Biodegradation of MTBE in reactors

Waul, Christopher Kevin

Publication date:
2007

Document Version
Publisher's PDF, also known as Version of record

DTU

Biodegradation of MTBE in Reactors

Christopher Kevin Waul

INSTITUTE OF ENVIRONMENT & RESOURCES

Biodegradation of MTBE in Reactors

Christopher Kevin Waul

Ph.D. Thesis
November 2007

Institute of Environment & Resources
Technical University of Denmark

Biodegradation of MTBE in Reactors

Cover: Torben Dolin & Julie Camilla Middleton
Printed by: Vester Kopi, DTU
Institute of Environment & Resources
ISBN 978-87-91855-35-1

The thesis will be available as a pdf-file for downloading from the institute homepage on: www.er.dtu.dk

Institute of Environment & Resources
Library
Bygningstorvet, Building 115,
Technical University of Denmark
DK-2800 Kgs. Lyngby

Phone:
Direct: (+45) 45 25 16 10
(+45) 45 25 16 00
Fax: (+45) 45 93 28 50
E-mail: library@er.dtu.dk

TABLE OF CONTENTS

PREFACE

This thesis is based on research done for a PhD project undertaken from October 2003 to November 2007 at the Institute of Environment & Resources, Technical University of Denmark (DTU). The supervisors were Professor Erik Arvin and Associate Professor Jens Ejbye Schmidt. The research was financed by a scholarship from DTU.

The thesis consists of a literature review book chapter on the subject, followed by seven journal and conference papers. The literature review book chapter has been published as follows:

> Waul C, Arvin E, Schmidt JE. 2007. Microbial degradation of MTBE in reactors. In: Barceló D, editor. Fuel Oxygenates. Series: The Handbook of Environmental Chemistry. Vol 5: Water Pollution, Springer. p 213-248 (The original publication is available at www.springerlink.com)

The journal and conference papers are as follows:

I) Waul C, Arvin E, Schmidt JE. 2007. Model description and kinetic parameter analysis of MTBE biodegradation in a packed bed reactor (submitted).

II) Waul C, Arvin E, Schmidt JE. 2007. Modeling the competitive effect of ammonium oxidizers and heterotrophs on the degradation of MTBE in a packed bed reactor (submitted).

III) Waul C, Arvin E, Schmidt JE. 2007. Effects of co-contaminants and factors influencing the startup time of MTBE degrading bioreactors. Third European Conference on MTBE, June 7- 8. Antwerp, Belgium. p 89-93.

IV) Waul C, Arvin E, Schmidt JE. 2007. Competition for oxygen and occupancy in a packed bed biofilm reactor between MTBE degraders, ammonium oxidizers and heterotrophs. Extended abstract accepted for the "Biofilm Technologies" conference, January 2008, Singapore.

V) Waul C, Arvin E, Schmidt JE. 2007. Estimation of the fraction of biologically active methyl *tert*-butyl ether degraders in a heterogeneous biomass sample. Biotechnology Letters. DOI 10.1007/s10529-007-9509-0.

VI) Waul C, Arvin E, Schmidt JE. 2007. Long term studies on the anaerobic biodegradability of MTBE and other gasoline ethers (submitted).

VII) Waul C, Christensen N, Mosbæk H, Arvin E, Schmidt JE. 2004. Fuel oxygenates toxicity to the anaerobic degradation process. Second European Conference on MTBE. Barcelona, Spain. p 51-55.

The papers are not included in this www-version but can be obtained from the library at the Institute of Environment & Resources, Bygningstorvet, Building 115, Technical University of Denmark, DK-2800 Kgs. Lyngby (library@er.dtu.dk).

ACKNOWLEDGEMENTS

My gratitude goes out to the following persons for their contribution to the completion of this thesis:

- My supervisors Professor Erik Arvin and Associate Professor Jens Ejbye Schmidt – they have given me dedicated guidance throughout the entire study.

- Associate Professors Nina Christensen and Hans Mosbæk. Hans has taught me MTBE analysis and Nina has advised me during the initial phase of the study.

- Henrik Spliid, Department of Informatics and Mathematical Modelling, DTU for advice on parameter estimation. Damien Batstone, now at the University of Queensland, Australia, and Willi Gujer, at the ETH Zürich, for advice on reactor modelling.

- The technical staff at E&R who have helped me with laboratory analysis and experimental setups.

- Julie Camilla Middleton from the graphic office for help with drawings in the book chapter.

- All my colleagues and fellow PhD students at E&R for their help, pleasant company and wonderful discussions.

- All my friends and family for motivation, support and patience.

Christopher Kevin Waul

Kgs. Lyngby
November 02, 2007

ABSTRACT

Methyl *tert*-butyl ether (MTBE) was originally introduced as an anti-knock agent into gasoline in the 1970s, both in Europe and the United States. Its use was primarily as an octane enhancer to replace lead in gasoline. Later on, it was also used as an oxygenate, up to 15% v/v, to accomplish a cleaner burning fuel with reduced emissions of carbon monoxide and hydrocarbons.

The problem with MTBE is that it has caused wide-scale contamination of groundwater supplies from accidental releases and leaking underground fuel tanks. Although MTBE is not very toxic, it has an obnoxious taste and odour, that of turpentine, noticeable at concentrations of about 2 ppb by some persons. In addition, it does not degrade very easily when it reaches groundwater aquifers, primarily because of the inability of the native organisms to accomplish this.

The main scope of the thesis is on using bioreactors for removal of MTBE from groundwater; it is intended to gain a further understanding of the possibilities and limitations of this remediation technique. The thesis is based on a literature review book chapter of the subject, followed by seven journal and conference papers. The subjects of the journal and conference papers have been summarized in five subtopics within this abstract, after the following paragraph: 1) mathematical modelling and implementation of a model for a laboratory MTBE degrading packed bed reactor (PBR) followed by model parameter estimation under dynamic conditions using least squares and parameter response surface methodologies; 2) model application to study the effects on the degradation of MTBE due to the competition between MTBE degraders, ammonium oxidisers, and heterotrophs for oxygen and reactor occupancy; 3) determination of the fraction of biologically active (BA) MTBE degraders in a heterogeneous biomass sample; 4) anaerobic biodegradability of MTBE and other gasoline ethers; and 5) toxicity of MTBE, *tert*-butyl alcohol (TBA) and other gasoline ethers to anaerobic organisms.

The literature review book chapter was based on six topics, identified as being of most interest and challenge, according to the scope of the thesis. These were as follows: 1) the role of biofilms or suspended biomass in the bioremediation of MTBE; 2) the suitability of different reactor types for MTBE bioremediation, and their operational characteristics; 3) the effects of process parameters for, e.g., oxygen, nutrients, toxicants, co-contaminants, temperature and pH on the degradation of MTBE in bioreactors; 4) the factors influencing the startup time of MTBE degrading reactors – these times have been reported in the range of 20 to over 200 days; 5) the role and potential for using cometabolic MTBE degrading cultures for bioremoval of MTBE in reactors; and 6) the use of mathematical models for MTBE degrading bioreactors in order to gain further understanding of the above mentioned areas of interest. Among the findings were as follows: MTBE can be removed in excess of 99% of efficiencies, and

down to concentrations as low as 1 ppb in the reactors studied; membrane bioreactors and fluidized bed reactors had the highest volumetric removal rates of all reactors studied, in the order of 1000 mg/(L.d); co-contaminants such as benzene, toluene, ethyl-benzene and xylenes (BTEX) will not cause toxicity problems in MTBE degrading reactors over the long term, even at concentrations at their solubility ranges; cometabolic MTBE degrading strains should be further researched for their application in reactors since they normally grow much faster than strains utilising direct metabolism.

The mathematical modelling and implementation of the physical and biological process occurring in a laboratory MTBE degrading PBR was carried out. This was then followed by estimation of the MTBE degrading parameters under dynamic experimental conditions. The response of the reactor to pulses of MTBE injected at its inlet was analysed using least squares and parameter response surface methodologies. The linear parameter uncertainty estimates for the half saturation constant (K_S) and the maximum growth rate (μ_{max}) were: $0 < K_S < 10$ mg COD/L and $0.12 < \mu_{max} < 0.25$ d^{-1}. Statistical analysis of the response surfaces proved them to be significant thus suitable for obtaining reliable model parameters.

The model was further used as a tool for addressing specific issues such as the effects on the removal of MTBE in bioreactors, due to the competition between MTBE degraders, ammonium oxidisers and heterotrophs for oxygen, and reactor occupancy. The competition between different organisms in the PBR was found to result in reduced and/or delayed degradation of MTBE whenever there are limitations for oxygen or occupancy in the biofilms. It was shown that when the total chemical oxygen demand of ammonium and/or BTEX fed to MTBE degrading reactors exceeded the quantity of available oxygen no MTBE degradation occurred. The results of the model further indicate that, contradicting findings in the recent literature about the effects of BTEX on the degradation of MTBE are mainly due to differences in the substrate and reactor conditions. The often long startup times of up to 200 days required for MTBE degrading reactors was due to the low μ_{max} of the MTBE degraders. Co-contaminants such as BTEXs may, however, allow fast startup of MTBE degrading reactors, since they can be used as a growth substrate for some MTBE degraders.

