Biodegradation of Ether Pollutants

17

Amie McElroy and Michael Hyman

Contents

A. McElroy
Department of Civil, Construction, and Environmental Engineering, North Carolina State University, Raleigh, NC, USA
e-mail: acmcelro@ncsu.edu

M. Hyman (✉)
Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC, USA
e-mail: mrhyman@ncsu.edu

R. J. Steffan (ed.), *Consequences of Microbial Interactions with Hydrocarbons, Oils, and Lipids: Biodegradation and Bioremediation*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-319-50433-9_27

Abstract

Ether-bonded compounds are often poorly biodegradable and can become important environmental pollutants. In this chapter, we summarize the microorganisms and key enzymes associated with the aerobic biodegradation of some of the most environmentally significant alkyl, chlorinated, branched, and cyclic ethers. Particular emphasis is placed on representative compounds from each of these groups including dimethyl ether (DME), bis(2-chloroethyl) ether (BCEE), methyl *tertiary* butyl ether (MTBE), and 1,4-dioxane (14D). The chapter emphasizes differences between growth-related and cometabolic ether biodegradation and the important roles that diverse oxygenase enzymes play in aerobic ether biodegradation.

1 Introduction

Ethers are defined as organic chemicals that contain an oxygen atom bonded to two alkyl or aryl groups (R–O–R′). Ethers are relatively stable and tend not to react with acids, bases, metals, or oxidizing/reducing agents although some classes of ethers (e.g., chloroalkyl ethers) can hydrolyze rapidly. Ethers lack strongly polarized O–H bonds and therefore do not hydrogen bond with themselves. Consequently, they tend to be volatile and have low boiling points. Conversely, the ether O atom possesses two lone electron pairs that enable ethers to form hydrogen bonds with water. This property makes smaller ethers highly water soluble. These lone electron pairs also make ethers good solvents for organic compounds. In combination, these properties make ethers a versatile class of organic compounds that are used in a wide variety of industrial processes.

In addition to their chemical stability, it has long been recognized that ether-bonded compounds are also often resistant to biodegradation and can therefore become recalcitrant environmental pollutants (White et al. 1996). From a historical perspective, the different groups of ethers examined in this chapter have been the focus of sequential waves of research as examples of these compounds either have posed important physiological and biochemical questions or have risen at different times to prominence as important pollutants. For example, this chapter starts with a discussion of the degradation of the simplest alkyl ether, dimethyl ether (DME). The role of DME as a potential growth-supporting substrate for aerobic methane-oxidizing bacteria and as a potential intermediate in the methane-oxidation pathway perplexed researchers for several years in the 1970s. Early studies of DME biodegradation therefore played a role in developing our understanding of both the basic physiology and enzymology of methane-oxidizing bacteria. These studies also helped highlight that some organics can act as growth-supporting substrates for microorganisms while others are only susceptible to fortuitous cometabolic degradation processes. This issue of growth-related (metabolic) and non-growth related (cometabolic) degradation is reiterated throughout this chapter. Studies with DME also highlighted the important role of nonspecific oxygenase enzymes

in aerobic ether degradation processes. This theme is also continued throughout this chapter.

Later sections of the chapter have focused on compounds such as methyl *tertiary* butyl ether (MTBE) and 1,4-dioxane (14D). At the turn of the millennium, MTBE became one of the most highly produced industrial chemicals due to its global use as a gasoline oxygenate. Biodegradation of this compound is particularly challenging as it contains both an ether linkage and a branched structure. Aerobic biodegradation processes for this compound subsequently became important as they offered inexpensive and reliable remediation approach for this compound in gasoline-impacted groundwater. Most recently, much effort has focused on understanding the aerobic biodegradation of 14D. As the most recent area of aerobic ether biodegradation research, our understanding of aerobic 14D-degrading microorganisms has been strongly impacted by modern "omic" approaches. An important driver of much of the research with compounds such as 14D is the identification of microbial processes that can be used to control and remediate environmental contamination by these chemicals. Like MTBE which has taste and odor thresholds in the low μg L^{-1} range (ITRC 2005), the challenge with aerobic 14D biodegradation is that biological systems need to be effective in the low to sub μg L^{-1} range to decrease the threat of this compound to human health. As growth-related microbial metabolism of ethers such as MTBE and 14D is challenging at these low concentrations, our understanding of cometabolic systems is particularly important for the development of effective biological treatment systems.

2 Alkyl Ethers

As indicated in the Introduction, ethers have many properties that make then useful as aprotic solvents. Alkyl ethers, and particularly diethyl ether (DEE), are therefore common reagents in organic chemistry. The simplest dialkyl ether, DME, is used as an aerosol propellant, a solvent, a fuel additive, a refrigerant, and as a substitute for liquefied petroleum gas (Semelsberger et al. 2006). DME is also a potential replacement diesel fuel as it has a high cetane number (55), generates low amounts of particulates, NO_x and CO, and can be used with only minor modifications to existing diesel engines (Semelsberger et al. 2006). DME is water-soluble (~46 g/L [~1 M]) (Hine and Mookerjee 1975) but does not hydrolyze under normal environmental conditions.

2.1 DME Metabolism

Historically, studies of the aerobic microbial metabolism of DME focused on methane-oxidizing bacteria (methanotrophs). The pathway of aerobic methane oxidation is now known to involve the initial hydroxylation of methane to methanol by one of two forms of methane monooxygenase (MMO). Methanol is then

sequentially oxidized to formaldehyde, formate, and CO_2 (Fig. 1). Both the particulate (pMMO) and soluble (sMMO) forms of MMO are nonspecific and can oxidize a wide variety of compounds that do not support the growth of methanotrophs (Higgins et al. 1979; Colby et al. 1977). In some of the earliest studies of methanotrophs, it was reported that some strains could grow on DME (Wilkinson 1971), and a pathway for methane oxidation in which DME was an integral intermediate was proposed (Wilkinson 1975). Degradation of DME without a direct role in methane oxidation was subsequently suggested to involve an initial hydroxylation of DME to form a hemiacetal (methoxymethanol) (Stirling and Dalton 1980). This hemiacetal could then either be oxidized to methylformate by alcohol dehydrogenases or could abiotically decompose to methanol and formaldehyde. Both abiotic and enzyme-catalyzed hydrolysis of methylformate would also be expected to generate methanol and formate (Fig. 2).

Although effects of DME on methanotrophs were investigated by others (Hazeu 1975; Ribbons 1975; Patel et al. 1976), the confusion surrounding actual growth of methanotrophs on DME and its role as an MMO substrate was only resolved when it was recognized that commercial sources of DME typically contain significant amounts of impurities (Stirling and Dalton 1980; Meyers 1982) including both methane (De Bont and Mulder 1974) and methanol (Stirling and Dalton 1980). With the development of purified sMMO preparations, it became clear that DME is an sMMO substrate that is oxidized to methanol and formaldehyde (Colby et al. 1977; Stirling and Dalton 1980). It was also established that methylformate but not DME can support the growth

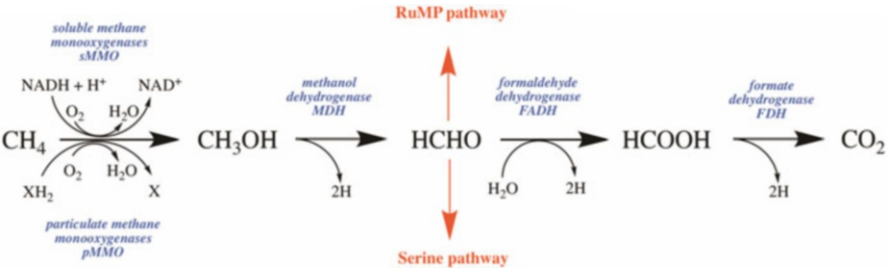

Fig. 1 Pathway of methane oxidation in methanotrophs

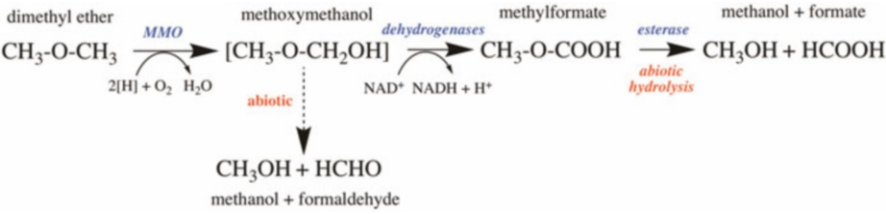

Fig. 2 *Biotransformations of DME by methanotrophs.* Solid lines represent enzyme-catalyzed reactions. Dashed lines represent abiotic reactions. The type of enzyme involved in each reaction is shown in italics. Undetected intermediates are shown in brackets

the model methanotroph *Methylococcus capsulatus* (Bath) and that methyformate can also be oxidized by sMMO (Stirling and Dalton 1980).

