75]
	1.4×10^{18}	[18]	1.2×10^9	[18]
	exp(– 95.4 kJ/mol/RT)		8.0×10^9 exp(– 4.6 kJ/mol/RT)	
	0.14	[66]	1.9×10^9	[66]
	0.003	[65]	$3.9 \pm 1.8 \times 10^9$	[76]
HCO_3^-			8.5×10^6	[73]
CO_3^{2-}			3.9×10^8	[73]
ETBE	1.98	[70]	2.7	[30]
			$2.80 \pm 0.38 \times 10^9$	[70]
			1.81×10^9	[77]
*t*BF	0.78	[66]	7.0	[66]
			4.1×10^8	[78]
			$1.2 \pm 0.4 \times 10^9$	[76]
			5.2×10^8	[79]
*t*BA	0.003	[80]	6.0×10^8	[73]
			7.6×10^8	[81]
TAME	1.28	[70]	$2.58 \pm 0.32 \times 10^9$	[70]
			2.37×10^9	[77]
DIPE	4.37	[70]	$2.98 \pm 0.28 \times 10^9$	[70]
			2.49×10^9	[77]
EtOH			1.9×10^9	[73]
H_2O_2			2.7×10^7	[82]
2-Methoxy-2-Methyl Propionaldehyde			3.0×10^9	[66]
Hydroxy-iso-Butyraldehyde			3.0×10^9	[66]
Methyl acetate			2.3×10^8	[73]
			1.2×10^8	[83]
Acetone			1.4×10^8	[84]
			1.1×10^8	[73]
Formaldehyde			1.0×10^9	[73]
Pyruvaldehyde			7.0×10^8	[73]
Acetic acid			1.0×10^8	[85]
Formic acid			2.2×10^9	[86]
Oxalic acid			5.3×10^6	[87]
Pyruvic acid			3.1×10^7	[88]

with the organic pollutant prevails. When raising the pH to 8.5, an elimination of 85% was observed. It was concluded that MTBE cannot directly react with ozone [62]. Karpel Del Leitner et al. [30] also observed an increase in

removal efficiency of MTBE from 30% at pH 2 to 80% at pH 8.0 (initial concentration 176 mg/L) with 528 mg/L ozone. Acero et al. [66] concluded from experiments in bi-distilled water that MTBE reacts primarily with OH radicals generated.

Natural water ingredients can have great influence on the elimination efficiency during ozonation. Natural organic matter competes with the pollutant for the available ozone. It may furthermore interfere in subsequent reactions, i.e., scavenge possible radical species formed during ozonation. Other radical scavenging species are carbonate and bicarbonate, which form radicals that are less reactive than the OH radicals (cp. Table 9). The latter influence was stated by Baus et al. [25, 62] who observed only 20% concentration reduction (initial concentration 10 μg/L) in highly alkaline tap water with 1 mg/L ozone compared to 40–50% in demineralized water at the same pH. At high initial concentration of pollutant, bicarbonate (HCO_3^-) shows, however, only limited influence on the reaction rates [30].

Mofidi et al. [67] observed a poor removal of MTBE at 200 μg/L initial concentration in groundwater in a pilot-scale ozone reactor (760 L): 20–25% at moderate ozone levels (1 and 2 mg/L) and up to 70% with higher ozone doses (4 mg/L). However, little to no elimination was observed at 2000 μg/L MTBE initial concentration with the same ozone doses. Mofidi et al. attribute the concentration effect to additional competition of formed by-products at high MTBE levels. Liang et al. [68] showed in a similar study with spiked groundwater at pilot-scale level (760 L semi-batch reactor) a comparable effect of initial MTBE concentration. At 200 μg/L initial concentration 10 mg/L ozone reduced the MTBE level to 97%, at 2000 μg/L only 53% elimination was achieved at the same ozone doses. In another study by Liang et al. [31] contaminated groundwater and surface water containing 20–75 μg/L MTBE was treated in a pilot-scale flow through reactor (3.8 L/min). They achieved a removal efficiency of 75 and 97% in the groundwater with 4 and 6 mg/L applied ozone, respectively; in the surface water the removal efficiency were 65 and 78%, respectively. No influence of inlet concentration in this concentration range was observed.

Acero et al. [66] observed in batch experiments a 28 to 39% elimination of MTBE (160 μg/L) in two surface waters (lake water) and one well water with an ozone dose of 2 mg/L. A change in pH in the natural waters from 7 to 8 led to a faster elimination but the removal was not more complete. The raw waters studied differed in DOC content and alkalinity. Acero et al. observed, that the NOM had a promoting effect on the ozonation, i.e., the ozone decomposition into OH radicals was accelerated. However, the NOM also acts as OH radicals scavenger thus limiting the promoting effect. During the ozonation experiment no significant impact of the alkalinity was observed. Baus et al. [25] observed no significant impact of the composition of different natural waters on the elimination of MTBE during ozonation.

Dionysiou et al. [69] showed in a pilot-scale flow through reactor (60 L) a maximum treatment efficiency of 70% (initial MTBE concentration 300 μg/L) in tap water at an ozone dose of 4.5 mg/L. Higher ozone doses showed no improvement.

ETBE proved to be a bit more amenable to removal by ozone than MTBE [30, 70]. Karpel del Leitner et al. [30] showed that ETBE is eliminated to 85% (starting from 204 mg/L with 648 mg/L ozone) at pH 2, whereas a change in pH to 8 results in a 99% removal.

Compared to MTBE the elimination of TAME, DIPE, and ETBE at 10 μg/L from a highly alkaline tap water showed no better removal, whereas in a softer surface water the elimination efficiencies were 60% for the alternative ethers compared to 40% for MTBE. This effect was attributed to the higher amount of OH radical scavenging species in the tap water [70].

Kerfoot et al. [71, 72] developed a new ozone sparging device which injects gaseous ozone in microbubbles resulting in a very efficient MTBE, TAME and *t*BA removal. MTBE and *t*BA were observed to degrade in similar reaction rates, whereas TAME reacts faster. Other intermediates such as *t*BF were found to be further oxidized at a slower rate. The authors claim to operate this system with lower costs than other on-site applications.

The elimination of MTBE and alternative ethers from water with pure ozonation is only possible if sufficient OH radicals are created. This is highly dependent on the type of water treated and high ozone doses are needed for a measurable concentration decline. These doses are higher than usually applied in drinking water treatment [25, 66].

6.3 Photochemical Treatment (UV Irradiation)

MTBE is not readily removed by pure UV irradiation regardless of the emitted wavelengths. Its structure does not include any dislocated electrons (double bonds or aromated ring structures) which could easily be extracted by UV photons. Therefore the formation of excited states needs very high energy doses, i.e., low wavelengths of irradiation. These wavelengths are, however, not applicable to water treatment options, since their penetration depths into the reactor are very short due to high water absorption.

Knowing this, the results of literature studies are explicable which found only very limited elimination of MTBE from any water type by pure UV irradiation [28, 33, 67, 70, 76, 89–93]. Mostly, the slight reduction in concentration observed (between 5 and 10%) could be attributed to the formation of radicals either by the homolysis of water or from other water ingredients. Mofidi et al. [67] explicitly stated that UV irradiation alone is not effective in removing MTBE.

6.4
Advanced Oxidation Processes (AOP)

6.4.1
General Remarks on Advanced Oxidation Processes

The oxidative elimination of MTBE during ozonation is solely based on the reaction with OH radicals as discussed in chapter 6.2. OH radicals are formed during the self decomposition of ozone, but they can also be artificially formed either from ozone or other oxidants. OH radicals exhibit the highest oxidation potential compared to H_2O_2, ozone or other oxidants (see Table 10). They react with organic compounds either by abstracting a H-atom or adding to a double bond yielding an organic radical. In the presence of dissolved oxygen, organic peroxyl radical are formed, which subsequently react with other water ingredients [33].

Advanced oxidation processes (AOP) can be classified using their manner of OH radical production. The most known AOP in water treatment is the combination of ozone with H_2O_2. Other AOP include the UV-induced AOP (UV/H_2O_2, UV/ozone, UV/TiO_2), Fenton and Photo-Fenton processes, sonolytically induced AOP and those AOP which create OH radicals directly from water by homolysis (gamma radiolysis/electron beam injection, VUV-irradiation). In the following chapters, each technology is discussed with respect to MTBE elimination.

AOP offer advantages compared to other treatment technologies [28]. They might result in a more complete destruction of MTBE since its high solubility enhances the effectiveness. When using ozone or UV light a beneficial side effect is disinfection. Furthermore, these technologies are already established in drinking water treatment. However, during advanced oxidation similar by-products are formed as with ozonation, the range of possible substances being even broader due to the unspecific reactions of OH radicals with the organic substances. The formation of bromate has to be considered in bromide containing waters if ozone-based AOP are to be applied.

Table 10 Redox potentials for different oxidants (from [94])

Oxidant	Redox potential [V]
OH radical	2.80
Ozone	2.07
H_2O_2	1.78
Potassium permanganate	1.69
Chlorine dioxide	1.56
Chlorine	1.36

Other water ingredients may significantly interfere with the performance of the AOP. Alkalinity, i.e., mainly carbonate and bicarbonate, act as an OH radical scavenger, though their inhibiting effect is dependent on the concentration. Their reaction rate constants are significantly lower than those of MTBE and other organic substances, but at high concentrations the actual reaction rate might be higher than that of MTBE removal. Natural organic matter (TOC) might also have an OH radical scavenging effect, similar to phosphate or sulphate. Iron, manganese or copper also react with OH radicals, but as scaling agents they are mainly responsible for fouling of UV systems.

6.4.2 The Ozone/H_2O_2 Process

The ozone/H_2O_2 process is used in water treatment installations in Europe to remove pesticides from drinking water [19].

If H_2O_2 is added during the ozonation of water, the removal of MTBE is significantly enhanced [18, 25, 30, 31, 62, 66–70]. This is due to the immediate formation of highly reactive OH radicals, which in turn are able to convert MTBE. H_2O_2 alone is not able to convert MTBE [33, 69].

An optimum H_2O_2 concentration for the conversion of ozone is a molar ratio of ozone/H_2O_2 of 2 : 1 as found by Hoigné [64, 95]. So most studies use this ratio though the results with lower ratios do not differ significantly and are only of interest for the optimization of a larger-scale process.

In demineralised water the elimination efficiency raised from 40% at low pH values, an initial ozone dose of 1 mg/L, and MTBE concentration of 10 μg/L to 100% with addition of H_2O_2 equimolar to ozone [25]. These results confirmed the findings of an earlier study of Karpel del Leitner et al. [30], only at lower concentrations. In that study a MTBE removal of 98% was observed with ozone/H_2O_2 compared to 80% with pure ozonation in the MTBE concentration range between 8 and 176 mg/L (528 mg/L ozone, ozone/H_2O_2 ratio 2 : 1).

Mofidi et al. [67] studied the oxidation of groundwater spiked with MTBE at 200 and 2000 μg/L with ozone doses ranging from 1 to 4 mg/L and an ozone/H_2O_2 weight-ratio of 1 : 1 in a pilot scale ozone reactor. The addition of H_2O_2 almost doubled the removal at low MTBE concentrations (from 20–25% to 40–50%), but the impact was much more decisive at an initial concentration of 2000 μg/L. While no significant elimination was observed with pure ozonation, up to 80% were removed at 4 mg/L ozone and H_2O_2.

Liang et al. [31, 68] showed, that in highly alkaline and bromide containing groundwater the ozone/H_2O_2 process at equimolar ratio converts MTBE more rapidly than pure ozonation. Moreover, at high initial concentrations of MTBE (2000 cp. to 200 μg/L), the ozone/H_2O_2 process proved to be more efficient. The combination of ozone and H_2O_2 was more effective and produced

fewer by-products than ozonation and was able to further oxidize formed intermediates. Similar observations were made by Mitani et al. [18] who showed that the addition of H_2O_2 increased the rate of mineralization.

The influence of NOM on the oxidation is less pronounced during the ozone/H_2O_2 process, since ozone is directly reacting with the H_2O_2 molecules and the promoting effect of NOM regarding OH radical formation is too slow to contribute [66]. Carbonate and bicarbonate, however, have a much higher impact if OH radicals are quickly generated due to their scavenging capacities. The inhibiting effect of carbonate and bicarbonate was also shown by Baus et al. [62, 70] who investigated three different water types with variations in DOC and alkalinity and found a higher elimination of MTBE at low DOC and low alkalinity.

The efficiency of the ozone/H_2O_2 process is, however, limited by the COD of the natural water. Sutherland et al. [27] observed no MTBE elimination in high COD groundwater at ozone dosages of up to 410 mg/L, and a molar ozone/H_2O_2 ratio of 2 : 1. Very high doses of ozone/H_2O_2 were needed in order to completely remove MTBE.

One large-scale device for the treatment of MTBE contaminated water by ozone/H_2O_2 is mentioned in scientific literature, the HiPOx advanced oxidation technology [96]. This device was used in a pilot-scale study with contaminated groundwater from Port Hueneme, California, where 748 μg/L MTBE were reduced to below 1 μg/L (average removal efficiency 99.87%) with 119 mg/L ozone and ozone/H_2O_2 weight ratio of 2 : 1. The system was designed for an enhanced and optimized mass transfer of ozone into water and thus shows low bromate formation. However, in this study, the bromate formation was above 10 μg/L due to the high bromide content in the raw water.

The combined ozone/H_2O_2 process was applied in situ to remediate a contaminated plume on a site in Delaware, containing MTBE, BTEX, *t*BA and TAME [97]. The authors report that most of the dissolved plume was remediated in the first 3 months of system operation.

Karpel del Leitner et al. [30] investigated the elimination of ETBE not only with ozone but also with the combination of ozone/H_2O_2. However, in the concentration range investigated (9.2 to 204 mg/L) in demineralized water at pH 8.0, no decisive improvement of the removal of ETBE could be observed under identical conditions with H_2O_2 addition, since the application of 528 mg/L ozone already resulted in a 99% reduction of ETBE.

Sutherland et al. [37] observed in a continuous plug flow reactor that other ethers (ETBE, DIPE, and TAME) are more efficiently removed than MTBE. Only *t*BA removal proved to be less efficient. These investigations resulted in lower calculated unit treatment costs for the alternative ethers (up to 64%) but higher costs for the treatment of *t*BA.

6.4.3
UV-Based Advanced Oxidation Processes: General Remarks

UV irradiation of drinking water is mostly applied in water treatment installations for disinfection purposes. However, the application of a UV-based AOP carries several advantages: besides the disinfecting properties, a chemical conversion of organic substances is induced.

In UV-induced AOP an aiding substance is added, which itself absorbs UV irradiation creating highly reactive OH radicals. For an efficient process design, the lamp's emission spectrum should overlap the maximum absorption range of the oxidant used. The process can be very inefficient, if this prerequisite is not considered.

Ozone can be used as a source for OH radicals since its absorption maximum lies in the UV light range between 240 and 280 nm wavelengths. H_2O_2 is more efficient in terms of OH radical production per photon absorbed. However, its molar absorption coefficient is lower than that of ozone at these wavelengths, only at lower wavelengths OH radicals are more effectively created [28]. Both combinations have been studied in literature for the reduction of MTBE.

The UV-based AOP can, however, be interfered by dissolved solids such as iron, calcium, magnesium. Iron not only absorbs UV light, dissolved iron takes also part in chemical oxidation and is thus a competitor for radicals. Other salts like calcium or magnesium salts act as a scaling agent of the lamps' quartz sheaths. Furthermore, high alkalinity inhibits the process by its OH radical scavenging characteristics [33]. Nitrate and nitrite limit the effectiveness of UV systems due to their light absorbance, and turbidity lowers the transmittance of UV irradiation.

One major disadvantage often mentioned is the lamp failure by sleeve cracking. The danger of mercury leaking into the water has to be considered. So far, this failing point is only rarely described in literature, main failing points are more often the electric surroundings or damage of the lamp during maintenance [28].

6.4.4
UV-Induced Advanced Oxidation Processes: UV/Ozone

Gurol et al. [98] conducted lab-scale experiments with a synthetic groundwater (adjusted pH and alkalinity) in which 123 mg/L initial MTBE concentration were fully eliminated by UV/ozone at an influent gaseous ozone concentration of 70 mg/L.

Garoma and Gurol [99] observed in pilot-scale experiments with a synthetic water (bidistilled water, adjusted alkalinity and pH) a 99% elimination of 80 mg/L MTBE within 20 min. The UV light source was a medium pressure mercury lamp and the applied ozone concentration at the inlet of the

demi-batch reactor ranged from 23 to 51 mg/L. The elimination efficiency of MTBE was enhanced by increasing UV light intensity, however, limited by the ozone dose in the range applied. An augmenting ozone concentration resulted in proportional higher efficiencies, since the UV intensity was high enough to convert all applied ozone into OH radicals. The degradation products formed during the AOP also consumed OH radicals, therefore the observed ratio of ozone absorbed per MTBE eliminated was higher at higher removal percentages. The variation of the alkalinity in the range of 2 to 8 mM showed no decisive impact on the removal efficiency.

Experiments in natural water (drinking water and surface water) also showed high efficiencies for MTBE removal [70, 100]. At low concentrations of MTBE (10 μg/L) the UV/ozone process showed the fastest removal rate in tap water compared to other AOP [70]. Graham et al. [100] showed with a pilot-scale reactor operating with a germicidal low-pressure UV lamp that the application of UV light to an ozone containing solution enhances the MTBE removal rate. Where pure ozonation yields 97.5% elimination of 1.2 mg/L MTBE, UV/ozone reaches 99.95% removal. The rate of elimination in the UV-based AOP is twice as high compared to that of pure ozonation. The authors stated that the basic mechanistic processes are the same in ozonation and the UV/ozone process, and the latter is only the acceleration of the former. The application of UV/ozone, however, yielded a higher conversion of organic carbon to inorganic carbon.

Garoma et al. [101] studied in the same experimental set-up as mentioned above the conversion of *t*BA as the main degradation product of MTBE. The elimination efficiency increased from 56% with pure ozonation to 99% with UV/ozone. An increase in UV light intensity and influent gaseous ozone concentration yielded an increase in *t*BA removal.

The application of the UV/ozone process has several advantages. It shows a higher removal efficiency than UV or ozone alone. However, if OH radicals are needed in large quantities, the UV/ozone process is limited by the solubility of ozone in water. Compared to H_2O_2, ozone is only poorly soluble in water. If low pressure mercury lamps are used, the system UV/ozone is more efficient in generating OH radicals than other UV-based AOP due to the higher molar extinction coefficient of ozone at 254 nm, which is the wavelength at which low pressure lamps emit. However, if the lamp type is changed, this advantage might not hold.

Moreover, since ozone is used as oxidant, the formation of bromate from bromide containing waters is most probable.

6.4.5 UV-Induced Advanced Oxidation Processes: UV/H_2O_2

The molar absorptivity of H_2O_2 is two orders of magnitude lower than that of ozone [28, 33]. Therefore, H_2O_2 must be present in much higher concen-

trations to generate the same number of OH radicals. However, H_2O_2 is much handier than ozone; it can easily be stored as a liquid, and it is less hazardous than ozone [33]. Furthermore, if UV/H_2O_2 is applied as advanced oxidation process, no bromate is formed in bromide containing waters opposed to ozone-based AOP [19, 102].

H_2O_2 undergoes a photolytically induced dissociation forming OH radicals. During the subsequent reactions with organic compounds as described in chapter Sect. 6.4.1, organic peroxyl radical are formed, which subsequently react with other water ingredients and might end in the formation of H_2O_2 [33]. This mechanism is independent on the lamp type used in the application, as long as the lamp emits UV light in the range of the absorption spectrum of H_2O_2. Most applications described in literature use medium pressure mercury lamps, only few apply low pressure mercury lamps. The advantage of medium pressure mercury lamps are their broad availability and their almost continuous emission spectrum in the UV-C range, where H_2O_2 absorbs maximally. For some lab-scale studies Xenon arc lamps were used as well [90].

MTBE removal by the UV/H_2O_2 process follows pseudo-first order kinetics [33, 76, 90, 102, 103]. Experiments in model water showed a fast elimination of MTBE [33]. In natural waters the reduction of MTBE is dependent on the concentration of other water ingredients, which may interfere either directly by competing with H_2O_2 for the UV light absorption, or indirectly via competition for OH radicals formed by the photolytic decomposition of H_2O_2 [27, 33, 102–104]. For example, Leong et al. [104] found a 99% MTBE elimination in natural well water, based on approx. 1000 µg/L initial MTBE concentration and 40 to 60 mg/L H_2O_2. MTBE is not reacting with H_2O_2 without any mediation by UV [28, 33, 69, 90, 91].

Suspended particles present in natural water may hinder the UV absorption by scattering the UV light [76]. Besides, a higher COD results in higher background UV-absorbance and thus the absorption efficiency of H_2O_2 is lowered and less OH radials are produced [27]. High nitrate contents may also hinder the absorbance efficiency for H_2O_2 [104]. On the other hand, water ingredients like carbonate (HCO_3^-/CO_3^{2-}) or other inorganic and organic compounds (NOM, phosphate ions) compete with MTBE for the OH radicals formed in the UV/H_2O_2 process.

But not only natural water ingredients can have a limiting effect on the process efficiency. Other organic pollutants such as BTEX compounds, which often occur jointly with MTBE if the contamination results from gasoline spills, also react with OH radicals and thus reduce the MTBE removal efficiency [19, 27, 102, 104]. Furthermore, most BTEX compounds absorb UV light and thus can also be converted by direct photolysis competing with H_2O_2 for the UV light [102].

The pH of the natural water is also of main influence. An increase in pH results in a shift of the HCO_3^-/CO_3^{2-} equilibrium towards higher CO_3^{2-} con-

tent. Since the reaction rate constant of CO_3^{2-} is two orders of magnitude higher than that of HCO_3^- the scavenging characteristic of the natural water rises accordingly [37]. Moreover, the pH also has an influence on the equilibrium of H_2O_2 and its corresponding base HO_2^-. The latter is more reactive towards OH radicals thus again increasing the OH radical scavenging properties of the water [37]. Therefore the energetic expense for the removal of MTBE is higher at higher pH values.

Additionally, the initial concentration of H_2O_2 is of influence on the process efficiency. If the H_2O_2 content is too high, H_2O_2 itself acts as an OH radical scavenger and lowers the process efficiency [27, 90, 91, 102]. However, if H_2O_2 concentration is too low, not enough OH radicals will be formed and the process remains ineffective [33, 70]. The H_2O_2 concentration thus has to be optimized depending on the water characteristics [70, 90].

*t*BA, a MTBE degradation product, is not as easily transformed as MTBE in the UV/H_2O_2 process [103, 104]. This is expected since its OH radical reaction rate constant is one order of magnitude lower than that of MTBE (see Table 9).

Stocking et al. mentions in a report for the California MTBE Research Partnership [28] several pilot studies for UV/H_2O_2 treatment of MTBE contaminated water. One is the Charnock Wellfield located in California. Here, medium-pressure UV lamps were implemented. However, costs were significantly higher and removal efficiencies lower than expected, and the formation of by-products required additional treatment. The authors mention furthermore two installations for wastewater treatment by UV/H_2O_2 systems treating 100 mg/L and 11 mg/L initial MTBE concentration, respectively. Only one full scale-drinking water treatment plant is so far reported located in Salt Lake City, Utah. Here also medium pressure UV lamps are implemented.

6.4.6
UV-Induced Advanced Oxidation Processes: UV/TiO_2 and UV-Enhanced TiO_2/H_2O_2

During illumination of TiO_2 with UV light, high-energy-state electron/hole pairs are formed on the surface of the catalyst. The formation occurs, however, only with photons exhibiting energy greater than the band gap energy of TiO_2; this means wavelengths below 380 nm. These electron/hole pairs can then either recombine or react with water molecules, hydroxide ions, or molecular oxygen adsorbed on the TiO_2 surface and result in the formation of highly reactive OH radicals, O_2^- radicals or HO_2 radicals. These reactive species can attack and oxidize organic contaminants present at or near the surface of TiO_2 [32, 89, 92, 93, 105].

The elimination of MTBE by the UV/TiO_2 process follows pseudo-first order reaction kinetics [105]. The limiting step of the reaction is the adsorption of MTBE onto TiO_2 surface where OH radicals are generated. Dark experiments showed that after 1 h less than 10% MTBE are adsorbed on the surface and saturation was achieved [89, 93]. The adsorption equilibrium is

dependent on solution pH; below a value of 6 the pH shows only small impact, at higher pH, however, higher adsorption occurs due to the change in surface potential (isoelectric point at neutral pH) [89, 105]. This is directly reflected in the elimination rates; at higher pH the rate is decisively higher.

The elimination efficiency is dependent on the dosage of TiO_2 as well. At TiO_2 concentrations below 500 mg/L there is an initial increase of the reaction rate with TiO_2 dosage, above that value the UV light is scattered by the TiO_2 particles and the efficiency decreases [105]. Moreover, the reaction rate increases linearly with UV light intensity (intensities varied between 215 and 586 mW/cm^2, MTBE concentration 750 mg/L, TiO_2 concentration 500 mg/L, pH 11) [105].

The addition of H_2O_2 in the UV/TiO_2 process enhances the efficiency of the process. H_2O_2 can act as an electron capturer preventing electron/hole pairs from recombining and concurrently forming OH radicals [93]. Zang and Farnood [93] and Hung [105] found independently pseudo-first order reaction kinetics for the elimination of MTBE with the combination UV/TiO_2/H_2O_2. The effect of H_2O_2 addition on the reaction rate, however, was observed controversially. Hung et al. mentioned an increase in reaction rate with added H_2O_2, whereas Zang et al. found a higher reaction rate in the UV/TiO_2 process. The addition of H_2O_2 at low concentrations to reaction mixtures with low TiO_2 concentrations led to a decrease in MTBE elimination rate, which increased again with higher H_2O_2 concentrations to reach the level of the reaction rate without H_2O_2 addition. Zang and Farnood contributed this phenomenon to competing mechanisms of H_2O_2 at the TiO_2 surface: H_2O_2 can act as an acceptor for electrons generated by the irradiated TiO_2 and form additional OH radicals. This contributes to the MTBE elimination. MTBE might furthermore be directly oxidized at positive holes on the TiO_2 surface; those, however, are scavenged by adsorbed H_2O_2. Thus the amount of MTBE directly oxidized on the TiO_2 surface is negligible. This is further supported by the low adsorption of MTBE on TiO_2 compared to H_2O_2. The OH radicals formed at the TiO_2 surface can, however, be scavenged by the H_2O_2 present. This is the main limiting effect during the addition of H_2O_2 to the UV/TiO_2. The direct adsorption of UV light by H_2O_2 creating OH radicals in the solution might be of influence, is, however, limited since at the wavelengths applied in the UV/TiO_2 the adsorption of H_2O_2 is small.

Natural water ingredients have a decisive impact on the performance of the UV/TiO_2 process. Sahle-Demessie et al. [32] found in a comparison of a model water with natural groundwater a decrease in reaction rate of one order of magnitude. Chloride ions, for example, do not only scavenge oxidizing radicals in the solution but can also block active sites on the catalyst surface thus deactivating the catalyst for oxidation of organics. Fe^{3+} ions may compete with molecular oxygen for the electron acceptor sites on the TiO_2. This latter effect was studied in detail by Klauson et al. [92] who added fer-

rous/ferric ions to the UV/TiO_2 process. At low $Fe^{2+/3+}$ concentrations an initial increase of MTBE elimination efficiency was observed with increasing $Fe^{2+/3+}$ concentrations, then a drop occurred at 1 mM with a subsequent gradual increase. The blockage of active sites at the TiO_2 surface by $Fe^{2+/3+}$ ions occurs only at higher concentrations; at lower concentration the direct oxidation at positively charged holes and the subsequent OH radical production is enhanced. The subsequent increase in elimination efficiency is due to the influence of Photo-Fenton processes in the bulk solution.

Sahle-Demessie et al. [32] observed a far better removal of BTEX with UV/TiO_2 than that of MTBE. They claim a necessary pre-treatment for natural water for an efficient removal of MTBE, including filtration, pH adjustment and H_2O_2 addition in small amounts. They furthermore propose a combined process of air stripping and gas phase oxidation by UV/TiO_2 for the treatment of MTBE contaminated water since the gas-phase oxidation rates of MTBE are orders of magnitude higher.