The fraction of biologically active (BA) MTBE degraders in a reactor is just as important for determination of the MTBE removal rates as knowledge of the kinetic parameters. MTBE degraders can often be outcompeted in reactors by other faster growing organisms; therefore, monitoring of this parameter can be used as a research tool to increase the fraction of BA MTBE degraders in reactors, through operation and design strategies. The fraction of BA MTBE degraders in a heterogeneous biomass sample was determined by a batch kinetic approach using biomass samples taken from the PBR. By modelling and parameter estimation of batch kinetic data this parameter was estimated to be about 10% of total volatile suspended solids (VSS). This value was

found to be plausible since literature studies indicate that biofilms may have as much as 50 – 90% of VSS in the extracellular polymer matrix.

The anaerobic biodegradability of MTBE and other gasoline ethers were investigated in long term studies of 2 years or more. Despite the use of several different types of inoculums and terminal electron accepting conditions, no biological removal of MTBE or the other ethers was found under anaerobic conditions. However, some batches using complexed Fe(III) as the electron acceptor showed MTBE removal of 30 – 60% in 2 months and production of TBA. The removal observed was attributed to being most likely as a result of abiotic processes and not biological. The complexed Fe(III) was found to create low pH's of 1 – 2 in the batches from the start of the experiment, this would have led to acid hydrolysis of MTBE to TBA.

In the final study presented in this thesis, the toxicity of MTBE, TBA and other gasoline ethers to anaerobic organisms was investigated. The results showed that the ethers were only significantly toxic at concentrations over 1 g/L. MTBE showed the least toxic effects among the ethers studied, while *tert*-amyl methyl ether had the highest. TBA, however, had a significantly lower toxicity compared to all the ethers studied. It was also pointed out that toxicity of these compounds will not be a likely reason for their recalcitrance observed in many degradation studies under anaerobic conditions.

SAMMENFATNING

Methyl-tertiær-butyl-ether (MTBE) blev oprindeligt introduceret i både Europa og USA i 1970'erne som et stof, der blev tilsat benzinen for at modvirke tændingsbanken. Stoffet blev primært brugt til at øge oktantallet som erstatning for bly. Senere blev MTBE også brugt som en oxygenat, op til 15% v/v, for dels at opnå et bedre brændstof og reducere emissioner fra kulmonoxid og kulbrinter.

Problemet med MTBE er, at det gennem utilsigtede udslip og utætte nedgravede benzintanke har været årsag til omfattende forurening af grundvand. Selv om MTBE ikke er særligt giftigt, så har det en ubehagelig terpentinagtig smag og lugt, som af nogle mennesker kan iagttages ved en koncentration på omkring 2 ppb. Dertil kommer, at det ikke nedbrydes særligt nemt, når det først har nået grundvandsmagasinerne, primært fordi de tilstedeværende organismer ikke formår at nedbryde det.

Omdrejningspunktet for denne afhandling er først og fremmest brugen af bioreaktorer til rensning af MTBE-forurenet grundvand. Intentionen er at få en bedre forståelse for mulighederne og begrænsningerne af denne rensningsteknik. Afhandlingen baserer sig på et indledende bogkapitel indeholdende et litteraturstudie om emnet, efterfulgt af syv artikel- og konferencemanuskripter. Temaerne i artikel- og konferencemanuskripterne gennemgås i fem undertemaer efter dette afsnit: 1) matematisk modellering og implementering af en MTBE-nedbrydende laboratorie packed bed reactor (PBR), herunder model parameter bestemmelse under dynamiske forhold ved anvendelse af mindste kvadraters metode og parameter response surface metodik; 2) modelanvendelse med henblik på bestemmelse af effekten på nedbrydning af MTBE af konkurrencen mellem MTBE nedbrydende bakterier, ammoniumoxiderende bakterier og heterotrofe bakterier om ilt og plads i filterporerne; 3) bestemmelse af den biologisk aktive fraktion (BA-fraktion) af MTBE-nedbrydere i en heterogen biomasseprøve; 4) den anaerobe bionedbrydelighed for MTBE og andre ethere, der anvendes i forbindelse med benzin; og 5) toksiciteten af MTBE, *tert*-butyl alcohol (TBA) og andre ethere, som anvendes i forbindelse med benzin, overfor anaerobe organismer.

Litteraturstudiet omfatter seks temaer, som kan identificeres som værende de mest interessante og udfordrende med henblik på afhandlingens omdrejningspunkt. Det drejer sig om følgende temaer: 1) biofilmenes eller den suspenderede biomasses rolle under den biologiske rensning af MTBE; 2) de forskellige reaktortypers egnethed til biologisk rensning af MTBE og deres operationelle karakteristika; 3) effekterne af procesparametre for fx ilt, næringsstoffer, toksiske stoffer, co-kontaminater, temperatur og pH-værdi på nedbrydning af MTBE i bioreaktorer; 4) faktorer, som påvirker opstartstider for reaktorer i hvilke MTBE nedbrydes – disse opstartstider har været rapporteret som værende mellem 20 og 200 dage; 5) rollen og potentialet for brugen af co-metaboliske MTBE-nedbrydende kulturer til biologisk nedbryding af MTBE in

reaktorer; og 6) brugen af matematiske modeller for MTBE-nedbrydende bioreaktorer for at opnå en bedre forståelse for de nævnte fokusområder. Der opnåedes bl.a. følgende resultater: MTBE kan nedbrydes med en renseeffekt på 99 % og ned til en koncentration på 1 ppb i de reaktorer, som indgik i undersøgelsen; membran-bioreaktorer og fluidized bed reaktorer havde den højeste volumetriske nedbrydningsrate af alle undersøgte reaktorer i størrelsesorden 1000 mg/(L.d); co-kontaminater som benzen, toluen, ethylbenzen og xylener (BTEX) vil ikke forårsage toksititetsproblemer i MTBE-nedbrydende reaktorer i det lange løb, ikke engang ved koncentrationer omkring deres opløselighed; co-metaboliske MTBE-nedbrydende organismer bør undersøges nærmere for deres anvendelse i reaktorer, eftersom de normalt vokser meget hurtigere end organismer, der anvender direkte metabolisme.

Der blev udført matematisk modellering af den fysiske og biologiske processer, som fandt sted i en MTBE-nedbrydende laboratorie packed bed reactor (PBR). Dette blev efterfulgt af kalibrering af de MTBE-nedbrydende organismers kinetiske parametre under dynamiske eksperimentelle betingelser. Output af reaktoren i forhold til pulser af MTBE, som blev injiceret i indløbet, blev analyseret under anvendelse af mindste kvadraters metode og parameter respons overflade metodik. De lineære parameter usikkerheds estimater for halvmætningskonstanten (K_S) og den maksimale vækshastighed (μ_{max}) var: $0 < K_S < 10$ mg COD/L og $0.12 < \mu_{max} < 0.25$ d^{-1}. Statistisk analyse af respons overfladerne viste, at de var signifikante og derfor anvendelige til at opnå troværdige modelparametre.

Modellen blev derudover brugt som et redskab til at addressere specifikke emner som fx effekterne på nedbrydning af MTBE i bioreaktorer som skyldes konkurrencen mellem MTBE-nedbrydere, ammoniumoxidanter og heterotrofe bakterier for ilt og plads i porestrukturen. Konkurrencen mellem forskellige organismer i packed bed reaktoren medførte reduceret og/eller forsinket nedbrydning af MTBE i de tilfælde, hvor der var begrænsning af ilt eller poreplads. Det blev vist, at når den samlede koncentration af kemisk oxygenforbrug (COD) af ammonium og/eller BTEX tilført til de MTBE nedbrydende reaktorer overstiger koncentrationen af ilt sker der ikke MTBE nedbrydning. Resultaterne fra modellen indikerer videre, at modstridende observationer i litteraturen omkring effekten af BTEX på nedbrydningen af MTBE hovedsagelig skyldes forskelle i substrat- og reaktorbetingelser. De ofte lange opstartstider på op til 200 dage, der kræves for MTBE nedbrydende bakterier, skyldes den lave μ_{max} for disse organismer. Co-contaminanter som BTEX kan imidlertid give anledning til hurtig opstart af MTBE nedbrydende reaktorer fordi de i nogle tilfælde kan bruges som vækstsubstrat for de MTBE-nedbrydende bakterier.