2.2 DME Cometabolism

Even though purified DME does not support the growth of methanotrophs, its high aqueous solubility and its effects as a substrate for MMO led to its use as an inhibitor of methane oxidation by free-living (Oremland and Culbertson 1992) and symbiotic methanotrophs (Fisher et al. 1987). DME is also an effective inhibitor of NH_3-dependent N_2O production by autotrophic nitrifying bacteria and has been used to discriminate between nitrifier- and denitrifier-dependent N_2O-production (Miller et al. 1993). These effects arise because DME is also a substrate for ammonia monooxygenase (AMO) in aerobic ammonia-oxidizing bacteria. This membrane-bound enzyme is closely related to pMMO and in the canonical ammonia-oxidizing bacterium *Nitrosomonas europaea*, AMO oxidizes DME to equimolar amounts of methanol and formaldehyde (Hyman et al. 1994).

2.3 Metabolism of Other Alkyl Ethers

Unlike DME, aerobic, growth-associated metabolism of diethyl ether (DEE) has been reported for a wide range of microorganisms including a hydrocarbon-oxidizing filamentous fungus, *Graphium* sp., (Hardison et al. 1997) and diverse bacteria including *Rhodococcus* (Bock et al. 1996; Kim and Engesser 2004; Kim et al. 2007, 2008; Moreno-Horn et al. 2005; Tajima et al. 2012), *Gordonia* (Kim et al. 2007), *Pseudonocardia* (Parales et al. 1994; Kohlweyer et al. 2000; Vainberg et al. 2006), and *Burkholderia* (Hur et al. 1997) strains. The toluene-oxidizing bacterium *B. cepacia* (*vietnamensis*) G4 expresses toluene-2-monooxygenase (T2MO) when grown on toluene (Newman and Wackett 1995). A mutant strain (PR1) that constitutively expresses T2MO grows well on DEE (Shields and Reagin 1992; Hur et al. 1997). Purified T2MO generates equimolar amounts of ethanol and acetaldehyde from DEE oxidation and this reaction is proposed to involve the subterminal oxidation of DEE to form an unstable hemiacetal (Hur et al. 1997). As strain G4 can grow on both ethanol and acetaldehyde but not DEE, the ability of strain PR1 to grow on DEE suggests DEE fails to induce expression of T2MO in the wild-type strain G4.

A DEE-metabolizing strain, *Rhodococcus* sp. DEE5151, grows on the homologous series of *n*-alkyl ethers up to *n*-heptyl ether (Kim and Engesser 2004). Like the oxidation of DEE by T2MO discussed above, it has been proposed that all of these ethers are also initially oxidized by monooxygenase-catalyzed reactions that target the subterminal carbons that form the ether bond. The enzyme responsible for this activity has not been identified at the molecular level but in vivo it is inhibited by glutaraldehyde and ethyl vinyl ether (Kim and Engesser 2005) and it can also O-dealkylate anisole and phenetole (Kim et al. 2008). A physiological and molecular

study of a wide variety of alkyl ether-degrading strains enriched from activated sludge suggests that alkyl ether-metabolizing activity is widely distributed but also often lost following cultivation in the absence of ethers, presumably due to the loss of catabolic plasmids (Kim et al. 2007). This study also suggests that the ability of strains to grow on DEE is also closely associated with the ability to grow on tetrahydrofuran (THF).

2.4 Cometabolism of Other Alkyl Ethers

Several microorganisms are known to cometabolically degrade DEE. For example, methanotrophs expressing sMMO can oxidize diethyl ether (DEE) to ethanol and acetaldehyde (Colby et al. 1977) and the same activity is also seen with *N. europaea* due to the activity of AMO (Hyman 2009). The oxidation of ethers such as *n*-propyl ether by AMO also gives rise to hydroxylated ethers rather than mixtures of alcohols and aldehydes (Hyman 2009).

3 Chloroalkyl Ethers

In this section, we examine several chloroalkyl ethers including bis(2-chloroethyl) ether (BCEE), 2-chloroethyl vinyl ether (2CEVE), bis(2-chloroisopropyl) ether (BCIP) (Fig. 3) that are included on the United State Environmental Protection Agency Priority Pollutant List (USEPA 2014). Our major focus is on the biodegradation of bis(2-chloroethyl)ether (BCEE) which is classified by USEPA as a probable human carcinogen (B2) (USEPA 2002). This ether is primarily used as an intermediate for pesticide synthesis, but it has also been used as a solvent for fats, waxes, greases, textiles, paints and varnishes (ATSDR 2017). BCEE is a semivolatile organic compound (vapor pressure 0.71 mm Hg at 20 °C). It is reasonably soluble in water (~10.4 g/L [0.073 M]) (Bednar et al. 2009) but undergoes a slow sequential hydrolysis to 2-(2-chloroethoxy) ethanol (2CEE) and diethylene glycol (DEG ($t_{1/2}$ = ~3 years at pH 7 and 25 °C)) (Payne and Collette 1989).

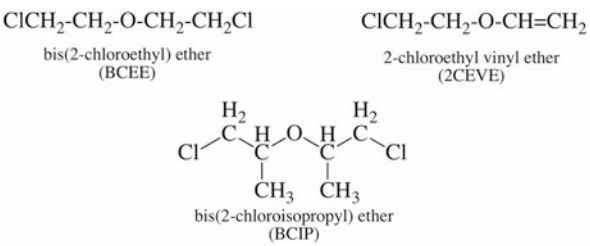

Fig. 3 Structures of representative chloralkyl ethers

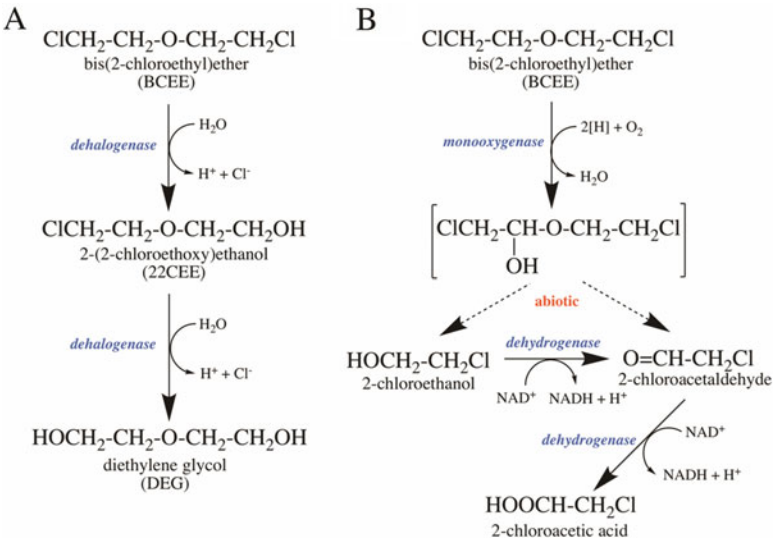

Fig. 4 *Initial reactions in the aerobic degradation of bis(2-chloroethyl)ether (BCEE). Panel A:* Initial reactions in metabolism of BCEE. *Panel B:* Initial reactions in cometabolic degradation of BCEE. Solid lines represent enzyme-catalyzed reactions. Dashed lines represent abiotic reactions. The type of enzyme involved in each reaction is shown in italics. Undetected intermediates are shown in brackets