All studies in literature concerning UV/TiO_2 were carried out with MTBE concentrations above 80 mg/L in order to study mechanistic effects. However, in drinking water purification these concentrations are never present, because they are much higher than any taste and odor thresholds of MTBE (10–30 μg/L). There was no literature found on the application of the UV/TiO_2 process at lower MTBE levels relevant for drinking water treatment.

6.4.7 Fenton Processes and Photo-Assisted Fenton Processes

Burbano et al. [82, 106] studied the elimination of MTBE with Fenton and Photo-Fenton processes.

The Fenton process consists of the reaction of ferrous ion (Fe^{2+}) with H_2O_2 generating OH radicals, hydroxid ions and ferric ions (Fe^{3+}). The ferric ion itself can further react with H_2O_2 in a Fenton-like reaction creating protons, HO_2 radicals and ferrous ion, the reaction rate and the reactivity of the HO_2 radical being smaller than that of the OH radical. In photo-assisted Fenton processes OH radicals and ferrous ions are created directly from aqueous Fe^{3+} complexes ($Fe(OH)^{2+}$) and additional Fenton reactions can subsequently take place [107].

Burbano et al. [82] found 91 to 97% elimination of 1 and 2 mg/L MTBE, respectively, in deionized water at pH 3.0. However, only 17 to 32% mineralization was observed. They found the reaction to be two-phased. After an initial pseudo-first order phase where a surge of OH radicals was created and no interfering by-products have yet been formed, the reaction rate reaches a turning point, after which the OH radicals further created also react with other water constituents. In this second part, Fenton-like reactions take place.

pH plays an important role during application of Fenton processes. At higher pH values, ferrous ion converts to ferric ion, and precipitation might

occur. The Fenton process shows its highest efficiency at pH 3.0. At neutral pH a MTBE elimination of only 10% was observed.

In another study, Burbano et al. [106] investigated the elimination of the MTBE by-products *t*BA, *t*BF, methyl acetate and acetone at a concentration of 0.0227 mM (1.3 to 2.3 mg/L). The reaction rates were slower than that of MTBE. The compounds containing a *tert*-butyl group were observed to be more susceptible to OH radical attack.

The applicability of the Fenton process in water treatment is limited to special cases, where the conditions might favor such a process. In drinking water production, for example, the pH value of the raw water usually varies between 6.5 and 8.5, rendering the feasibility of the process impossible.

6.4.8 Sonolytically Induced Advanced Oxidation Processes

If water is treated with ultrasonic waves, small micro-bubbles are created. Those cavitation bubbles collapse violently with adiabatic heating creating temperatures up to 5000 K and pressures of 975 bars. Under these conditions the thermal dissociation of water forms H and OH radicals. H radicals combine with present oxygen to form HO_2 radicals. These and other intermediate radicals can then further react with other water ingredients. Organic pollutants are converted either by the radicals' attack or they might undergo direct pyrolytic reactions in the cavitation bubbles [108–111].

Kim et al. [109] showed that MTBE is rapidly eliminated during ultrasound application. The by-products observed resulted predominantly from OH radical attack and were thus similar to those found in the other AOP. If the reaction mixture was nitrogen saturated instead of oxygen-saturated, the variety of products broadened because of the formation of nitrogen-containing reactive species. Under Argon saturated conditions more pyrolytic products were observed.

MTBE is eliminated with pseudo-first order reaction kinetics [108–111]. The reaction rate is dependent on the frequency and power density of the ultrasound. At higher frequency, the elimination of MTBE is much faster. For each frequency the power density shows an optimum, since the interaction of and influence on cavitation bubble size, collapse time, transient temperature and internal pressure is very complex. Initial MTBE concentration was also observed to be of influence; the reaction rate decreased with increasing MTBE concentration. This indicates that the reaction is limited by OH radical diffusion.

The influence of natural water ingredients such as HCO_3^- and CO_3^{2-} was found to be negligible over the concentration range studied (1–2 mM). The addition of NOM also has negligible effect on the elimination rate of MTBE [108]. That indicates that the major reaction takes place inside the micro-bubble, not in the bulk solution of the aqueous phase [111].

The combination of ozone with ultrasound was reported to yield an enhancement of the reaction rates between 1.5 to 3.9 times [110, 111]. It also results in an increase in removal efficiency compared to the ozone/H_2O_2 process of a factor of 1.1. During sonolysis of ozone-containing water the ozone is sonolytically decomposed into molecular and atomar oxygen. The latter reacts with water again forming OH radicals [111], thus enhancing the elimination rate of MTBE.

Lifka and Ondruschka [110] yielded an even higher elimination rate by the addition of H_2O_2 to the ultrasound/ozone system.

TAME, DIPE and ETBE were investigated in the study by Kim et al. [109]. They showed similar elimination rates. If pyrolysis were the main elimination mechanism, the volatility and thus the partitioning into the gas phase during cavitation would be of much influence. MTBE, ETBE, TAME and DIPE have different vapor pressures (345, 183, 105, 222 mbar, respectively [109]), however, under the studies' conditions no decisive difference in elimination rates could be observed—another hint for the predominance of the radical-induced elimination path over direct pyrolysis. The addition of ozone to the reaction mixture resulted in an enhancement of the elimination rate for ETBE by the factor of 1.5 and for TAME of 2.1 [110]. The mixture of different ethers proved to yield a slower reduction rate than the ethers alone.

6.4.9 Water Treatment With Ionizing Irradiation

Treatment of water with ionizing irradiation leads to the direct formation of highly reactive species from water molecules. These include oxidizing compounds like OH radicals or hydrogen peroxide (H_2O_2), reducing compounds like hydrogen radicals (H radicals) and aqueous electrons (e^-_{aq}), as well as molecular hydrogen (H_2) and protons (H^+). The relative number of each species formed per 100 eV (the yield) for the generated species are: 2.7 for OH-radicals, 2.6 for H_2O_2, 2.6 for H-radicals, 2.6 for e^-_{aq}, 0.7 for H_2, and 2.6 for H^+.

Hsieh et al. [112] observed a decrease in dose constant (analogon to the first-order rate constant based on the absorbed energy in Gy^{-1}) with increasing MTBE concentration during the irradiation of deionized water spiked with 150 µg/L MTBE by a ^{60}Co-γ source. This implies that the degradation of MTBE is limited by the OH radical formation. The main reacting species is the OH radical due to the highest reaction rate constant with MTBE compared with those of hydrogen radicals or aqueous electrons. If benzene is added, the MTBE elimination rate decreases with increasing benzene concentration. This is due to the reaction of benzene not only with OH radicals but also with hydrogen radicals forming by-products (e.g., phenol) which themselves compete for OH radicals. The influence of cupric ions is limited to the reaction with aqueous electrons at higher concentrations, therefore MTBE elimination is not hindered by the presence of those ions.

Cooper et al. [113] irradiated "natural" water (groundwater and wastewater after RO treatment) spiked with 200 µg/L MTBE with irradiation from a 20 kW electron accelerator. The radical scavenging influence of TOC was demonstrated resulting in a decrease of removal efficiency of MTBE. The spiking of other organic compounds also resulted in a decrease in removal efficiency. During irradiation protons are formed which lower the pH of the solutions thus pushing the equilibrium HCO_3^-/CO_3^{2-} towards higher HCO_3^- content which reacts more slowly with OH radicals than CO_3^{2-}. Therefore the influence of alkalinity on the removal of MTBE is only marginal.

This process is only of scientific interest, since the irradiation of water during drinking water production with ionizing irradiation is so far unheard of and not permitted in most countries.

6.4.10 Other Oxidative Treatment Options

6.4.10.1 Oxidation With Sodium Persulfate

Sodium persulfate ($Na_2S_2O_8$) is a radical-based oxidant. The reactive species formed during oxidation include OH and SO_4^- radicals. Huang and Couttenye [114] showed that the elimination of MTBE with this oxidant follows pseudo-first-order kinetics. However, the reaction rates during oxidation with persulfate are decisively lower than for the oxidation with other oxidants, ranging between 0.13 and 5.8×10^{-4} s^{-1}. The activation energy (24.5 ± 1.6 kcal/mol) is adversely affected by pH (2.5 to 11.0) and ionic strength (0.11 to 0.53 M) whereas temperature and persulfate concentration may significantly accelerate MTBE elimination. The oxidation with persulfate was, however, found to be ineffective at ambient temperature and pressure [115].

However, heat-assisted persulfate oxidation was able to completely convert MTBE and its major reaction products *t*BA, *t*BF, acetone, and methyl acetate.

6.4.10.2 Oxidation With Permanganate

Permanganate oxidation of organic compounds can occur via several different reaction pathways: electron exchange, hydrogen atom abstraction and direct donation of oxygen [116]. The resulting pathway is subject to reaction conditions and the molecule reacting.

Damm et al. [116] investigated MTBE elimination with permanganate in lab-scale experiments in buffered, decarbonized ultrapure water at initial MTBE concentrations of approx. 120 mg/L and molar permanganate: MTBE ratios between 6.6 and 117.3. MTBE was eliminated up to 99.9% with initial rates between 0.2 and 1.4 mg/L h. The reaction was found to follow

pseudo-first order kinetics and the reaction rate constant was determined to 3.49×10^{-5} L/mol s. The reported half-life values of MTBE in permanganate solution are much longer than those of other organic compounds, e.g., trichloroethylene or phenol. Furthermore, between pH values of 5.6 and 9.9 no significant impact on MTBE elimination was noticed. However, no complete mineralization could be observed, intermediates such as *t*BF and *t*BA were formed.

6.4.10.3
Oxidation By Bifunctional Aluminum

Oxygen alone is not efficient in oxidizing MTBE or other ether compounds [117]. However, by a reductive activation of molecular oxygen with bifunctional aluminum, oxygen is converted to reactive atomar oxygen and water via formation of superoxide (O_2^{*-}) and peroxide (O_2^{2-}). Bifunctional aluminum is prepared by sulphating zero-valent aluminum with sulphuric acid leading to a dual functionality of simultaneously decomposing both reductively and oxidatively degradable contaminants in the presence of oxygen.

Lien and Zang [117] investigated the elimination of MTBE and TAME with bifunctional aluminum. After 6 h a complete elimination of MTBE with initial concentration of 35 mg/L was observed in the presence of oxygen. Without oxygen only 40% elimination was achieved, however, no reaction by-products were observed, indicating that the elimination was only due to the sorption of MTBE onto the catalyst. Without bifunctional aluminum no reaction took place. The sulphuric acid treatment is decisive for the performance, since aluminum without sulfation only yielded 25% conversion of MTBE.

A 90% TAME elimination was achieved after 24 h (initial concentration 20 mg/L).

The reaction followed pseudo-first order kinetics for both ethers, however, the formed by-products (*t*BA, *t*BF and *t*AF) were not following pseudo-first order. Here the reaction was overlaid by sorption effects.

Conclusively, this process is able to oxidize organic pollutants under reducing conditions.

6.4.10.4
Oxidation With Perfluorinated Alumina Bonded Phases

The limited elimination potential of ozone for MTBE can be catalytically enhanced. A possible catalyst is a perfluorinated alumina bonded phase. Perfluorooctanoic acid (PFOA) may be used as catalyst. Kasprzyk-Hordern et al. [118] showed that the addition of this catalyst results in an increase in elimination efficiency when treating MTBE and ETBE. In that study, an aqueous extract of gasoline was used containing 20 mg/L of ETBE and 2.5 mg/L

MTBE besides BTEX compounds. With catalysed ozonation the elimination increased from 46.8% to 90% for MTBE and from 47.7% to 80% for ETBE. Despite a measurable adsorbability of the PFOA for the ethers (192 μg MTBE/g PFOA), the elimination could be predominantly attributed to chemical oxidation since the initial concentration was two orders of magnitude higher. Experiments in natural water showed an increase in efficiency during catalytic ozonation compared to distilled water, which the authors contributed to the higher pH present in the experiments. However, the enhancement of the ozonation by addition of PFOA proved to be only significant with ethers, not as much with BTEX.

6.5 Formation of By-Products

The major advantage of chemical oxidation processes for water purification is concurrently its main disadvantage: the chemical conversion of the pollutant down to CO_2 and H_2O. If such total mineralization is achieved, the process is working efficiently and no adverse effects are created. However, as soon as no total mineralization is achieved, the formation of by-products has to be considered; they have to be determined, analyzed and assessed for their environmental damage potential.

Since it is unanimously agreed that MTBE is eliminated via OH radicals, many studies have been performed in order to elucidate the mechanistic pathway of the conversion. OH radicals react with organic molecules by the abstraction of hydrogen from C – H or O – H bonds, via hydroxyl group addition to unsaturated carbon bonds or the interaction with N-, P-, and S-containing bonds [32]. Since in the MTBE molecule no unsaturated carbon bonds and no N-, P-, or S-atoms are present the initial reaction has to concentrate on the abstraction of H atoms or the cleavage of the C – O bond.

In Fig. 1 the main reaction products for the conversion of MTBE are on display. Literally all literature studies mention the formation of *tert*-butyl formate (*t*BF) and *tert*-butyl alcohol (*t*BA) as main products. These products result from an attack on the methoxy group (O – CH_3) of the MTBE molecule. This pathway is observed to be the main reaction path, Hsieh et al. [112] showed that 71% of the MTBE is eliminated via an attack on this side of the molecule. The other 29% account for an attack on the *tert*-butyl-group, yielding 2-methoxy-2-methyl propionaldehyde as the primary intermediate as well as methyl acetate and acetone [18]. Besides *t*BF and *t*BA formaldehyde and acetone are found as major reaction by-products resulting from the attack on the methyl group. *t*BF and *t*BA themselves undergo further reactions with OH radicals leading to different aldehydes and their corresponding acids (cp. Fig. 1).

The different treatment processes show the same reaction intermediates, however, they differ in the extent of their formation. During ozonation the

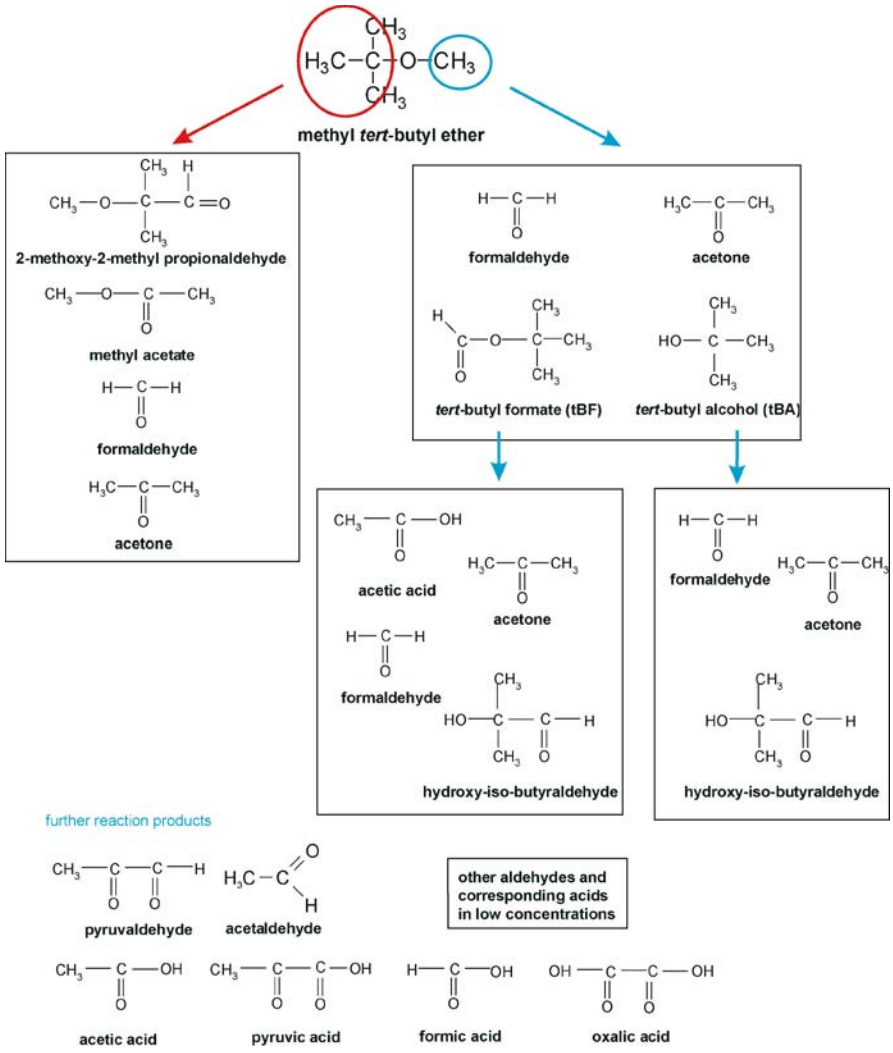

Fig. 1 Oxidative Reaction Products of MTBE (after [18, 66, 103, 117])

formation of *t*BF is dominant, whereas during ozone/H_2O_2 treatment the primary intermediates are further degraded [18, 68]. The addition of H_2O_2 yields a higher mineralization rate [18]. The addition of a catalyst during ozonation (i.e., perfluorinated alumina bonded phase) also results in a higher amount of more stable intermediates such as acetone and carboxylic acids [118]. Sahle-Demessie et al. [32] and Xu et al. [89] found reaction pathways and by-product formation during UV/TiO_2 treatment to be a function of reaction conditions (initial MTBE concentration, pH, MTBE: oxidant ratio, dissolved ions, oxidant activity) and reactor design (light intensity, contact

time, contacting method). During treatment with the Fenton process, a high amount of Fenton reagent is necessary to reach mineralization [106]. Furthermore, Burbano et al. [106] showed that simply structured compounds as acetone or methyl acetate are less susceptible to OH attack than those including *tert*-butyl group. The Fenton process proved to be more efficient at low pH values which was attributed to a higher oxidation potential of the OH radicals at low pH values (2.65–2.80 V at pH 3.0; 1.90 V at pH 7.0) [82]. However, this trend was not observed in other AOPs, possibly due to other factors such as the influence of adsorption by the pH [89].

One major transformation pathway of *t*BF to *t*BA was claimed to be via simple hydrolysis in aqueous solution [18, 68, 82, 109, 111, 117]. However, the OH radical may also attack the *t*BF molecule and convert *t*BF directly [82].

Almost no treatment method investigated in literature yielded total mineralization. The reaction end products most often consist of carboxylic acids, acetone and other stable organic molecules.

For the other fuel oxygenates detailed studies on the reaction pathways are not as abundantly available in literature. However, the similarity in chemical structure implies similar reaction by-products. In the case of TAME, *tert*-amyl formate and *tert*-amyl alcohol were observed instead of *t*BF and *t*BA, and their subsequent degradation products; however, acetone and methyl acetate were observed as well [117]. The attack on the methoxy group was observed to be the major pathway, corresponding to the MTBE elimination. During the elimination of ETBE the same reaction by-products were observed as with MTBE, with the exception of *tert*-butyl acetate which was formed instead of *t*BF [30].

Formation of Bromate and Nitrite

The use of ozonation either as disinfection step or for the removal of organic compounds carries one disadvantage: the possible formation of bromate from bromide containing waters. Bromate is formed through complex reactions between ozone, bromide, TOC and hydroxyl radicals [119–123]. Since bromate is a suspected human carcinogen it is regulated in the Drinking Water Directive of the European Union [124]. Drinking water directives of several countries are in the process of implementing this limit; so for example in Germany, where the limit in drinking water is set to 10 μg/L from 2008. Therefore the excessive use of ozone in order to get rid of organic pollutants such as MTBE is counterproductive and leads to an increased risk for consumer's health [67].

In various studies concerning ozonation and ozone-based AOP the formation of bromate is observed [31, 62, 66–68]. The extent of bromate formation is dependent on the ozone exposure. Higher ozone doses usually result in higher bromate concentrations. However, if the ozone demand of the water itself is high, the ozone exposure for bromide is smaller and less bromate is formed [31, 62, 66]. During experiments with the combined process

ozone/H_2O_2 less bromate formation is observed [31, 66, 67]. This is due to the interference of H_2O_2 in the bromate formation pathway and to the scavenging of ozone by the H_2O_2 molecules. Other parameters influencing the bromate formation are pH and presence of carbonate: Formed carbonate radicals contribute to bromate formation. Therefore a careful optimization of the treatment process dependent on the nature of the raw water is necessary [66]. Furthermore, AOP which use ozone as oxidant have to consider their bromate formation potential.

The application of UV irradiation on nitrate containing water may result in the formation of nitrite, especially if low wavelengths are admitted. However, in the studies investigated for this review, no information was given on that topic. Since nitrite is a parameter regulated in the Drinking Water Directive of the European Union (limit 0.5 mg/L) [124], for drinking water applications the possible formation of nitrite has to be taken into account and its occurrence in drinking water has to be prevented.

6.6 Comparison of Different Advanced Oxidation Processes

In order to compare different AOP, a figure-of-merit was developed by IUPAC [125] describing the expense of energy necessary for the elimination of organic pollutants. This parameter is called EE/O (electrical energy per order). It denotes the electrical energy consumed during the concentration reduction of the organic pollutant for one order of magnitude in one cubic meter. Different approaches for batch and flow-through operation are available in literature, however, the resulting values provide a basis for the comparison of different AOP and different modes of operation. The EE/O parameter is said to be independent of initial concentration, however, this assumption has to be relativated. In Table 11 EE/O values are summarized as found in literature.

The values calculated in literature differ widely. However, it becomes clear that for the removal of MTBE one has to consider EE/O values between 1 and 9 kWh/m^3 order, both in lab-scale and pilot-scale operation. There is not much difference between the application of ozone/H_2O_2 or UV/H_2O_2 process. Typical EE/O values for other contaminants range from 0.5–1.3 kWh/m^3 order for BTEX, 2.6–2.7 kWh/m^3 order for atrazine or 0.5–1.6 kWh/m^3 order for 1,4-dioxane. This difference could be expected, since MTBE is much less efficiently eliminable than BTEX.

The EE/O values as reported in literature are dependent on the MTBE inlet concentration in contrast to the assertion of Bolton et al. [125]. This effect may be due to the enhanced formation of by-products at higher MTBE concentrations which compete for the OH radicals during the reaction [65, 104]. The increase in EE/O during concurrent MTBE and *t*BA or BTEX removal backs this assumption up [19, 104].

Table 11 EE/O values of different oxidative processes for MTBE treatment

Matrix	Process	Scale	MTBE conc.	EE/O [kWh/m^3 order]	Refs.
Tap water	Ozone/H_2O_2	Lab-scale	100 mg/L	0.9–1.7	[29]
Groundwater	Ozone/H_2O_2	Lab-scale	50–300 mg/L	2.98–6.93	[65]
Tap water	Ozone/H_2O_2	Lab-scale	1–327 mg/L	0.26–4.73	[65]
Groundwater	Ozone/H_2O_2	Lab-scale	20–830 mg/L	0.91–29.80	[65]
7 different Groundwater	Ozone/H_2O_2	Pilot	0.029–2.3 mg/L	1.3–8.2	[27]
5 different Tap water	Ozone/H_2O_2	Pilot-scale	100–1000 μg/L	1.9 (pH 7) 8.8 (pH 9)	[37]
Drinking Water	UV/H_2O_2	Lab-scale	80–85 000 μg/L	0.18–7.5	[102]
Well water	UV/H_2O_2	Lab-scale	200–1000 μg/L	0.6–0.8	[104]
Groundwater	UV/H_2O_2	Pilot-scale	0.029–2.3 mg/L	1.2–8.6	[27]
4 different Groundwater	UV/H_2O_2	Pilot-scale	0.029–2.3 mg/L	13–247	[27]
Well water	UV/H_2O_2	Pilot-scale	650–1300 μg/L	1.16	[104]
Tap water	UV/H_2O_2	Pilot-scale	100–1000 μg/L	1.5 (pH 7) 3.8 (pH 9)	[37]

Table 12 EE/O values for different oxidative processes for treatment of alternative oxygenates (after [37])

Matrix	Process	Compound	Scale	Concentration	EE/O [kWh/m^3 order]
Tap water	Ozone/H_2O_2	ETBE	Pilot-scale	100–1000 μg/L	1.2 (pH 7) 1.8 (pH 9)
Tap water	Ozone/H_2O_2	TAME	Pilot-scale	100–1000 μg/L	1.8 (pH 7) 1.7 (pH 9)
Tap water	Ozone/H_2O_2	DIPE	Pilot-scale	100–1000 μg/L	1.1 (pH 7) 3.1 (pH 9)
Tap water	Ozone/H_2O_2	*t*BA	Pilot-scale	100–1000 μg/L	4.1 (pH 7) 9.1 (pH 9)
Tap water	UV/H_2O_2	ETBE	Pilot-scale	100–1000 μg/L	1.6 (pH 7) 2.5 (pH 9)
Tap water	UV/H_2O_2	TAME	Pilot-scale	100–1000 μg/L	1.3 (pH 7) 2.0 (pH 9)
Tap water	UV/H_2O_2	DIPE	Pilot-scale	100–1000 μg/L	1.3 (pH 7) 1.9 (pH 9)
Tap water	UV/H_2O_2	*t*BA	Pilot-scale	100–1000 μg/L	2.5 (pH 7) 9.0 (pH 9)

During UV/H_2O_2 treatment the increase in H_2O_2 concentration lowers the EE/O value until 100 mg/L initial H_2O_2 concentration. Above this concentration the EE/O increases again because H_2O_2 competes for OH radicals by itself [102].

Table 11 also shows the impact of natural water ingredients on the energetic expense for MTBE removal. If the water matrix contains substances which compete for OH radicals (alkalinity, NOM) or which lower the extent of OH radical formation (absorption of UV irradiation), the EE/O value for achieving a certain remediation goal will increase [65]. The influence of pH on EE/O is shown in a study from Sutherland et al. [37]. The ozone/H_2O_2 process exhibits a higher EE/O at higher pH values since at higher pH values more CO_3^{2-} and HO_2^- is present which act as OH radical scavengers.

Compared to MTBE, the alternative ethers are more easily and efficiently removed [37]. Only the removal of *t*BA is less efficient. The EE/O values for ETBE, TAME, DIPE and *t*BA are given in Table 12. A correlation was found of the EE/O with the reaction rate constant k_{OH}.

7 Membrane Processes

Membrane processes used in water treatment applications normally reduce to ultra-, nanofiltration or reverse osmosis. The application of these membranes for the purification of water contaminated with MTBE has so far been objective of only a few studies. Due to the small molecular weight and size, MTBE is not likely to be removed by ultrafiltration membranes normally used in drinking water treatment.

However, it might be retained by nanofiltration or reverse osmosis processes [20]. The nanofiltration process is usually applied for softening of drinking water. Naraghi et al. [126] found that MTBE is removed more efficiently than smaller molecules such as tetrachloroethylene and trichloroethylene during nanofiltration. A removal efficiency of 88% was achieved at a MTBE feed concentration of 5 μg/L. This indicates that MTBE is already removed on the surface of the membrane since the pore size of the nanofiltration membrane used in this study was smaller than the molecular size of MTBE. Additionally membrane surface properties like zetapotential and hydrophobicity play an important role. Lipp et al. [127] confirmed these findings with a different nanofiltration membrane. At similar inlet MTBE concentrations (5–10 μg/L) 88–99% retention is achieved, depending on the operating conditions. However, large-scale applications have to be thought over carefully because large membrane areas have to be implemented due to the low transmembrane flux inherent to this membrane process. Moreover, nanofiltration removes MTBE not selectively but also other water compounds resulting in a permeate that requires further treatment steps to be constringent

with the drinking water standards. Additionally, the resulting concentrate has to be treated before discharge.

Another application of membrane processes for MTBE removal studied in literature is the pervaporation, which represents an alternative to the stripping process discussed in chapter 3. Hydrophilic pervaporation is commonly used for the absolutation of alcohol which forms an azeotropic mixture at high ethanol content [128]. By the vaporization of the mixture through a thin membrane layer, the azeotrope is cleaved and water is evaporated leaving the pure ethanol in the retentate.

Organophilic pervaporation enhances the mass transport of volatile organic compounds (VOC) from liquid (water) to gas phase. The VOC molecules dissolve in the membrane material, diffuse through it and evaporate on the back side of the membrane. The driving force of the process is maintained with a partial vacuum or sweeping gas. The evaporation is thus not only dependent on Raoult's law but also influenced by the affinity of the membrane material for the VOC and the diffusivity of the substance in the membrane material.