Størrelsen af den biologisk aktive fraktion (BA-fraktionen) af MTBE-nedbrydere i en reaktor er lige så vigtig til bestemmelse af MTBE-nedbrydningsraterne som viden om de kinetiske parametre. MTBE-nedbrydere kan ofte blive udkonkurreret i reaktorer gennem andre hurtigere voksende organismer. Derfor kan monitorering af den

biologisk aktive fraktion bruges som et forskningsværktøj til at forøge BA-fraktionen for MTBE-nedbrydere i reaktorer gennem drifts- og designstrategier. På basis af modellering og parameterestimationer på MTBE nedbrydningsdata fra batch-forsøg, hvor der anvendtes biomasseprøver taget fra PBR (i 0,2 m dybde) til inokulering i batchene, blev BA-fraktionen af MTBE-nedbrydere bestemt til omkring 10% af den samlede biomasse bestemt som glødetab (VSS). Denne værdi skønnes rimelig, idet litteraturstudier indikerer, at biofilm kan have helt op til 50-90 % af deres VSS bundet i form af ekstracellulære polymerer.

Den anaerobe bionedbrydelighed for MTBE og andre ethere, der anvendes i forbindelse med benzin, blev undersøgt i langtidsstudier med en varighed på 2 år eller mere. På trods af brugen af flere forskellige typer inokuleringer og terminale elektron-acceptorer blev ingen biologisk nedbrydning af MTBE eller andre ethere fundet under anaerobe forhold. Nogle af batchene, hvor der blev anvendt komplekseret Fe(III) som elektronacceptor, viste imidlertid en MTBE-nedbrydning på 30 – 60 % indenfor 2 måneder og en omdannelse til *tert*-butyl alcohol (TBA). Den observerede nedbrydning kan højst sandsynligt relateres til at være et resultat af abiotiske processer. Det blev konstateret, at det komplekserede Fe(III) skabte lave pH-værdier på 1 – 2 i batchene fra begyndelsen af eksperimentet. Dette kan føre til syrehydrolyse af MTBE til TBA.

I den sidste undersøgelse, som præsenteres i denne afhandling, blev toksiciteten af MTBE, TBA og andre ethere, som anvendes i forbindelse med benzin, undersøgt i forhold til anaerobe organismer. Det fremgik af resultaterne, at disse ethere kun var signifikant toksiske ved koncentrationer over 1 g/L. MTBE fremviste de mindst toksiske effekter blandt de undersøgte ethere, mens *tert*-amyl methyl ether havde den største effekt. TBA havde imidlertid en signifikant lavere toksicitet sammenlignet med alle de andre ethere, der indgik i undersøgelsen. Det blev også påpeget, at toksiciteten af disse komponenter ikke ville være en sandsynlig grund til deres unedbrydelighed observeret i mange nedbrydningsstudier under anaerobe forhold.

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 (Deeb et al. 2000; EFOA 2005; Mays 1989; Morgenroth and Arvin 2003).

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 (Johnson et al. 2000; White 2001).

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 (Davis and Erickson 2004; Du et al. 1998). However, the threshold value does vary a lot, for instance a value of 2 – 2.5 ppb was reported by Fiorenza and Rifai (Fiorenza and Rifai 2003). MTBE is not retarded by aquifer material, and in addition, it has a high solubility of approximately 50 g/L at room temperature (Davis and Erickson 2004). 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 (Deeb et al. 2003; Keller et al. 1998). In Denmark, the limit value is also set at 5 µg/L, but preferable below 2 µg/L (Juhler and Felding 2003).

The tertiary structure of MTBE leads to a steric hindrance to an enzymatic attack on the molecule (White et al. 1996). Compounds with ether bonds are also generally relatively stable (Fayolle et al. 2001). 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 (Hanson et al. 1999; Salanitro et al. 1994). MTBE which has volatilised to the atmosphere will decompose readily there by the action of free radicals (Howard et al. 1991; Japar et al. 1991). 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 (Davis and Erickson 2004). 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) (Prince 2000). MTBE has a low affinity for sorption to the organic phase. Application of GAC sorption processes 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 (Li et al. 2003). 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 (Ferreira et al. 2006; François et al. 2002; Hanson et al. 1999; Hatzinger et al. 2001; Hristova et al. 2003; Mo et al. 1997).

Many other aerobic strains are also able to use MTBE in cometabolic reactions with other substrates (Garnier et al. 1999; Hyman et al. 1998; Johnson et al. 2004; Liu et al. 2001; Steffan et al. 1997). 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 (Rittmann and McCarty 2001). There is a strong correlation between organisms which can degrade and grow on branched alkanes, and their ability to cometabolise a structurally analogous compounds such as MTBE. Simple branched alkanes are abundant in gasoline and therefore the application of cometabolic cultures for remediation of gasoline impacted MTBE plumes in reactors is an interesting prospect (Hyman et al. 2000).

Degradation under methanogenic conditions and with the use of nitrate, sulphate and ferric iron has been shown to a limited extent (Bradley et al. 2001; Finneran and Lovley 2001; Pruden et al. 2005; Somsamak et al. 2001; Sulflita and Mormile 1993; Yeh and Novak 1994). 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 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 (Bryers and Characklis 1989). Several studies using biofilm reactors in experimental systems have shown that MTBE can be removed down to less than 1 µg/L (Morrison et al. 2002; Vainberg et al. 2002; Wilson et al. 2002).

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 (Arvin et al. 2004). 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." (Characklis and Marshall 1989). 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 (Characklis 1989; Trulear and Characklis 1982).

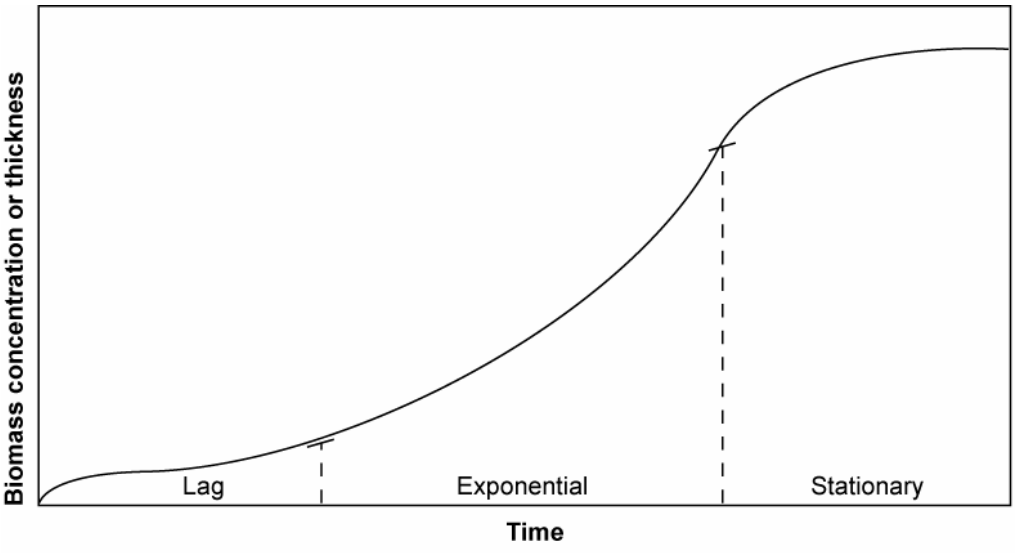


Fig. 1: The growth stages of a biofilm. The plot can be divided into three phases: (1) lag, (2) exponential and (3) stationary or plateau (modified from (Characklis 1989)**).**

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 in the order of 10 days at 20 °C (Fortin et al. 2001; Waul et al. 2007c) compared to a few hours for general heterotrophs growing on easily degradable compounds (Henze et al. 1997). 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 (Christensen and Characklis 1989; Henze et al. 1997). The higher biomass concentration that can 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 (Characklis and Marshall 1989). 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 (Bryers and Characklis 1989).

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 (Deeb et al. 2000; Stocking et al. 2000; Zein et al. 2006a). The sludge age within the system will be greatly increased, while still maintaining a relatively short hydraulic retention time.

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 (Characklis et al. 1989). 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 (Henze et al. 1997):

$$\frac{S_O}{S_{MTBE}} = \frac{D_{MTBE}}{D_O \, V_{O,MTBE}} \tag{1}$$

where S_O and S_{MTBE} are the dissolved bulk oxygen and MTBE reactor concentrations, respectively, in chemical oxygen demand (COD) units; $V_{O,MTBE}$ is the stoichiometric coefficient for oxygen and MTBE; D_O and D_{MTBE} are the oxygen and MTBE diffusion

coefficient, respectively. D_O and D_{MTBE} are estimated as 1.7×10^{-4} and 0.6×10^{-4} m^2/d respectively (Henze et al. 1997). While $V_{O,MTBE}$ is equal to 1.07 $gCOD_{MTBE}/gO_2$ and was deduced from the stoichiometric expression for mineralization of MTBE.