3.1 BCEE Metabolism

The most detailed study of aerobic BCEE biodegradation to date involves *Xanthobacter* sp. ENV481. This aerobic bacterium was isolated by enrichment culture using BCEE as a sole source of carbon and energy and it can grow on both BCEE and 2CEVE (McClay et al. 2007). The stoichiometry of chloride (Cl^-) production and detected organic metabolites, as well as growth on putative intermediates, suggest the pathway of BCEE degradation involves two initial dehalogenation reactions that generate 2-(2-chloroethoxy) ethanol (22CEE) and diethylene glycol (DEG) as sequential intermediates (Fig. 4A). Degradation of BCEE to 22CEE and Cl^- also occurs in the absence of oxygen, indicating that oxygenases are not involved in this initial reaction. Haloalkane dehalogenases from other microorganisms are known to dechlorinate both BCEE and 2CEVE (Janssen et al. 1988; van den Wijngaard et al. 1993). It is likely that a similar enzyme is responsible for initiating the growth-supporting metabolism of both of these ethers in strain ENV481. Two *Ancylobacter aquaticus* strains (AD25 and AD27) have been described that can both grow on 2CEVE (van den Wijngaard et al. 1993). Like the degradation of BCEE by strain ENV481, the degradation pathway for 2CEVE in these strains is initiated by hydrolytic dehalogenases and does not appear to involve oxygenases.

3.2 BCEE Cometabolism

Unlike *Xanthobacter* sp. ENV481, oxygenase activity is clearly involved in BCEE degradation by *Pseudonocardia* sp. ENV478 (McClay et al. 2007). Strain ENV478 does not grow on BCEE but can rapidly degrade this ether after growth on propane. It also more slowly degrades BCEE after growth on THF (Vainberg et al. 2006). The enzyme(s) responsible for propane-oxidation in strain ENV478 have not been identified. However, growth of strain ENV478 on THF has been linked to THF monooxygenase (THFMO) through transcriptional studies of the gene encoding a hydroxylase component of this enzyme (*thmB*) and through the inhibition of growth on THF in cells expressing an antisense *thmB* transcript (Masuda et al. 2012). Degradation of BCEE by THF-grown cells of strain ENV478 leads to the stoichiometric accumulation of 2-chloroethanol and smaller amounts of 2-chloroacetic acid. Based on these products, BCEE degradation by THF-grown strain ENV478 likely involves an initial THFMO-catalyzed reaction that generates an unstable hemiacetal that subsequently decomposes to 2-chloroethanol and 2-chloroacetaldehyde. The further dehydrogenase-catalyzed oxidation of both of these compounds can then led to production of 2-chloracetic acid (Fig. 4B). The same metabolites are also observed during the cometabolic degradation of BCEE by *Rhodococcus* sp. DTB after growth on DEE (Moreno-Horn et al. 2005) (see below).

3.3 Metabolism of Other Chloroalkyl Ethers

Most of our understanding of the microbial metabolism of BCIP is derived from studies of *Rhodococcus* sp. DTB. This strain was isolated from a mixed culture developed to treat BCIP in a fixed bed reactor (Hauck et al. 2001). Strain DTB generates both 1-chloro-2-propanol and chloroacetone during BCIP degradation. The kinetics of production of these metabolites and the delayed but stoichiometric release of Cl^- suggests that cleavage of the ether bond is the initial step in a pathway that eventually fully mineralizes BCIP (Moreno-Horn et al. 2003). Degradation of BCIP is strongly inhibited by methimazole which suggests a role for a flavin-dependent monooxygenase in this initial reaction. Degradation of BCIP also generates a third product identified as 1′-chloro-2′-propyl-3-chloro-2-prop-1-enyl-ether; a dichloro vinyl ether (DVE) (Garbe et al. 2004). This metabolite is also degraded to 1-chloro-2-propanol and chloroacetone by living, but not heat-killed cells. Together, these results suggest that in addition to a monooxygenase-catalyzed oxidative ether cleavage reaction, BCIP degradation by strain DTB can also involve an initial monooxygenase-catalyzed desaturation reaction to form the DVE. This is followed by a second enzyme-catalyzed hydrolytic reaction to form an unstable hemiketal that subsequently decomposes to release 1-chloro-2-propanol and chloroacetone (Fig. 5). The relative contributions of the pathways involving oxidative scission of the ether bond and the formation of the DVE to the overall degradation of BCIP are not known. Strain DTB also catalyzes other interesting reactions with chloroalkyl ethers.

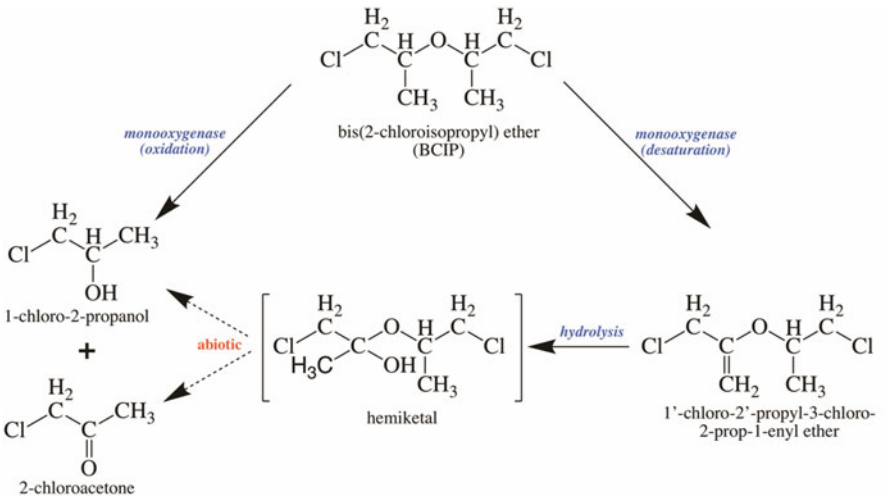

Fig. 5 *Oxidation and desaturation reactions in the biodegradation of bis(2-chloroisopropyl) ether (BCIP) biodegradation.* Solid lines represent enzyme-catalyzed reactions. Dashed lines represent abiotic reactions. The type of enzyme involved in each reaction is shown in italics. Undetected intermediates are shown in brackets

For example, the degradation of BCIP and its DVE metabolite are both enantioselective and the bacterium preferentially degrades (*S*)-configured ether-bonded carbons in these compounds (Garbe et al. 2006). It is likely that this enantioselectivity is conferred by the monooxygenase responsible for initiating BCIP degradation.

3.4 Cometabolism of Other Chloroalkyl Ethers

Strain DTB discussed above also grows on DEE and DEE-grown cells can then degrade several chlorinated and non-chlorinated ethers that otherwise do not support growth of this strain (Moreno-Horn et al. 2005). These include BCEE, bis(4-chloro-*n*-butyl) ether (BCBE), 1,2-dichloroethyl-ethyl ether, 2-chloroethyl-ethyl ether, 1,3-dichloro-2-propy-1′-chlor-2′-propyl ether, THF, and phenetole. γ-Butyrolactone is a product of both THF and BCBE degradation by strain DTB. In the case of THF oxidation, THF can potentially be initially oxidized by a monooxygenase to form 2-hydroxytetrahydrofuran and γ-butyrolactone can then be formed by the further dehydrogenase-catalyzed oxidation of this intermediate. Conversely, during BCBE degradation, it is thought an initial monooxygenase-catalyzed scission generates mixtures of 4-chlorobutanol and 4-chlorobutyraldehyde. The hydrolytic dehalogenation of these intermediates and their further oxidation by dehydrogenases generates 4-hydroxybutyrate which can then lactonize to form γ-butyrolactone (Moreno-Horn et al. 2005).