In the case of MTBE, the application of this treatment method offers the advantage of lower air-to-water ratios than with air stripping. Keller and Bierwagen found air-to-water ratios between 6–56 with high removal efficiencies (between 80 to 99%) [36, 128].

The membrane material is decisive for the removal efficiency. Urkiaga et al. [129] tested different commercially available membranes on their adsorption characteristics for MTBE showing that silicone rubber was more efficient than polyether-polyamide block-copolymer membranes (PEBA) and polyoctyl methyl siloxane membranes (POMS) at MTBE concentrations of 250 mg/L and 1250 mg/L. The studies by Keller et al. [128] and Vane et al. [130] used polypropylene and silicone rubber membranes, respectively.

The removal efficiency is influenced by operating conditions such as temperature, vacuum pressure, liquid and gas flow rate, and influent MTBE concentration.

All studies were conducted at very high initial MTBE concentrations (between 10 to 900 mg/L) compared to concentrations typically found in ground and surface waters apart from contaminated remediation sites. In the concentration range studied, however, no influence of the initial MTBE concentration on the MTBE flux through the membrane could be observed [130].

From the above-mentioned studies it can be concluded that the desorption step (influenced by the gas flow rate or vacuum pressure) is not mass transfer limiting for MTBE pervaporation in the permeate pressure and gas flow rate range studied [128, 129]. Only the pilot-scale study by Vane et al. [130] showed a small decline in removal efficiency (6%) at higher permeate pressures.

Contradicting results were found concerning the liquid flow rate. Urkiaga et al. [129] used plate modules with PEBA membranes and showed an increase in the removal efficiency with increasing flow rate due to a minimiza-

tion of the polarization layer (laminar layer on the surface of the membrane) on the liquid side which is a major diffusion barrier for the VOC molecules. Keller and Bierwagen [128] observed a decrease in removal efficiency with increasing liquid flow rate. One possible explanation might be the different module design, since Keller used a hollow fibre module made of polypropylene, but no detailed information are given on this topic in either reference. No effect on the removal efficiency was found in the bench-scale study by Vane et al. [130], who used silicone rubber membranes in a plate module. The authors concluded that MTBE pervaporation is membrane diffusion controlled, i.e., that the membrane resistance towards MTBE is not located in the liquid boundary layer mass transfer. Only in the pilot-scale part of the study an influence of liquid flow rate was observed showing that in larger membrane installations and modules the influence of the boundary layer has to be taken into account.

Temperature influence is unanimously described as enhancing removal efficiency with higher temperature due to the increase in Henry's law constant and diffusion coefficient in water and the membrane material with temperature. An inhibiting effect on the removal efficiency with temperature is solely the decreasing sorption in the membrane material with increasing temperature [36, 128–130].

Compared to other VOC, which might be removed by pervaporation (e.g., toluene), MTBE has a much lower overall mass transfer coefficient [130]. However, in comparison with *t*BA, MTBE exhibits a much higher selectivity and overall mass transfer. In pilot-scale experiments the removal of *t*BA in single pass was found to be $6.7 \pm 1.2\%$ whereas MTBE was eliminated to 68%.

8 Elimination of MTBE in Waterworks

Investigations regarding MTBE elimination in waterworks are mostly focused on raw water that is highly contaminated with MTBE and other VOC. These can often be counted among remediation technologies in respect to concentration range. Mostly, there are special installations build for treating highly contaminated groundwater which is afterwards subjected to conventional drinking water treatment.

The already-mentioned studies of Rockaway Township, NJ, USA [1], and the installation in Kansas, USA, [38] fall in this category.

Stocking et al. identified two water supplies in the US which use air stripping as treatment technology. Inlet MTBE concentrations of 96 and 900 μg/L were removed to above 95% in both cases. The second installation had two air stripping towers operating in series with an air-to-water ration of 175 : 1.

In the US state of New Jersey, four installations are described that were examined on their MTBE elimination potential [131]. Originally designed for

the removal of VOC and other organic compounds, they also had to cope with rising MTBE concentrations. Two installations worked with air stripping towers, which were extremely efficient at removing BTEX, TCE or PCE, however, less efficient at removing MTBE. The applied air-to-water ratio was 11 and 23 : 1 at the first installation, resulting in an MTBE removal of 43% (starting from 2.8 μg/L) and 71 : 1 at the second installation with 76% MTBE removal (from 6–11 mg/L). Another waterworks for the treatment of groundwater removed PCE and TCE below detection limits, however, the MTBE concentrations in in- and effluent remained the same. In 1998, a surface water treatment plant removed MTBE in concentrations between 0.8 to 2.3 μg/L to above 62% and 25% using ozonation with subsequent activated carbon filtration.

Stocking et al. describe a full-scale drinking water treatment in Salt Lake City, Utah, USA, where MTBE contaminated water is treated by a UV/H_2O_2 system using medium-pressure lamps. However, no detailed description of the efficiency of the system, operating conditions or concentration ranges is given.

In the USA, more installations are reported with respect to MTBE elimination than in Europe. This is mainly due to the higher concentrations in which MTBE is found in the aquatic environment. In Europe, the used percentage of MTBE in gasoline is lower and gasoline stations have been obliged to higher standards for underground storage tanks for many years now. Therefore, LUST (leaking underground storage tanks)—the main reason for high groundwater contaminations in the US—are much less frequently detected and the groundwater contaminations are lower. Furthermore, if raw water resources are such highly contaminated with MTBE, the main course of action in Europe is to consider a closure of the source if other resources are available. Only limited studies are therefore available for drinking water treatment with concurrent MTBE removal. Baus et al. [25, 62], Achten et al. [16], and Kolb et al. [60] report results from measurements in waterworks in Germany. In spot samples, an overall elimination of 30% was achieved by aeration at initial concentrations of 0.1 to 0.4 μg/L [25]. With 0.5 mg/L ozonation and subsequent activated carbon filtration the calculated average efficiency was 60% during monitoring over a period of 2 years (max. initial concentration 0.2 μg/L) [25, 62]. In this case, raw water was taken from a bank filtration site. In another waterworks 40% elimination was achieved with ozonation and activated carbon filtration [25]. Achten et al. [16] measured similar concentrations in bank filtrated water and tap water from the waterworks treating this bank filtrate. The waterworks uses aeration, ozonation and activated carbon filtration as treatment steps, however, no detailed description of the operating conditions are given. Kolb et al. [60] conducted a survey on the occurrence of MTBE in several drinking waters all over Germany. From 50 cities tested, 40% of the waters were found positive for MTBE in the concentration range between 17 and 712 ng/L. 89% of the positive samples were from water suppliers which use bank filtrated water.

9
Assessment of the Treatment Options for Drinking Water Production

Subsoil passage is not efficient in removal of MTBE and other oxygenates, rendering riverbank filtration as treatment step in drinking water production ineffective. Therefore, natural attenuation cannot take care of the MTBE problem in respect to drinking water production. For remediation purposes where no drinking water resources are affected and the MTBE concentration is much higher, it may still be a viable option.

It is possible to remove MTBE and other ethers by aeration; however, high air-to-water ratios are required and an additional off-gas treatment has to be implemented. These aspects may increase the operational costs for water treatment significantly. Especially at low MTBE concentrations, high expense is required for sufficient elimination. However, aeration proves to be the most robust process and is least affected by water quality variations [27].

Adsorptive elimination of MTBE and its substitutes has great prospects, however, the activated carbons commonly used in waterworks exhibit only limited adsorption capacities for the ethers. The application of alternative adsorption materials (e.g. zeolites or resins) shows high potential, but as long as these new materials are too expensive to be applied in large quantities, their use will not expand into drinking water production.

Chemical oxidation as treatment option for MTBE contaminated water has drawn most attention in literature. It appears that every possible oxidation technique was investigated regarding its MTBE removal potential, no matter how scientific and far-fetched it may seem. For drinking water application ozonation is the most important technology since it is already widely used. However, pure ozonation cannot reliably reduce MTBE concentrations. Advanced oxidation processes such as ozone/H_2O_2 or UV-induced AOP (UV/ozone and UV/H_2O_2) are more effective and are already implemented. However, the elimination is only achieved with high amounts of operating resources making the removal very expensive compared to other organic compounds. Moreover, with the application of chemical oxidation, the formation of by-products has to be considered. One of the major reaction by-products of MTBE and the other ethers is *t*BA, which is regarded toxic. The formation of *t*BA even at low levels thus creates more problems.

The removal of MTBE by membrane micro- or ultrafiltration is highly ineffective due to the molecule's small size. Only nanofiltration showed removal potential. However, the process is very elaborate and expensive in terms of equipment and operating conditions (low transmembrane flux, high membrane area). Moreover, the resulting water needs further treatment in order to comply with drinking water standards, and the concentrate has to be treated before discharged.

An application of pervaporation for drinking water production has not been implemented so far. It might be a treatment option for highly contaminated water in competition to aeration.

Aeration systems, activated carbon filters, and AOP are the treatment options that are currently implemented for MTBE removal. However, implementation and operation of these systems are always considered to be more expensive and less efficient compared to the application for the removal of BTEX and other VOC.

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Toxicological Review of Methyl- and Ethyl-*tertiary*-Butyl Ethers

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Abstract Metabolism and kinetic studies have shown that the overall elimination of ETBE and MTBE from blood in volunteers was multiphasic, (two or four phases in the case of ETBE and two or three phases for MTBE). The half-lives varied in different experiments and ranged from a few minutes for early phases up to a terminal half-life of 33 h in one experiment each with ETBE and MTBE. The kinetic data obtained from experiments with rats exposed to ETBE are restricted to a statement that the apparent half-life of elimination of ETBE from blood is about 0.8 h, but it is not clear if this only refers to an initial half-life. Some guidance may be possible from the known behaviour of MTBE. Its elimination from rat blood appears to be biphasic, with an initial half-life of less than 1 h and second half-lives ranging from 37 h to 92 h (reviewed in McGregor 2006). Elimination occurs in exhaled air (mainly unchanged ethers) and urine (metabolites).

The main circulating metabolite is TBA formed by oxidation of ethers by cytochrome isoenzymes, while the main urinary metabolites are 2-hydroxyisobutyrate and 2-methyl-1,2-propanediol. The half-life of TBA in the blood of volunteers exposed to the ethers is about 8 to 12 h. Comparable measurements have not been made in rodents. Exposure of volunteers to the ethers at concentrations of up to 25 or 50 ppm (106 or 212 mg/m^3) for 2 h produced a slight impairment (3.2 to 4.4%) in pulmonary function, but other measures and subjective reports show little effect of exposure. Non-human experimental studies have not revealed significant general toxicity, neurotoxicity, toxicity to reproduction or genotoxicity. Neither MTBE nor ETBE is acutely toxic following oral, dermal or inhalation administration or an eye irritant, while MTBE, but not ETBE is considered to be a skin irritant. Sensitisation has not been demonstrated with either compound in guinea-pig maximisation tests.

Other studies of systemic toxicity of MTBE and ETBE were largely restricted to a nephropathy in male rats that was associated with the accumulation of hyaline droplets that immunohistochemically stained for α_{2u}-globulin. Apparently, the same type of nephropathy occurs in TBA-treated rats. In addition, MTBE exposure during a two-year study in rats led to exacerbation in males of the spontaneously occurring chronic progressive nephropathy (CPN), even to the most severe or "end-stage" in some cases. The effects of MTBE, ETBE and TBA on renal tubule cells are weak, specific to male rats, and not observed in mice of either sex. They are not necessarily due to metabolically generated TBA alone, although this metabolite, which is common to both ethers, does persist in the blood of rats at higher concentrations and for a longer time than the parent ethers. In vitro studies with MTBE demonstrated its specific binding to kidney proteins from male rats and that it interacts with α_{2u}-globulin. Under the conditions of these experiments, metabolism of MTBE to TBA was not likely to be significant. It is reasonable to predict that similar experiments with ETBE would produce similar results. The available data on ETBE are not extensive, but they support the hypothesis that the mode of action is dependent on α_{2u}-globulin nephropathy, which is widely considered to be of no human relevance. In CD-1 mice of both sexes there was a minimal renal nephropathy, but this occurred in all groups, including controls, at frequencies that varied between 27% and 64% with little indication of a dose-response relationship. It is unlikely to have been caused by ETBE treatment. The only other effect of note was treatment-associated bone marrow congestion in female rats exposed to 1750 ppm (7420 mg/m^3) ETBE for 3 months. There was no accompanying effect on the haematopoietic cell population, and an increase in mean corpuscular volume was not considered clinically relevant. No similar effect was reported for male rats or for mice of either sex.

With regard to carcinogenicity, low incidences of renal tubule cell adenomas were found in male rats treated with MTBE. This effect appears to be associated with exacerbation of CPN to end-stage as well as α_{2u}-globulin nephropathy induction. Both conditions are male rat specific. TBA also induces adenomas of the renal tubule cells and this response is also associated with α_{2u}-globulin nephropathy. Neither of the conditions predisposing to renal tubule cell neoplasia has human relevance. ETBE has not been tested for carcinogenicity, but results from short-term studies suggest that it would also induce kidney tumours by the same modes of action as MTBE.

Thyroid follicular cell adenomas were increased in female mice treated with TBA, but this result lacks any independent supporting evidence from a number of studies in mice and rats. There was no evidence for a hepatic effect of TBA within this mouse carcinogenicity study; therefore, no internal evidence exists for a hormonal mechanism of thyroid follicular cell induction. No thyroid neoplasms were increased in the carcinogenicity studies of MTBE.

1 Introduction

Methoxy-2-methylpropane, or methyl *tertiary*-butyl ether (MTBE) (CAS No. 1634-04-4) and 2-ethoxy-2-methylpropane, or ethyl *tertiary*-butyl ether (ETBE) (CAS No. 637-92-3) are produced and used as fuel oxygenates in gasoline. Fuel oxygenates are oxygen-rich compounds that act as octane enhancers, with the additional benefit of making gasoline burn more completely, thereby reducing exhaust emissions. Introduction of oxygenates into motor vehicle fuel has brought as benefits the substitution of lead, which is toxic and non-degradable, and hence the possibility of using three-way catalytic converters in motor vehicle engines. The result is reduced exhaust emissions, particularly of carbon monoxide, unburned hydrocarbons, polycyclic aromatics, oxides of nitrogen and particulate carbon. Other benefits from the use of oxygenates are the maintenance of high octane numbers in fuels with reduced content of aromatics, including benzene, and reduction in the fuel vapour pressure (Reid vapour pressure), so that vapour emissions during refuelling are reduced [1].

Oxygenates can be blended into gasoline in two forms: alcohols (such as methanol or ethanol) or ethers. However, ethers have certain advantages over alcohols which, when added to gasoline, tend to make the blend very volatile and water soluble, possibly creating problems in the fuel distribution system and vehicle engine. The technical characteristics of MTBE and ETBE (Table 1) suggest they have comparable usefulness as fuel oxygenates, but MTBE was

Table 1 Basic properties of ETBE compared with MTBE (data from http://www.efoa.org)

Properties	ETBE	MTBE
CAS No.	637-92-3	1634-04-4
Molecular weight	102.2	88.2
Boiling point	73.1°	55.3
Melting point	– 94°	– 108°
Density (g/cm^3)	0.745	0.741
Oxygen content (% wt)	15.7	18.2
Research octane number	118	118
Motor octane number	102	101
Vapour pressure (hPa20 °C)	128	270
Water solubility (g/L)	23.7	42
Log Pow	1.28	1.06
Odour detection in water (avg., μg/L)	49	95
Taste detection in water (avg., μg/L)	47	134
Henry's law constant ($Pa\,m^3/mol$)	140	43.8
Conversion factor [ppm → mg/m^3]	4.24	3.60

developed earlier and is more widely used as a consequence. The much lower water solubility of ETBE in comparison with MTBE is considered an advantage because its mobility in ground water will be lower, should leakage of oxygenated fuel from underground storage tanks occur. However, the lower oxygen content of ETBE means that to provide the 2.0 to 2.7% oxygen content of reformulated gasoline, an ETBE content of 13 to 17% is required, compared with 11 to 15% MTBE.

It is important to understand the impact these compounds can have on human health because of the potential for exposure to the general public. The toxicology of MTBE and ETBE has been reviewed previously [2–6]. ETBE is currently also under review for risk assessment by the European Union (rapporteur Member State, Finland). Since the structures of ETBE and MTBE are very similar, it is expected that there will also be similarities in their toxicological properties. Sometimes, this may allow gaps in the database for ETBE, which has been less extensively studied, to be tentatively supplemented with data derived from studies with MTBE.

2
Metabolism and Kinetics

2.1
Human Metabolism and Kinetics

Studies on the metabolism of MTBE and ETBE have demonstrated an initial oxidation by human liver microsomal enzymes to formaldehyde and acetaldehyde, respectively, and *tertiary*-butyl alcohol (TBA) as a metabolite common to both ethers (Fig. 1 for the metabolic pathways). Comparison of MTBE and ETBE metabolism in a battery of human cytochrome P450 (CYP) enzymes expressed in human B-lymphoblastoid cells indicated that CYP2A6 was the most active [7–10]. CYP2E1, which can metabolise diethyl ether, was much less active in MTBE and ETBE metabolism. Some human variants of CYP2A6, obtained from people who claimed to be sensitive to MTBE, were about 17% less active than the wildtype in oxidising ETBE and 33% less active than the wildtype in oxidising MTBE [9]. Whether this is enough to explain the reported interindividual sensitivity to MTBE (see below) is unclear. If this polymorphism does play a role in the inter-individual differences, then it would suggest that the sensitivity is to MTBE itself, rather than a metabolite.

TBA generated by the oxidation of these two ethers can be further metabolised, first to 2-methyl-1,2-propanediol and then to 2-hydroxyisobutyrate. These are the major urinary metabolites of MTBE and ETBE, but 2-hydroxyisobutyrate is also measurable in significant amounts in all urine samples from unexposed volunteers (and rats) [11]. This compound is formed endogenously as a product of branch-chained amino-acid degrada-

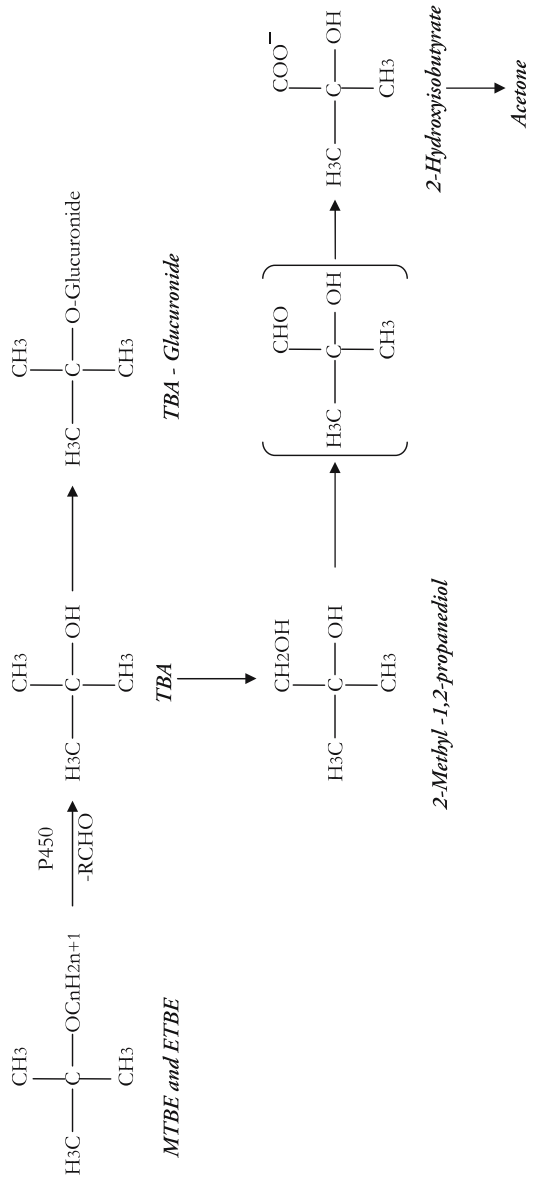

Fig. 1 Metabolism of MTBE and ETBE in mammals

tion and ketogenesis [12]. Minor urinary metabolites of MTBE and ETBE are TBA and its glucuronide conjugate; there may also be trace amounts of TBA sulphate formed [11, 13].

It could be considered that the aldehydes produced in the metabolism of these ethers would be important in their toxicology; however, this seems un-

likely. No measurements have been reported on the blood concentrations of formaldehyde, after exposure to MTBE or of acetaldehyde after exposure to ETBE. Formaldehyde is an endogenous metabolic product of *N*-, *O*- and *S*-demethylation reactions within cells. Circulating formaldehyde concentrations of around 2.6 ± SE 0.14 μg/g blood, range, 2.05–3.09 μg/g (i.e., about 70 μM) are normal in human beings unexposed to external sources of formaldehyde [14, 15] and this concentration was not significantly altered in the blood of volunteers immediately after exposure by inhalation to 1.9 ppm [2.3 mg/m^3] formaldehyde for 40 min. The absence of an increase is because formaldehyde reacts rapidly and spontaneously combines with reduced glutathione to form *S*-hydroxymethylglutathione, a detoxification mechanism that can divert the toxic chemical into biosynthetic pathways [16–18]. Acetaldehyde is one of a broad variety of aldehydes that are irreversibly oxidised to carboxylic acids by NAD-linked aldehyde dehydrogenases (ALDHs). Normal physiological concentrations of acetaldehyde are typically 0.4 to 2.5 μM [19–21] and it is rapidly detoxified, almost exclusively by mitochondrial ALDH-2 in human tissues [22–24].

MTBE is well and rapidly absorbed following human oral and inhalation exposure and it is also absorbed through the skin [25–28], all of which may be anticipated to be likely routes of human exposure resulting from contaminated air and water. These studies are summarised in Table 2. Other smaller or less detailed studies, at least from the point of view of kinetics [29–33], are described in [5]. In contrast, the kinetics and metabolism of ETBE in volunteers (Table 3) has been studied only after inhalation exposure [11, 25, 27].

The exposures used in the kinetic experiments on volunteers encompass the blood concentrations of MTBE found in a number of occupationally exposed and consumer subject studies, as described below: e.g., 0.2–37.0 μg/L) in Alaska [34] and < 0.05–37 μg/L in Connecticut [29]. A special situation is that of patients undergoing gall-bladder perfusion with MTBE for the dissolution of stones, where blood concentrations were about 1000-fold higher than those in workers exposed by inhalation [33].

Pulmonary retention (net respiratory uptake) of MTBE in volunteers exposed to concentrations ranging from 5 to 75 ppm is around 40%, while pulmonary retention of ETBE over an exposure range of 5 to 50 ppm is about 26% [35]. About 7–9% of the inhaled MTBE is reversibly taken up by the mucous membranes of the upper airways [31]. The internal dose calculated from the area under the inhaled air concentration and alveolar breath concentration curves, after inhalation exposure to 1.7 ppm for 15 min [26], averaged 197±50 μg for all subjects (about 3.86 μg/kg bw).

On the basis of tissue/air partition coefficients measured in vitro [36, 37], it appears likely that both MTBE and ETBE are extensively distributed in human tissues. The highest concentrations of MTBE in blood are found at the end of inhalation or dermal exposure periods, whereas they are found within a few minutes of oral, bolus exposures [28].

Table 2 Kinetics of MTBE and TBA in Human Subjects

Refs.	Subjects	Exposure to MTBE	Concentration in blood	Comments
[25]	Ten men aged 23–51 years and weighing 70–90 kg	Inhalation of 5, 25 or 50 ppm [18, 90 and 180 mg/m^3] MTBE during 2 h of light exercise (50 W) on three occasions separated by at least two weeks	1.4, 6.5 and 15 μmol/L [120, 570 and 1100 μg/L] to the three concentrations, respectively at end of exposure. Elimination of MTBE in urine in two linear phases: $T_{1/2}$ of 20 min and 3 h.	Pulmonary retention ranged from 32 to 42% at 5, 25 or 50 ppm. Blood concentration of TBA increased slowly during exposure and remained high for several hours after exposure. Renal clearance of TBA was low, suggesting extensive blood protein binding or extensive tubular reabsorption.
[26]	3 men + 3 women	Inhalation of 1.7 ppm MTBE (from vapourised 15 LV% MTBE gasoline mixture) for 15 min.	MTBE C_{max} about 5 μg/L at end of exposure $T_{1/2}$ M & F: 0.62 & 0.29; 1.23 & 0.95; and 14 8 & 33.2 h TBA C_{max} about 5–10 μg/L at 2–4 h $T_{1/2}$ M & F: 8.0 & 10.5 h	About 65% absorbed; accumulated percentage exhaled 40% at 1 h and 69% at 8 h. Triphasic elimination. Maximum urinary concentrations of MTBE immediate; TBA after 6–8 h.
[27, 39]	3 men + 3 women	Inhalation, 4 h to 4 ppm or 40 ppm Ingestion 5 mg (0.067 mg/kg) 15 mg (0.201 mg/kg) (4 weeks between each exposure)	MTBE C_{max} 1.9 ± 0.4 μM at end of exposure $T_{1/2}$: 1.3 ± 0.2 h; and 2.3 ± 0.3 h TBA C_{max} 2.6 ± 0.3 μM et end of exposure $T_{1/2}$: $6.5\,h \pm 2.1$ h MTBE C_{max} 6.7 ± 1.6 μM at end of exposure $T_{1/2}$: 1.2 ± 0.2 h; and 2.4 ± 0.6 h TBA C_{max} 21.8 ± 3.7 μM at end of exposure $T_{1/2}$: $5.3\,h \pm 2.1$ h	Elimination of MTBE from blood is biphasic after inhalation, triphasic after ingestion

Table 2 (continued)

Refs.	Subjects	Exposure to MTBE	Concentration in blood	Comments
			MTBE C_{max} 0.10 ± 0.03 μM at 1 h (first sample at 1 h after dosing for all ingestion measurements) $T_{1/2}$: 0.8 ± 0.1 h; 1.8 ± 0.3 h; and 8.1 ± 3.0 h TBA C_{max} 0.45 ± 0.13 μM at 1 h $T_{1/2}$: 8.1 h ± 1.6 h MTBE C_{max} 0.69 ± 0.25 μM at 1 h $T_{1/2}$: 0.7 ± 0.2 h; 1.2 ± 0.3 h; and 3.7 ± 0.9 h TBA C_{max} 1.82 ± 0.63 μM at 1 h $T_{1/2}$: 8.5 h ± 2.4 h	
[28]	14 men	Inhalation MTBE, 3.1 ppm (11.2 mg/m^3) for 1 h Oral MTBE in salty drink, 11 mg/L: dose 0.15 mg/kg bw Dermal MTBE in water, 65 mg/L declining to 51 mg/L for 1 h	MTBE C_{max} 0.28 ± 0.02 μM at 1.0 h. $T_{1/2}$ 0.03 ± 0.01 h; 0.98 ± 0.09 h; 5.23 ± 0.56 h TBA C_{max} 0.19 ± 0.01 μM at 4.0 h MTBE C_{max} 0.17 ± 0.027 μM at 0.25 h. $T_{1/2}$ 0.25 ± 0.09 h; 1.7 ± 0.35 h; 6.95 ± 1.11 h TBA C_{max} 0.23 ± 0.03 μM at 0.75 h MTBE C_{max} 0.05 ± 0.0005 μM at 1.08 h $T_{1/2}$ 0.09 ± 0.02 h; 2.11 ± 0.20 h; 6.72 ± 0.64 h TBA C_{max} 0.06 ± 0.004 μM at 7.0 h	

Table 3 Kinetics of ETBE in human subjects

Refs.	Subjects	Exposure to MTBE	Concentration in blood	Comments
[35]	8 men aged 21–41 years and weighing 70–97 kg (mean 82 kg)	Inhalation of 5 ppm [21 mg/m^3] ETBE during 2 h of light exercise (50 W); exposures separated by two weeks Inhalation of 25 ppm [106 mg/m^3] ETBE during 2 h of light exercise (50 W); exposures separated by two weeks Inhalation of 50 ppm [212 mg/m^3] ETBE during 2 h of light exercise (50 W); exposures separated by two weeks	ETBE C_{max} 1.1 μM at end of exposure $T_{1/2}$: 1.8 ± 0.1 min; 20 ± 11 min; 2.1 ± 0.6 h and 33 ± 6.0 h (4 phases) TBA C_{max} not measured ETBE C_{max} 5.4 μM at end of exposure $T_{1/2}$: 1.2 ± 0.8 min; 15 ± 9.1 min; 1.5 ± 0.5 h and 24 ± 9.7 h (four phases) TBA C_{max} 6.9 μM 0.5 h after end of exposure $T_{1/2}$: 12 ± 2.2 h ETBE C_{max} 10.2 μM at end of exposure $T_{1/2}$: 2.0 ± 1.3 min; 19 ± 9.3 min; 1.5 ± 0.2 h and 27 ± 11 h (four phases) TBA C_{max} 12 μM 0.5 h after end of exposure $T_{1/2}$: 12 ± 1.2 h	Inhaled ETBE at the three concentrations, respectively, were 0.58, 2.9 and 5.8 mmol. Pulmonary retention of ETBE ranged from 32 to 34% at 5, 25 or 50 ppm. Blood concentration of TBA increased slowly during exposure and remained high for several hours after exposure. Blood acetone increased toward the end of exposure and reached a maximum about 1.5 h later, with a net mean of about 75 μM. Kinetics of ETBE and TBA linear up to 50 ppm. Urinary excretion of ETBE and TBA less than 1% ETBE uptake.
[11]	3 men and 3 women aged 26–31 years and weighing 58–83 kg (mean 67.5 kg)	Inhalation of 4.5 ppm [19 mg/m^3] ETBE during 4 h; exposures separated by four weeks Inhalation of 40.6 ppm [172 mg/m^3] ETBE during 4 h; exposures separated by four weeks	ETBE C_{max} 1.3 ± 0.7 μM at end of exposure $T_{1/2}$: 1.1 ± 0.2 h TBA C_{max} 1.8 ± 0.2 μM at end of exposure $T_{1/2}$: 8.2 h ± 2.2 h ETBE C_{max} 12.1 ± 4.0 μM at end of exposure $T_{1/2}$: 1.1 ± 0.1 h; and 6.2 ± 3.3 h (two phase) TBA C_{max} 13.9 ± 2.2 μM $T_{1/2}$: 9.8 h ± 1.4 h	Average received doses of ETBE 121 μmol and 1092 μmol. Main urinary metabolites were 2-hydroxy-isobutyrate and 2-methyl-1,2-propane diol.