Solving expression 1, the following is obtained: $S_O = 0.33S_{MTBE}$. 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: $S_O > 0.33S_{MTBE}$, 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.

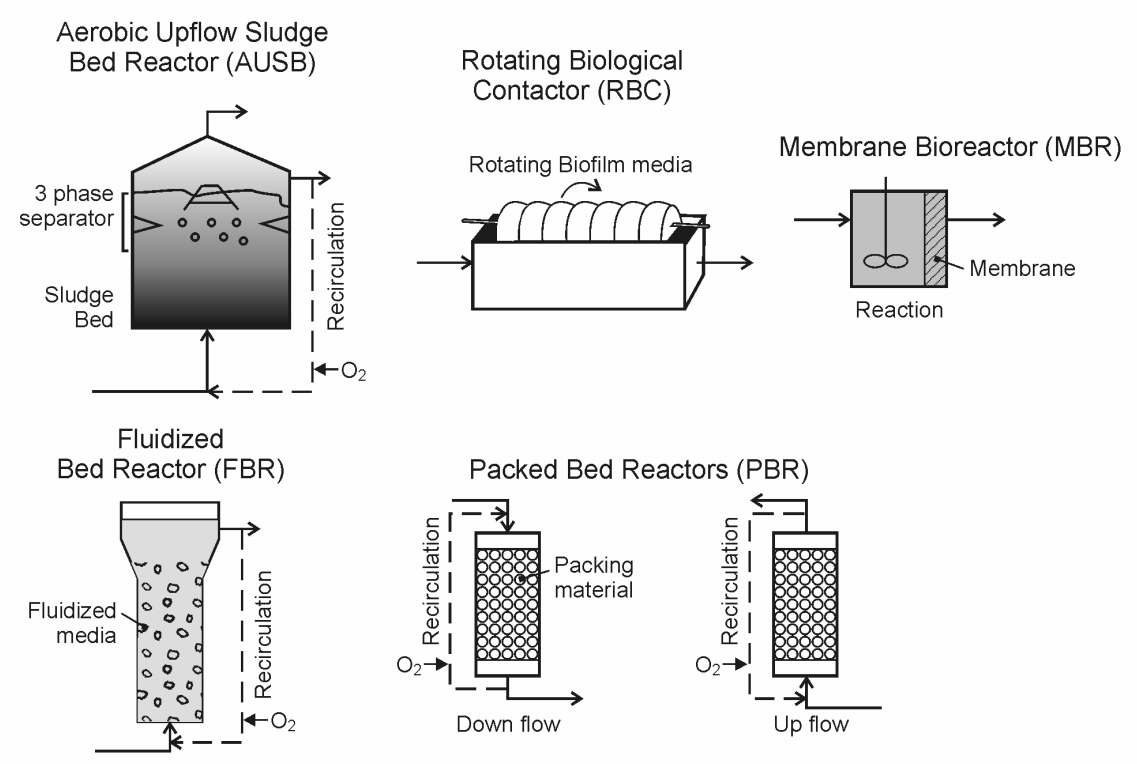


Fig. 2: Reactor types suitable for MTBE biodegradation.

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.

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 (Tchobanoglous et al. 2003). 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 (Speece 1996; Van Loosdrecht et al. 2002). One of the key requirements for application of the AUSB for MTBE

bioremediation, however, will depend on the ability of the MTBE bacteria to agglomerate and form dense granules (Schmidt and Ahring 1996). The granules also need to attain a settling velocity in the range of 40 – 100 m/h to function properly inside a reactor (Versprille Ir 2002).

It has also been reported that many facilities manufacturing MTBE successfully use the activated sludge process for treating MTBE and TBA at high concentrations (Li et al. 2003). 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 (Bryers and Characklis 1989). 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 (Jördening and Buchholz 2005).

The biomass yield coefficient (Y) for MTBE is very low, only about 0.1 − 0.2 g VSS/g MTBE (Fortin et al. 2001; Salanitro et al. 1994). 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 (Bryers and Characklis 1989; Jördening and Buchholz 2005). 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 (Pruden et al. 2003; Stringfellow and Oh 2002), 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 (Li et al. 2003).

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 (Steffan et al. 2000). 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 (Morrison et al. 2002); 2) an internal hollow fibre membrane (Steffan et al. 2000); and 3) a porous polyethylene, 0.48 cm thick membrane with pore size of 18 – 28 μm (Wilson et al. 2002; Zein et al. 2006a; Zein et al. 2004; 2006b). 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 (Zein et al. 2006b). 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 1a – c 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.

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 10 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.

Table 1a: Packed bed reactor applications.

Reactor Description	Influent characteristics	Operational data	Treatment efficiency	Startup time/days	Comments	References
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	Kharoune et al. (2001)
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)	Arvin et al. (2003)
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*; removal is comparatively low	Acuna-Askar et al.(2000); Jin and Englande Jr (1998)
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	Liu et al. (2006)
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	Arvin et al. (2004); Nielsen et al. (2002)
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% till 2.5 mg/(L.h)		Simultaneous nitrification	Morales et al. (2000)
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	Prandi et al. (2002)

Table 1b: Fluidized bed reactor applications.

Reactor description	Influent characteristics	Operational data	Treatment efficiency	Startup time/days	Comments	References
***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	Stringfellow and Oh (2002)
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	Stringfellow and Oh (2002)
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 225days; instantaneous removal of BTEX. Reactor seeded with PM1 type culture from membrane reactor	Pruden et al. (2003)
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 strain ENV735 taken from a membrane bioreactor	Steffan et al. (2000)
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	O'Connell (2001)
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	O'Connell (2001)
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	Vainberg et al. (2002)

Table 1c: Membrane bioreactor applications.

Reactor description	Influent characteristics	Operational data	Treatment efficiency	Startup time/days	Comments	References
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	Pruden et al. (2001)
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	Wilson et al. (2002)
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	Morrison et al. (2002)
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	Zein et al. (2006b)
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 ENV735 culture; infinite SRT first 160 days; MTBE removal rate was 1008 mg/(L.d) at 1 day HRT	Steffan et al. (2000)
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	Zein et al. (2004)

3.5 Process Comparison and Summary

Table 2 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 2: 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:

$$C_5H_{12}O + 6.961O_2 + 0.078H^+ + 0.078NO_3^- \rightarrow 0.078C_5H_7NO_2 + 4.61CO_2 + 5.78H_2O$$
$$C_5H_{12}O + 7.11O_2 + 0.078OH^- + 0.078NH_4^+ \rightarrow 0.078C_5H_7NO_2 + 4.61CO_2 + 5.922H_2O$$

The biomass composition is $C_5H_7NO_2$, taken from McCarty (1972), and the Y is taken as 0.1 g VSS/g MTBE or 0.078 mol VSS/mol MTBE (Fortin et al. 2001; Pruden et al. 2003). 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 (Longmuir 1954). 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 3 shows some reported values for the K_s of dissolved oxygen during MTBE degradation.

Table 3: 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)	References
Mixed culture		0.9	Park and Cowan (1997)
Vapour phase biofilter consortium		3	Koenigsberg et al. (1999)
Vapour phase biofilter consortium		0.16	Wang (2003)
BC-1	1		Salanitro et al. (1998)

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 (Tchobanoglous et al. 2003). 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 (Eweis et al. 1997; Wang 2003). 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 (François et al. 2002; Piveteau et al. 2001).

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: 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 (Koenigsberg et al. 1999).

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 (Deeb et al. 2001).

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 (Church et al. 1999).

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 (Stringfellow et al. 2000). 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 (Wang 2003).

4.2.2 Competition for Reactor Occupancy, Oxygen and Nutrients

The maximum growth rate reported (μ_{max}) for aerobic BTEX degrading and nitrifying organisms at 25 °C lies in the range 3 – 9 d^{-1} (Goudar and Strevett 1998) and about 0.6 – 1 d^{-1} (Henze et al. 1997) respectively. These μ_{max}'s are over an order of magnitude higher than that reported for MTBE (Fortin et al. 2001). 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 (Waul et al. 2007a; 2007d).

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 (Waul et al. 2007a; 2007d). 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 (Krag and Arvin 2004).

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 (Pruden 2002; Pruden et al. 2003; Sedran et al. 2002; Vainberg et al. 2002; Zein et al. 2006b). In some studies BTEX was shown to have an enhancing effect on the degradation of MTBE (Deeb et al. 2001; Eweis et al. 1998b; Pruden 2002). 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 (Eweis et al. 1998a). 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 will 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 adaptation. 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 (Deeb et al. 2000). 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 (Shim and Yang 1999). 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 (Arcangeli and Arvin 1994). Toluene concentrations at 6 mg/L showed no inhibitory effects on its own degradation in a fixed film aerobic reactor in another study (Arcangeli and Arvin 1992).