4 Branched Alkyl Ethers

The branched alkyl ethers considered in this section have all be used to one extent or another as gasoline oxygenates. In the United States, ether oxygenates were initially added to gasoline in the 1980s to compensate for the loss of octane rating following the removal of tetraethyl lead during the transition to lead-free gasoline (ITRC 2005). Branched ethers and alcohols were subsequently used to meet Federal requirements for oxygen that aimed to improve air quality by decreasing exhaust emission of partially oxidized hydrocarbons and CO (ITRC 2005). The main focus of this section is on the biodegradation of methyl *tertiary* butyl ether (MTBE) but also extends to the structurally- and functionally-related compounds, ethyl *tertiary* butyl ether (ETBE), *tertiary* amyl ether (TAME), and diisopropyl ether (DIPE) (Fig. 6). The aerobic biodegradation of MTBE and ETBE generates *tertiary* butyl alcohol (TBA) as an intermediate while biodegradation of TAME generates *tertiary* amyl alcohol (TAA). The biodegradation of these alcohols is also briefly addressed in this section.

4.1 MTBE Metabolism

Our understanding of the aerobic microbial growth-related metabolism of MTBE is based primarily on studies with three bacteria: *Hydrogenophaga flava* ENV735, *Methylibium petroleiphilum* PM1, and *Aquincola tertiaricarbonis* L108. *H. flava* ENV735 is a H_2-oxidizing bacterium that grows slowly and inefficiently (0.4 g/g) on MTBE (Hatzinger et al. 2001). The bacterium also generates TBA from MTBE and

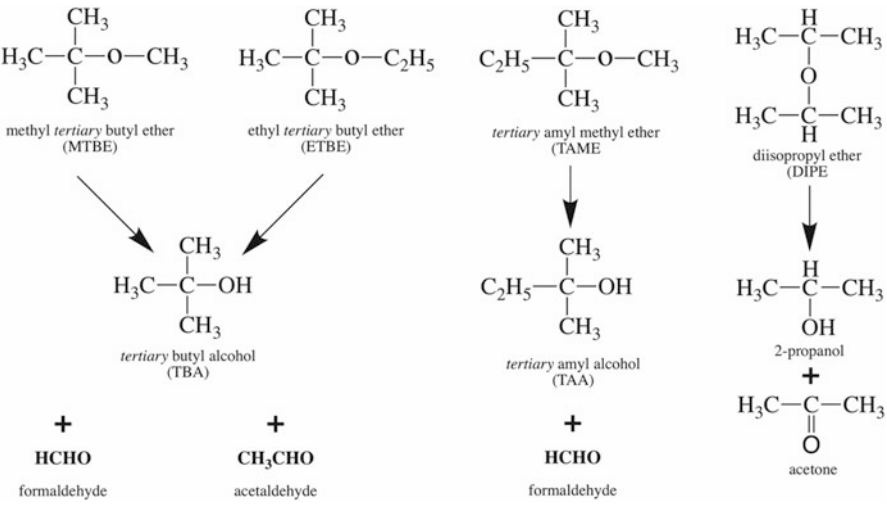

Fig. 6 Structures of the four main branched ether oxygenates and their corresponding major metabolites

can grow on TBA alone. MTBE and TBA are apparently oxidized by two different enzymes as MTBE-oxidizing activity is constitutive and sensitive to the cytochrome P450 inhibitor, aminobenzotriazole, whereas TBA oxidation is inducible and insensitive to aminobenzotriazole. Strain ENV735 also generates 2-hydroxyisobutyric acid (2HIBA) during MTBE oxidation and can grow on this compound as a sole source of carbon and energy (Hatzinger et al. 2001). The enzymes thought to be responsible for MTBE and TBA degradation in this strain have not been identified but both are reasonably anticipated to be oxygenases.

Like *H. flava* ENV735, *M. petroleiphilum* PM1 also grows slowly and inefficiently (<0.2 g/g) on MTBE (Hanson et al. 1999). This strain can also grow on methanol, ethanol, toluene, benzene, and ethylbenzene (Nakatsu et al. 2006). The genome of *M. petroleiphilum* PM1 consists of a ~4 Mb circular chromosome and a large (~600 kb) circular plasmid (pPM1) (Kane et al. 2007). Many of the genes encoding enzymes proposed to be involved in the MTBE oxidation pathway are located on pPM1. These include *mdpA*, *mdpE,* and *mdpJK* which encode an MTBE-oxidizing alkane hydroxylase-like monooxygenase, a hemiacetal-oxidizing dehydrogenase, and a TBA-oxidizing oxygenase, respectively. Although *tertiary* butyl formate (TBF) has not been observed as an intermediate in MTBE oxidation by *M. petroleiphilum* PM1, it has been proposed that the first enzyme (MpdA) oxidize MTBE to a hemiacetal. This hemiacetal then rapidly abiotically decomposes to form TBA and formaldehyde or is enzymatically oxidized to TBF by MpdE. The hydrolysis of TBF then releases TBA and formate. The TBA is then further oxidized to 2-methyl-1,2-propanediol (MPD) by MpdJK (Hristova et al. 2007) (Fig. 7).

The MTBE-oxidizing monooxygenase (MpdA) is an AlkB-like alkane hydroxylase. Genes encoding other enzymes typically associated with AlkB-like alkane hydroxylases such as an AlkG-like rubredoxin, an AlkT-like rubredoxin reductase, and a putative AlkS-like transcriptional regulator (MdpC) do not occur as a discrete operon but candidate genes all reside within a 10 kb locus on pPM1 (Schmidt et al. 2008). Another MTBE-degrading *Methylibium* isolate, strain R8, also contains *mdpA*. A ^{13}C-protein stable isotope probing analysis of a mixed MTBE-degrading culture that contained PM1-like bacteria characterized 23 ^{13}C-labeled proteins including MpdA, MdpJ, and MdpK (Bastida et al. 2010). This analysis also detected several other as yet uncharacterized proteins (MpeB0532-MpeB0535) whose genes are strongly up regulated in MTBE-grown cells of strain PM1 (Hristova et al. 2007).

A. tertiaricarbonis L108 grows on MTBE, TBA, and 2HIBA (Lechner et al. 2007; Müller et al. 2008). Among these, MTBE supports the lowest maximal growth rate (0.045 h^{-1}), and growth yield (~0.5 g/g) (Müller et al. 2007). This bacterium must sustain high rates of MTBE utilization to overcome the high minimum substrate concentration and maintenance energy barrier required for growth on this ether (Müller et al. 2007). The initial oxidation of MTBE in strain L108 is catalyzed by a 43 kDa cytochrome P450 (EthB) that was first identified in *Rhodococcus ruber* IFP 2001 (Chauvaux et al. 2001). The *eth* operon in strain L108 lacks the transcriptional regulator, *ethR* found in the *ethRABCD* operon in strain IFP 2001 (Chauvaux et al. 2001). This leads constitutive expression of the *eth*-gene-encoded cytochrome P450