The studies of Dekant et al. [27, 38], Amberg et al. [39] and Prah et al. [29] are particularly valuable because they permit comparisons to be made of the inhalation and oral routes of exposure using approximately the same MTBE doses; however, the first time of blood sampling described in the former two publications was 1 h after dosing, which imposes certain limitations on the description of the early kinetics. Nevertheless, it is clear from the Dekant–Amberg study that maximum blood concentrations of both MTBE and TBA are higher following inhalation than oral exposure. Prah et al. [28] confirmed the higher maximum MTBE concentration in blood after inhalation compared with oral exposure, but the maximum concentrations of TBA were similar by the two exposure routes. No similar route comparison is possible for ETBE. After similar "low" exposures for 4 h to MTBE or ETBE (4 ppm and 4.5 ppm, respectively) maximum concentrations of the ethers in blood were similar, whereas "high" exposures for 4 h (40 ppm and 40.6 ppm, respectively) led to ETBE concentrations in blood almost two-fold higher than for MTBE, and the maximum concentration of TBA from MTBE was about 50% higher than TBA from ETBE. These results suggest that MTBE is more rapidly metabolised than ETBE after inhalation exposure. Data from the two independent studies of ETBE kinetics were very similar in terms of maximum concentrations of the ether and TBA in blood and the respective $T_{1/2}$ values [11, 35].

Blood concentration of TBA increases gradually during inhalation exposure, reaching maximum concentrations 2–4 h later than MTBE, but after oral exposure the delay is no more than about 30 min and high concentrations tend to persist for up to 7 h following either route. In the only human dermal absorption study of MTBE [28], the maximum blood concentration of TBA was not reached until 6 h after that of MTBE. Elimination of MTBE from blood following inhalation exposure was observed to be either biphasic [27, 38] or triphasic [26, 28], but the discrepancy is possibly an artefact of the sampling times used. The first $T_{1/2}$ is a few minutes after C_{max} is reached, the second is about 1–2 h and the third is several hours after C_{max}. In their study of ETBE, Nihlén et al. [35] described four phases of elimination, the first two being of a few minutes duration, whereas Amberg et al. [11] described a single ETBE elimination phase. Once again, this discrepancy is most probably due to methodological differences. Elimination from blood after acute oral exposure to MTBE was triphasic in both studies [27, 28, 38] with $T_{1/2}$ values of about 0.25–0.8 h, 1–2 h and 7–8 h. In the only study of dermal exposure, elimination of MTBE was also triphasic, with $T_{1/2}$ values similar to those found after acute oral exposure. These results demonstrate rapid reductions in blood concentrations of both MTBE and ETBE, but elimination of TBA from blood is slower, with $T_{1/2}$ values of about 4–10 h (from MTBE) and 6–14 h (from ETBE). Because of its high water solubility and blood:air partition of 462 [28], little TBA is excreted in exhaled air. Samples of urine collected from the volunteers before exposure

contained low concentrations of TBA (2.3 μmol), 2-methyl-1,2-propanediol (4.7 μmol) and 2-hydroxyisobutyrate (131 μmol) but not of ETBE [11, 27]; however, these concentrations increased significantly following exposure to ETBE. After subtraction of the background, the concentrations of ETBE, TBA, 2-methyl-1,2-propanediol and 2-hydroxyisobutyrate were about 0.3, 5.1, 13.6 and 130 μmol, respectively, after exposure to 4.5 ppm ETBE and 0.9, 22.6, 96.6 and 522.6 μmol, respectively, after exposure to 40.6 ppm ETBE. The $T_{1/2}$ values for the appearance in urine of TBA and 2-methyl-1,2-propanediol were about 10–12 h and the $T_{1/2}$ value for 2-hydroxyisobutyrate was 17–28 h [11, 27, 39].

Respiratory excretion as a percentage of the respiratory ETBE uptake ranged from 45 to 50%. In volunteers, the kinetics of ETBE and TBA were linear up to an exposure to ETBE of 50 ppm (212 mg/m^3).

Interspecies comparisons of the time-course of elimination of MTBE and ETBE and their quantified metabolites show that excretion of ETBE is more rapid in rats (reviewed below). Nonetheless, the earlier half-lives of elimination of both ETBE (phase 1 of Amberg et al. [11] or the combined phases 1–3 of Nilhén et al. [35] and MTBE were 1 to 2 h in human blood, although there is a tendency for a longer terminal half-life in human blood for ETBE (24–33 h) [35] than for MTBE (2–8 h) in different studies reviewed by McGregor [5], as predicted from their blood/air and olive oil/blood partition coefficients. TBA formed from ETBE also is rapidly cleared from human blood, with half-lives of 8 to 12 h [35], which are similar to those of TBA derived from MTBE. Comparison of the concentrations of recovered urinary metabolites after exposure to ETBE and MTBE suggests that the extents of metabolic transformation of these two compounds in man are similar and there are no significant sex differences [11]. They are also similar in rats. In view of the rapid exhalation of ETBE in man, and the rapid elimination of its metabolites in urine, it is unlikely that there would be accumulation of ETBE or its metabolites in human tissues. Finally, the major human metabolites of ETBE (and MTBE) are also formed endogenously, and the expected low exposures to ETBE from the environment are not likely to alter significantly the body burden of these compounds. Consequently, it can be predicted that the generation of toxic responses in people exposed under realistic conditions are unlikely.

2.2 Non-Human Metabolism and Kinetics

The metabolic pathways in rats of MTBE and ETBE to TBA and formaldehyde and acetaldehyde, respectively, with further oxidation of TBA to 2-methyl-1,2-propanediol and then 2-hydroxyisobutyrate is similar to the pathway described above for man [11, 13, 39]. It has also been suggested that acetone could be formed from TBA and 2-hydroyisobutyrate [13, 40]. Since diethyl

ether is a substrate for CYP2E1, it was surmised that this enzyme would also be the catalyst for MTBE metabolism and, indeed, an early study did show a partial contribution of this acetone/ethanol inducible isozyme. Brady et al. [41] found that microsomes from the livers of Sprague-Dawley rats treated with acetone or phenobarbital, to induce CYP2E1 and CYP2B1, respectively, metabolised MTBE more rapidly than microsomes from untreated rats. Savolainen et al. [42] found there was almost no effect on hepatic cytochrome enzyme concentrations and only a minor induction of kidney cytochrome enzymes following exposure of rats to up to 300 ppm MTBE vapours for 2–15 weeks. There was, however, a transient, dose-dependent increase in UDP-glucuronosyltransferase activity in liver, which was observed at two weeks but not at 15 weeks; the maximum increase was three-fold the control level at 300 ppm. Neither ETBE nor TBA had a significant effect on enzyme activity at a dose of 400 mg/kg body weight/day for four days by intraperitoneal (i.p.) injection [47]. Brady et al. [41] found that much more intense MTBE treatment (1 or 5 ml/kg bw i.p.) resulted in a 50-fold increase in liver microsomal pentoxyresorufin dealkylase activity 18 h later, although there was no change in *N*-nitrosodimethylamine demethylase activity, suggesting an increase in Cyp2b1, but not in Cyp2e1 activity. These trends in activity agreed with immunoblot analysis, which showed an increase in Cyp2b1 but no change in Cyp2e1 levels, while Turini et al. [43] demonstrated that extremely high doses of ETBE (2 ml/kg bw on two consecutive days) induced CYP2B1 and CYP2E1 in rat liver. The metabolism of 1 mM MTBE was inhibited 35% by monoclonal antibodies against Cyp2e1. However, Hong et al. [9, 44] demonstrated that *Cyp2e1*-null mice were unable to metabolise *N*-nitrosodimethylamine, whereas they continued to metabolise both MTBE and ETBE. Therefore, the balance of evidence is that Cyp2e1 plays a minor role, if any, in the oxidation of MTBE in rodents.

Comparison of the metabolic activity towards both MTBE and ETBE of microsomes prepared from nasal mucosae (separately from olfactory and respiratory epithelium), liver, lung, kidney and olfactory bulb of male Sprague-Dawley rats [45] showed that the highest activities were in the olfactory mucosa and these were almost 50-fold higher than that found in liver. Activity was inhibited 87% by 50 μM coumarin, suggesting an involvement of a cytochrome enzyme corresponding to the human CYP2A6. No detectable activity was found in microsomes prepared from lungs, kidneys or olfactory bulb; however, the analytical method used (head-space analysis for TBA) probably did not have the sensitivity required to demonstrate the virtual absence of activity. Also, although the specific activity may be very much higher in olfactory mucosa than in liver, the latter has a greater metabolic capacity, simply because of the size difference.

Rat liver microsomes metabolise TBA via oxidative demethylation to formaldehyde and acetone. It was presumed that hydroxyl radicals were involved in the reaction [40]. However, the K_m of 30 mM is very high and

this seems to preclude this pathway as being functionally relevant. The generation of formaldehyde in MTBE metabolism is a point of major toxicological interest, because of the reactivity of the compound and because it is mutagenic. Casanova and Heck [46] demonstrated that the metabolism of MTBE to formaldehyde in hepatocytes prepared from female CD-1 mice, male $B_6C_3F_1$ mice and male Fischer 344 rats approached saturation at concentrations below 0.33 mM. Also, formaldehyde, which is a naturally occurring endogenous substance, is metabolised extremely rapidly to formate, which is largely incorporated in the one-carbon pool. Circulating concentrations of formaldehyde in F344 rats exposed by inhalation to 14.2 ppm formaldehyde for 2 h, 2.25±0.07 μg/g blood, was not different from the concentrations of 2.24 ± 0.07 μg/g blood found in unexposed rats [15]. These authors also attempted to follow formaldehyde production in the metabolism of MTBE, evaluated by measuring the formation of DNA–protein cross-links and RNA–formaldehyde adducts. No increases were detected at concentrations of MTBE up to 6.75 mM. Induction of MTBE metabolism did not change the yield of either of these adducts, and no species or strain differences were seen.

The kinetics and disposition of [^{14}C]MTBE in rodents have been studied to varying extents after intravenous, intraperitoneal, oral, dermal and inhalation administration [25, 42, 47]. Design features of these experiments are listed in Table 2. The metabolism and kinetics of ETBE have been studied in rats and mice exposed by inhalation [11, 48–50], but not by other routes of exposure.

In order to permit comparison with other dose routes, Miller et al. [47] calculated that the doses achieved by inhalation of MTBE were 242 and 4709 mg/kg bw after single exposures to 400 and 8000 ppm and 220 mg/kg bw on day 15 of repeated exposure to 400 ppm, assuming 50% lung retention. At the end of the 6 h nose-only inhalation exposure periods, the quantities of radioactivity in the blood of rats increased from C_{max} values of 37 μg ETBE-equivalents/g at 500 ppm to 156 μg ETBE-equivalents/g at 2500 ppm, but again there was no further significant increase following exposure to 5000 ppm. In the blood of mice, the C_{max} values increased from 154 μg ETBE-equivalents/g at 500 ppm to 481 μg ETBE-equivalents/g at 1750 ppm, but there was no further significant increase following exposure to 5000 ppm [48, 49]. The ETBE study of Amberg et al. [11] used much lower exposure levels (4.5 and 40.6 ppm), which allowed comparisons with the human exposures these authors used, but are not relevant for the exposures in the rodent toxicity studies.

Only in the Miller et al. [47] experiments were possible sex differences in the kinetics and disposition of MTBE studied (in rats), but they found, with few exceptions, that they were insignificant. Plasma concentrations of MTBE in female rats were generally lower than in males, but the difference was statistically significant only after i.v. dosing. Significant sex differences in MTBE kinetics were also seen after a single 6 h inhalation of 400 ppm, but not after repeated inhalation exposures. Comparison of the AUCs for MTBE after i.v.

and oral administration indicated that MTBE is rapidly and completely absorbed from the gastrointestinal tract, while dermal absorption represented approximately 16 and 34% of the low and high doses, respectively. Following inhalation or dermal exposures, the plasma concentrations remained at about the same level until exposure was ended, when there were immediate reductions. Maximum plasma concentrations of MTBE were reached almost immediately ($\leq$ 15 min) after dosing by the i.v. or oral routes, after 2 h of dermal application and 6–7 h after the beginning of inhalation exposure. The C_{max} values for MTBE in blood were 14 000 and 493 000 μg/L after single 6 h inhalation exposures to 400 and 8000 ppm, respectively, but only 9000 μg/L after multiple exposures to 400 ppm. This lower value was associated with a significantly lower AUC (6700 μg/h/L) than after the single exposure to 400 ppm (84 300 μg/h/L), suggesting self-induction of MTBE metabolism. The initial $T_{1/2}$ values were all between 0.45 and 0.79 h, except for after dermal applications of 40 and 400 mg/kg bw, for which $T_{1/2}$ values were about 2 h. Second phase $T_{1/2}$ values for MTBE were about 92 h and 37 h for low and high doses, respectively. Plasma concentrations of TBA lagged behind those of MTBE, so that the C_{max} was reached after 1–2 h of i.v. or oral dosing, 4 h of dermal exposure and 6–7 h of the start of inhalation exposure. C_{max} values for TBA in blood were about 40 000 μg/L after both single and multiple exposures to 400 ppm and about 500 000 μg/L after exposure to 8000 ppm. The respective AUC values for multiple and single exposures to 400 ppm were 127 000 μg/h/L and 404 000 μg/h/L. Initial $T_{1/2}$ values for TBA were about 1 h after i.v. or oral dosing, about 2 h after dermal exposure, about 3.3 h after single inhalation exposures and about 1.8 h after repeated inhalation exposure.

Tissue distribution is likely to be extensive in rats, just as it is expected to be so in human tissues. Tissue/air partition coefficients measured in vitro on F344 rat tissues [51] show that the blood/air partition coefficient was 11.5 and the rat fat/air coefficient was 116. Thus, MTBE is moderately soluble in blood and most other tissues, but it is 7–10 times more soluble in fat tissue and its concentration in male rat kidney is about 6 times higher, due to specific binding [52]. This property is discussed below. All tissue concentrations of MTBE and TBA were directly inhalation exposure related up to 300 ppm at all sample times up to 15 weeks. The brain/blood ratios were consistently about 1, while the perirenal fat/blood ratios were about 10 [42]. Pharmacokinetic analysis of experimental data for MTBE in F344 rats suggested that the apparent volume of distribution roughly corresponded to the body weight, with the exception that the volume of distribution was higher after dermal exposure [47].

Pulmonary excretion accounted for means of 23%, 38% and 69% of the 50, 100 and 500 mg/kg bw i.p. doses of MTBE in mice. More than 90% of the MTBE excreted through the lungs was eliminated within 3 h [53]. At a dose comparable with the low i.p. dose in mice, 60% of a 40 mg/kg bw i.v. dose in rats was excreted in exhaled air, about 90% of that proportion appear-

ing within the first 3 h and consisting of about 97.4% unmetabolised MTBE, 1% TBA and 1.6% carbon dioxide [47]. Most of the radioactivity recovered after dermal exposure was in the dermal wash (77% and 70% of the 40 and 400 mg/kg bw doses, respectively). This left relatively little for disposition elsewhere and only about 8% and 19%, respectively, of the exposure, or about 53% of the received dose after either exposure, was recovered in exhaled air. Inhalation by rats of repeated 400 ppm, single 400 ppm and 8000 ppm MTBE exposures resulted in 17%, 21% and 54% of these respective doses appearing in exhaled air. Unchanged MTBE constituted 66%, 69% and 79% of the radioactivity excreted in exhaled air, while TBA constituted the remainder [47]. Most of the remaining radioactivity was in urine, while recovery from faeces was only about 2% of the i.v. injection and less than 1% of any of the other treatments. Radioactivity remaining in the carcass was about 0.4% of the i.v. dose and about 0.1% of the dermal treatment. Much higher proportions of the delivered radioactivity (means of 4–13%) were found in the carcasses after inhalation treatment, but most of this radioactivity was recovered from skin and most probably artefactual due to contamination by urine. In rats exposed by inhalation, 2-hydroxyisobutyrate accounted for about 70% of the total urinary excretion, 2-methlpropane-1,2-diol accounted for about 14% and two unidentified metabolites accounted for about 10% and 5% [47].

Thus, by all routes of exposure studied, exhaled air is the major route of excretion of unchanged MTBE and for a substantial amount of TBA. In view of the carcinogenicity test conducted by oral, gavage administration, it is unfortunate that disposition studies are not available following oral dosing.

Direct administration of TBA by i.v. injection to male and female F344 rats showed a saturation of elimination at 300 mg/kg bw [54]. After a dose of 37.5 mg/kg bw to males, clearance was about 54 ml/h and the elimination $T_{1/2}$ was 3.6 h, whereas after a dose of 300 mg/kg bw clearance was 25 ml/h and $T_{1/2}$ was 5 h. Nose-only exposures of male and female F344 rats and CD-1 mice to concentrations ranging from 500 to 5000 ppm ETBE (2120 to 21 200 mg/m^3) labelled on the *tertiary* carbon, i.e., $(CH_3)_3[^{14}C]COCH_2 \cdot CH_3$, for 6 h on a single occasion led to more than 90% of the radioactivity being eliminated within 48 h. The major elimination routes were via kidneys in urine and lungs in exhaled air. After exposure of rats to 500 ppm [^{14}C]-ETBE, most radioactivity was in urine, whereas after exposure to 5000 ppm, most radioactivity was found in the volatile organics fraction of exhaled air. This observation was reproducible in two laboratories [48, 50]. As exposure concentrations increased from 500 to 1750 ppm for rats, the proportion of label eliminated in urine decreased from 60 to 38% of the total, while the proportion eliminated in lung increased from 37 to 58% [48]. In mice, although there was a trend similar to that found in rats, the quantities of radioactivity in urine and exhaled air after exposure to 5000 ppm were approximately the same [49, 50]. As exposure concentrations increased from 500 to 1750 ppm for mice, the proportion of label eliminated in urine decreased

from 74 to 46% of the total, while the proportion eliminated in lung increased from 10 to 42% [49]. In the experiments of Amberg et al. [11], the exposure concentrations were chosen as direct comparisons with those used in their human studies, i.e., 4.5 and 40.6 ppm; therefore, direct comparison with the results from the other rodent studies, in which far higher exposure concentrations were used, is not useful. Effects of repeated exposure to ETBE on ETBE metabolism were studied in rats whole-body exposed to unlabelled ETBE for 13 days and then nose-only exposed to [^{14}C]-ETBE for 6 h on a single occasion. At a concentration of 5000 ppm, the major route of elimination shifted from exhalation (the major route at this concentration after a single exposure) to the urine [50], suggesting that there had been induction of metabolism.

After the end of single exposures, the quantity of ETBE exhaled by rats decreased rapidly in the 5000 ppm exposure group from about 300 to 325 μmol to about 50 to 70 μmol within 5 h, while the level of TBA exhaled in the same group increased to a maximum of about 20 μmol in females and 55 μmol in males at 3 h and then remained relatively stable up to 16 h. Repeated exposure of rats before the single exposure to [^{14}C]-ETBE led to a more rapid fall in ETBE exhalation and the exhalation of a greater quantity of TBA, which was particularly noticeable in males. In mice, there was a rapid decrease in ETBE exhaled following all exposure concentrations. TBA was present in exhaled air at the earliest sample time (1 h after exposure), and its concentration in air decreased more slowly than that of ETBE [50].

In both rats and mice, urinary levels of TBA were greater than ETBE, even after single exposures, but prior exposure of rats (particularly males) to ETBE for 13 days led to an increase in the urinary excretion of TBA similar to the observations made on exhaled air. Both 2-methyl-1,2-propanediol and 2-hydroxyisobutyric acid were identified in the urine of male and female rats and mice. The faeces and exhaled $^{14}CO_2$ are minor elimination pathways for radioactivity derived from [^{14}C]-ETBE in rats and mice [50]. These data are consistent with saturation of metabolism and urinary secretion following single exposures to high concentrations and adaptation upon multiple exposures. The manner of such postulated adaptation is unknown; the only data available on self-induction of ETBE metabolism is after four days intraperitoneal dosing of rats with 400 mg/kg body weight that was ineffective and two days oral dosing of rats with 2 ml/kg body weight (1490 mg/kg body weight) that resulted in a two-fold increase in *p*-nitrophenol hydroxylase activity and a six-fold increase in pentoxyresorufin *O*-depentylase activity [43]. The dose in the latter of these experiments was close to the daily dose of about 1590 mg/kg body weight used in the 13-day inhalation experiment of Borghoff and Asgharian [50][1] and provides evidence that the shift in elimi-

[1] 21.2 mg/L × 0.2 L/min × 0.26* × 360/0.25 kg** = 1587 mg/kg bw where * = pulmonary retention factor for ETBE; ** = actual bw of male rats in the experiment.

nation route observed on multiple exposure to ETBE was due at least in part to self-induction of ETBE metabolism at a high exposure concentration.

F344 NH rats exposed at the much lower concentrations of 4.5 ppm and 40.6 ppm for 4 h on a single occasion [11] received doses[2] of 2.0 μmol and 17.8 μmol, respectively. The body weights of these rats ranged from 210 to 240 g for males and 190 to 220 g for females. The blood ETBE C_{max} values at these exposure levels were 1.0±0.7 μM and 5.3±1.2 μM, respectively, and the apparent half-life of elimination of the higher ETBE dose from blood was 0.8±0.2 h, but the half-life of TBA was not determined and it is not clear whether the ETBE half-life in rat blood was an initial or terminal value.

TBA concentrations in blood at the end of the exposure period were 5.7±0.8 μM and 21.7±4.9 μM, respectively. TBA was also present in the blood of non-exposed rats at a concentration of 1.1±0.7 μM. The total urinary metabolites corrected for background occurrence constituted 50% (1.1±0.7 μM) and 53% (10.9±3.1 μM) of the received low and high doses, respectively. All ETBE metabolites measured (TBA, 2-methyl-1,2-propanediol and 2-hydroxybutyrate) were quantifiable even in unexposed rats (Table 3). The major urinary metabolite at both exposure levels was 2-hydroxyisobutyrate. In contrast, the quantity of TBA hardly differed from background and 2-methyl-1,2-propanediol was excreted in small quantities in urine.

2.3 Health Effects

2.3.1 Human Health Effects

There has been no field or epidemiological study of populations exposed to ETBE. This is in contrast to MTBE, which has seen a longer and more wide-spread usage. The health effects of MTBE exposure in small human populations and controlled inhalation chamber experiments with volunteers have been reviewed [5]. While there might be justification for an assumption that the outcome of studies on ETBE would not be very different, caution is always necessary before acceptance of this assumption: a minimum database for comparison is a basic requirement.

Oxygenated fuel containing 15% by volume MTBE was introduced in Fairbanks, Alaska, USA in mid-October, 1992 as a measure to reduce the high carbon monoxide production in that area (TWA of 10.3 mg CO/m^3 over an 8 h period). By late November 1992 more than 150 people had reported symptoms, such as headache, nausea and eye, nose and throat irritation, to a local hotline and the sale of oxygenated fuel was discontinued by mid-December

[2] Received dose is a calculation based on an alveolar ventilation rate of 0.169 l/min and a retention of 0.26 in rats [25].

1992 on instructions from the state authorities. Subsequent to these reports a number of preliminary, hypothesis-generating studies were conducted and reported as conference proceedings. An early publication of the phenomenon also recorded air and blood concentrations of MTBE during and after the use of oxygenated fuels, in addition to the symptoms described [34].

It was recognised by the authors of these early studies (reviewed in 5) that the observations they recorded would have to be followed by more rigorously designed studies. This requirement was all the more necessary because the effects included in a list of seven "key" symptoms apparently characteristic of MTBE exposure tended to be non-specific descriptions of malaise. These were: headache, eye irritation, burning sensation in the nose and throat, cough, nausea or vomiting, dizziness and disorientation.

However, the most searching study conducted so far [55], which was on people who self-reported as sensitive (SRS) to MTBE, did not confirm the existence of the particular key set of symptoms. This study also found no effect of MTBE on neurobehavioural or psychophysiological performance, and while there might have been an increase in non-specific symptoms recorded by SRS subjects exposed to gasoline containing 15% MTBE, there was no increase in response to gasoline containing 11% MTBE. The lower volatility of ETBE would be predicted to produce study results of even less clarity, assuming that the toxicities of the two chemicals are similar.

2.3.2 Controlled Human Studies

Three well-conducted, controlled exposure studies have investigated the effect of pure MTBE on symptoms and objective measures of irritation and performance amongst healthy subjects. In addition, there has been one similar study of effects of MTBE in gasoline on subjects that have reported themselves as being particularly sensitive to MTBE. There has also been one study of ETBE.

Using a double-blind cross-over design, Prah et al. [56] exposed 19 men and 18 women to 1.39 ppm [5.0 mg/m^3] MTBE for 1 h on several occasions, each separated by at least one week. There was no significant effect upon neurobehavioural performance, headache, nasal irritation or odour intensity, and no evidence of ocular inflammation. The numbers of inflammation mediators and neutrophils were not significantly different in the nasal lavage fluid of control and treated volunteers. Similar results were obtained by Cain et al. [30] among 22 men and 21 women exposed to clean air, 1.7 ppm [6 mg/m^3] MTBE or 7.1 ppm mixture of 17 volatile organic compounds for 1 h. There were no effects upon neurobehavioural performance and no increases were observed in symptoms such as irritation, headache and mental fatigue, eye irritation (by tear-film breakup, eye redness) and nasal inflammation (by measurement of polymorphonuclear neutrophilic leukocytes). Even at the more elevated expo-

sures used by Nihlén et al. [57], only minimal acute effects or no effects were observed after inhalation by 10 men of 5, 25 or 50 ppm [18, 90 and 180 mg/m^3] MTBE during 2 h of light (50 W) physical exercise on three occasions, with an interval of at least two weeks between exposures. The only rating noted was that of solvent smell, which increased greatly as the subject entered the chamber and with exposure concentration. Ocular changes (redness and tear film break-up time, conjunctival epithelial damage and blinking frequency) and nasal measurements such as peak expiratory flow, acoustic rhinometry to assess nasal swelling (5 and 25 ppm) and levels of inflammatory markers in nasal lavages (50 ppm) were evaluated. No effect on any of the eye measurements was associated with exposure to MTBE. Although some nasal changes where reported and there were significant increases in nasal airway resistance after exposure, they were not related to the concentration.