Formaldehyde, a well known microbial inhibitor, is produced as an intermediate in the microbial degradation of MTBE (Deeb et al. 2000). 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 (Waul et al. 2004). 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 (Vainberg et al. 2002).

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 (Rittmann and McCarty 2001). 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 (Schroeder et al. 2000; Zaitsev et al. 2007).

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 (Nielsen and Petersen 2001; Sedran 2004; Sedran et al. 2002; Zaitsev et al. 2007). Operation of MTBE degrading bioreactors way outside of the normal optimal range of pH is likely to affect the process (Eweis et al. 1997). 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 (Vainberg et al. 2002; Wilson et al. 2002). 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 (Waul et al. 2007a; 2007d).

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) (Steffan et al. 2000). 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 (Morrison et al. 2002; Pruden 2002). 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 (Pruden et al. 2003; Vainberg et al. 2002).

Model simulations showed that by increasing the initial seed concentration of MTBE degrading biomass in bioreactors by 10 times, the startup time could be reduced by 50 – 100 days (Waul et al. 2007a).

5.2 Co-contaminants

Previously in this review, 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 mixed reactors would be increased with higher inlet concentrations of co-contaminants (Waul et al. 2007a).

It has been reported that some BTEX compounds may stimulate the growth of MTBE degraders (Deeb et al. 2001; Eweis et al. 1998b; Pruden 2002). 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 gVSS/gTBA, the TBA grown cells have also been shown to degrade MTBE (François et al. 2002). The MTBE degrading strain PM1 isolated by Hanson et al. (1999) is reported to be capable of rapid growth on ethanol and TBA (Schroeder et al. 2000). 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 (Hyman and O'Reilly 1999; Hyman et al. 2000; Liu et al. 2001).

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 (Steffan et al. 2003; Steffan et al. 1997; Steffan et al. 2000). 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 (Hernandez-Perez et al. 2001). 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 (Koenigsberg et al. 1999).

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 (Stringfellow and Oh 2002).

In cometabolism, both the primary substrate and the cometabolic substrate are competing for the same enzyme system. The presence of the primary substrate 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 of the primary substrate than when degraded alone (Garnier et al. 1999; Liu et al. 2001). 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 (Corcho et al. 2000; Liu et al. 2001; Smith et al. 2003a; 2003b; Steffan et al.

1997). 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 are 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. (2000) reported values ranging from 10.56 – 44 mg/L for nine different strains, while Smith et al. (2003b) 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 (Cowan and Park 1996; Fortin et al. 2001).

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 (Henze et al. 1987). All growth process rates 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 mg COD/L). Ammonium is fully nitrified while MTBE is mineralised by oxygen. For a full description of the model and its implementation see Waul et al. (2007c).

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 reactor'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 (Morrison et al. 2002; Wilson et al. 2002).

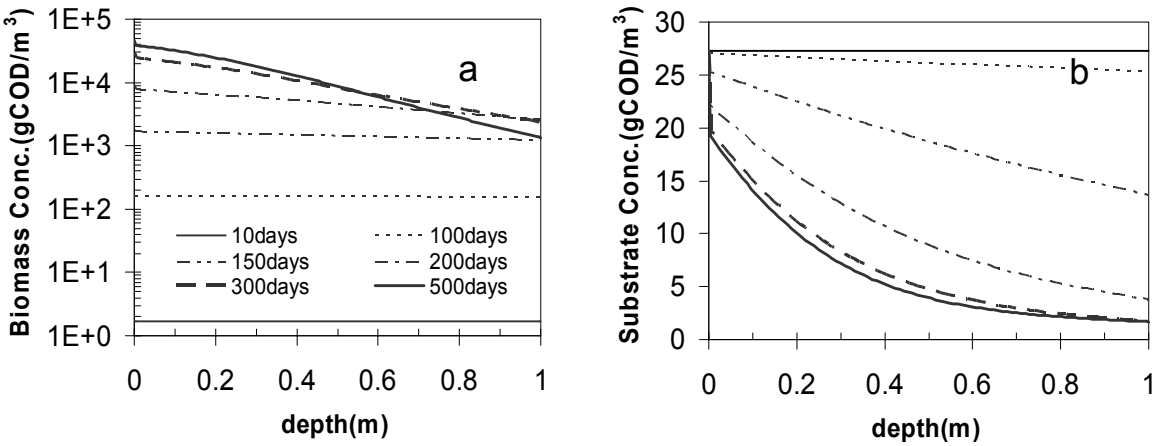


Fig. 3: The modelled dynamics of (a) biomass concentration plotted on a log scale and (b) substrate concentration for MTBE as a function of reactor depth in a packed bed reactor.

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 4 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 et al. (2007c) 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}.

Table 4: Model parameters for MTBE degradation.

Parameter	Symbol	Units	Value	References
Maximum growth rate	μ_{max}	d^{-1}	0.1 (T = 30 °C); 0.86 (T = 30 °C); 0.07 – 0.5	Cowan and Park (1996); Fortin et al. (2001); Wilson (2003)
Half saturation constant	K_s	mg COD/L	4.1; 15.63	Cowan and Park (1996); Fortin et al. (2001)
Decay constant	b	d^{-1}	0.001 (T = 30 °C); 0.12 (T = 30 °C)	Cowan and Park (1996); Fortin et al. (2001)
Yield coefficient	Y	g VSS/g MTBE	0.11; 0.21 – 0.28; 0.18; 0.1 – 0.14	Fortin et al. (2001); Hanson et al. (1999); Pruden et al. (2003); Salanitro et al. (1994)

The value for b reported by Fortin et al. (2001) is considered very low; in well tested models such as the activated sludge model 1 (ASM1), the b value is about 10% of the μ_{max} values (Henze et al. 1987). Based on the data of Hanson et al. (1999), 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 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 (Vainberg et al. 2002). 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 maximum 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 (S_O) should be as follows: $S_O > 0.33S_{MTBE}$, on a COD basis, where S_{MTBE} is the bulk MTBE reactor concentration.

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.

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 (Kharoune et al. 2001; Waul et al. 2007b).

REFERENCES

Acuna-Askar K, Englande Jr AJ, Hu C, Jin G. 2000. Methyl tertiary-butyl ether (MTBE) biodegradation in a batch and continuous upflow fixed-biofilm reactors. Water Sci Tech 42(5-6):153-161.

Arcangeli J-P, Arvin E. 1992. Toluene biodegradation and biofilm growth in an aerobic fixed-film reactor. Appl Microbiol Biotechnol 37:510-517.

Arcangeli J-P, Arvin E. 1994. Kinetics of toluene degradation in a biofilm system under denitrifying conditions. Water Sci Tech 29(10-11):393-400.

Arvin E, Krag R, Karlson U. 2003. Development of the MTBE degradation rate in a biofilter. First European Conference on MTBE. Dresden, Germany: Dresden University of Technology. p 88-94.

Arvin E, Nielsen LK, Tully AG, Albrechtsen H-J, Mosbæk H. 2004. MTBE removal by biofiltration in water works. IWA Leading Edge Conference. Prague, Czech Republic. p 120-121.

Bradley PM, Chapelle FH, Landmeyer JE. 2001. Effect of redox conditions on MTBE biodegradation in surface water sediments. Environ Sci Technol 35:4643-4647.

Bryers JD, Characklis WG. 1989. Biofilms in water and wastewater treatment. In: Characklis WG, Marshall KC, editors. Biofilms. New York: John Wiley and Sons. p 671-696.

Characklis WG. 1989. Biofilm processes. In: Characklis WG, Marshall KC, editors. Biofilms. New York: John Wiley & Sons. p 195-231.

Characklis WG, Marshall KC. 1989. Biofilms: a basis for an interdisciplinary approach. In: Characklis WG, Marshall KC, editors. Biofilms. New York: John Wiley & Sons. p 3-15.

Characklis WG, Turakhia MH, Zelver N. 1989. Transport and interfacial transfer phenomena. In: Characklis WG, Marshall KC, editors. Biofilms. New York: John Wiley & Sons. p 265-340.

Christensen BE, Characklis WG. 1989. Physical and chemical properties of biofilms. In: Characklis WG, Marshall KC, editors. Biofilms. New York: John Wiley & Sons. p 93-130.

Church CD, Tratnyek PJ, Pankow JF, Landmeyer JE, Baehr AL, Thomas MA, Schirmer M. 1999. Effects of environmental conditions on MTBE degradation in model column aquifers Proceedings of the Technical Meeting of USGS Toxic Substances Hydrology Program. Charleston, SC. p 93-101.

Corcho D, Watkinson RJ, Lerner DN. 2000. Cometabolic degradation of MTBE by a cyclohexane-oxidising bacteria. In: Wickramanayake GB, Gavaskar AR, Alleman

BC, Magar VS, editors. The Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds. Monterey, California, May 22-25: Battelle. p 183-189.