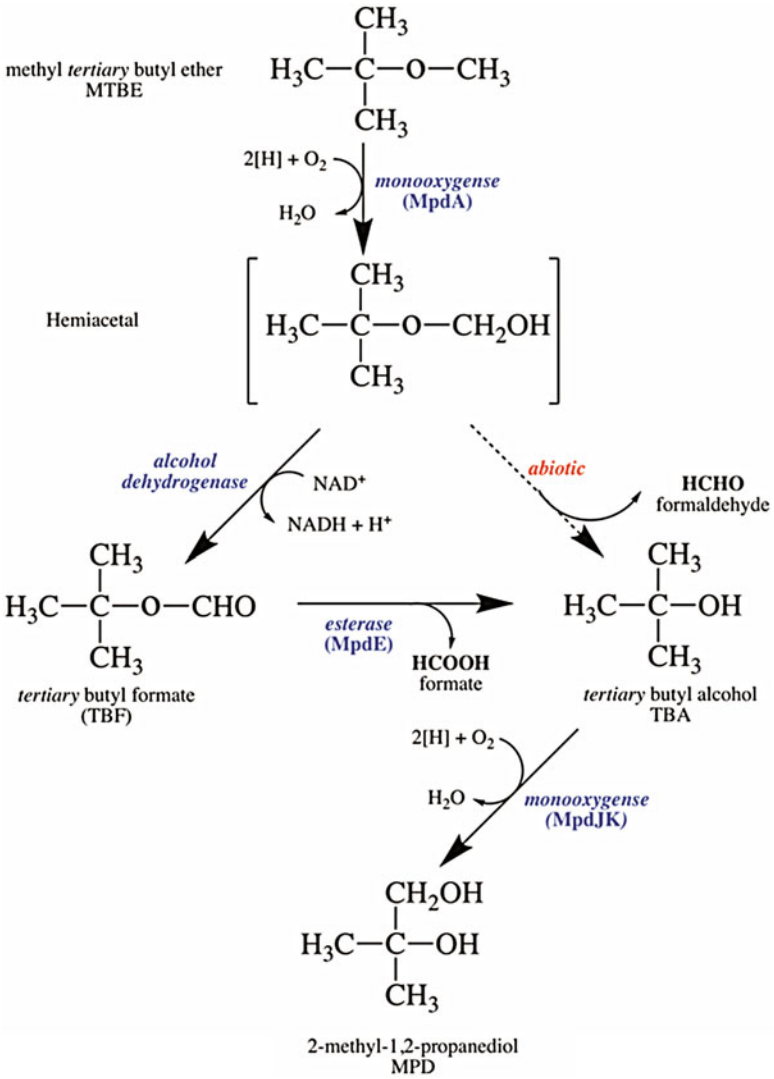

Fig. 7 *Initial reactions in the metabolism and cometabolism of methyl tertiary butyl ether.* Solid lines represent enzyme-catalyzed reactions. Dashed lines represent abiotic reactions. The type of enzyme involved in each reaction is shown in italics. Undetected intermediates are shown in brackets

system and MTBE-degrading activity in strain L108 (Schuster et al. 2013; Lechner et al. 2007). The other genes in the *eth* operon encode a ferredoxin reductase (EthA [~45 kDa]), a [2Fe-2S]-like ferredoxin (EthC [~12 kDa]), and a ~10 kDa protein of unknown function (EthD). The further oxidation of TBA to 2MPD by both strains L108 and PM1 is catalyzed by Rieske nonheme mononuclear iron oxygenase that consists of a 55 kDa hydroxylase (MdpJ) and a 38 kDa reductase (MdpK).

4.2 MTBE Cometabolism

The aerobic cometabolic degradation of MTBE has also been extensively studied and this activity occurs in diverse bacteria including *Pseudomonas*, *Pseudonocardia*, *Rhodococcus*, and *Mycobacterium* strains, as well as some fungi. In all cases, the initial step in MTBE oxidation appears to involve nonspecific oxygenases and leads to the production of TBA. For example, *Pseudomonas* strains that can grow on short chain ($<C_{10}$) *n*-alkanes have frequently been shown to oxidize MTBE (Garnier et al. 1999, 2000; Smith et al. 2003b; Morales et al. 2009; Smith and Hyman 2010; Salazar et al. 2012; Li et al. 2016). There are multiple lines of evidence that indicate this activity is catalyzed by AlkB-containing alkane hydroxylases. For instance, in *P. putida* GPo1, MTBE-oxidizing activity is observed in *n*-octane-grown cells and a gratuitous inducer, dicyclopropylketone (DCPK), also promotes MTBE-oxidizing activity in cells grown on alkane-free media (Smith and Hyman 2004). A similar effect of DCPK has been observed with other *Pseudomonas* strains (Bravo et al. 2015). Oxidation of MTBE in DCPK-induced cells of strain GPo1 is also inhibited by *n*-alkane substrates for alkane hydroxylase (C_3–C_{10}) and 1,7-octadiyne, a mechanism-based inactivator of alkane hydroxylase in this strain (Smith and Hyman 2004; Katapodis et al. 1984). The form of alkane hydroxylase in strain GPo1 is typically found in *Pseudomonas* strains that grow on shorter chain ($<C_{10}$) *n*-alkanes (Vomberg and Klinner 2000; Belhaj et al. 2002) and several *Pseudomonas* strains that contain genes that are identical to *alkB* in strain GPo1 all oxidize MTBE but not TBA (Smith and Hyman 2010). Conversely, MTBE-oxidizing activity is not associated with the more common forms of alkane hydroxylase that oxidize longer chain *n*-alkanes ($>C_{10}$) (Smith and Hyman 2010).

Pseudonocardia sp. ENV478 and *P. tetrahydrofuranoxidans* K1 both oxidize MTBE after growth on THF, and both strains generate TBA which is not further oxidized (Vainberg et al. 2006; McKelvie et al. 2009). In both cases, MTBE oxidation has been attributed to the activity of THFMO. In the case of strain ENV478, this is the same enzyme implicated in the degradation of BCEE. This monooxygenase is encoded by the *thm* operon (*thmADBC*) and the holoenzyme consists of two hydroxylase components (α- [ThmA, 60 kDa] and β- [ThmB, 38 kDa]), a small coupling protein (ThmC, 13 kDa) and a flavin-containing reductase (ThmD, 40 kDa) (Thiemer et al. 2003).

Several bacteria including *Rhodococcus ruber* ENV425, *Mycobacterium* sp. ENV421, and *Mycobacterium vaccae* JOB5 oxidize MTBE after growth on propane (Steffan et al. 1997; Smith et al. 2003a). These strains can be distinguished from microorganisms such as *P. putida* GPo1, *Pseudonocardia* ENV478, and *P. tetrahydrofuranoxidans* K1 discussed above as they do further oxidize TBA. The MTBE-degrading activity of strain ENV425 is inhibited by the cytochrome P450 inhibitors, CO and aminobenzotriazole. However, a subsequent molecular study did not find a role for a cytochrome P450 in propane-induced activities in strain ENV425 (Fournier et al. 2009). A recent genome-enabled proteomic analysis indicates propane-grown cells of strains ENV425 express two different soluble diiron monooxygenases (SDIMOs) (Tupa and Masuda 2018b, c). One of these is a Group 6

SDIMO, while the other is a Group 5 SDIMO. In contrast, propane-grown cells of ENV421 express a single Group 6 SDIMO, a membrane-bound, pMMO-like, copper-containing monooxygenase (CuMMO), and a cytochrome P450 (Tupa and Masuda 2018a, c). The role of these individual enzymes in propane, MTBE, and TBA oxidation by these strains has not been established. Similar Group 6 SDIMOs have recently been detected in other gaseous hydrocarbon-oxidizing strains such as *Rhodococcus* sp. strain BCP1 (Cappelletti et al. 2015) and have also recently been implicated in 14D degradation (He et al. 2017a, b; Deng et al. 2018a; Bennett et al. 2018).

The enzyme(s) responsible for MTBE oxidation in strain JOB5 have not been determined although MTBE- and TBA-oxidizing activity is observed in cells previously grown on a wide range of alkanes ($<C_{10}$) (House and Hyman 2010). In propane-grown cells of strain JOB5, TBF is an initial product of MTBE oxidation. Production of TBF has been proposed to involve the further alcohol-dehydrogenase-catalyzed oxidation of a hemiacetal generated from the initial monooxygenase-catalyzed oxidation of a C–H bond in the methoxy group of MTBE (Smith et al. 2003a). Mechanistically, this is the same pathway proposed for MTBE oxidation by strain PM1 (Fig. 7). However, unlike strain PM1, kinetic and inhibition studies using alkanes and acetylene suggest the same monooxygenase is responsible for the oxidation of both MTBE and TBA in strain JOB5 (Smith et al. 2003a). This enzyme is also thought to be responsible for initiating the oxidation of propane and other short chain alkanes ($<C_8$).