The most recent chamber study of MTBE [55, 58] compared the symptoms experienced by 12 subjects who had reported themselves as sensitive to MTBE (self-reported sensitive, SRS) and 19 subjects not in this category in a double blind, repeated measurement controlled exposure study. The two groups of subjects were exposed for 15 min on each occasion to clean air, gasoline, gasoline + 11% MTBE and gasoline + 15% MTBE. What sets this study apart from earlier controlled exposure studies is that MTBE exposure was in gasoline matrix, a situation that might modify the responses of the subjects and is more typical of public exposure. Neurobehaviour performance during a driving simulation task and psychophysiological responses (including heart and respiration rate) were applied before, during and immediately after exposure. Questionnaires to record symptoms (including, but not confined to the "key" symptoms supposedly associated with MTBE) were also completed before and after exposure. Mean concentrations of MTBE in blood at the end of the exposure period ranged from 0.13 μg/L after clean air and 0.22 μg/L after gasoline alone to 1.23 μg/L after gasoline + 11% MTBE and 1.75 μg/L after gasoline + 15% MTBE. The corresponding mean concentrations of TBA were 0.86, 1.04, 2.34 and 2.63 μg/L. There were no significant differences in responses to any of the exposures in neurobehavioural or psychophysiological tests by either group. Responses to the symptom questionnaires are summarised as follows:

- SRSs symptom scores were significantly higher than those of the controls before any of the exposures were experienced;
- All SRSs and controls symptom scores increased following all exposures (including clean air), indicating that participation in the procedures was, by itself, affecting the responses of the participants;
- The controls' symptom scores were not differentially increased following exposure to any of the gasoline atmospheres, in comparison with clean air;
- Relative to controls, the SRSs' symptom scores were significantly higher after exposure to gasoline + 15% MTBE in comparison with exposure to

clean air or gasoline + 11% MTBE. However, there was no significant difference in symptom scores between SRSs exposed to gasoline alone and after exposure to gasoline + 15% MTBE.

Among both SRS and control subjects, the existence of a so-called MTBE-specific list of key symptoms suggested by the epidemiological literature was not confirmed.

Odour did not seem to be a factor that might have biased the results and so the possibility that increased odour perception of MTBE in gasoline (a suggestion made, with some justification as described earlier, in relation to the original Alaskan observations) is not a valid criticism of this study. In conclusion, the study did not support a simple dose-response relationship for MTBE exposure, although there may be some evidence for a threshold amongst SRSs.

The authors note that the number of SRS subjects who were healthy and willing to participate in an exposure study was small relative to the numbers cited by community groups as adversely affected by MTBE. Thus, there was likely to have been a self-selection bias that the authors suggest would have been in the direction of deterring even more sensitive individuals from participating. They also state that time constraints prevented the use of additional neurobehavioural tests or other objective markers (e.g., tear film) that may have been more sensitive. However, tear film break-up tests had been incorporated in the chamber studies of [30, 56, 57] in which no effect of exposure to MTBE was observed on this parameter.

The human toxicity database for ETBE currently consists of a single, small but well-conducted inhalation chamber study [59] that is described below. Eight healthy men were exposed by inhalation to 0, 5, 25 or 50 ppm (0, 21.2, 106 and 212 mg/m^3) ETBE during 2 h of light (50 W) physical exercise on four occasions, with an interval of at least two weeks between exposures. Measurements were performed the day before exposure, during the exposure day and on the mornings of the two following days. The acute effects of these exposures were studied by means of subjective ratings and objective measurements of ocular and mucous membrane irritation and changes in lung function. The rating of solvent smell increased greatly as the subjects entered the chamber and with exposure concentration, but decreased with time spent in the chamber. Significantly increased ratings of discomfort in the throat and airways were reported during and after exposure to 50 ppm ETBE. While the average highest questionnaire responses related to discomfort to the eyes and nose, difficulty in breathing, fatigue, nausea, dizziness and intoxication were recorded at the 50 ppm level, no concentration-effect correlation was seen and the ratings were not significantly different from the 0 ppm exposure. Furthermore, these ratings corresponded to something between "not at all" and "hardly at all" (investigators' categories). Ocular changes (redness and tear film break-up time, conjunctival epithelial damage and blinking frequency) showed no effect that was associated with exposure

to ETBE. Nasal measurements (peak expiratory flow, acoustic rhinometry to assess nasal volume and nasal lavage examination for cell numbers and levels of inflammatory markers) were generally unchanged by exposure. The only exception was a decrease in nasal volume at all exposure levels, compared to the pre-exposure measurement, but there was no correlation with ETBE exposure concentrations. Slightly impaired pulmonary function was observed at 25 ppm and 50 ppm, at which concentrations forced vital capacity and vital capacity were significantly reduced compared with pre-exposure values. The reductions were about 3.2 to 4.4%. While this study had the benefit of high exposure levels of ETBE, the number of subjects was small. Just as some people have reported themselves as particularly sensitive to MTBE it would not be surprising if a sub-population with particular sensitivity to ETBE should, eventually, also be revealed. In the case of MTBE, the existence of this sub-population was identified only after large numbers of the public had been exposed in particular circumstances. It is unlikely that the discovery would have been made on the basis of volunteer studies. In conclusion, these reassuring ETBE data may be accepted, but with some caution.

2.3.3
Non-Human Studies on Health Effects

2.3.3.1
Acute and General Effects

Both MTBE and ETBE have low acute toxicity in laboratory animals with single dose oral and dermal LD50 values well in excess of the limit concentration of 2000 mg/kg bw and 4 h-inhalation LC50 values in excess of 6000 mg/m^3 [60–65]. In none of these studies was there any death and no treatment related pathology was recorded.

Although MTBE is not classified as an eye irritant [3], there have been small effects noted in several of the studies conducted [66–69]. Among three studies for eye irritation in rabbits conducted to OECD protocols, the balance of the evidence is that ETBE is not an eye irritant. In one study, some conjunctival redness and chemosis was recorded in all rabbits soon after application of the undiluted material [70], but in two other studies in rabbits, conjunctival responses were minimal and no iridial or corneal effects were observed [71, 72].

The skin irritancy of MTBE has been demonstrated in most studies [66, 67, 73–75] whereas ETBE is not a skin irritant according to studies conducted to OECD guidelines [76–78]. No sensitising potential was demonstrated for MTBE [66, 67, 79] or ETBE in a maximisation test in guinea pigs [80].

The most typical clinical signs induced by ETBE in rodents in repeated inhalation exposure experiments were sedation and ataxia, followed by rapid recovery after removal to air. No exposure related deaths occurred in groups

of F344 rats exposed by inhalation for 6 h/day, 5 days/week to ETBE concentrations up to either 4000 ppm (16 960 mg/m^3) for 4 weeks [81] or 5000 ppm (21 200 mg/m^3) for 13 weeks [82][3]. Although there were several deaths among CD-1 mice exposed for 13 weeks [82] (Medinsky et al. 1999), they were not treatment related.

2.3.3.2 Neurotoxicity

Ataxia and hypoactivity have been common observations in several rodent studies using MTBE exposure concentrations of 8000 or 3000 ppm. Indications of dose-related CNS depression were noted in Sprague-Dawley rats exposed to 250, 500 or 1000 ppm MTBE, 6 h/day, 5 days/week for 13 weeks [85] and profound anaesthesia was observed in Sprague-Dawley rats treated orally with MTBE doses of 1200 mg/kg bw [86]. The behaviour of F344 rats exposed to 0, 800, 4000 or 8000 ppm [0, 2900, 14 000 or 29 000 mg/m^3] MTBE for 6 h was evaluated 1, 6 and 24 h later using a functional observational battery (FOB), measures of motor activity and a neuropathological evaluation. At 1 h after exposure, rats exposed to 8000 ppm and, to a lesser extent 4000 ppm, showed a variety of sensorimotor changes indicative of CNS depression. The most frequent findings were ataxia, duck-walk, increased lachrymation, laboured breathing, decreased muscle tone, lowered body temperature and decreased hind-leg grip strength. No changes were observed 6 or 24 h after exposure. A 13-week study at the same exposure levels demonstrated that these effects were neither persistent nor cumulative. The body and brain weights of rats at 8000 ppm were decreased, but no histological changes were seen in the brain or peripheral nervous tissue. No effects were observed at 800 ppm in either study [87]. No effect on brain acetylcholinesterase activity was observed in rats exposed to 300 ppm MTBE for 15 weeks [42].

Transient sedation and mild-to-moderate ataxia, which is a sign of narcosis, have also been observed in F344 rats and CD-1 mice during or shortly after inhalation exposure to ETBE concentrations of 4000 or 5000 ppm in 4 and 13 week studies [81, 82, 88]. These effects were not noted during oral exposures of F344 and Sprague-Dawley rats used in reproduction studies [89–91], although transient, excessive salivation was observed at daily doses of 250 mg/kg bw and greater. Dorman et al. [88] (1997) evaluated the neurotoxicity of ETBE in F344 rats exposed up to 5000 ppm [21 200 mg/m^3] ETBE for 6 h/day on 65 occasions over a 14 week period. No significant differences in motor activity were observed in any group, although ataxia was a common finding immediately following exposure in male rats of the

[3] This reference is based on data reported in its primary form in the following unpublished, but GLP-compliant reports [83, 84]. To avoid the impression that there are more studies than is the case, no reference to these reports will be made unless they contain information considered pertinent that is not to be found in Medinsky et al. [82].

5000-ppm group. No effects of exposure were observed in the FOB at least 18 h after the rats had been last exposed, and there were no clinical signs of toxicity. No gross or microscopic abnormalities were found in the central, peripheral or autonomic nervous systems of rats from the 5000-ppm group, and there were no exposure-related eye abnormalities. In addition to these specific neurotoxicity studies in adult rats, reflex development was examined in the progeny of F0 and F1 generation Sprague-Dawley rats treated orally by gavage with ETBE doses up to 1000 mg/kg bw/day for 10 weeks. No effects were found [91]. Both compounds induced transient ataxia and sedation, effects that are common amongst ethers, including diethyl ether [92].

2.4 Non-Neoplastic Pathology

2.4.1 Kidney Pathology

A common finding in rats in repeated exposure studies with MTBE has been various manifestations of kidney pathology. In a two-year study, exposure-related increase in the incidence and severity of renal changes associated with chronic progressive nephropathy (CPN) was seen at 3000 and 8000 ppm MTBE in male and to a lesser extent in female F344 rats [93, 94]. In this experiment, CPN was the major cause of death in male rats. These renal lesions were associated in both sexes with secondary lesions that included fibrous osteodystrophy, hyperplasia of the parathyroids and mineralization in numerous tissues [94]. In contrast, it was reported that there was no treatment-related non-neoplastic pathology (in any organ) in Sprague-Dawley rats treated for two years with MTBE delivered orally by gavage at doses up to 1000 mg/kg bw and then observed until their natural death [95–97]. Given that CPN is a common occurrence in aging rats and that the condition is often aggravated by treatment, this is a surprising result. In a two year drinking water study of TBA in F344 rats the incidence of nephropathy was 100% or very close at all doses levels, including controls and other manifestations of renal pathology were also observed [98, 99]. The longest study so far of ETBE involved exposure of rats for up to 13 weeks to 0, 500, 1750 or 5000 ppm (0, 2120, 7420 or 21 200 mg/m^3) ETBE, which resulted in increased kidney weights in males of the higher two dose groups by 10% and 19%, respectively [82]. In this same study, nephropathy characterised by regenerative foci occurred in all groups of male rats with terminal incidences of 4/11, 10/11, 11/11 and 11/11, respectively. There were no effects on kidney weights and no treatment-related effects on renal histopathology in Sprague-Dawley rats exposed to 4000 ppm (16 960 mg/m^3) ETBE 6 h/day, 5 days/week for 4 weeks [81], whereas renal effects were found in another short-term inhalation study in which hyaline droplets were observed after one week [82].

No reason for this discrepancy has been proposed. The only indications of kidney effects after oral administration of ETBE comes from a reproduction study in which F_0 and F_1 generation Sprague-Dawley rats were treated with ETBE by gavage at doses of 500 and 1000 mg/kg bw/day for 10 weeks [91]. In the two dose groups and both generations of this study, male rats developed significant increases in absolute and relative (to body weight) kidney weights. No microscopic changes were reported in the lower dose group, but at 1000 mg/kg bw/day, acidophilic droplets of slight to moderate severity were noted in the renal tissue of most males of the F_1 generation. There was no report of similar lesions in females.

Hyaline or protein droplet accumulation was observed in the proximal convoluted tubules of male F344 rats exposed by inhalation to MTBE concentrations of 4000 and 8000 ppm for three months [100] and 1500 and 3000 ppm for 10 days [101]. The histological changes were slightly more severe at higher doses, even in the short-term study (Prescott-Mathews et al. 1997) and, in addition to protein droplet accumulation, were characterised in male rats by epithelial cell necrosis, karyomegaly within the renal tubules and, at the highest dose, by exfoliation of renal tubule epithelial cells into the lumen. These lesions were not found in females of any group or male control rats. Similar observations were made in male Sprague-Dawley rats dosed orally by gavage with 1428 mg/kg bw MTBE for 2 weeks [86], 440 mg/kg bw for one month [102] and 250 mg/kg bw for 10 days [103] as well as in male F344 rats exposed to TBA in their drinking water at concentrations of 10 and 20 mg/ml for three months and 5 mg/ml for 15 and 24 months. Angular, crystalline structures were associated with the hyaline droplets within the renal tubule epithelium and tubule lumina. Even at 15 months, renal papilla mineralization was observed in all males of the 5 mg/ml group. At two years, foci of linear mineralisation in the renal medulla were significantly increased in males of the highest TBA exposure group. The incidences in the 0, 1.25, 2.5 and 5.0 mg/ml groups were 0/50, 5/50, 24/50 and 46/50, respectively [98]. This lesion has been specifically and consistently reported as a long-term consequence of hyaline droplet nephropathy [104]. As was found in MTBE experiments, hyaline droplet accumulation was not observed in female rats dosed with TBA. Decreases have been observed in hyaline droplet accumulation at 1750 ppm [98, 105]. Assuming that the droplets were due to α_{2u}-globulin, possible explanations for this observation include an oestrogen-like suppression of hepatic α_{2u}-globulin synthesis [106] or a non-specific hepatotoxicity at a high dose.

Immunohistochemical analysis by Swenberg and Dietrich [107] of the kidney slides from the subsequently published Lington et al. [100] MTBE study demonstrated the presence of α_{2u}-globulin in the protein droplets found in renal tubules, but there was no exposure-response relationship for either α_{2u}-globulin or the presence of α_{2u}-globulin-positive proteinaceous casts at the junction of the proximal tubules and the thin limb of the Henlé loop. Similar

analysis of kidney slides in the study of MTBE by Prescott-Matthews et al. [101] and TBA by Borghoff et al. [105] also revealed some increases of α_{2u}-globulin that did not appear to be linearly exposure-related; however, gel and anion-exchange chromatography of the kidney cytosol in both studies failed to demonstrate accumulation of any protein other than α_{2u}-globulin. In addition, the concentration of α_{2u}-globulin in homogenised kidney cytosol, measured using an ELISA with mouse monoclonal antibody, was significantly increased by about 60% in male rats exposed to 1750 ppm TBA, whereas the concentrations in male rats of the lower exposure concentration groups were not different from the controls [105]. The concentrations in female rats were very low in all groups. While this result correlates with the non-specific protein staining, it does not correlate well with the α_{2u}-globulin immunohistochemical staining result. It is conceivable, however, that the cytosolic measurement of α_{2u}-globulin by ELISA is a more quantitative and reproducible technique than immunohistochemical staining. Oral administration of [^{14}C]MTBE at 750 mg/kg bw for four consecutive days to male and female F344 rats induced a mild increase in renal α_{2u}-globulin concentration in male rats, but the total radioactivity recovered in kidney samples was similar in male and female rats. A slightly greater percentage of MTBE or MTBE metabolite-derived radioactivity co-eluted with the total protein fraction from the treated male rat kidney cytosol compared to the female [108]. Supportive evidence for the binding of MTBE to a male rat-specific protein that is probably α_{2u}-globulin comes from in vitro studies with kidney homogenates demonstrating the much greater, saturable, partitioning of MTBE to male rat compared with female rat preparations that can be greatly reduced by heat denaturation and the competitive inhibition of binding by *d*-limonene [109].

Some of the analytical methods used (gel filtration, equilibrium dialysis and anion-exchange chromatography) in the MTBE studies of Poet and Borghoff [109] and Prescott-Matthews et al. [108] failed to demonstrate that the association of radioactivity was with α_{2u}-globulin or any other protein. In both cases, the authors suggested that the supposed anomalous results were due to a combination of the weak binding affinity of MTBE with α_{2u}-globulin and its high vapour pressure resulting in losses during tissue homogenisation and subsequent processing. This explanation seems likely because the in vivo interaction of the less volatile TBA with α_{2u}-globulin has been demonstrated in experiments in which male and female F344 rats were given a single gavage ^{14}C-TBA dose of 500 mg/kg bw [110]. At 12 h after dosing, the concentrations of TBA-equivalents in renal cytosol were higher in males than in females and both gel filtration and ion-exchange chromatography showed that the radioactivity in male rat renal cytosol co-eluted with α_{2u}-globulin. Furthermore, the radioactivity in renal cytosol of rats dosed with either labelled MTBE [108] or labelled TBA [110] could be displaced from the low molecular weight protein fraction of male rat kidney by *d*-limonene oxide, which has a particularly high affinity to α_{2u}-globulin. There was no effect on female rat samples.

Cell proliferation was measured as the osmotic pump-delivered bromodeoxyuridine (BrdU) labelling index (LI) in experiments with both MTBE and TBA. LI was increased in the renal cortex of all MTBE treated male groups (lowest dose 400 ppm) and in the outer part of the medulla in the two highest male exposure groups of F344 rats exposed to MTBE for 10 days [101]. After exposure of F344 rats to 3000 ppm MTBE for either 5 days or 4 weeks, ELISA-analysis of kidney cytosol showed clear positive correlations of both MTBE concentration and labelling index with α_{2u}-globulin concentration. No such effect was observed in female rats at any time or dose and there was no increased DNA synthesis in male rats after 4 weeks exposure followed by a 16-day recovery period [94]. Exposure to TBA in the Borghoff et al. [105] study was very clearly dose related to statistically significant increases in LI in epithelial cells of the renal cortex of male rats at all doses, but there was no response in female rats. The results, however, also show an apparent dislocation of cell proliferation from α_{2u}-globulin accumulation, since proliferation occurred at doses lower than those at which protein accumulation was recognised. Therefore, while there is no doubt that hyperplasia is specific to the male sex amongst rats, and this does not occur in mice, the role of α_{2u}-globulin accumulation at low TBA doses in this process remains to be demonstrated. It may be that the cell proliferation observed at the low doses would not have been sustained, if exposures had continued beyond 10 days, in contrast to the proliferation observed at higher doses when protein accumulation occurred. The discrepancy could also reflect a difference in sensitivities of the methods employed and/or the time course of the different responses, the one (DNA synthesis) involving a multi-day infusion of label and the other (α_{2u}-globulin detection) capturing a single early time-point in a chronic process.

After two years exposure, examination of step-sectioned kidneys showed that the increased LI in the short-term experiments has progressed to a persistent renal tubule hyperplasia. In the two-year drinking water study of TBA, hyperplasia was observed in 14/50, 20/50, 17/50 and 25/50 ($p < 0.01$) male rats of the 0, 1.25, 2.5 and 5.0 mg/ml drinking water dose groups, respectively. In contrast, in females receiving up to 10.0 mg/ml TBA, renal tubule hyperplasia developed in a single rat in the highest dose group, probably representing an incidental finding [98, 99].

The results obtained with TBA are remarkably similar to those obtained with MTBE. The similarities suggest very reasonably that the α_{2u}-globulin nephropathy of MTBE in male rats is due to TBA, although the similarity could simply be what might be expected of two independently acting, weakly active compounds.

Other non-neoplastic effects observed in the urinary system are inflammation and hyperplasia of the transitional epithelium of the kidney (rats) or bladder (rats and mice) following treatment with TBA [98]. These effects were observed in 13-week studies at 20 and 40 mg/ml in both sexes of both species. In the two-year studies with TBA, transitional cell hyperplasia was increased

in the kidney of male rats of the 2.5 mg/ml dose and clearly so in female rats of the 10 mg/ml dose level, while in mice the increases were observed only in the urinary bladder at 20 mg/ml in both sexes. There was no indication of progression of severity or emerging neoplasia from these tissues in either species in the two-year studies.

In this same study, nephropathy characterised by regenerative foci occurred in all groups of male rats with terminal incidences of 4/11, 10/11, 11/11 and 11/11, respectively. Regenerative foci were not observed in any group after 1 week of exposure, but by 4 weeks, the mean numbers of regenerative foci/two kidney sections/rat were 0, 0, 2 and 4, which by week 13 had increased to 2, 11, 17 and 34. Mallory-Heidenhain staining demonstrated dose-related increases in severity of protein droplet accumulation in the renal proximal tubules at all observation times (weeks 1, 4 and 13). These protein droplets were also immunoreactive for α_{2u}-globulin, as demonstrated using the method of Prescott-Matthews et al. [101]. This finding suggests that an interaction of ETBE or TBA with α_{2u}-globulin may be one mechanistic basis for the nephropathy. DNA synthesis (infusion for 3.5 days with BUdR from osmotic pumps) in the renal proximal tubules was significantly increased in male rats of the 5000-ppm group after one week, which persisted through weeks 4 and 13. Increases in the two lower-dose groups occurred only in week 13.

In female F344 rats, relative kidney weights were significantly increased after 10 days of inhalation exposure to 1750 ppm TBA [105] and, in the 13 week study of ETBE [82], small (2%) but significant increases in kidney weight also occurred in female rats. There was no microscopic evidence of treatment-related increases in nephropathy and the use of the Mallory-Heidenhain stain did not reveal any renal proximal tubule protein droplet accumulation in any of the ETBE-treated groups. In this experiment, DNA synthesis was significantly increased in female rat kidney, but the pattern of dose and time relationships with response were quite different from those observed in male rats. Significant increases were observed in all dose groups after the first week, but this response was maintained in week 4 only in the 5000-ppm group and had completely disappeared by week 13. Thus, a sustained response in cell proliferation as occurred in male rats of the 5000-ppm ETBE group did not occur in female rats of any group.

2.4.2 Testis Pathology

In Sprague-Dawley rats dosed by gavage with MTBE for 28 days, relative testicular weight increased significantly only at 1500 mg/kg bw [103]. No testicular pathology was reported in a two-year exposure in F344 rats treated via drinking water with TBA [98] and no significant testicular changes related to treatment have been reported in CD-1 or $B_6C_3F_1$ mice exposed to MTBE or TBA, respectively [93, 98]. However, a dose-related increase in interstitial

cell hyperplasia was noted in a two-year inhalation study with MTBE in F344 rats [93]. The incidences in the 0, 400, 3000 and 8000 ppm groups, respectively, were 16/37, 20/44, 20/44 and 36/41. A weaker dose relationship was also seen in the incidences of seminiferous tubule atrophy and mineralisation in this study. Although no similar effects were originally reported in the study in Sprague-Dawley rats treated by gavage with MTBE [95, 96] in a later evaluation [97] it was reported that interstitial cell (Leydig cell) hyperplasias (focal and diffuse combined) occurred in 4/60, 8/60 and 9/60 rats of the 0, 250 and 1000 mg/kg bw groups, respectively.

Increases in the percentages of seminiferous tubules with spermatocyte degeneration were observed in F344 rats in a 13-week inhalation study at ETBE exposure concentrations of 1750 and 5000 ppm (7420 and 21 200 mg/m^3) [82]. These exposures would have provided received doses of about 555 and 1590 mg/kg bw per day[4] for 5 days per week. Using the seminiferous tubules staging scheme proposed by Hess [111], most tubules with spermatids that had degenerated during development were in stages IX to XIII. Seminiferous tubules in stages I to VIII from rats exposed to 1750 ppm or 5000 ppm ETBE had fewer spermatocytes than the controls. No effect was found at 500 ppm. Clearly, such an effect could seriously compromise studies of effects on reproductive outcome. Consequently, a toxicity test was undertaken prior to a multi-generation reproduction study of ETBE. In this toxicity test, both F344 and Sprague-Dawley rats were used. The detailed examination of the testes from F344 rats treated orally by gavage with ETBE for 12 weeks revealed minimal degeneration of the seminiferous tubules in 7/12 controls and 6/12 rats treated with 1000 mg/kg body weight/day and minimal vacuolation of the seminiferous tubules in one F344 rat of each of these groups. In the Sprague-Dawley rats exposed in the same way, there was minimal desquamation of spermatocytes and minimal degeneration of seminiferous tubules in a single control rat [89]. Thus, the earlier report of effects of a higher dose of ETBE on the testes was not supported by these results with two strains of rats. Furthermore, non-neoplastic testicular changes were not reported in either the F0 or F1 generations of the 10-week premating segments of a reproduction study with Sprague-Dawley rats, in which there was particularly careful histological examination of the testes [91]. Furthermore, no testicular toxicity (or neoplasia) has been reported following TBA exposures for up to two years [98, 99].

2.4.3 Liver Pathology

Liver weight increases have been observed in many experiments involving exposure of rats and mice to MTBE. In F344 rats that received MTBE

[4] e.g., 21.2 mg/L × 0.2 L/min × 0.26* × 360/0.25 kg** = 1587 mg/kg bw where * = pulmonary retention factor for ETBE; ** = mean body weight of male rats in the experiment.

vapour concentrations of 0, 800, 4000 and 8000 ppm 6 h/day 5 days/week for 13 weeks, liver weights were significantly increased at all doses in males (8%, 20% and 39%) and in the mid and high doses in females (13% and 15%) [87, 100]. Liver weights were also significantly increased in mid- and high-dose female F344 rats similarly exposed, but in a two-year study, by 20% and 42%, respectively. Because of the high mortality associated with CPN in male rats, any changes in liver weights of males could not be assessed. Oral dosing of Sprague-Dawley rats with MTBE led to liver weight or relative (to body weight) liver weight increases at doses from 200 mg/kg bw in 13 week experiments [86, 112] and 1000 mg/kg bw in a 4 week study [103], but de Peyster et al. [113] found no such increases in male Sprague-Dawley rats dosed with MTBE by gavage up to 800 mg/kg bw for 4 weeks. Male F344 rats that received TBA in their drinking water up to 20 mg/ml for 13 weeks or 5 mg/ml for 15 months did not develop absolute increases in liver weight, although relative (to body weight) increases were found at 5 mg/ml and higher for 13 weeks and at 5 mg/ml for 15 months. Liver weight changes in female F344 rats exposed to TBA were more erratic, absolute weight increases being observed at all TBA concentrations from 2.5 mg/ml for 13 weeks, but there were no absolute weight increases after 15 months exposure to 5 mg/ml (indeed, there was a significant reduction at 1.25 mg/ml the lowest concentration) [98]. Generally, there were no reported histological changes associated with the liver weight increases in rats, even after two years exposure of F344 rats to MTBE by inhalation [94] or Sprague-Dawley rats to MTBE by gavage [95]. However, hepatocellular hypertrophy in Sprague-Dawley rats was reported at a dose of 500 mg/kg bw for 4 weeks [103] and Zhou et al. [112] reported that electron microscopy of hepatic cells of all treated groups showed nuclear condensation, fat droplet and lysosome accumulation in cells, and smooth endoplasmic reticulum disintegration. While there have been these observations in rats, liver changes were more generally observed in mice.