Cowan RM, Park K. 1996. Biodegradation of the gasoline oxygenates MTBE, ETBE, TAME, TBA and TAA by aerobic mixed cultures. Proceedings of the 28th Mid-Atlantic Industrial and Hazardous Waste Conference. Buffalo, New York. 15 - 17 July 1996. p 523-530.

Davis LC, Erickson LE. 2004. A review of bioremediation and natural attenuation of MTBE. Environ Prog 23(3):243-252.

Deeb RA, Chu K-H, Shih T, Linder S, Suffet I, Kavanaugh MC, Alvarez-Cohen L. 2003. MTBE and other oxygenates: environmental sources, analysis, occurrence, and treatment. Environ Eng Sci 20(5):433-447.

Deeb RA, Hu H-Y, Hanson JR, Scow KM, Alvarez-Cohen L. 2001. Substrate interactions in BTEX and MTBE mixtures by an MTBE-degrading isolate. Environ Sci Technol 35:312-317.

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

Du JT, Abernathy CO, Donohue J, Mahfouz A, Khanna K. 1998. A drinking water advisory: consumer acceptability and advice and health effects analysis on methyl tertiary-butyl ether (MTBE). Toxicologist 42(1-S):A 1123.

EFOA. 2005. MTBE resource guide. Brussels, Belgium: The European Fuel Oxygenate Association.

Eweis JB, Chang DPY, Schroeder ED, Scow KM, Morton RL, Caballero RC. 1997. Meeting the challenge of MTBE biodegradation. Proceedings of the 90th Annual Meeting and Exhibition of the Air & Waste Management Association. Toronto, Canada.

Eweis JB, Schroeder ED, Chang DPY, Scow KM. 1998a. Biodegradation of MTBE in a pilot-scale biofilter. In: Wickramanayake GB, Hinchee RE, editors. The First International Conference on Remediation of Chlorinated and Recalcitrant Compounds. Montery, California, May 18-21: Battelle. p 341-346.

Eweis JB, Watanabe N, Schroeder ED, Chang DPY, Scow KM. 1998b. MTBE degradation in the presence of other gasoline components. National Groundwater Association Conference. Anaheim, California.

Fayolle F, Vandecasteele J-P, Monot F. 2001. Microbial degradation and fate in the environment of methyl tert-butyl ether and related fuel oxygenates. Appl Microbiol Biotechnol 56:339-349.

Ferreira NL, Maciel H, Mathis H, Monot F, Fayolle-Guichard F, Greer C. 2006. Isolation and characterization of a new Mycobacterium austroafricanum strain, IFP 2015, growing on MTBE. Appl Microbiol Biotechnol 70(3):358-365.

Finneran KT, Lovley DR. 2001. Anaerobic degradation of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA). Environ Sci Technol 35(9):1785-1790.

Fiorenza S, Rifai H. 2003. Review of MTBE biodegradation and bioremediation. Bioremed J 7(1):1-35.

Fortin NY, Morales M, Nakagawa Y, Focht DD, Deshusses MA. 2001. Methyl tert-butyl ether (MTBE) degradation by a microbial consortium. Environ Microbiol 3(6):407-416.

François A, Mathis H, Godefroy D, Piveteau P, François F, Monot F. 2002. Biodegradation of methyl tert-butyl ether and other fuel oxygenates by a new strain, Mycobacterium austroafricanum IFP 2012. Appl Environ Microbiol 68(6):2754-2762.

Garnier PM, Auria R, Augur C, Revah S. 1999. Cometabolic biodegradation of methyl t-butyl ether by Pseudomonas aeruginosa grown on pentane. Appl Microbiol Biotechnol 51:498-503.

Goudar CT, Strevett KA. 1998. Comparison of relative rates of BTEX biodegradation using respirometry. J Indus Microbiol Biotech 21:11-18.

Hanson JR, Ackerman CE, Scow KM. 1999. Biodegradation of methyl tert-butyl ether by a pure bacterial culture. Appl Environ Microbiol 65(11):4788-4792.

Hatzinger PB, McClay K, Vainberg S, Tugusheva M, Condee CW, Steffan RJ. 2001. Biodegradation of methyl tert-butyl ether by a pure bacterial culture. Appl Environ Microbiol 67(12):5601-5607.

Henze M, Grady Jr. CPL, Gujer W, Marais GvR, Matsuo T. 1987. Activated sludge model no.1 (IAWPRC Scientific and Technical Report No. 1). London: IAWPRC.

Henze M, Harremoës P, Jes la Cour J, Arvin E. 1997. Wastewater treatment: biological and chemical processes. Heidelberg: Springer.

Hernandez-Perez G, Fayolle F, Vandecasteele J-P. 2001. Biodegradation of ethyl t-butyl ether (ETBE), methyl t-butyl ether (MTBE) and t-amyl methyl ether (TAME) by Gordonia terrae. Appl Microbiol Biotechnol 55(1):17-121.

Howard PH, Boethling RS, Jarvis WF, Meylan WM, Michalenko EM. 1991. Handbook of environmental degradation rates. Chelsea, Michigan: Lewis Publishers Inc. 653-654 p.

Hristova K, Gebreyesus B, Mackay D, Scow KM. 2003. Naturally occurring bacteria similar to methyl tert-butyl ether (MTBE)-degrading strain PM1 are present in MTBE-contaminated groundwater. Appl Environ Microbiol 69:2616-2623.

Hyman MR, Kwon P, O'Reilly KT. 1998. Cometabolism of MTBE by alkane-utilizing microorganisms. In: Wickramanayake GB, Hinchee RE, editors. The First International Conference on Remediation of Chlorinated and Recalcitrant Compounds. Monterey, California, May 18-21: Battelle. p 321-326.

Hyman MR, O'Reilly KT. 1999. Physiological and enzymatic features of MTBE-degrading bacteria. In: Alleman BC, Leeson A, editors. Proceedings of the Fifth International In Situ and On-site Bioremediation Symposium. Monterey, California, May 18-21: Battelle. p 7-12.

Hyman MR, Taylor C, O'Reilly KT. 2000. Cometabolic degradation of MTBE by iso-alkane-utilizing bacteria from gasoline-impacted soils. In: Wickramanayake GB, Gavaskar AR, Alleman BC, Magar VS, editors. The Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds. Monterey, California, May 22-25: Battelle. p 149-155.

Japar SM, Wallington TJ, Rudy SJ, Chong TY. 1991. Ozone-forming potential of a series of oxygenated organic compounds. Environ Sci Technol 25:415-420.

Jin G, Englande Jr. AJ. 1998. Carbon tetrachloride biodegradation in a fixed-biofilm reactor and its kinetic study. Water Sci Tech 38(8-9):155-162.

Johnson E, Smith CA, O'Reilly KT, Hyman MR. 2004. Induction of methyl tertiary butyl ether (MTBE)-oxidizing activity in Mycobacterium vaccae JOB5 by MTBE. Appl Environ Microbiol 70(2):1023-1030.

Johnson R, Pankow J, Bender D, Price C, Zororski J. 2000. MTBE: to what extent will past releases contaminate community water supply wells? Environ Sci Technol 34:210A-217A.

Jördening H-J, Buchholz K. 2005. High rate anaerobic wastewater treatment. In: Jördening H-J, Winter J, editors. Environmental biotechnology: concepts and applications. Weinheim: Wiley-VCH. p 135-162.

Juhler R, Felding G. 2003. Monitoring methyl tertiary butyl ether (MTBE) and other organic micropollutants in groundwater: results from the Danish national monitoring program. Wat Air Soil Poll 149:145-161.

Keller A, Froines J, Koshland C, Reuter J, Suffet I, Last J. 1998. Health & environmental assessment of MTBE, summary and recommendations. UC TSR&TP report to the governor of California. Davis, California.

Kharoune M, Pauss A, Lebeault JM. 2001. Aerobic biodegradation of an oxygenates mixture: ETBE, MTBE and TAME in an upflow fixed-bed reactor. Water Res 35(7):1665-1674.

Koenigsberg S, Sandefur C, Mahaffey W, Deshusses MA, Fortin NY. 1999. Peroxygen mediated bioremediation of MTBE. In: Leeson A, Alleman BC, editors. The Fifth International In Situ and On-site Bioremediation Symposium: Battelle. p 13-18.

Krag R, Arvin E. 2004. Up-scaling of MTBE removal in a biological filter. Second European Conference on MTBE. Barcelona, Spain. p 88-91.

Li T, Patel RU, Ramsden DK, Greene J. 2003. Ground water recovery and treatment. In: Moyer E, Kostecki PT, editors. MTBE Remediation Handbook. Massachusetts: Amherst Scientific Publishers. p 289-327.

Liu CY, Speitel Jr. GE, Georgiou G. 2001. Kinetics of methyl t-butyl ether cometabolism at low concentrations by pure cultures of butane-degrading bacteria. Appl Environ Microbiol 67(5):2197-2201.