Similar effects of acetylene on both MTBE and TBA oxidation and similar kinetics and patterns of metabolite excretion and degradation have been observed in *Mycobacterium* strains that have been reported to grow on MTBE and TBA as a sole source of carbon and energy (François et al. 2002, 2003; Ferreira et al. 2006). However, these studies have typically used high initial MTBE and TBA concentrations and very high initial inoculum levels and have not shown an unequivocal relationship between MTBE removal and increases in culture density or any other potential measurement of growth. Although more compelling evidence is available for the growth of these strains on TBA, it has been suggested that the enzyme responsible for initiating both MTBE and TBA oxidation in these strains and strain JOB5 is an AlkB-like alkane hydroxylase (Ferreira et al. 2007). This seems unlikely as the MTBE-oxidizing AlkB-alkane hydroxylases characterized to date do not further oxidize TBA at any physiologically relevant rate. Molecular studies of AlkB-like alkane hydroxylase expression in strain JOB5 also indicate these enzymes are not expressed in propane-grown cells (Sharp et al. 2010). In contrast to the high MTBE and TBA concentrations used in the studies described above, *n*-butane-grown cells of an *Arthrobacter* strain (ATCC 27778) and propane-grown cells of strain ENV425 have been shown to oxidize low concentrations of MTBE (100–800 μg L^{-1}) (Liu et al. 2001). Similar to strain JOB5, MTBE oxidation by *Arthrobacter* sp. ATCC 27778 is sensitive to inhibition by alkanes (e.g., *n*-butane), TBA, and acetylene (Liu et al. 2001). The enzyme(s) responsible for the oxidation of MTBE in ATCC 27778 have not been identified.

The filamentous fungus, *Graphium* sp., grows on gaseous *n*-alkanes (Curry et al. 1996), DEE (Hardison et al. 1997), and THF (Skinner et al. 2009) and mycelia grown on propane, *n*-butane, or DEE all slowly degrade MTBE (Hardison et al. 1997). With *n*-alkane-grown mycelia, TBF can initially represent as much as 95% of the MTBE oxidized and its hydrolysis product, TBA, is not further oxidized at significant rates (Hardison et al. 1997; Skinner et al. 2008). Evidence that TBF production from MTBE involves rapid enzymatic oxidation of an initial monooxygenase-generated hemiacetal is supported by the observation that propane- and THF-grown mycelia can rapidly generate methylformate from concentrated mixtures of formaldehyde and methanol (Skinner et al. 2009). RNAi silencing of posttranscriptional translation of a single cytochrome P450-encoding gene severely inhibits growth of this fungus on *n*-alkanes and THF and suggests this gene encodes an enzyme with a broad hydrocarbon- and ether-oxidizing capabilities (Trippe et al. 2014). Another fungus, *Agrocybe aegerita*, excretes an extracellular heme-containing peroxygenase that can also oxidize MTBE (Kinne et al. 2009). A similar enzyme is also produced by a variety of other fungi (Hofrichter and Ullrich 2014). The well-characterized camphor-oxidizing cytochrome $P450_{cam}$ in *P. putida* CAM (ATCC 17453) can also oxidize MTBE to TBA but does not further oxidize TBA but ether-oxidizing activity does not appear to be widespread activity among these heme-containing enzymes (Steffan et al. 1997).

4.3 Metabolism of Other Branched Alkyl Ethers

Many of the bacteria described above that metabolize MTBE also metabolize other branched alkyl ethers. For example, *M. petroleiphilum* PM1 can grow on both MTBE and TAME, but not ETBE (Schuster et al. 2012). As both MTBE and ETBE oxidation are expected to generate TBA as a key metabolite, this growth substrate range suggests the ether substrate range of MdpA, like other AlkB-containing alkane hydroxylases, is limited to methyl ethers (van Beilen et al. 1994; Katapodis et al. 1984). Similarly, in addition to growth on MTBE, TBA, and 2HIBA, *A. tertiaricarbonis* L108 can also grow on ETBE, TAME, and TAA (Lechner et al. 2007; Müller et al. 2008). The initial multicarbon metabolites generated from ETBE and TAME by the EthB cytochrome P450 are assumed or known to be TBA plus acetaldehyde, and TAA, respectively (Müller et al. 2008; Schuster et al. 2013). It is further assumed that these products are derived from unstable monooxygenase-generated hemiacetal intermediates. The further oxidation of TBA to 2MPD involved in both MTBE and ETBE metabolism by strain L108 and PM1 involves an MpdJK-catalyzed monooxygenation reaction. The analogous products of TAA oxidation would be expected to be various diols. However, the immediate product of TAA degradation by these strains is 2-methyl-3-buten-2-ol which indicates the further degradation of TAA involves a desaturase rather than a monooxygenase activity of MdpJK (Schuster et al. 2012).

Although some alkyl ether-metabolizing bacteria have broad growth substrate ranges, other strains are more restricted. For example, some of the earliest studies of ether oxygenate degradation described the isolation and characterization of *Rhodococcus ruber* IFP 2001 and *R. zopfii* IFP 2002 (Fayolle et al. 1998). Strain IFP 2001 generates stoichiometric amounts of TBA during growth on ETBE suggesting that it grows on the 2-carbon fragment released during ETBE oxidation, rather than TBA. Although strain IFP 2001 does not grow on MTBE or TAME, resting ETBE-grown cells readily oxidize both compounds without a lag phase and generate near stoichiometric amounts of TBA and TAA, respectively (Hernandez-Perez et al. 2001). As indicated earlier, molecular studies of the ETBE-oxidizing system in strain IFP 2001 led to the identification of the *eth* operon and the role of *ethB*-encoded cytochrome P450 in ether oxygenate biodegradation. Transcriptional analyses have demonstrated that ETBE but not MTBE or TAME induces expression of the *eth* genes in strain IFP 2001 (Malandain et al. 2010). It is therefore not clear whether the principal restriction on the ability of strain IFP to grow on methyl ether oxygenates (MTBE and TAME) is a lack of *eth* gene induction or an inability to utilize the C_1 fragments generated from their oxidation.

Several bacterial strains have been reported to grow aerobically on DIPE. These include *Rhodococcus ruber* 219 (Bock et al. 1996) and *Pseudonocardia* sp. ENV 478 (Vainberg et al. 2006). The intermediates generated from DIPE oxidation and the oxygenase enzymes potentially involved in their biodegradation are currently not known. Although *A. tertiaricarbonis* L108 has not been described to grow on DIPE, it can constitutively oxidize this ether and acetone has been reported as a major metabolite (Schuster et al. 2013). Based on other oxygenase-catalyzed reactions, it is likely that DIPE is initially oxidized to a hemiacetal that then decomposes to form 2-propanol and acetone. In the case of strain L108, it is likely that 2-propanol is further oxidized to acetone which accumulates due to lack of a pathway for its further metabolism.

4.4 Cometabolism of Other Branched Alkyl Ethers

The AlkB-containing alkane hydroxylases found in *P. putida* GPo1 (Smith and Hyman 2004) *P. mendocina* KR1 (Smith et al. 2003b) and other closely related Pseudomonads (Smith and Hyman 2010) appear to be restricted to methyl ethers as cells expressing this enzyme can oxidize MTBE and TAME but are largely unreactive towards ETBE and the *tertiary* alcohols, TBA and TAA. A mixed culture containing *Pseudomonas* strains has been described that can mineralizes MTBE, ETBE, TAME, TBA, and TAA in the presence of C_3–C_7 *n*-alkanes (Morales et al. 2009). A *P. citronellolis* strain isolated from this culture expresses a very similar AlkB-containing alkane hydroxylase to the enzyme in *P. putida* GPo1 (Bravo et al. 2015) and it is likely that this mixed culture contains separate TBA-metabolizing strains rather than Pseudomonads with full MTBE-mineralizing capabilities. In addition to MTBE, other strains including THF-grown *P. tetrahydrofuranoxidans* K1 (McKelvie et al. 2009) and propane-grown *R. ruber*

ENV425 (Steffan et al. 1997) can also oxidize ETBE and TAME. Unlike strain K1 which does not appear to oxidize *tertiary* alcohols at any significant rate (McKelvie et al. 2009), propane-grown strain ENV425 can also oxidize both TBA and TAA (Steffan et al. 1997). It is likely both of these alcohols are further oxidized to diols through monooxygenase activity.