Inhalation exposures of $B_6C_3F_1$ mice to MTBE for short periods (3 days or 3 weeks) have produced erratic changes in liver weights [114], whereas inhalation of MTBE at concentrations of 3000 ppm or 8000 ppm for 4 weeks or longer induced liver weight increases in CD-1 mice [93, 94]. In this latter study, the 8000 ppm dose mice showed centrilobular hepatocellular hypertrophy after one month, which was more severe in the males than in the females and, after 18 months, hepatocellular hypertrophy was increased in males of the 3000 ppm and 8000 ppm groups and females of the 8000 ppm group. Moser et al. [115] showed that the activity of pentoxyresorufin-*O*-dealkylase, a marker enzyme for the CYP 2B family which is commonly induced by many tumour promoters, was increased by 5- and 14-fold after 3 and 21 days of exposure to MTBE, respectively. 7-Ethoxyresorufin activity was increased 2- to 3-fold at both 3 and 21 days. In mice implanted with osmotic pumps releasing BrdU, there were slight increases in liver cell proliferation after exposure of $B_6C_3F_1$ mice to 8000 ppm MTBE for 3 days, but not 21 days in one study [114]

and in male and female CD-1 mice exposed to 8000 ppm and in females exposed to 3000 ppm MTBE for 5 days, but not 28 days in another study [93, 94]. The loss of the proliferative response after the longer exposure periods may indicate an adaptive response of the liver cells.

In the drinking water study of TBA in mice [98, 99], there were no absolute liver weight changes in males or females exposed for 13 weeks to TBA concentrations up to 40 mg/ml. However, relative liver weights were increased in males from 20 mg/ml and in females at 40 mg/ml. Organ weights were not available from the two-year study in mice. The incidence of hepatic steatosis was significantly increased in male mice of the 20 mg/ml group, but not in the 5 or 10 mg/ml groups. Steatosis is a relatively mild, non-specific condition caused by abnormal fatty acid metabolism. There were no other remarkable hepatic changes in male or female mice. Although clear cell foci were more common in all treated male mice than in controls, there was no dose-related response. No histopathological findings in the liver were mentioned that would correlate with the increased relative liver weights in the three-month mouse study [98].

Liver weights were increased by ETBE treatment in studies of 4 to 13 weeks duration that included Sprague-Dawley and F344 rats [81, 82, 91] and CD-1 mice [82]. In one of the studies with Sprague-Dawley rats, ETBE was delivered orally [91]; otherwise, compound delivery was by inhalation.

No microscopic changes were found in rat liver in the inhalation studies [81–83], whereas, following oral administration of ETBE at 1000 mg/kg bw for 10 weeks, centrilobular hypertrophy was observed in 3/25 rats [91]. Mice exposed by inhalation to 0, 500, 1750 or 5000 ppm ETBE (0, 2, 120, 7450 or 21 200 mg/m^3) for 13 weeks showed exposure related increased incidences ($p < 0.05$, one-sided Fisher's exact test) of centrilobular hypertrophy at the highest dose level (males: 0/15, 0/15, 2/15, 8/10; females: 0/13, 2/15, 1/15, 9/14), but all other hepatic histological changes were minimal and not related to exposure. In mice implanted with BUdR-releasing osmotic pumps, the proportion of liver cells engaged in DNA synthesis were significantly increased in males of the 1750- and 5000-ppm exposure groups after 1 and 4 weeks, but not after 13 weeks, while in female mice, the proportion of liver cells engaged in DNA synthesis was significantly increased in weeks 1 and 13, but not in week 4 [82, 84]. These observations appear to be similar to the mitogenic effects reported in mice exposed to MTBE or unleaded gasoline. In these latter cases, the number of cells in S-phase DNA synthesis was increased in female $B_6C_3F_1$ mice at three days, but not at 21 days; the experiment was not continued beyond that time [114]. The hepatic changes observed in rats in inhalation experiments were consistent with an adaptive response to ETBE exposure. The same suggestion could be made for the multigeneration study in rats [91], but this cannot be confirmed because blood chemistry investigations that could have provided evidence of toxicity were not undertaken. In mice, the evidence for a purely adaptive response is weaker. In addition to

hypertrophy, increased hepatocellular DNA synthesis was observed in the 13 week inhalation study in mice [82]; however, there was no mention of altered hepatic foci that could have indicated a more serious response.

2.4.4 Thyroid Pathology

There have been no reports of morphological changes in the thyroids of rats and mice exposed to MTBE in studies varying in length from 14 days to more than 24 months [85–87, 93, 100, 102]. In some of these studies, however, the thyroid does not appear to have been examined microscopically (e.g., the one month studies in rats and mice by Chun et al. [93] and in rats by IITRI [102]; 14 day and three month studies by Robinson et al. [86]; and a three month study by Zhou and Ye [112], although there may have been study of thyroid function in some of these publications (see Endocrine Effects, below). Exposure of $B_6C_3F_1$ mice and F344 rats to TBA in drinking water for two years produced no toxicity that was reported [98, 99] and there was no hyperplasia in a 13-week study that might have indicated a response to short-term increases in TSH. Nevertheless, following exposure to TBA for two years at concentrations of 0, 5, 10 and 20 mg/ml, respectively, the incidences of hyperplasia were increased at all doses, but while they were dose-related in females (19/58, 28/60, 33/59 and 49/59), in males there were no increases in the middle and high dose mice above that observed in the low dose (5/60, 18/59, 15/59, 18/57). The follicular cell hyperplasia consisted of foci with increased numbers of closely packed follicular epithelial cells, sometimes with minimal papillary folds, that were morphologically indistinguishable in treated mice from those occurring in the control group mice. The focal distribution is not that expected of a hyperplastic response to perturbed control of the pituitary-thyroid hormonal axis. This mechanism often involves induction of hepatic microsomal enzymes [116, 117], but in neither the three month nor the 24 month studies was there liver hypertrophy or other evidence of liver enzyme induction that might have presaged perturbation of thyroid hormone status. No treatment-related non-neoplastic effects on the thyroid were noted in the inhalation study of methanol in mice [118].

2.4.5 Pathology of Other Organs and Tissues

Dose-related increases in the incidence of mineralisation of various tissues, including stomach, mammary gland, testes, lungs and kidneys, were found in F344 rats treated with MTBE for two years [93].

In the two-year gavage study of MTBE in Sprague-Dawley rats, an increase of dysplastic proliferation of lymphoreticular tissues from various body sites occurred in 3/60, 16/60 and 12/60 females in the 0, 250 and 1000 mg/kg

bw dose groups, respectively [97]. Dysplastic changes are occasionally associated with neoplastic transformation. The higher incidence of hyperplastic cells seen in the low rather than in the high dose group was interpreted by the authors to have been caused by progression to lymphoma and leukaemia in the high dose group. Unfortunately, there is no detailed description of the lung pathology. This is an important omission because lung seems to have been involved in most (16/21) of the females with haemolymphoreticular tumours (see below) and these may have arisen in peribronchial and perivascular lymphoid tissue [97]. These types of tumour in rat lung may be related to an increased amount of lymphoid tissue in the lungs due to chronic pulmonary inflammation [119–121].

A treatment-associated bone marrow congestion was observed in female rats exposed to 1750 or 5000 ppm (7420 or 21 200 mg/m^3) ETBE for 3 months [82]. There was no accompanying effect on the haematopoietic cell population, and an increase in mean corpuscular volume was not considered clinically relevant. No similar effect was reported for male rats or for mice of either sex that had been treated in the same way.

Hyperplasia has been seen in the parathyroid of male F344 rats treated with MTBE for two years, with incidences of 5/43, 12/38, 23/39 and 37/47 in the 0, 400, 3000 and 8000 ppm groups, respectively [93]. This response is likely due to hyperparathyroidism, which is commonly seen in cases where the parathyroid compensates in hypocalcaemia caused by, for example, chronic renal failure [122]. Adenomata in the two high dose groups (3/39 at 3000 ppm, 1/47 at 8000 ppm) were probably associated with the reported hyperplasia of parathyroid cells.

Exposure of CD-1 mice to 0, 400, 3000 or 8000 ppm MTBE by inhalation 6 h/day, 5 days/week for 18 months produced a statistically significant decrease in the incidence of cystic endometrial-cell hyperplasia in the higher two dose groups, whereas no effect was observed on the uteri of F344 rats similarly exposed, but for up to 24 months [93, 94]. Since the effect was observed in mice at both concentrations, this is likely to be a real species difference and could indicate an antioestrogenic effect.

2.4.6 Endocrine Changes

Total L-thyroxine (T4) and thyroid-stimulating hormone (TSH) were significantly increased in male CD-1 mice subjected to whole body MTBE vapour exposure at 8000 ppm, 6 h/day, 5 days/week for 4 weeks, but no effect was observed at 3000 ppm [93]. The biological significance of this alteration is unknown, because no parameter in clinical pathology indicated an effect in this experiment. In female mice there was a dose-related decrease in total T4 at day 5, which was also seen in the 3000 ppm group at day 31. In Sprague-Dawley rats treated orally by gavage with MTBE for 28 days [103], serum

triiodothyronine (T3) was significantly increased at 1000 and 1500 mg/kg bw, but in contrast to the mouse experiment, there was no change in serum T4 or TSH concentrations.

Moser et al. [114] found that in $B_6C_3F_1$ mice that were exposed to 7814 ppm [28 000 mg/m^3] MTBE 6 h/day, 5 days/week for 3 or 21 days the relative uterine weight was decreased in both treated groups. They also showed that the rate of 17β-oestradiol metabolism to water-soluble metabolites was significantly increased in hepatocytes isolated from female $B_6C_3F_1$ mice treated by gavage with MTBE at 1800 mg/kg bw per day for three consecutive days.

Exposure of $B_6C_3F_1$ mice to 8000 ppm MTBE resulted in decreased weights of the uterus, ovaries and pituitary after 3 days, 3, 16 and 32 weeks, increased length of the oestrous cycle (numbers of days in both oestrus and non-oestrus stages) after 32 weeks; decreased number of uterine glands and decreased uterine glandular and luminal epithelial DNA synthesis after 3 days, or 3, 16 or 32 weeks; and decreased number of epithelial layers in the cervix and vagina at all times [114, 123]. In mice exposed to MTBE, serum oestrogen levels were not affected. Furthermore, neither MTBE nor TBA inhibited the binding of [^{3}H]17β-oestradiol to a recombinant human receptor and treatment of HepG2 cells with MTBE did not induce oestrogen receptor activity or antagonise a maximally inducing dose of 17β-oestradiol [123]. The authors suggested that these results indicate that MTBE-induced endocrine effects are not mediated by the oestrogen receptor. It may also be noted that many of the changes reported by Moser et al. [123] are indicative of an antioestrogenic effect.

On the basis of the probably incorrect assumption (see below) that increases in Leydig cell adenoma incidences in F344 and Sprague-Dawley rats were exposure related, studies have been made on the effects of MTBE on the metabolism of testosterone and associated hormones. Dose related, reproducible reductions of at least 50% were observed in circulating testosterone in Sprague-Dawley rats within 4–5 h of treatment with MTBE at 1000 mg/kg bw and higher; however, the reductions were not sustained upon continuing at the same or reduced dose levels for 4 weeks [113]. While body weight reductions alone (as occurred in this study) can decrease testosterone levels [124], this would not explain the very rapid reduction observed after the first dose. The authors considered whether the recovery in testosterone levels might have been due to self-induction of MTBE metabolism and compensatory rise in luteinising hormone (LH), but there seemed to be no effect on LH (or prolactin) at 4 weeks and there was a slight, but significant reduction in LH at 2 weeks. In castrated rats, there was no evidence of an effect of MTBE on LH or pituitary weight.

Treatment with MTBE decreased aromatase activity in microsomes prepared from liver and testis, but contrary to expectations, circulating concentrations of 17β-oestradiol were increased, while effects on LH were vari-

able [113]. MTBE also reduced circulating dihydroxytestosterone concentrations [103]. This might be expected if 5α-reductase activity was inhibited, but this effect would also have been expected to increase LH concentration, which has not been demonstrated. In vitro, basal and gonadotropin-stimulated testosterone production was inhibited in isolated Leydig cells from Sprague-Dawley rats exposed to 50 mM MTBE by about 50%, or TBA by about 73% [113]. These authors calculated that this concentration of MTBE would be about 2.5-fold higher than that experienced by testicular tissues of rats in the two-year MTBE inhalation study and suggested that repeated testosterone synthesis inhibition is a likely mechanism of Leydig cell adenoma induction. Although an interesting suggestion, it is noted that, at least in the in vitro study, TBA had a similar and perhaps more potent effect than MTBE (although its volatility may have played a role in this difference). This study has been extended to include ETBE, which was found to inhibit gonadotropin-stimulated testosterone release to almost the same extent as MTBE (Stanard et al. 2003, abstract). They also reported that when ETBE was administered orally by gavage at a dose of 1800 mg/kg body weight per day for 14 days to groups of 12 SD rats, serum mean testosterone concentration 1 h after the final dose was 44% lower than in the controls, although the difference was not statistically significant. The relevance of these observations to any possible testicular toxicity is unclear, given the lack of reproducibility in several studies of effects on spermatocytes attributed to ETBE in one study. Furthermore, no testicular toxicity (or neoplasia) has been reported for TBA, even after exposures for up to two years [98, 99] and the reported Leydig cell neoplasia associated with MTBE is in doubt (see below).

Adrenal weights were increased in female SD rats dosed orally with 1200 mg MTBE/kg bw for 13 weeks [86] and in both male and female F344 rats exposed by inhalation to 4000 or 8000 ppm MTBE for 13 weeks [87, 100]. In the latter study, there were significantly elevated blood concentrations of corticosterone of more than 3–5 fold the controls in both sexes in the 8000 ppm group. Corticosterone showed a trend towards increases during the CD-1 mouse and F344 rats carcinogenicity studies with MTBE at concentrations of 3000 and 8000 ppm. However, there was more than a two-fold decrease in corticosterone in the male rats of the 8000 ppm group at the end of the two-year period that correlated with disruption of the zona reticularis [94].

2.5 Toxicity to Reproduction

MTBE has been tested by inhalation and ETBE has been tested by gavage for effects upon reproduction and prenatal development. The experiments with MTBE were a one-generation study at concentrations up to 2900 ppm, 6 h/day for 12 and 3 weeks before mating of male and female Sprague-Dawley (CD) rats, respectively [125] and a two-generation study in Sprague-Dawley

rats at concentrations up to 8000 ppm, 6 h/day for 10 weeks before mating, throughout mating and gestation and then from day 5 of lactation. F1 rats were exposed from day 28 for 4 weeks [126]. The developmental studies were: one study in Sprague-Dawley rats exposed for 6 h/day on gestation days 6–15 to concentrations up to 2500 ppm [127]; two studies in CD-1 mice exposed for 6 h/day on gestation days 6–15 to concentrations up to 2500 ppm [127] or up to 8000 ppm (Bevan et al. 1997b); and one study in New Zealand white rabbits exposed for 6h/day on gestation days 6–18 to concentrations up to 8000 ppm [128].

The one- and two-generation studies showed that MTBE does not cause significant or specific toxicity to reproduction in Sprague-Dawley rats. In the two-generation study, adults exposed to 8000 ppm showed ataxia, hypoactivity and reduced body weights. At 3000 ppm these same effects were less severe or transient. F1 generation rats had increased liver weights in both sexes in the 8000 ppm group and in males of the 3000 ppm group, but the histological appearance was unchanged. The number of dead F2 generation pups was increased on post-natal day 4 and the growth of both F1 and F2 pups in the 8000 and 3000 ppm groups was decreased during the pre-weaning period.

The developmental studies of MTBE in rats and rabbits showed that exposure to 8000 ppm reduced body weight gain and food consumption in adult rabbits and a transient reduction in food consumption in adult rats, but there were no effects upon foetal development. Mouse was the only species of the three tested that showed any adverse effects of exposure upon development and these only in the study using the higher exposure concentrations [128]. The maternal effects were hypoactivity and ataxia at 8000 and 4000 ppm and reduced food consumption at 8000 ppm. Late resorptions and dead foetuses and the incidence of cleft palate were significantly increased at 8000 ppm, while foetal body weight was lower, and the incidences of skeletal variations (poorly ossified phalanges, vertebral arches and centra, reduced number of caudal segments) were increased at 4000 and 8000 ppm. Post-implantation deaths were seen mainly among male offspring. Dodd and Kintigh [129] had previously shown that female Fisher-344 rats and CD-1 mice exposed to 8000 ppm MTBE have markedly elevated corticosterone concentrations when compared to controls. Thus, maternal stress may have played a role in the formation of cleft palates, the only malformation that was increased in the mice and to which they are known to be susceptible.

The reproduction study was a two-generation study using daily ETBE doses of 0, 500 and 1000 mg/kg bw for 10 weeks before mating of male and female Sprague-Dawley rats [89, 91]. The doses and strain of rat were selected on the basis of a preliminary study using F344 and Sprague-Dawley strains. The latter was preferred because of the generally higher fecundity in comparison with the F344 strain. The concern that ETBE might be damaging seminiferous tubules in F344 rats [82] was the reason for the strain comparison, but the effect was not confirmed [89]. Dosing of both sexes con-

tinued throughout the mating period, gestation, and lactation (i.e., a total of 15 weeks for both sexes). The prenatal developmental study was conducted in Sprague-Dawley rats dosed orally by gavage at 0, 250, 500 and 1000 mg/kg bw/day from day 5 to 19 inclusive, *post-coitum*.

The two-generation study showed that ETBE does not cause significant or specific toxicity to reproduction in Sprague-Dawley rats. Effects of treatment recorded for the adult rats included dose-related ptyalism, reductions in body weight gain in those rats receiving 1000 mg/kg bw/day and, to a smaller extent, 500 mg/kg bw/day. Liver and kidney weights were increased in F0 males, as discussed above ("Nonneoplastic Effects"). F1 pup body weights were unaffected by treatment and a small reduction in F2 pups on *post-partum* days 1 through 4 in the 1000-mg/kg bw dose group was not statistically significant. Two F2 pups of the 1000-mg/kg bw dose group were born with gross external malformations (absence of a tail and, in one of them, anal atresia), but this low incidence was not considered unusual and furthermore, neither of these malformations was observed among a total of 566 pups from dams treated at the same dose level in the dose-range finding experiment [89] or the prenatal developmental study [90]. Thus, systemic toxicity in adults was limited to weight gain reductions at the higher two dose levels and there were no effects at any dose level of ETBE on fertility, gonadal function, reproductive performance, parturition and lactation or postnatal development to weaning or sexual maturity.

The prenatal developmental study of ETBE in rats showed that exposure to 1000 mg/kg body weight/day reduced body weight gain by 11%, whereas 500 mg/kg body weight and lower dose levels had no effect. There were no treatment-related effects on the gestational parameters or foetuses at any dose level. No experiments to investigate possible effects on prenatal development have been conducted in other species.

2.6 Carcinogenicity

MTBE has been tested for carcinogenicity in F344 rats and CD-1 mice exposed by inhalation [93, 94, 130] and orally by gavage in Sprague-Dawley rats [95–97]. Its major and more persistent metabolite, TBA, has been directly tested by oral, drinking-water exposure in F344 rats and B_6F_1 mice [98, 99]. There is a single study in which ETBE was tested for its carcinogenic potential [131], but for reasons given elsewhere [6] it is not considered reliable and will not be discussed further. The dose levels used in these experiments are given in Table 4 and the significant data are given in Table 5.

A feature of the inhalation study of MTBE in male F344 rats [93, 94] that could complicate tumour evaluation was the large increase in mortality diagnosed as due to CPN. This led to termination of exposure and withdrawal of rats from the experiment at different times according to group. Deaths due to

Table 4 Doses administered to rats and mice in carcinogenicity studies

Chemical	species	Exposure route, dose & duration	Refs.
MTBE	Rat, F344, 50/sex/dose	Inhalation, 0, 400, 3000, 8000 ppm, 6 h/day [0, 40, 310, 830 mg/kg be for males; 0, 60, 450, 1190 mg/kg bw for females] [a], 5 days/week, 104 weeks	[93, 94]
	Rat, Sprague-Dawley, 60/sex/dose	Gavage, 0, 250, 1000 mg/kg bw/day 4 days/week, 104 weeks, then maintained for lifetime, (last rat died in week 166)	[95–97]
TBA	Rat, F344, 60/sex/dose	Drinking water, 0, 1.25, 2.5, 5.0 mg/ml [0, 85, 195, 420 mg/kg bw] for males; 0, 2.5, 5.0, 10.0 mg/ml [0, 175, 330, 650 mg/kg bw] for females	[98, 99]
MTBE	Mouse, CD-1, 50/sex/dose	Inhalation, 0, 400, 3000, 8000 ppm, 6 h/day [0, 210, 1550, 4150 mg/kg bw for males; 0, 250, 1850, 4950 mg/kg bw for females] [b] 5 days/week, 80 weeks	[94, 130]
TBA	Mouse, B6C3F1, 60/sex/dose	Drinking water, 0, 5, 10, 20 mg/ml [0, 535,1035, 2065 mg/kg bw for males; 0, 510, 1015, 2105 mg/kg bw for females], 104 weeks	[98, 99]

[a] Assuming 6 L inhaled/hour, pulmonary retention of 40% for MTBE, 500 g bw for males and 350 g bw for females

[b] Assuming 1.8 L inhaled/hour, pulmonary retention of 40% for MTBE, 30 g bw for males and 25 g bw for females

CPN in the 0, 400, 3000 and 8000 ppm groups, respectively, were 2/17, 8/23, 16/24 and 28/28. Only the 0 and 400 ppm groups continued on test for the scheduled 104 weeks, when survival rates were 26% and 12%, respectively. The 3000 and 8000 ppm dose groups were withdrawn at weeks 97 and 82, respectively, when survival rates were 16% and 18%, respectively. There were also severe effects on body weight and body weight gain in the 8000 ppm group. CPN was increased to a lesser extent in female rats and all groups of female rats remained on test for 104 weeks, when survival rates were 60%, 54%, 47% and 50%, respectively. These same publications describe the responses of CD-1 mice to inhalation of MTBE at the same concentrations as used for rats. Amongst the 8000 ppm group males, there was decreased survival that was attributed to a slightly increased obstructive uropathy. Dysuria or urine retention was previously reported as the cause of death in CD-1 mice, accounting for nearly 30% of all males dying in that study [52]. Survival rates after 18 months in the 0, 400, 3000 and 8000 ppm groups, respectively, were 67%, 78%, 65% and 51% in male mice and 73%, 82%, 77% and 67% in female mice. Body

Table 5 Selected tumour incidences in rats chronically exposed to MTBE or TBA

Chemical/ Refs.	Proliferative lesion	Exposure 0		Exposure 1		Exposure 2		Exposure 3	
		Male	Female	Male	Female	Male	Female	Male	Female
MTBE/[93]	Renal tubule adenoma	1/50	0/50	0/50	0/28	5/50	1/39	3/50	0/50
	Renal tubule carcinoma	0/50	0/50	0/50	0/28	3/50[a]	0/39	0/50	0/50
MTBE/[96]	Renal tubule adenoma	1/60	0/60	1/60	0/60	–	–	2/60	0/60
	Renal tubule carcinoma	0/60	0/60	0/60	0/60	–	–	0/60	0/60
TBA/[98]	Renal tubule adenoma	1/50	0/50	3/50	0/50	4/50	0/50	3/50	0/50
	Renal tubule adenoma	1/50	0/50	3/50	0/50	4/50	0/50	3/50	0/50
	+ carcinoma	8/50 (step section)		13/50		19/50		13/50	
MTBE/[93]	Leydig cell adenoma	32/50		35/50		41/50		47/50	
MTBE/[97]	Leydig cell adenoma	2/60 (26)**		2/25		–		11/32	
TBA/[98]	Leydig cell adenoma	34/50		31/50		31/50		33/50	
MTBE/[93]	Large granular lymphocytic leukaemia[b]	33/50	22/50	22/48	14/29	20/48	15/34	3/50	16/50
MTBE/[96, 97]	Lymphomas/leukaemias	10/60 (10/59*)	2/60 (2/58**)	9/60 (9/59)	6/60 (6/51)	–	–	7/60 (7/58)	12/60 (12/47)
TBA/[98]	Mononuclear cell leukaemia	30/50	22/50	28/50	20/50	24/50	18/50	23/50	13/50

Table 5 (continued)

Chemical/ Refs.	Proliferative lesion	Exposure 0		Exposure 1		Exposure 2		Exposure 3	
		Male	Female	Male	Female	Male	Female	Male	Female
MTBE/[93]	Mammary adenoma, fibroadenoma or carcinoma	1/45	8/50	0/36	4/27	2/35	9/32	0/44	8/49
MTBE/[97]	Mammary fibroma, fibroadenoma or carcinoma	2/60	25/60	3/60	20/60	–	–	4/60	16/60
TBA/[98]	Mammary adenoma, fibroadenoma or carcinoma		17/50		16/50		12/50		10/50
MTBE/[93]	Pancreatic islet adenoma or carcinoma	4/49	2/49	3/44	0/24	1/42	0/27	2/48	0/49
MTBE/[97]	Pancreatic islet adenoma	4/60	1/60	2/60	1/60	–	–	2/60	2/60
TBA/[98]	Pancreatic islet adenoma or carcinoma	17/50	2/50	15/49	1/50	9/49	4/48	6/49	1/50

[a] These carcinomas were diagnosed as of extra-renal origin, upon re-evaluation [132]

[b] Large granular lymphocytic leukaemia as used by Chun et al. [93] is the same as mononuclear cell leukaemia as used by NTP [98]. This non-T, non-B cell neoplasm is not the same as the lymphomas/leukaemias as used by Belpoggi et al. [95, 97]

weight gains were also reduced in the 8000 ppm group male and female mice by 16% and 24%, respectively. Mortality-adjusted analysis was not performed on tumour incidence data in either the rat or the mouse study and while this would not have been important in the case of female mouse or female rat tumour analysis, the same cannot be said for male rat tumours in particular, for which late on-set tumours would be more likely in the control and low dose groups. Also, mice in the intermediate and low doses were not subjected to complete histopathological examination unless they died spontaneously or were killed when moribund.

The gavage study of MTBE in 1 ml olive oil at 4-times weekly doses of 0, 250 and 1000 mg/kg bw in Sprague-Dawley rats [95–97] was not so severely affected by toxicity, although low tolerance to the high dose was the justification for the unusual dosing schedule. There were no treatment related clinical signs of toxicity or body weight changes. Male survival rates were similar in all dose groups up to week 88, when the rate was about 50%. However, between weeks 104 and 136, the survival rates in the 0, 250 and 1000 mg/kg bw dose groups were < 5%, about 30% and 45–15%, respectively. These variations also would have complicated tumour incidence evaluation, particularly in the case of non-lethal tumours arising late in life. The last rat died in week 166. Because the rats were allowed to live out their natural lifespan and mortality-adjusted analyses were not performed, estimates of effective group numbers and tumour incidences are difficult to analyse. Late on-set tumours would be more likely in the two treated groups than in the controls.

In the two-year drinking water studies on TBA, survival rates of the male rats receiving the two higher doses were significantly reduced at week 101 [98, 99]. Survival rates in the 0, 1.25, 2.50 and 5.00 mg/ml drinking water groups of male rats, respectively, were 20%, 17%, 7% and 3% and in the 0, 2.5, 5.0 and 10.0 mg/ml drinking water groups of female rats, respectively, were 48%, 47%, 43% and 28%. Mean body weights of male rats were similar through about week 65, after which there were dose-related reductions in the respective treated groups at week 101 of 15%, 18% and 24% in male rats and 2%, 5% and 24% in female rats. Only the highest body weight reduction is notable in females. Also, since there were no significant body weight differences between groups during the first 16 months of the study, the later weight changes would not appear to be in response to toxicity. The same publications report the drinking water study conducted on $B_6C_3F_1$ mice, in which the same TBA concentrations of 0, 5, 10 and 20 mg/ml were received by males and females. At week 101, survival rates in the four respective groups were 57%, 73%, 68% and 38% in male mice and 48%, 47%, 43% and 28% in female mice. The survival reduction in male mice was statistically significant. Mean body weights in the treated groups of male mice were similar to their controls at the end of the study; however, the mean body weights of the 20 mg/ml group males were 5–10% lower than the controls from about week 9 until about week 90. Female mice of this dose group had 10–15% lower body weights than the controls

from about week 13 until the end of the study. Statistical analyses of tumour incidences in both rats and mice used survival-adjusted procedures.