Liu S-J, Jiang B, Huang G-Q, Li X-G. 2006. Laboratory column study for remediation of MTBE-contaminated groundwater using a biological two-layer permeable barrier. Water Res 40:3401-3408.

Longmuir IS. 1954. Respiration rate of bacteria as a function of oxygen concentration. Biochemistry 57:81-87.

Mays MA. 1989. The use of oxygenated hydrocarbons in gasoline and their contribution to reducing urban air pollution. Pure Appl Chem 61(8):1373-1378.

McCarty PL. 1972. Energetics of organic matter degradation. In: Mitchell R, editor. Water Pollution Microbiology. New York: Wiley. p 91-118.

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

Morales M, Deshusses MA, Revah S. 2000. Microcosm and column studies on the biodegradation of methyl tert-butyl ether (MTBE) in soil water systems. Paper 795. Proc. Annual Meeting and Exibition of the Air and Waste Management Association (AWMA). Pittsburgh.

Morgenroth E, Arvin E. 2003. The European perspective to MTBE as an oxygenate in fuels. In: Rapport D, Lasley W, Rolston D, Nielsen O, Qualset C, Damania A, editors. Managing for Ecosystem Health. Boca Raton, Florida: Lewis Publisher. p 1447-1456.

Morrison JR, Suidan MT, Venosa AD. 2002. Use of membrane bioreactor for biodegradation of MTBE in contaminated water. J Environ Eng 128(9):836-841.

Nielsen LK, Petersen AG. 2001. Removal of MTBE in a Danish water works - with emphasis on the biological degradation of MTBE in the sand filter. [MSc]. Copenhagen: Technical University of Denmark.

Nielsen LK, Tully AG, Albrechtsen H-J, Mosbæk H, Arvin E. 2002. Fjernelse af MTBE i danske vandværker. Copenhagen: Miljøstyrelsen (In Danish).

O'Connell JE. 2001. Fluidized bed bioreactor for MTBE and TBA in water. In: Magar VS, Gibbs JT, O'Reilly KT, Hyman MR, Leeson A, editors. The Sixth

International In Situ and On-Site Bioremediation Symposium. San Diego, California, June 4-7: Battelle. p 91-98.

Park K, Cowan RM. 1997. Effects of oxygen and temperature on the biodegradation of MTBE. Proceedings of the 213th ACS National Meeting, Division of Environmental Chemistry. San Francisco, California. p 421-424.

Piveteau P, Fayolle F, Vandecasteele J-P, Monot F. 2001. Biodegradation of tert-butyl alcohol and related xenobiotics by a methylotrophic bacterial isolate. Appl Microbiol Biotechnol 55:369-373.

Prandi A, Romano M, Bottarelli M, Armanini S. 2002. Trickling filter decontamination of MTBE from groundwater: 15 field applications. In: Gavaskar AR, Chen ASC, editors. Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds. Monterey, CA p2H-28.

Prince R. 2000. Biodegradation of methyl tertiary-butyl ether (MTBE) and other fuel oxygenates. Crit Rev Microbiol 26(3):163-178.

Pruden A. 2002. Biodegradation of methyl tert-butyl ether [PhD]. Cincinnati: University of Cincinnati.

Pruden A, Sedran MA, Suidan MT, Venosa AD. 2003. Biodegradation of MTBE and BTEX in an aerobic fluidized bed reactor. J Environ Eng 47(9):123-128.

Pruden A, Sedran MA, Suidan MT, Venosa AD. 2005. Anaerobic biodegradation of methyl tert-butyl ether under iron-reducing conditions in batch and continuous-flow cultures. Water Environ Res 77(3):297-303.

Pruden A, Suidan MT, Venosa AD, Wilson GJ. 2001. Biodegradation of methyl tert-butyl ether under various substrate conditions. Environ Sci Technol 35(21):4235-4241.

Rittmann BE, McCarty PL. 2001. Environmental biotechnology: principles and applications. New York: McGraw-Hill.

Salanitro JP, Chou CS, Wisniewski HL, Vipond TE. 1998. Perspectives on MTBE biodegradation and the potential for in-situ aquifer bioremediation. Southwestern Regional Conference of the National Ground Water Association.

Salanitro JP, Diaz LA, Williams MP, Wisniewski HL. 1994. Isolation of a bacterial culture that degrades methyl t-butyl ether. Appl Environ Microbiol 60(7):2593-2596.

Schmidt JE, Ahring BK. 1996. Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactor. Biotechnol Bioeng 49:229-246.

Schroeder ED, Scow KM, Converse BM, Scarano J, Watanabe N, Romstad K. 2000. Experience with laboratory and field-scale ex situ biodegradation of MTBE.

Extended abstract prepared for the USEPA/API Workshop on the Biodegradation of MTBE. Cincinati, OH. p 341-346.

Sedran MA. 2004. MTBE and BTEX biodegradation in a porous pot and a fluidized bed [PhD]. Cincinnati, USA: University of Cincinnati.

Sedran MA, Pruden A, Wilson GJ, Suidan MT, Venosa AD. 2002. Effect of BTEX on degradation of MTBE and TBA by mixed bacterial culture. J Environ Eng 128(9):830-835.

Shim H, Yang S-T. 1999. Biodegradation of benzene, toluene, ethylbenzene, and o-xylene by a coculture of Pseudomonas putida and Pseudomonas fluorescens immobilized in a fibrous-bed bioreactor. J Biotech 67:99-112.

Smith CA, O'Reilly KT, Hyman MR. 2003a. Characterization of initial reactions during the cometabolic oxidation of methyl tert butyl ether by propane-grown Mycobacterium vaccae JOB5. Appl Environ Microbiol 69(2):796-804.

Smith CA, O'Reilly KT, Hyman MR. 2003b. Cometabolism of methyl tertiary butyl ether and gaseous n-alkanes by Pseudomonas mendocina KR-1 grown on C_5 to C_8 n-alkanes. Appl Environ Microbiol 69(12):7385-7394.

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

Speece RE. 1996. Anaerobic biotechnology for industrial wastewaters. Nashville, TX: Archae Press.

Steffan RJ, Farhan YH, Condee CW, Drew S. 2003. Bioremediation at a New Jersey site using propane-oxidising bacteria. In: Moyer E, Kostecki PT, editors. MTBE Remediation Handbook. Amherst, Massachusetts: Amherst Scientific Publishers. p 503-516.

Steffan RJ, McClay K, Vainberg S, Condee CW, Zhang D. 1997. Biodegradation of the gasoline oxygenated metyl tert-buty ether, ethyl tert-butyl ether, and tert-amyl methyl ether by propane-oxidizing bacteria. Appl Environ Microbiol 63(11):4216-4222.

Steffan RJ, Vainberg S, Condee CW, McClay K. 2000. Biotreatment of MTBE with a new bacterial isolate. In: Wickramanayake GB, Gavaskar AR, Alleman BC, Magar VS, editors. The Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds. Monterey, California, May 22-25: Battelle. p 165-173.

Stocking AJ, Deeb RA, Flores AE, Stringfellow WT, Talley J, Brownell R, Kavanaugh MC. 2000. Bioremediation of MTBE: a review from a practical perspective. Biodegradation 11(2-3):187-201.

Stringfellow WT, Hines RD, Cockrum DK, Kilkenny ST. 2000. Factors influencing biological treatment of MTBE in fixed film reactors. In: Wickramanayake GB,

Gavaskar AR, Alleman BC, Magar VS, editors. The Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds. Monterey, California, May 22-25: Battelle. p 175-181.

Stringfellow WT, Oh K-C. 2002. Initiation of MTBE biotreatment in fluidized-bed bioreactors. J Environ Eng 128(9):852-861.

Sulflita JM, Mormile M. 1993. Anaerobic biodegradation of known and potential gasoline oxygenates in the terrestrial subsurface. Environ Sci Technol 27(5):976-978.

Tchobanoglous G, Burton F, Stensel H. 2003. Wastewater engineering: treatment and reuse/Metcaff and Eddy, Inc. New York: McGraw-Hill.

Trulear MG, Characklis WG. 1982. Dynamics of biofilm processes. Wat Pollut Control 54:1288-1301.

Vainberg S, Togna AP, Sutton PM, Steffan RJ. 2002. Treatment of MTBE-contaminated water in fluidized bed bioreactor. J Environ Eng 128(9):842-851.

Van Loosdrecht MCM, de Kreuk MK, Heijnen JJ. 2002. Aerobic granular sludge formation. Papers of the Farewell Seminar of Dr.ir. Look Hulshoff Pol. Wageningen, The Netherlands. p 81-96.

Versprille Ir AI. 2002. Granular sludge in full-scale UASB and EGSB reactors: facts and figures. Papers of the Farewell Seminar of Dr.ir. Look Hulshoff Pol. Wageningen, The Netherlands. p 27-40.