5 Cyclic Ethers

Cyclic ethers are oxygen-containing heterocyclic compounds. The simplest cyclic ether is ethylene oxide (1,2-epoxyethane). This highly reactive gas is widely used as a chemical feedstock and as a sterilizing agent. The model cyclic ether considered in this section is 14D. This compound was extensively used as a stabilizing agent in commercial formulations of chlorinated solvents such as 1,1,1-trichloroethane (Mohr et al. 2010). It is also found in cosmetics, detergents, and shampoos as a byproduct of manufacturing processes involving ethylene oxide (USEPA 2016). 14D is a likely human carcinogen with an increased one in one million cancer risk associated with lifetime consumption of drinking water at a concentration of 0.35 µg/L (USEPA 2017). The other cyclic ethers considered in this section include THF, tetrahydropyran (THP), 1,3-dioxane (13D), and 1,3-dioxalane (13DO) (Fig. 8).

5.1 14D Metabolism

A wide variety of microorganisms are known to grow on 14D as a sole source of carbon and energy. These include fungi such as *Cordyceps sineies* (Nakamiya et al. 2005) and representatives of the following bacterial genera; *Acinetobacter* (Huang et al. 2014; Zhou et al. 2016), *Afipia* (Sei et al. 2013a), *Mycobacterium* (Kim et al. 2009; Sei et al. 2013a), *Rhodanobacter* (Pugazhendi et al. 2015), *Rhodococcus* (He et al. 2014; Inoue et al. 2016, 2018), *Pseudonocardia* (Parales et al. 1994; Mahendra

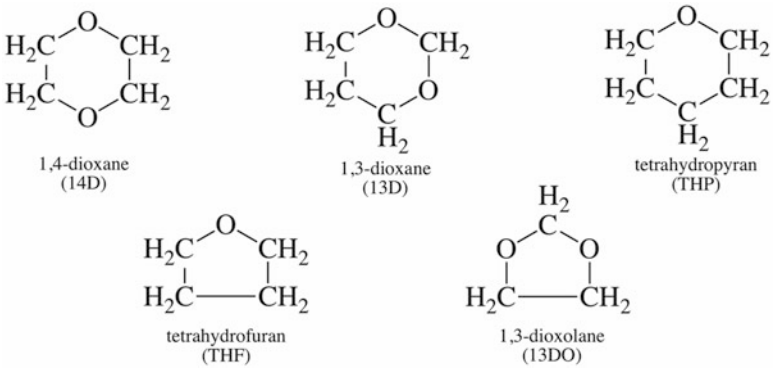

Fig. 8 Structures of representative cyclic ethers

and Alvarez-Cohen 2005; Matsui et al. 2016; Inoue et al. 2016; Yamamoto et al. 2018), and *Xanthobacter* (Jin et al. 2012; Chen et al. 2016).

The best-characterized 14D-metabolizing bacterial strains at the molecular level are *Pseudonocardia dioxanivorans* CB1190 and *Mycobacterium dioxanitrophicus* PH-06. Strain CB1190 (ATCC 55486) was isolated a mixed culture enriched from a 14D-contaminanted sludge. The culture was initially grown on THF and slowly adapted to 14D (Parales et al. 1994). This bacterium is most likely a THF-metabolizing strain that acquired a stable mutation affecting a regulatory, transport, or enzymatic feature that now allows it to grow, albeit slowly (30 h generation time) (Parales et al. 1994) and inefficiently (0.09 g/g) (Mahendra and Alvarez-Cohen 2005), on 14D. The genome of strain CB1190 consists of a circular 7.1 Mb chromosome with three plasmids (pPSED01 (192 kb), pPSED02 (137 kb), and pPSED03 (15 kb)) (Sales et al. 2011). Among several other multicomponent monooxygenases encoded in the genome, a complete operon encoding THFMO is present on pPSED02. This operon is nearly identical to the operon encoding this enzyme in other strains including *Rhodococcus* sp. YLL (He et al. 2014; Yao et al. 2013), *Pseudonocardia* ENV478 (Masuda et al. 2012), and *P. tetrahydrofuranoxidans* K1 (Bennett et al. 2018) that can all cometabolically degrade 14D after growth on THF but do not grow on 14D.

The role of THFMO in initially oxidizing 14D and THF by strain CB1190 and *P. tetrahydrofuranoxidans* K1 has been confirmed by heterologous expression of their respective THFMO gene clusters (*thmADBC*) in *Rhodococcus jostii* RHA1 (Sales et al. 2013). It has also been determined that THFMO does not participate in the further oxidation of a key 14D-derived metabolite, 2-hydroxyethoxyacetic acid (2HEAA) (Sales et al. 2013). Transcription-based studies of *thm* gene expression in response to 14D, THF, and inhibition of THFMO activity by acetylene also support a role for THFMO in 14D metabolism by strain CB1190 (Gedalanga et al. 2014). Genes encoding THFMO hydroxylase subunits have been suggested to be useful biomarkers for detecting the potential for 14D biodegradation in field samples and in these studies THFMO in strain CB1190 has been referred to as DXMO (Li et al. 2013; Gedalanga et al. 2016). This nomenclature may have been introduced to suggest that THFMO expressed by CB1190 is somehow different from other THFMOs as it is associated with an organism that can grow on 14D. If so, this is misleading because all THFMOs currently known are structurally and catalytically almost indistinguishable.

Mycobacterium PH-06 was originally isolated from a 14D-contaminated river sediment by enrichment culture using 14D as the enrichment substrate (Kim et al. 2009). The genome of the bacterium consists of a circular chromosome (7.6 Mb), with four plasmids (156, 153, 106, and 70 kb). The genome encodes several monooxygenases including a group 6 SDIMO and a copper-containing monooxygenase (CuMMO) that are closely located on plasmid 3. RNAseq analyses indicate that expression of all components of both of these enzymes is greatly increased in cells grown on 14D as compared to glucose-grown cells (He et al. 2017b). This suggests both enzymes may have a role in 14D metabolism. The Group 6 SDIMO has been further characterized and transcriptional analyses have shown

that all four genes encoding this enzyme (*prmABCD*) are expressed as a single polycistronic transcript when cells are exposed 14D, THF or propane (Deng et al. 2018a). This SDIMO has also been heterologously expressed in *M. smegmatis* mc^2-155 and shown to oxidize 14D, THF, and propane (Deng et al. 2018a). In contrast, the CuMMO that is co-expressed with the SDIMO has not been further characterized so it is currently not clear whether the propane and cyclic ether-degrading activities of strain PH-06 can be attributed to just one or multiple monooxygenases. A role of the CuMMO in propane oxidation is likely as similar enzymes have been detected in other gaseous alkane-oxidizing bacteria including *Mycobacterium* sp. NBB4 (Coleman et al. 2012), *Nocardiodes* CF8 (Sayavedra-Soto et al. 2011), and several marine bacteria identified by stable isotope probing (Redmond et al. 2010)

The SDIMO detected in strain PH-06 has been called a propane monooxygenase, presumably based on its ability to oxidize propane (Deng et al. 2018a). Like the use of DXMO to describe THFMO discussed above, this, nomenclature is likely to cause confusion as a group 5 SDIMO that is found in strains with typically weak and narrow gaseous alkane-oxidizing activities such as *R. jostii* RHA1 (Sharp et al. 2007), *Gordonia* TY-5 (Kotani et al. 2003), and others is also called propane monooxygenase. A Group 5 SDIMO propane monooxygenase is also co-expressed with THFMO during 14D degradation by strain CB1190 but is not thought to be directly involved in cyclic ether biodegradation by this bacterium (Gedalanga et al. 2014).