2.7 Renal Tubule Cell Neoplasms

The incidence of renal tubular-cell tumours (Table 5) was increased in male F344 rats at the two higher inhalation doses of MTBE [93, 94]. The combined adenoma and carcinoma incidence in males at the intermediate dose of MTBE (8/50) was statistically significantly increased and was outside the range of the historical controls. The lower incidence in the highest dose group is almost certainly a result of the reduced survival and shorter experiment time for that group. A re-assessment of the kidney pathology [132] confirmed that there was a modestly increased incidence of renal tubule cell adenomas in male rats of the mid- and high-dose MTBE groups, but there were no carcinomas. The carcinomas originally reported [93] were of extra-renal origin and therefore should not enter the statistical analysis of renal tumours. It was also found that CPN was severely exacerbated in the mid- and high-dose groups of males. Furthermore, 85% of the treatment group adenomas occurred in male rats with end-stage (i.e., the most severe stage) CPN, with the remainder in rats exhibiting the next most severe grade of CPN.

In male F344 rats exposed to TBA in drinking water at 0, 1.25, 2.5 and 5 mg/ml, the standard evaluation (a single, sagittal and a single transverse section) revealed aggregate adenoma and carcinoma incidences of 1/50, 3/50, 4/50 and 3/50, respectively, but a significant increase in the incidence of these tumours was achieved for the middle dose only when the results of step sectioning of the kidney were included. The additional step sectioning at 1 mm intervals combined with the standard sectioning provided aggregate tumour incidences (adenomas and carcinomas combined) of 8/50, 13/50, 19/50 ($p < 0.01$) and 13/50, respectively. Statistical significance was also achieved ($p < 0.05$) at the high dose when one tumour found at an interim sacrifice was included in the total [98, 99]. It should be noted that step sectioning has transformed a hitherto rare or unusual tumour type in control rats into a common one. Also, these additional benign tumours are very small. Female rats and male and female mice exposed to MTBE by inhalation or TBA by drinking water showed no significant increase in the incidence of renal tumours. Neither male nor female rats nor mice exposed by inhalation to methanol (as a "prodrug" for formaldehyde), or rats exposed to methanol in drinking water showed any increase in renal tumour incidence [118, 133].

Male rat kidney secretes several milligrams of α_{2u}-globulin a day, which is normally cleared in urine via glomerular filtration, but a substantial proportion of it is reabsorbed. A protein similar to α_{2u}-globulin has not been identified in human kidneys [134]. α_{2u}-nephropathy syndrome follows from an overload in renal tubule cells of reabsorbed α_{2u}-globulin that has been

altered by the binding of a xenobiotic chemical, rendering it more resistant to hydrolysis by lysosomal enzymes [52]. This leads to an accumulation of hyaline droplets and injury to the proximal convoluted tubule. There is a sequence of events that is characteristic of α_{2u}-globulin nephropathy syndrome that usually includes granular cast formation in the outer medulla and increased severity or incidence in CPN. In longer-term studies, linear papilla mineralisation, urothelial hyperplasia, increased cell proliferation and ultimately tubular neoplasia may be seen. Other criteria applied to identify the α_{2u}-globulin syndrome include an affinity of the xenobiotic for α_{2u}-globulin, an absence of genotoxicity and an inability to produce these symptoms in female rats. These criteria have been used by IARC and US EPA to distinguish the male rat specific mechanism from others and to reach a judgment as to whether the tumours observed have relevance for human health [135, 136]. However, of 38 chemicals believed to cause α_{2u}-globulin nephropathy, all of the (more stringent) IARC criteria were met by only a few (*d*-limonene, unleaded gasoline, sodium barbital and its metabolite diethylacetylurea, 1,4-dichlorobenzene and isophorone) [137]. Clearly, in-

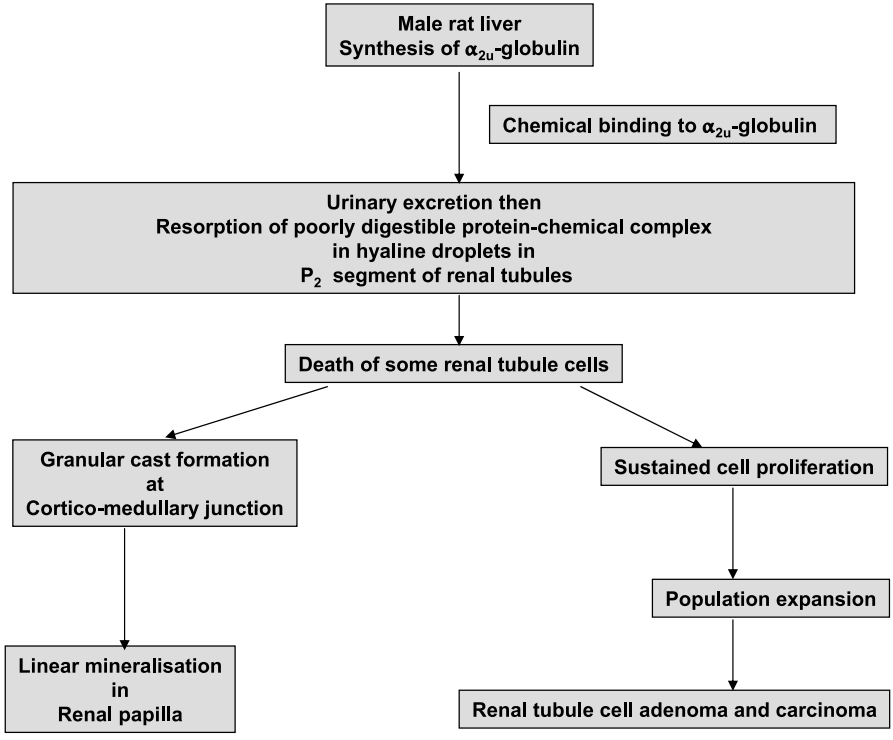

Fig. 2 Process of induction of α_{2u}-globulin nephropathy in male rats

clusion in such a list is partially dependent on the research effort applied to a particular compound.

It is postulated that high levels of MTBE and TBA result in α_{2u}-globulin nephropathy in male rat kidney (see Non-neoplastic Effects) and exacerbate the development of CPN to end-stage, both of which can lead to the formation of kidney tumours [132]. The postulated steps in these processes are outlined in Figs. 2 and 3. Whether these chemicals have similar, independent actions, or whether MTBE acts because it is metabolised to TBA is not entirely clear, although some of the in vitro studies would suggest that both can interact with α_{2u}-globulin. Independent assessment of the NTP microscope slides from the TBA study (G. Hard, personal communication) confirms that linear mineralization of the papilla (LPM) was present in the majority of high-dose males (5 mg/mL) to a mild or moderate degree of severity. It was also present to a lesser extent (minimal to moderate severity) in most of the mid-dose males (2.5 mg/mL), but in none of the low-dose males. LPM was also observed at the 15-month time-point in both mid- and high-dose groups at an equivalent severity to that present in the 2-year study, indicating that it was a long-standing change. LPM is believed to represent the accumulation of cell debris from granular casts higher up in the nephron that becomes lodged and calcified at the U-bend in the loop of Henlé. The NTP report implies an absence of granular casts in the 13-week toxicity study, as might be expected because granular casts appear to be of limited persistence; they have not been recorded at 2-year time-points in NTP studies. On the other hand,

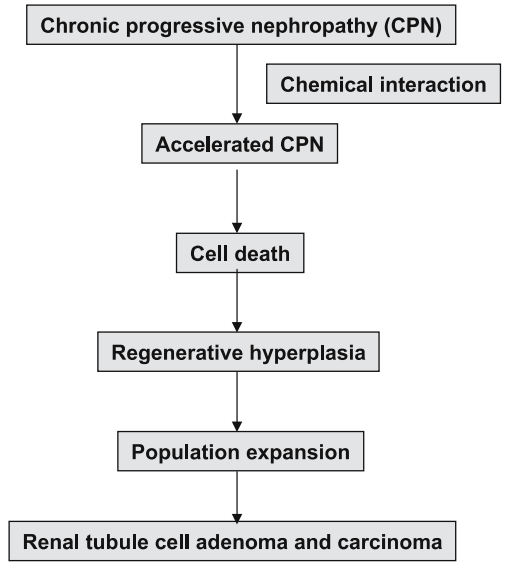

Fig. 3 Involvement of chronic progressive nephropathy in rat renal tubule cell neoplasia

LPM appears to be a more permanent indicator that has correlated strongly with renal tumour development associated with all other compounds suggested so far to act through the α_{2u}-globulin pathway. The evidence therefore is in support of the α_{2u}-globulin nephropathy-based MOA for both MTBE and TBA, but there is, in addition, evidence for CPN involvement in the MTBE tumours.

Because the α_{2u}-globulin pathway is male rat specific and α_{2u}-globulin does not occur in man, this MOA is widely regarded as having no human relevance. Similarly, the CPN of rats does not appear to have a human equivalent and so the exacerbation of this condition in rats by chemicals also is regarded as lacking human relevance [138]. No specific kidney disease that is totally confined to the aging kidney has been identified in humans [139]. Also, whereas progression of CPN in rats can be ameliorated by reducing their protein intake, the prevailing view is that diseases causing chronic renal failure in man show negligible benefit from a low-protein diet [140].

2.8 Leydig Cell Neoplasms

The incidence of Leydig cell adenomas (Table 6) was significantly increased in two studies, one with F344 rats and one with Sprague-Dawley rats treated with MTBE [93–97]. The increases occurred in the 3000 and 8000 ppm inhalation groups of F344 rats and in the 1000 mg/kg bw gavage dosing group of Sprague-Dawley rats. In the latter, the increase was significant only when the number of tumour-bearing rats was counted against the number of rats alive at the time of observation of the first tumour. This restriction reduces the biological significance of the result because the rats were allowed to survive until their natural death, but the method of statistical analysis did not allow for survival differences between groups. Survival was for longer periods in the high dose group; therefore the number of tumour-bearing rats would be expected to be greater in this group, even in the absence of treatment, because Leydig cell tumours in rats are strongly age-associated neoplasms. Typically, they are first observed at about 12 months [141–144]. The problem of different longevities in the groups of rats used in this study has been addressed (Dr. B. Beck, Gradient Corp. 2007, personal communication) by conducting separate Poly-3 analyses for the most extreme scenarios of survival age and tumour incidence on the reasonable assumption that the true data fall somewhere within this range. Definitions of statistical significance were *p*-values of 0.01 for pair-wise comparisons and 0.005 for trend tests of common tumours, as recommended to control for the overall false-positive error rate that can arise from multiple comparisons of tumour incidences in the large number of tissue and organ sites examined in carcinogenicity tests [145]. These methods led to the conclusion that MTBE does not cause a statistically significant increase in Leydig cell tumours in SD rats.

Table 6 Results of genotoxicity studies conducted with MTBE, ETBE and TBA

Test system	Results[a]	Dose[b] (LED or HID)	Refs.
Methyl *tertiary*-Butyl Ether			
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538 reverse mutation	–	74 000 μg/plate	[66, 208]
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538 reverse mutation	–	10 000 μg/plate	[209, 210]
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA102 reverse mutations	–	5000 μg/plate	[211]
Salmonella typhimurium TA100, TA98, TA104, TA1535, reverse mutation	–	7400 μg/plate	[212]
Salmonella typhimurium TA102, reverse mutation	+	750 μg/plate	[196]
Salmonella typhimurium TA100, TA98, reverse mutation	–	100 μg/plate	[213]
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA102 reverse mutations	–	5000 μg/plate	[197]
Saccharomyces cerevisiae D4, gene conversion	–	74 000 μg/mL	[66]
Drosophila melanogaster, sex-linked recessive lethal mutations	–	0.3% feed	[214]
Single cell gel electrophoresis (comet) assay, human HL-60 leukaemia cells in vitro	+	1 mM	[215]
Unscheduled DNA synthesis, primary cultures of rat hepatocytes	–	10 000 μg/mL	[216]
Unscheduled DNA synthesis, primary cultures of rat hepatocytes	–	10 000 μg/mL	[217]
Gene mutation, mouse lymphoma L5178Y cells, *tk* locus in vitro	+	740 μg/mL	[66, 205]
Gene mutation, Chinese hamster V79 cells, *hprt* locus in vitro	–	2500 μg/mL	[218]
Micronucleus test, mouse NIH/3T3 cells in vitro	–	14 000 μg/mL	[213]
Unscheduled DNA synthesis, male and female CD-1 mouse hepatocytes in vivo	–	8000 ppm inh, 6 h/d × 2 d	[214]
DNA strand breakage, Sprague-Dawley rat peripheral lymphocytes (Comet assay), in vivo	+	800 mg/kg bw/d, po ×28	[219]
Gene mutation, *hprt* locus, CD-1 mouse spleen lymphocytes in vivo	–	1000 mg/kg bw/d, po 5 d/week, 3 weeks	[220]
Micronucleus formation, male and female CD-1 mouse bone-marrow cells in vivo	–	8000 ppm inh, 6 h/d × 2 d	[214]
Micronucleus formation, male and female Swiss-Webster mouse bone-marrow cells in vivo	–	1750 ip × 1	[212]
Chromosomal aberrations, male Sprague-Dawley rat bone-marrow cells in vivo	–	280 mg/Kg bw ×5	[221]

Table 6 (continued)

Test system	Results[a]	Dose[b] (LED or HID)	Refs.
Chromosomal aberrations, male and female Fischer 344 rat bone-marrow cells in vivo	–	8000 ppm inh, 6 h/d × 5 d	[214]
Ethyl *tertiary*-Butyl Ether			
Salmonella typhimurium TA1535, TA1537, TA1538, TA98, reverse mutation	–	500 μg/plate	[222]
Salmonella typhimurium TA100, TA98, TA1535, TA97, reverse mutation	–	10 000 μg/plate	[223]
Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100, reverse mutation	–	5000 μg/plate	[224]
Gene mutation, Chinese hamster V79 cells, *hprt* locus in vitro	–	5000 μg/mL	[225]
Chromosomal aberrations, Chinese hamster ovary CHO cells in vitro	–	5000 μg/mL	[226]
Micronucleus formation, male and female OF-1 mouse bone marrow cells in vivo	–	5000 mg/kg bw ×1	[227]
Micronucleus formation, male and female CD-1 mouse bone marrow cells in vivo	–	5000 ppm (21 200 mg/m^3) 6 h/day × 5	[228]
***Tertiary*-Butyl Alcohol**			
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538 reverse mutation	–	10 000 μg/plate	[229]
Salmonella typhimurium TA102, reverse mutation	+	750 μg/plate	[196]
Salmonella typhimurium TA102 reverse mutations	–	5000 μg/platec	[197]
Single cell gel electrophoresis (comet) assay, human HL-60 leukaemia cells in vitro	+	1 mM	[215]
Gene mutation, mouse lymphoma L5178Y cells, tk locus in vitro	–	5000 μg/mL	[207]
Sister-chromatid exchange, Chinese hamster ovary CHO cells in vitro	–	5000 μg/ml	[98]
Chromosomal aberrations, Chinese hamster ovary CHO cells in vitro	–	5000 μg/ml	[98]
Micronucleus formation, male and female $B_6C_3F_1$ mouse peripheral blood cells in vivo	–	40 mg/ml drinking water for 13 weeks	[98]

[a] + Significant response; – No significant response
[b] LED, lowest effective dose (for significant responses); HID, highest ineffective dose (for responses that are not significant)

There are also difficulties in ascribing the increased incidence in the inhalation study to treatment. As in the case of other non-life-threatening endocrine tumours in rats, such as phaeochromocytomas and mammary tumours, Leydig cell tumours occur at variable and occasionally very high

incidences in different strains. In F344 rats, the incidence of Leydig cell adenomas is quite variable and very high at 24 months of age. The historical control frequencies in this strain recorded in US NTP studies range from 74% to 98%, while Leydig cell carcinomas are relatively rare (one recorded among 1352 control rats) [146]. Also, the historical control incidences in the laboratory performing this particular study were 86% and 91% [93]. Therefore, the incidences in the concurrent control (64%) and 400 ppm exposure (70%) groups were lower than expected, for unidentified reasons. Treatment of rats with either TBA or methanol did not result in an increase in Leydig cell adenomas [98, 99, 118, 133]; therefore, if the tumour increase is not simply due to chance or an artefact of experimental design, then it would appear that only MTBE itself, not a metabolite, could be responsible.

Certain strains of rats, particularly F344, are more susceptible to the development of Leydig cell adenomas than mice or man, but progression to carcinoma in rats is rare. Features of rats that are likely to contribute to this susceptibility to adenoma induction have been reviewed [147]. Of particular interest are (a) the 13-fold higher density of LH receptors in Leydig cells of rats compared with man [148]; (b) rat Leydig cells respond to human chorionic gonadotropin (a hormone equivalent to LH) by hyperplasia [149], whereas human Leydig cells only respond by hypertrophy [150, 151] and (c) rat Leydig cells contain gonadotropin-releasing hormone (GnRH) receptors, perhaps uniquely, since they are not present in man [152] or mouse [153].

Experimentally induced Leydig cell neoplasia has been very well reviewed on several occasions [147, 154–156]. Six modes of action for chemical induction of these tumours in rodents (more usually rats) have been published, the fundamental factor in these modes of action being increased LH secretion stimulating Leydig cell activity. These cells synthesise testosterone that either directly or after conversion to oestradiol by aromatase can feedback and inhibit LH secretion. Disruption of this feedback loop is frequently the mode of action of Leydig cell adenoma inducers. The six recognised modes of action are: testosterone biosynthesis inhibition [157]; androgen receptor antagonism [158–160]; aromatase inhibition [147, 161]; 5α-reductase inhibition [162]; dopamine agonism [163]; and peroxisome proliferation [164, 165], but none of these is a likely mode of action for MTBE. Most of these modes of action depend upon feedback increases in circulating LH to stimulate Leydig cell hyperplasia, whereas in the case of MTBE such increases in hormone concentration have not been demonstrated [103, 113]. In the case of peroxisome proliferation as a possible mode of Leydig cell adenoma induction by MTBE, no such proliferation has been demonstrated [113] and this mechanism typically also involves liver tumour induction in the same species.

A number of chemicals are associated with increases in Leydig cell adenoma incidence in rats, but not in mice, for example, clofibrate, fenofibrate, gemfibrozil, JP-4 jet fuel, lansoprazole, *d*-limonene, oxazepam, methylclofenapate, tetrachloroethylene and trichloroethylene [157, 166–170]. Perox-

isome proliferation is a common (although not universal) mode of action of these chemicals.

Substances that have been associated with increases in Leydig cell tumours in mice are mainly chemicals with clear oestrogenic properties, e.g., diethylstilboestrol, methoxychlor, tri-*p*-anisylchloroethylene, stilboestrol, 17β-oestradiol, oestradiol ester, tamoxifen and triphenylethylene [171–179]. Other compounds active in mice are finasteride, a 5α-reductase inhibitor that also induces Leydig cell hyperplasia, but not adenomas, in Sprague-Dawley rats [170, 180] and *N*-nitrosodiethylamine [181]. In contrast to its effects in mice and Syrian and European hamsters [182], 17β-oestradiol treatment of rats does not induce Leydig cell tumours and inhibits the appearance of the spontaneous tumours in the F344 strain [184].

Prenatal exposure of mice to oestrogens, such as ethinyl oestradiol or DES, can result in a high frequency of cryptorchidism and impairment of spermatogenesis and Leydig cell hyperplasia [184, 185]. A proportion of these mice go on to develop testicular tumours. Surgically produced cryptorchidism also induces Leydig cell tumours in mice whereas in rats surgically induced cryptorchidism inhibits Leydig cell tumour formation [186] and can even prevent Leydig cell hyperplasia. The chronic administration of diethylstilbestrol to F344 rats also inhibits Leydig cell tumour formation [186]. Postnatal exposure of some mouse strains to DES implanted subcutaneously induces Leydig cell tumours, e.g., BALB/C is susceptible, while C3H is not [187]. In addition, transgenic mice that overexpress aromatase have increased oestrogen production and a changed hormone milieu that appears to lead to the induction of Leydig cell tumours [188]. Other than these oestrogenic exposures, the only agent that has induced Leydig cell tumours in mouse is cadmium chloride, injected subcutaneously. No increases were recorded in the incidences of any type of testicular tumour in the mouse studies with MTBE, TBA or methanol.

Another clear difference between rats and mice is that testicular tumours of any type are unusual in control mice and, even at 24–32 months of age, the range in a number of strains has been reported as from 0% to 6% [146, 189, 190]. Some of these characteristics of mice are shared at least in part by man and it appears that mouse is the better model for human testicular neoplasia [5, 156].

2.9 Lymphomas and Leukaemia

A statistically significant increase in the incidence of lymphomas and leukaemia (Table 5) was observed in female Sprague-Dawley rats treated by gavage with an MTBE dose of 1000 mg/kg bw (2/60 versus 12/60, $p < 0.01$) [95]. No significant increase was observed in females of the 250 mg/kg bw dose group or in males of either MTBE dose group in this study. Precise

historical control tumour incidence data were not given, but it was stated in the earlier of the two reports that the historical incidence of these tumours is below 10% for female rats, which was clearly exceeded by the high dose response. In this study, control tumour incidence was 3.3% and therefore well within the stated upper limit of the historical controls. The report also states that the high dose male incidence, which was slightly lower than that of the male controls, remained within the expected fluctuations in the historical controls. However, there does seem to be a possibility that the female control group incidence of these neoplasms could have been at the low end of the historical range. Studies from the same laboratory that both antedate and post-date the MTBE experiment cite female control group lymphoma and leukaemia incidences that can vary widely, for example, 2% [191]; 0.7% [192]; 13% and 20% [133]; 7% [193]. Similar wide variations occur in male rats from the same laboratory. In lifetime studies, lymphomas occur spontaneously with variable and sometimes high incidence (depending on the strain of rat or mouse), which makes the interpretation of these tumours difficult. There exist, therefore, some doubts regarding the reported significant result, particularly in view of the lack of confirmation of the observation in male rats within the same MTBE experiment or in F344 rats in the inhalation study with MTBE [93]. Furthermore, no increased incidence of leukaemia or lymphoma was observed in mice exposed to MTBE by inhalation [130] or in either rats or mice exposed to TBA in drinking water [98, 99].

The pooling of different lymphoreticular neoplasms for statistical analysis in the Belpoggi et al. [95, 97] study is not a feature that detracts from the conclusions of the authors. The various lymphoreticular neoplasms involved (lymphoblastic lymphoma, lymphoblastic leukaemia and lymphoimmunoblastic lymphoma) are solid and circulating forms of the same lymphoid neoplasm, so any distinction on this basis would be artificial. Furthermore, they are all derived from B cells, so there is a biological justification for maintaining them as a group.

The most frequently found neoplasm in both the 250 and 1000 mg/kg bw gavage MTBE exposure groups was lymphoimmunoblastic lymphoma, with a proportion of > 85% of the combined incidence localised in the lungs. Leukaemia and lymphomas should usually be expected to arise in lymphoid tissue of some kind. They may subsequently move to other tissues, but there should never be an instance where the frequency of these neoplasms is greater in any single, non-lymphoid tissue within a group of rats than in lymphoid tissues. The authors assumed that they arose from peribronchial and perivascular lymphoid tissue [97]. Such neoplasms have been known to arise from the increased amount of lymphoid tissue in the lungs due to chronic pulmonary inflammation [119–121]. The significance of chronic inflammatory changes in the lungs for the occurrence of pulmonary haemolymphoreticular dysplasias and neoplasias was not addressed and this cannot be done on the basis of the publications because of the lack of detailed description of

lung pathology. Nelson [120] showed that the elimination of chronic respiratory disease from rats could reduce the incidence of lymphosarcomas almost to zero. Sinkeldam et al., [194] found a high incidence of lymphoreticular tumours of the peribronchial tissue in high dose male Wistar rats treated with acesulfame-K. Upon re-testing in the same strain, but now cleaned and bred under SPF conditions, chronic respiratory disease was absent and no lymphoreticular tumours were seen. Thus, the tumours found in the first experiment appeared to be unrelated to treatment with acesulfame-K, but was associated with the occurrence of peribronchial and perivascular lymphoid accumulations in response to chronic inflammation of the lungs [195].

An interesting difference between the studies with Sprague-Dawley and F344 rats is that the haematopoietic neoplasms involved are quite different. Those of Sprague-Dawley rats are B-cell derived, while the common neoplasm in F344 rats are non-B-, non-T-cell derived. These spontaneous neoplasms of F344 rats arise in spleen. B-cell neoplasms were not increased except in the Sprague-Dawley rat experiment.

No effect on any haematopoietic neoplasms has been found in CD-1 mice treated with MTBE or in $B_6C_3F_1$ mice treated with TBA or methanol. Thus, the response described by Belpoggi et al. has not been described in another rat study with MTBE, in a mouse study with MTBE or in either rats or mice treated with the more persistent of the primary metabolites of MTBE. The only support for their result is the drinking water study of methanol conducted in rats within the same laboratory [133].

2.10 Liver Cell Neoplasms

An increase in hepatocellular adenoma incidence (Table 5) was observed in female CD-1 mice exposed to 8000 ppm MTBE by inhalation (2/50 versus 10/50, $p = 0.014$, one-sided Fisher's exact test). In the same experiment, no increase in adenomas was observed in female mice exposed to 3000 ppm MTBE or in male mice at any concentration level. The historical incidence of adenomas for female CD-1 mice in this laboratory ranged up to 4% [94]. The incidence of hepatocellular carcinomas was higher in male mice of the 8000 ppm concentration group, but not significantly so (12/49 versus 16/49) and there was no significant increase in these male mice of hepatocellular adenomas and carcinomas combined. The historical incidence of adenomas for male mice adenomas and carcinomas combined ranged up to 33%. Thus, the incidence of adenomas in the highest dose female mice was in excess of the historical controls, while the highest combined incidence of adenomas and carcinomas in male mice was within the upper limit of the historical controls. The incidence of hepatocellular adenomas or carcinomas was not increased by treatment of male $B_6C_3F_1$ mice with TBA in drinking water [98] or in either sex by methanol (which would generate formaldehyde within

the mouse tissues) administered by inhalation [118]. In female $B_6C_3F_1$ mice treated with TBA at a concentration of 20 mg/ml the incidence of hepatocellular adenomas or carcinomas was significantly lower than in the controls, an effect that the authors attributed to an unusually high control group incidence (25/59 or 42%). High incidence of hepatocellular neoplasms in general appear to be associated with increased body weight [146]. The mean historical control group incidence for hepatocellular carcinomas in female mice in the NTP database at the time of the study was 49/239 (20.5%), with a range of 8–42% [98]. The same high body weight explanation presumably applies equally to the low and middle dose group female mice, in which the incidences were similar to that of the controls. While there was a clear reduction in body weight in the high dose female mice that correlates with the lower tumour incidence, the body weight differential in male mice was small and was unlikely to account for the highly significant reduction ($p = 0.0078$, Fisher's exact test, one-tailed) in the incidence of hepatocellular adenomas in the high dose group. Carcinoma incidence was not significantly affected. It is considered that the variations observed are typical of those that occur in rodent carcinogenicity studies and no interpretation of a beneficial effect of exposure to TBA is intended. No significant effect on liver tumour incidence was observed in rats exposed to MTBE, TBA or methanol [93, 95, 96, 98, 133].

In spite of the weak evidence for a neoplastic effect in liver, MTBE has been tested for later stage effects in carcinogenesis because unleaded gasoline, which has anti-oestrogen effects similar to those seen with MTBE (effects of which include decreased uterine, ovary and pituitary weights, reduction in uterine glands and in the number of cervical and vaginal epithelial layers), produced an increase in the incidence of liver tumours in mice that had been treated previously with *N*-nitrosodiethylamine (NDEA) [115]. This study also demonstrated that liver tumour formation in mice was secondary to the interaction of components of unleaded gasoline with the oestrogen receptor. MTBE, however, does not appear to react with this receptor [123]. To test whether MTBE might have an effect similar to that of unleaded gasoline, $B_6C_3F_1$ mice (the same strain as used in the unleaded gasoline experiment) were given a single i.p. injection of NDEA and then exposed to 8000 ppm MTBE vapour for up to 32 weeks. Generally, all MTBE-exposed mice had increased liver weights and hypertrophy of the centrilobular and mid-zonal areas. There were no significant differences in hepatic microsomal enzyme activity or hepatocyte proliferation between control and NDEA treated mice after MTBE exposure. After 32 weeks of MTBE exposure, the mean volume fraction and mean volume of the initiated hepatic foci decreased by about 25% as compared to controls and is therefore contrary to what would have been expected of a substance having an enhancing effect late in the neoplastic process [115]. Thus, this experiment provided no support for MTBE as an enhancer of preneoplastic effects following treatment with NDEA.