Wang X. 2003. From microorganisms to engineered systems: a laboratory study on the bioremediation of MTBE contaminated groundwater [PhD]. California, USA: University of California, Riverside.

Waul C, Arvin E, Schmidt JE. 2007a. Effects of co-contaminants and factors influencing the startup time of MTBE degrading bioreactors. Third European Conference on MTBE, June 7- 8. Antwerp, Belgium. p 89-93.

Waul C, Arvin E, Schmidt JE. 2007b. Long term studies on the anaerobic biodegradability of MTBE and other gasoline ethers (submitted).

Waul C, Arvin E, Schmidt JE. 2007c. Model description and kinetic parameter analysis of MTBE biodegradation in a packed bed reactor (submitted).

Waul C, Arvin E, Schmidt JE. 2007d. Modeling the competitive effect of ammonium oxidizers and heterotrophs on the degradation of MTBE in a packed bed biofilm reactor (submitted).

Waul C, Christensen N, Mosbæk H, Arvin E, Schmidt JE. 2004. Fuel oxygenates toxicity to the anaerobic degradation process. Second European Conference on MTBE. Barcelona, Spain. p 51-55.

White GF, Russell NJ, Tidswell EC. 1996. Bacterial scission of ether bonds. Microbiological Reviews 60:747-758.

White J. 2001. Commentaries and perspectives: California UST program connection to MTBE problems. Environ Foren 2:185-187.

Wilson GJ, Pruden A, Suidan MT, Venosa AD. 2002. Biodegradation kinetics of MTBE in laboratory batch and continuous flow reactors. J Environ Eng 128(9):824-829.

Wilson JT. 2003. Aerobic in situ bioremediation. In: Moyer E, Kostecki PT, editors. MTBE Remediation Handbook. Amherst, Massachusetts: Amherst Scientific Publishers. p 243-264.

Yeh CK, Novak JT. 1994. Anaerobic biodegradation of gasoline oxygenates in soils. Water Environ Res 66(5):744-752.

Zaitsev GM, Uotila JS, Häggblom MM. 2007. Biodegradation of metyl *tert*-butyl ether by cold-adapted mixed and pure bacterial cultures. Appl Microbiol Biotechnol 74:1092-1102.

Zein MM, Pinto PX, Garcia-Blanco S, Suidan MT. 2006a. Treatment of groundwater contaminated with PAHs, gasoline hydrocarbons, and methyl tert-butyl ether in a laboratory biomass-retaining bioreactor. Biodegradation 17:57-69.

Zein MM, Suidan MT, Venosa AD. 2004. MTBE biodegradation in a gravity flow, high-biomass retaining bioreactor. Environ Sci Technol 38(12):3449-3456.

Zein MM, Suidan MT, Venosa AD. 2006b. Bioremediation of groundwater contaminated with gasoline hydrocarbons and oxygenates using a membrane-based reactor. Environ Sci Technol 40(6):1997-2003.

APPENDICES

I) Waul C, Arvin E, Schmidt JE. 2007. Model description and kinetic parameter analysis of MTBE biodegradation in a packed bed reactor (submitted).

II) Waul C, Arvin E, Schmidt JE. 2007. Modeling the competitive effect of ammonium oxidizers and heterotrophs on the degradation of MTBE in a packed bed reactor (submitted).

III) Waul C, Arvin E, Schmidt JE. 2007. Effects of co-contaminants and factors influencing the startup time of MTBE degrading bioreactors. Third European Conference on MTBE, June 7- 8. Antwerp, Belgium. p 89-93.

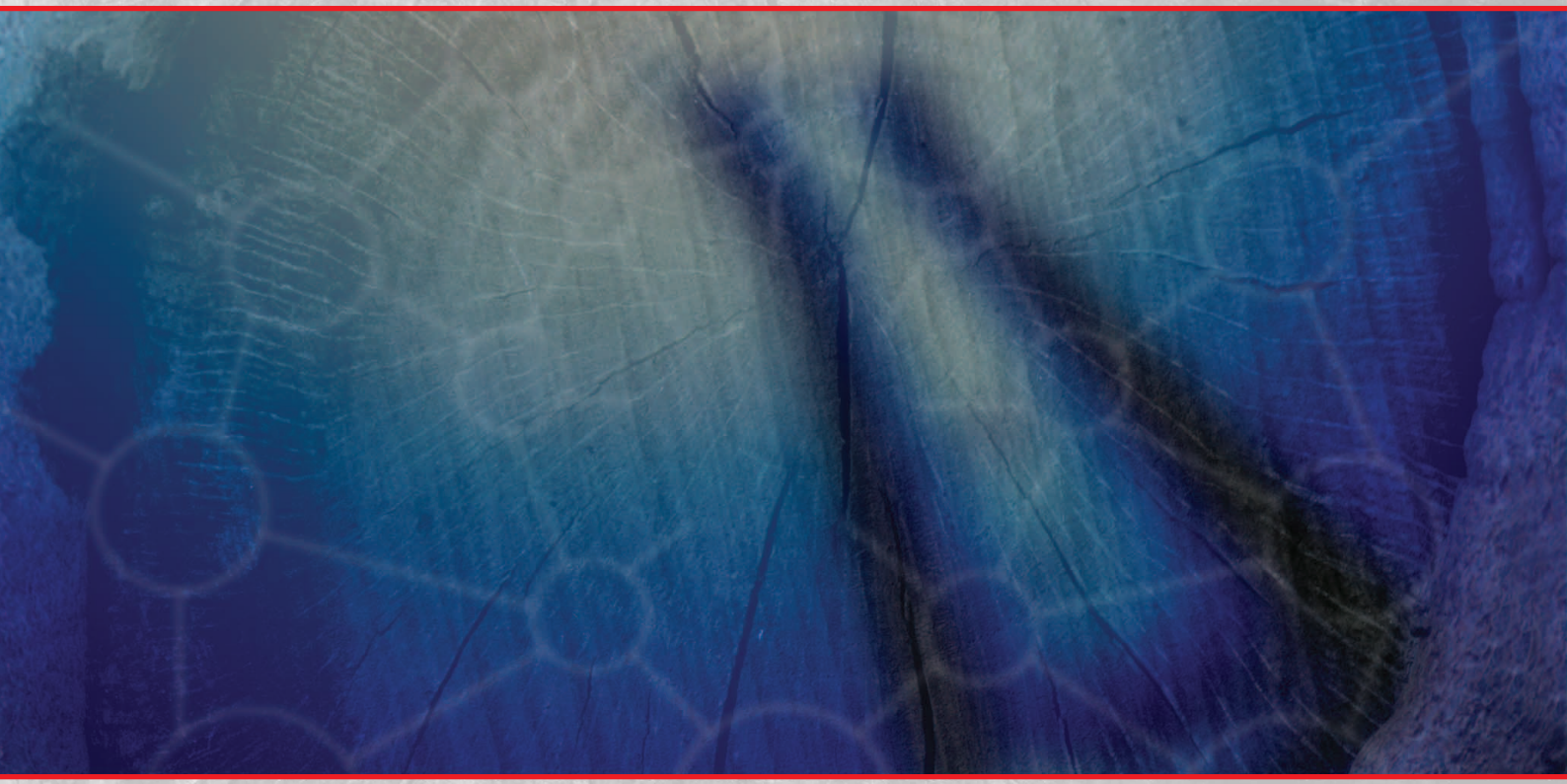
IV) Waul C, Arvin E, Schmidt JE. 2007. Competition for oxygen and occupancy in a packed bed biofilm reactor between MTBE degraders, ammonium oxidizers and heterotrophs. Extended abstract accepted for the "Biofilm Technologies" conference, January 2008, Singapore.

V) Waul C, Arvin E, Schmidt JE. 2007. Estimation of the fraction of biologically active methyl *tert*-butyl ether degraders in a heterogeneous biomass sample. Biotechnology Letters. DOI 10.1007/s10529-007-9509-0.

VI) Waul C, Arvin E, Schmidt JE. 2007. Long term studies on the anaerobic biodegradability of MTBE and other gasoline ethers (submitted).

VII) Waul C, Christensen N, Mosbæk H, Arvin E, Schmidt JE. 2004. Fuel oxygenates toxicity to the anaerobic degradation process. Second European Conference on MTBE. Barcelona, Spain. p 51-55.

The papers are not included in this www-version but can be obtained from the library at the Institute of Environment & Resources, Bygningstorvet, Building 115, Technical University of Denmark, DK-2800 Kgs. Lyngby (library@er.dtu.dk).



Institute of Environment & Resources
Technical University of Denmark
Bygningstorvet, Building 115
DK-2800 Kgs. Lyngby

Phone: +45 4525 1600
Fax: +45 4593 2850
e-mail: reception@er.dtu.dk

Please visit our website www.er.dtu.dk

ISBN 978-87-91855-35-1