2.11 Thyroid Follicular Cell Neoplasms

In $B_6C_3F_1$ mice treated with drinking water containing TBA concentrations of 0, 5, 10 or 20 mg/ml, the incidence of thyroid follicular-cell adenoma (Table 5) was significantly increased in females at the high dose (1/60 versus 9/59, $p = 0.039$). The overall incidence in this dose group included one mouse with bilateral adenomas. Such an event would normally be expected either after exposure to a very potent carcinogen or in circumstances where genetic factors in the animal population were important. The historical control incidence for follicular cell adenomas in female mice in NTP studies at the time was 8/238 (2.2%), range, 0–5%, a range that was exceeded in the highest dose group. No carcinomas had been recorded in historical control group female mice. No significant increase was observed in follicular cell adenomas in male mice, although one of two tumours found in the high dose group was a carcinoma. These weak indications of a tumourigenic effect occurred against a background of hyperplasia that was more common in both males and females of the two higher dose groups. Follicular cell adenomas of the thyroid gland were distinguished from hyperplastic foci by their more complex structure and greater variation in size, nucleus to cytoplasm ratio, or nuclear chromatin pattern [98, 99]. In contrast, no increases in thyroid follicular cell neoplasms were observed in male or female CD-1 mice treated with MTBE by inhalation [94, 130]. The MTBE concentrations used in this study would have delivered internal doses of TBA, upon metabolism, at least as high as those experienced by mice in the TBA drinking water study (Table 4). No increases in thyroid tumour incidences were observed in rat carcinogenicity studies with MTBE [93–96] or TBA [98, 99].

2.12 Genotoxicity

A large majority of the genotoxicity assays conducted with MTBE, ETBE and TBA found no significant effects (Table 6). None of the studies with ETBE produced significant responses. Significant responses in genotoxicity tests have been reported in five studies of either MTBE or TBA, each using a separate type of test system, but the data they provide are very weak and are counterbalanced by a number of negative studies using the same dosing and testing methods or assaying the same endpoint. Except for mutagenicity in mouse lymphoma cells, none of the studies indicating significant activity has been independently verified. Two of the assays producing significant responses will be discussed here because of the importance they command in regulatory mutagenesis (*Salmonella typhimurium* TA102 and mouse lymphoma TK+/– mutation assays); however, all significant effects of MTBE and TBA have been discussed elsewhere [4, 5] with the conclusion that they do not provide convincing evidence for genotoxicity of either MTBE or TBA.

MTBE and TBA were reported to be mutagenic in *Salmonella typhimurium* TA102 with a maximum response at about 2000 μg/plate [196]; however, one other study of MTBE and two of TBA, all compliant with Good Laboratory Practice (GLP), using doses up to 5000 μg/plate and both DMSO and aqueous vehicles, reported no significant increase in mutations from MTBE or TBA in TA102 [197]. In addition, MTBE has been tested in seven Ames design assays using *S. typhimurium* TA98 and TA100, six using TA1535, five using TA1537, four using TA1538 and one each using TA102 and TA104, while TBA has been subjected to one assay with TA100, TA98, TA1535, TA1537 and TA1538. None of the results from these assays indicated increased numbers of mutations. Budroe et al. [198] has asserted, however, that prior to 1999 the assays used were not specifically sensitive to oxidative damage.

Observations relevant to an interpretation of these data are [1] that strain TA102 is not uniquely sensitive to either reactive oxygen species or aldehydes, since strain TA100 (used in many of the studies) also reacts with these chemical species [199]; and [2] the scavenging property of DMSO is not an important factor in reducing sensitivity to either reactive oxygen species or aldehydes [199, 200]. In addition, TBA is itself regarded as an oxygen radical scavenger, not a generator of oxidative damage. TBA is reported to inhibit prostaglandin synthesis by scavenging needed hydroxyl radicals [201] as well as to protect DNA from the effects of radiation [202–204]. Thus, apart from the non-GLP, unsubstantiated study reported by Williams-Hill et al. [196], there is no evidence of mutagenicity in bacterial systems for MTBE or TBA.

Two reports have shown that MTBE is mutagenic in the mouse lymphoma cell line L5178Y TK+ / – in the presence, but not the absence of rat liver S9 [66, 205] and one of these [205] demonstrated that the activity was due to formaldehyde. This conclusion is reinforced by the observation that methanol, a source of formaldehyde similar to MTBE upon oxidation, is active in the same assay only in the presence of S9 [206], while TBA is not active at concentrations up to 5000 μg/ml [207]. In vivo, formaldehyde is rapidly detoxified and taken up into one-carbon metabolism by an enzymatic mechanism that first requires reaction of formaldehyde with sulphydryl groups such as reduced glutathione or cysteine, but neither of these chemicals is a component of Fisher's medium formulation (used by Mackerer et al. [205]). Consequently, the normal detoxification mechanism is at least partially compromised in vitro and the mutagenicity observed is an artefact.

3 Conclusions

Early human studies on gasoline containing up to 15% MTBE were more in the nature of reports of complaints from communities, rather than well-controlled epidemiological of experimental designs. The weaknesses inherent

in these early approaches were recognised by the investigators involved, but their reports nevertheless stimulated much public concern and debate. Seven key symptoms were identified as being specifically associated with exposure to MTBE and there emerged a particular subset within human populations who were believed to be sensitive to these symptoms, while other people appeared to be unaffected.

It became clear that resolution of the controversy would require special efforts to be made. Controlled environment studies were conducted in which volunteers were exposed to likely or greatly exaggerated atmospheric concentrations of MTBE. While symptoms were sometimes recorded, the early reports of malaise could not be confirmed in the chamber studies. Therefore, these studies became progressively focussed on those people who reported themselves as being sensitive to MTBE. The most recent and complex study [55] compared responses of SRSs and non-SRSs after exposure, not to MTBE in air, but to MTBE in a gasoline matrix in air. In this respect, the study is unique. Its conclusions were that there was no effect on neurobehavioural or psychophysiological performance, but there may have been an increase in symptoms recorded by SRS subjects exposed to gasoline containing 15% MTBE, but not to gasoline containing 11% MTBE. The symptoms recorded did not confirm the existence of the particular key set of symptoms suggested by early community studies and the dose relationship with the frequency of symptoms recorded was not simple. This last effect was presumably due to chance differences in variation within the groups that permitted, for example, a significant difference between gasoline + 15% MTBE and gasoline + 11% MTBE, but not between gasoline + 15% MTBE and gasoline alone. Even in this study there may have been bias in subject selection, since it is possible that people even more sensitive than the recruited SRSs could have been reluctant to participate in this study. Indeed, this and several other studies have suffered from low participation rates. Therefore, the current situation with regard to symptomology is that a single, well-designed, objective study has not confirmed the existence of a special set of MTBE-related symptoms and evidence for an especially sensitive population rests on a less than satisfactory dose-response relationship. It remains, however, a single study and its conclusions and uncertainties require reinvestigation in order to define whether there is or is not a small proportion of human populations who are highly sensitive to the development of symptoms of malaise induced by MTBE, even if their behaviour and psychophysiological performance remains unaffected. It is now more than five years since this publication and there appears to be no indication that there will be any follow-up efforts to obtain the elusive evidence that may confirm or refute the existence of particularly sensitive individuals.

Metabolism and kinetic studies have shown that the initial elimination of MTBE from blood was similar in rodents and human volunteers, but later phase elimination was much slower in rodents. Furthermore, the overall elimination of TBA is much more rapid in man than in rodents. These data

indicate that internal exposure to more elevated metabolite concentrations will be for shorter periods in exposed people than in rodents. If it can be assumed that the internal doses required to elicit a particular dynamic response is similar in man and rodents, then it might be predicted that man would be less susceptible at any given exposure level of MTBE. MTBE is regarded as a skin irritant, but neither MTBE nor ETBE is an eye irritant or a sensitizer. Non-human experimental studies have not revealed significant neurotoxicity (although CNS depression does occur at very high concentrations), toxicity to reproduction or genotoxicity. Non-specific toxicity was observed in multigeneration studies in rats at MTBE concentrations of 3000 ppm and higher, exposure to 400 ppm being without significant effect. In developmental studies in rats, mice and rabbits, mouse was the only species in which an adverse effect was found. Cleft palate was increased at 8000 ppm in one study in which there were also increases in skeletal variations at 4000 ppm, whereas these effects were not observed at lower doses or in another study in which the highest vapour concentration tested was 2500 ppm. Markedly elevated cortisone concentrations have been measured in CD-1 mice exposed to 8000 ppm MTBE, so it is likely that the cleft palates were an example of the common murine response to stress and not an issue for concern, given the lack of effect in the other two species.

With regard to carcinogenicity, there is some evidence for carcinogenicity in rats, but there is unlikely to be any human relevance in these observations. Eight long-term studies relevant to MTBE have been conducted in rats and mice: three with MTBE itself, two with its major persistent, primary metabolite, TBA, and three with methanol, which has been viewed here as a particularly good model for the unique internal generation of formaldehyde, the other primary metabolite of MTBE. In this respect, methanol serves to mimic MTBE, although the rat enzymes involved in formaldehyde generation are different. Some increases in tumour incidence have been observed in most of these studies, but consistency of outcome was lacking and even some degree of replication was observed in only three cases. These were:

1. renal tubule cell adenoma in male rats exposed to MTBE in one study and TBA in another, but not to MTBE in a third study;
2. Leydig cell adenoma in male rats exposed to MTBE in two studies, but not to either TBA, in one study, or methanol in two;
3. B-cell derived lymphoma/leukaemia in female rats exposed to MTBE in one study and methanol in another within the same laboratory, but not MTBE or methanol (or TBA) in other studies. No other lymphoreticular neoplasm was increased in any study.

Conclusions reached from the foregoing discussions of the various neoplasms are as follows.

The effects of MTBE and TBA on male rat renal tubule cells are weak, sex specific and not observed in mice of either sex. A re-evaluation of the MTBE

study reduced the incidence of these tumours so it was barely significant. The available data strongly support the hypothesis that the mode action is dependent on α_{2u}- globulin nephropathy and exacerbation of chronic progressive nephropathy, both of which are considered of no human relevance.

Although there were increases in Leydig cell tumour incidence in two experiments, the evidence is not clear that MTBE has induced Leydig cell adenomas in rats, because of the lower than expected control group incidence in one study and confounding by inter-group age differences in the other. Statistical re-analysis by methods that appropriately took account of the longevity differences in the latter led to the conclusion that there was no significant increase in Leydig cell tumour incidence. In addition, no testicular tumours were induced by MTBE in mice or by either TBA or methanol in rats or mice. A comparison of characteristics of testicular cancer in man with those in rats and mice suggest that mice provide the better model for the disease, although some reservations must be retained because Leydig cell tumours are very rare in man, a factor that makes their study particularly difficult. However, the balance of evidence is strongly in favour of the apparent tumour increases in rats being of no human relevance.

An effect of MTBE on the incidence of lymphoreticular neoplasms has not been clearly demonstrated. In a gavage study with MTBE, an increased incidence was observed in female rats but no effect was observed in male rats. The effect could be in response to local pulmonary conditions (e.g., infections), rather than to MTBE itself (which was administered by gavage), since it is in the lungs that most of these neoplasms were found. In conclusion, on one hand, there is doubt regarding the pathogenesis of these neoplasms while, on the other, if the response was truly to MTBE or its metabolite, formaldehyde, it could be a C_{max} effect at which normal defence mechanisms had been overwhelmed; this would be an extremely unlikely occurrence during human exposure.

Hepatocellular adenomas were significantly higher in female CD-1 mice exposed to 8000 ppm MTBE, but there was no supporting evidence either from male mouse adenomas or adenomas and carcinomas combined within the same study or from mice or rats of any of the other studies considered relevant to an evaluation of MTBE carcinogenicity. The scientific basis for an assumption of carcinogenicity is, therefore, extremely weak and is not an adequate basis for considering liver as a target for neoplastic effects of any human significance.

Thyroid follicular cell adenomas were increased in female mice treated with TBA, but this result lacks any independent supporting evidence from a number of studies in mice and rats. MTBE has been shown in other studies to induce hepatic hypertrophy and can induce certain liver enzymes, including UDP-glucuronosyltransferase, so it is possible that this hepatic effect could accelerate the biliary excretion of thyroid hormones, which in turn would stimulate TSH release and hyperplasia within the thyroid. How-

ever, there was no evidence for a hepatic effect of TBA within this mouse carcinogenicity study; therefore, no internal evidence exists for a hormonal mechanism of thyroid follicular cell induction. In the absence of evidence of thyroid oncogenicity (benign tumours only were increased) from studies with MTBE itself, this result is not strong evidence for an oncogenic response.

Decreased incidences were found in some neoplasms. These included fibroma and fibroadenoma of the mammary gland in female Sprague-Dawley rats treated with MTBE; pancreatic islet adenoma or carcinoma in male rats treated with TBA; Harderian gland adenoma and carcinoma in male mice and hepatocellular adenomas and adenomas and carcinomas combined in male mice also treated with TBA. These findings received no supporting evidence from other studies and are therefore considered to be chance findings. It is considered that the variations observed are typical of those that occur in rodent carcinogenicity studies and no interpretation of a beneficial effect of exposure to TBA is intended.

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MTBE: WHO Guidelines and Taste and Odour Issues for Drinking Water

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Abstract The World Health Organization (WHO) Guidelines for drinking water quality are one of the most important sources of advice on the safety and acceptability of drinking water around the world. They are used as the basis for standards in a substantial part of the world and are respected for their independence and transparency. WHO bases its evaluations on international peer-reviewed evaluations where possible, but will also use peer-reviewed documents prepared by member states. In the case of methyl *tert*-butyl ether (MTBE), several such documents exist that provide the basis for an international consensus on the science surrounding water contamination by MTBE. The toxicology of MTBE indicates that while it induces tumours in rodents, there is doubt as to the significance to humans and the mechanism appears to be a high-dose, non-genotoxic phenomenon. MTBE can be detected in water by taste and odour at low concentrations. WHO considered that it was unnecessary to set a health-based guideline value, since any such value would be substantially above the concentration at which MTBE could be detected by taste and odour.

Keywords Drinking water · WHO Guidelines · Taste and odour · MTBE

1 Introduction: The WHO Guidelines for Drinking Water Quality

The World Health Organization (WHO) is the primary arm of the United Nations dealing with health. It is apolitical and provides a vital source of advice and assistance that can be applied across the world. WHO is greatly respected and, as a consequence, has a considerable influence in most countries. WHO is strengthened by its network of regional offices, which provide an unsur-

passed network that can provide a local dimension to WHO's operations and initiatives.

The Sanitation and Health Division is responsible for the key areas of drinking water and sanitation. These remain vital areas for WHO with more than 40% of the world's population, some 2.6 billion people, not having access to basic sanitation and more than one billion with no access to safe sources of drinking water. This is a hugely important area for improving not only health but also the economic development of developing countries. It is estimated that for every US dollar invested in water and sanitation improvements, the resultant economic benefits will return between $3 and $4. Governments such as that in Thailand see the provision of safe and good drinking water as a cornerstone of their development strategies. An important part of this process is drinking water quality, which covers microbiological contamination, chemical contamination and acceptability.

The first edition of the Guidelines for Drinking Water Quality was published in 1984. Prior to this International and European Standards had been published. The change to the name Guidelines was a recognition that WHO provides a basis for setting standards but the Guidelines have no formal legal force; standards are the responsibility of the state. In addition, the Guidelines emphasise the need for member states to take into account local geographical and socio-economic considerations in adapting the Guidelines for setting standards. The first edition concentrated on microbial quality, which remains the highest priority, but increased the range of chemical substances that were covered. These included guideline values for a number of substances based on criteria for consumer acceptability. The second edition of the Guidelines was published in 1993 and reflected the evolution of the Guidelines in providing an increasingly helpful and practical source of advice for member states. Among the changes was an increase in the number of chemicals that were considered, although there was still a major emphasis on microbial quality and emerging pathogens. A significant change was that guideline values were only developed for substances on a health basis. No formal guideline values were set on the basis of consumer acceptability because acceptability can vary significantly under different circumstances. However, there was extensive discussion of consumer acceptability, which was still seen as a vital component of good water quality. Water that is not acceptable or is less acceptable in terms of taste, odour or appearance can drive consumers to abandon safe supplies and turn to alternative supplies which may be less safe from a microbiological point of view or which may be much more expensive. It can also significantly undermine consumer trust in the water supply and water supplier. In the development of the second edition WHO increased both the transparency of the process and the representation of developing countries in the expert groups. There is always difficulty in producing guidelines that are to provide a basis for such a wide range of countries, from highly developed western nations to countries with only very limited development.

Having established a 10-year revision process, the revision of the Guidelines continued with the objective of developing the third edition. This was launched at the end of September 2004 [1]. The third edition represents a significant development in thinking that has also been mirrored by members of the international water community, including suppliers and regulators, in looking to move from a system for ensuring drinking water quality by measuring an increasing number of parameters in the final product to one that is much more based on prevention and assurance of quality at all times. This is particularly important for microbial pathogens since the measurement of indicator organisms means, by necessity, sampling only very small amounts of the water supplied, although even a short breakdown in the barriers against pathogens can lead to significant outbreaks of waterborne disease. The third edition is based around the concept of water safety plans providing an integrated catchment-to-tap approach, with the emphasis on identifying threats and dealing with them by both prevention at source and by managing the drinking water process to ensure that the system can deliver safe water at all times. The Guidelines still contain a significant number of guideline values, which are an important part of the verification process and provide a vital means of assessing the risks to health, although the acceptability of risk will vary between different member states, from the risk averse developed countries to those with more pressing and immediate problems. One notable feature of the third edition is that a number of guideline values for chemicals have increased as new scientific data have reduced the uncertainty.

One of the changes associated with the third edition of the Guidelines is that there is a network of supporting documents on a range of topics, including groundwater and the importance of groundwater contamination and a protocol for managing chemicals in drinking water. These documents provide an important source of more detailed information and advice that can be used by authorities and water suppliers in member states. They are also of potential importance to industry in helping to develop environmental strategies to make the use of their products more sustainable.

To avoid the difficulties involved in carrying out large revisions of the Guidelines, WHO has instigated a process of rolling revision. The availability of the Internet has allowed a process of continuous updating, although there will be a 10 year consolidation into a version which will be available in hard copy and on CD. The rolling revision process is reflected in annual addenda to the Guidelines. Methyl *tert*-butyl ether (MTBE) was considered as part of the first addendum and is now included in the Guidelines. In developing guideline values for chemicals WHO has laid down broad policy approaches to the methods used and the assumptions that are incorporated. It is considered that achieving the highest level of transparency is most important in maintaining the scientific independence and credibility of the Guidelines. To this end experts who are invited to participate in the process are expected to attend

as experts in their own right and not as representatives of a particular organisation. In addition, the third edition has seen the introduction of web posting and a period of public consultation for background documents before the final task group considerations. These periods for comment are important because they provide an opportunity for new data to be considered and for additional data on use and occurrence to be made available. This process will continue in the future and will increasingly provide a greater level of transparency, although there are some difficulties in managing the process for specific contaminants that are the subject of intense international scrutiny and often pressure group activity.

Substances are selected for consideration in the Guidelines on the basis of the following criteria:

- There is credible evidence of occurrence of the chemical in drinking water, combined with evidence of actual or potential toxicity; or
- The chemical is of significant international concern; or
- The chemical is being considered for inclusion or is included in the WHO Pesticide Evaluation Scheme (WHOPES) programme (approval programme for direct application of pesticides to drinking water for control of insect vectors of disease).

To this end the information may come from the scientific literature or member states may ask for guidance from WHO. Where possible WHO will base its considerations on one of the internationally recognised and peer-reviewed documents developed by the International Programme on Chemical Safety (IPCS) and take into account evaluations by other WHO organisations, such as the International Agency for Research on Cancer (IARC), the Joint Expert Committee on Food Additives and Contaminants (JECFA) and, for pesticides, the evaluations by the Joint Meeting on Pesticide Residues (JMPR). Where such evaluations are not available, or cannot be generated in a suitable time frame, other international peer-reviewed documents developed by member states can provide the basis for a background document, but this does not include accepting proposed standards that may include policy considerations applicable to that state. In considering MTBE, WHO has a significant advantage in that there is an existing Environmental Health Criteria document from IPCS and MTBE has also been considered by IARC. There are also considerable data available on the taste and odour characteristics of MTBE in drinking water.

In setting guideline values, WHO does not seek to have an ever increasing list of guideline values that may not be necessary for protection of health. In cases where the health-based value is well above the concentrations normally found in drinking water no formal guideline is presented, although the health-based value is discussed in the summary and in the background document. Consumer acceptability is also considered and the published information of the particular aspect of consumer acceptability that is of importance

is discussed. This is necessary for substances that can cause problems for acceptability at low concentrations, since it is important to provide a sound basis for reassuring member states and consumers about the health consequences where there is a problem with acceptability.

The Guidelines are a major influence on drinking water standards and regulation in most parts of the world, and most member states rely heavily on the Guidelines for the development of their national standards. The European Union Directive on drinking water quality, for example, is largely based on the WHO Guidelines as an independent source of scientifically based advice. This is actually enshrined in the Directive and with the expansion of the European Union will affect most of Europe. The Guidelines are the primary source of advice throughout South America, Eastern Europe, Asia and Australasia. With drinking water being a major part of the international strategy for health, the Guidelines are only likely to become more important in the future. An indication of the influence of the Guidelines is that volume 1 with the summary and recommendations is WHO's best-selling document. The free access to the Internet version has increased the use even further, and the Guidelines are set to remain the most important source of advice and guidance for member states.

2 MTBE in the Guidelines

MTBE was considered by WHO at the request of several member states because of concerns regarding potential contamination of drinking water sources and the need to maintain consumer confidence. A number of member states had identified problems in which MTBE had been found in drinking water, primarily as a consequence of the release of unleaded gasoline from storage facilities and gas stations. Because it is a high profile chemical it is particularly important that the process not only takes an appropriate international scientific consensus, using the best scientific data, but also is seen to have followed that process. Several internationally recognised peer-reviewed documents were available. These included a toxicological review and risk assessment carried out by IPCS [2] and an evaluation of the potential carcinogenicity by IARC [3]. There were also peer-reviewed evaluations available that had been prepared by member states. There was, therefore, a significant body of data that had been assessed for its quality, and that provided a sound basis for consideration of the health effects of MTBE through drinking water and provided adequate data to enable the determination of a health-based value [4].

3 Taste and Odour

One of the features of MTBE is its distinctive taste and odour. This has been both a benefit and a disadvantage. The taste and odour means that it can be readily identified in the case of accidental contamination of a water source, but when that water source is groundwater the volatility of MTBE is not a route of dissipation as it is in surface waters. This means that it can be present for extended periods of time. In addition, groundwater sources usually undergo much less treatment than surface-derived drinking water. The potential for MTBE to impart a taste or odour, particularly one that is considered to be unpleasant, is of great significance in terms of consumer acceptability.

A number of studies have been carried out to determine the levels at which odour can be detected in drinking water. These studies are normally carried out in a laboratory setting with trained panellists in order to maximise the sensitivity of the study. This is important because the number of panellists must be highly restricted, but there is a wide variation in the sensitivity within the population and it is not necessarily possible to cover the full range. Set against this is the fact that under normal circumstances of use individuals would not be routinely seeking to detect odour in water, much water is drunk cold, which reduces the potential for the volatilisation of odorous substances, while other water is boiled resulting in increased loss of such substances before consumers drink the water. In addition, while every effort is made to exclude extraneous odours in the laboratory, this is not the case when the consumer uses the water. Odour and taste are closely linked and in most circumstances both odour and taste thresholds are determined in laboratory studies. In many cases one is clearly lower than the other but with MTBE the data are mixed. This may reflect the volatility of MTBE and its release in the mouth.

WHO considered taste and odour in some detail [4]. Taste and odour thresholds reported in four studies considered by the US Environmental Protection Agency (USEPA) [5] were 24–135 $\mu g\,l^{-1}$ for taste and 15–180 $\mu g\,l^{-1}$ for odour. These studies were by: Young et al. [6], in which the geometric means for taste and odour were 48 and 34 $\mu g\,l^{-1}$, respectively; the American Petroleum Institute [7], in which calculated threshold values were 39 $\mu g\,l^{-1}$ for taste, 45 $\mu g\,l^{-1}$ for odour detection and 55 $\mu g\,l^{-1}$ for odour recognition, and subjects described the taste of MTBE in water as "nasty", bitter, nauseating and similar to rubbing alcohol; Prah et al. [8], in which the concentration of MTBE in distilled water that was identified as having an odour by 50% of the study participants was 180 $\mu g\,l^{-1}$; and Dale et al. [9], in which the range for 60% probability of detecting the odour of MTBE in odour-free water was 43–71 $\mu g\,l^{-1}$, whereas the corresponding range for taste was 24–37 $\mu g\,l^{-1}$. A recent study specifically designed to set an odour threshold for MTBE in drinking water used a panel of 57 people and a protocol based on the American Society for Testing and Materials (ASTM) method E679-91 [10]. Eight

concentrations of MTBE in water ranging between 2 and 100 $\mu g\,l^{-1}$ were used with a 1.75 step factor. The geometric mean detection threshold for the 57 subjects and the recommended odour threshold was 15 $\mu g\,l^{-1}$. This was similar to the odour threshold determined by Young et al. [6].

4
Overall Evaluation of Potential Toxicity

Unusually there have been three lifetime studies in rodents that are available to assess the long-term toxicity and carcinogenicity of MTBE. WHO [4] based its evaluation on the IARC and IPCS assessments and these concluded that, although caution should be exercised in extrapolating the results of these studies to humans, the finding of a dose-related increase in lymphomas and leukaemias in female rats in the oral study could not be simply dismissed in spite of limitations in the study. Increased testicular interstitial Leydig cell adenomas observed in the oral study and in the rat inhalation study were only apparent at the highest dose in the oral study and were within the historical control range for the inhalation study. It was also considered that this type of tumour may not be relevant to humans in view of the fact that it can be influenced by hormonal status and there are significant differences between humans and rats in this respect. Other tumours were also considered to be of questionable relevance to humans.

There is an extensive range of studies on the genotoxicity of MTBE and WHO noted that with one exception these studies have been negative. There is some question over the mechanism of this one positive study, and WHO considered that the weight of evidence suggests that MTBE is not genotoxic and that the mechanism of action of MTBE is more likely to be non-genotoxic than genotoxic.

IPCS [2] in its evaluation concluded that MTBE should be considered to be a rodent carcinogen, but that it is non-genotoxic with carcinogenicity only apparent at high levels of exposure that also produce other adverse effects. IARC [3] classified MTBE in group 3, not classifiable as to its carcinogenicity to humans, based on limited evidence in experimental animals and inadequate evidence in humans.

5
WHO Evaluation

In view of the toxicological evidence and the evidence on taste and odour WHO decided that it was not necessary to develop a health-based guideline value for MTBE, since any such guideline value would be significantly above the concentration at which it would be detected by odour. The evaluation

was based on the lowest concentration that could be detected in the study by Young et al. [6], which was 15 $\mu g\,l^{-1}$.

In view of this conclusion, it is not anticipated that member states would set numerical standards for MTBE, although most have a requirement that taste and odour should be acceptable to consumers. However, the introduction of water safety plans (WSPs) means that the hazard to drinking water, in terms of acceptability, should be identified and controls put in place to prevent the spillage or leakage of gasoline containing MTBE. In this respect the Guidelines have taken a much more proactive approach to the prevention of contamination of drinking water sources. Since the removal of MTBE by drinking water treatment, particularly from groundwater, is potentially both difficult and expensive, the preventive approach advocated by WSPs is practical and appropriate. In view of the enormous influence of the WHO Guidelines around the world, it is likely that the approach advocated in the Guidelines will be widely adopted.

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Subject Index