The immediate product of 14D oxidation by the different monooxygenase associated with 14D oxidation in both strains PH-06 and CB1190 is thought to be 1,4-dioxane-2-ol (Kim et al. 2009; Mahendra et al. 2007). This metabolite may undergo ring chain tautomerism to 2-hydroxyethoxyacetaldehyde or may be further oxidized to a lactone (1,4-dioxanone) that can then undergo reversible hydrolysis and lactonization to generate 2-hydroxyethoxyacetic acid (2HEAA) (Fig. 9). This metabolite has been detected in several studies of microbial 14D degradation (Vainberg et al. 2006; Mahendra et al. 2007; Sales et al. 2013; Deng et al. 2018a) and it has been suggested that another monooxygenase-catalyzed reaction is required to further oxidize 2HEAA (Mahendra et al. 2007). Heterologous expression studies have indicated that if needed, this reaction is not catalyzed by THFMO (Sales et al. 2013). Although 1,4-dioxane-2-ol is also thought to be an initial product of 14D degradation by *Acinetobacter baumannii* DD1, 1,4-dioxene has been detected as a minor metabolite (Huang et al. 2014). 1,4-Dioxene has also been observed in studies of 14D degradation by *Xanthobacter flavus* DT8 and has been suggested to be a dehydration product of 1,4-dioxane-2-ol (Chen et al. 2016). 2HEAA has not been detected as a metabolite of 14D degradation by strain DT8 which suggests that production of 1,4-dioxene may be indicative of a 2HEAA-independent pathway of 14D degradation.

5.2 14D Cometabolism

Given the structural similarities between 14D and THF, it is not surprising that many THF-metabolizing strains can cometabolically degrade 14D. Bacterial strains that

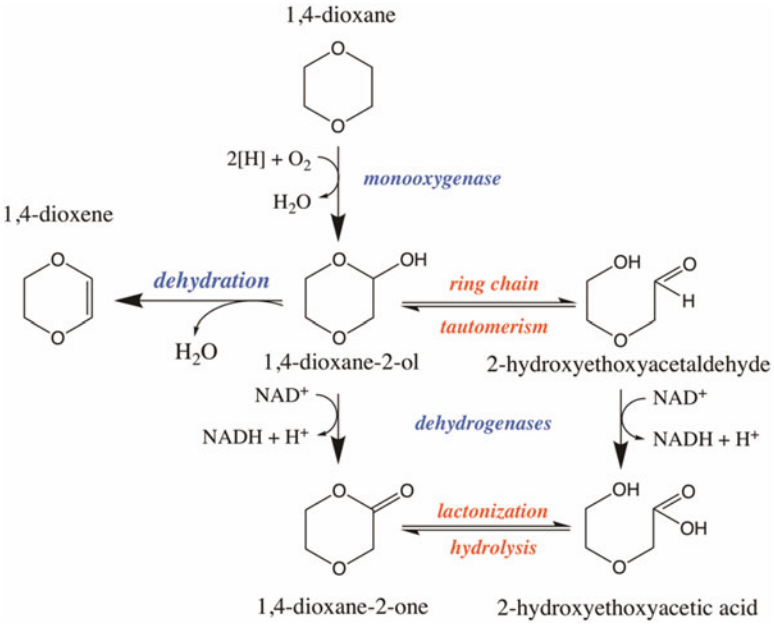

Fig. 9 *Initial reactions in the metabolism and cometabolism of 1,4-dioxane.* Solid lines represent enzyme-catalyzed reactions. Dashed lines represent abiotic reactions. The type of enzyme involved in each reaction is shown in italics. Undetected intermediates are shown in brackets

cometabolically degrade 14D after growth on THF include *Rhodococcus ruber* strains 219 (Bock et al. 1996), T1, and T5 (Sei et al. 2013b), and *Pseudonocardia* isolates including strains ENV478 (Vainberg et al. 2006), K1 (Mahendra and Alvarez-Cohen 2006; Bennett et al. 2018), and others (Inoue et al. 2016). The filamentous fungus *Graphium* sp. also slowly oxidizes 14D after growth on THF (Skinner et al. 2009) through the activity of a cytochrome P450 (Trippe et al. 2014).

Due to its own human toxicity, THF is not a useful substrate for stimulating microbial processes in the environment. Consequently, there is considerable current interest in other substrates that can stimulate cometabolic degradation of 14D. Three model toluene-oxidizing strains expressing either toluene-2-, -3-, or -4-monooxygenase can all oxidize high ($\leq$500 mg L^{-1}) initial concentrations of 14D at faster initial rates than cells of strain CB1190 grown on 14D (Mahendra and Alvarez-Cohen 2006). This activity has also been confirmed with cloned and heterologously expressed forms of these enzymes. *Methylosinus trichosporium* OB3b expressing sMMO has also been reported to degrade 14D although this activity was not sustainable (Mahendra and Alvarez-Cohen 2006). A subsequent study using purified sMMO has not confirmed 14D-degrading activity for this enzyme (Hatzinger et al. 2017). In the same study, an ethane-metabolizing strain (*Mycobacterium* ENV482) was isolated from a site where ethane had been demonstrated to stimulate cometabolic degradation of low concentrations of ethylene dibromide. This bacterium can also oxidize 14D after growth on ethane. *M. vaccae* JOB5 has also been reported to oxidize 14D after growth on propane (Burback and

Perry 1993; Mahendra and Alvarez-Cohen 2006; Lan et al. 2013) and other alkanes including *n*-butane, *n*-pentane, isobutane, and isopentane (Lan et al. 2013). The enzyme(s) responsible for the oxidation of 14D and these alkanes has not been identified but cells grown on these substrates are also able to oxidize both MTBE and TBA (House and Hyman 2010). The most recent report of a pure culture capable of propane-dependent cometabolic degradation of 14D describes an *Azoarcus* strain, DD4 (Deng et al. 2018b). When spiked into groundwater, this strain was able to oxidize 14D to very low concentrations (<0.4 μg L^{-1}). Although the genome of this bacterium has not been described and transcriptional studies have not been conducted, the 14D-degrading activity has been tentatively attributed to a Group 5 SDIMO, propane monooxygenase. This bacterium was also shown to concurrently degrade 1,1-dichloroethene which is a common co-contaminant often encountered in 14D-contaminanted groundwater.

5.3 Metabolism of Other Cyclic Ethers

In addition to 14D, many of currently known 14D-metabolizing strains also grow on other ethers. For example, strain CB1190 can also grow on both cyclic (THF, THP, 13D, 2-methyl-1,3-dioxane) and alkyl ethers (DEE and butyl methyl ether) (Parales et al. 1994). Among the cyclic ethers, many 14D-metabolizing microorganisms can grow on the 14D isomer, 13D (Nakamiya et al. 2005; Huang et al. 2014, Sei et al. 2013a; Kim et al. 2009) and the structurally similar compound, THP (Nakamiya et al. 2005; Sei et al. 2013a). The enzymes responsible for initiating the degradation of these ethers has not be unequivocally identified in most cases although it is assumed that the enzymes responsible for initiating the metabolism of these compounds are the same as the enzymes used to initiate 14D metabolism.

5.4 Cometabolism of Other Cyclic Ethers

Overall, little attention has been paid to the cometabolic biodegradation of cyclic ethers other than 14D. *M. vaccae* JOB5 has been shown to oxidize trimethylene oxide, THF, THP, 13D, and 13DO after growth on propane, *n*-butane, *n*-pentane, isobutane, and isopentane (Lan et al. 2013). During the degradation of THF by propane-grown cells, near stoichiometric amounts of γ-butyrolactone accumulate and are subsequently consumed. This is thought to reflect the initial oxidation of THF to 2-hydroxytetrahydrofuran followed by the rapid further oxidation of this intermediate to γ-butyrolactone by alcohol dehydrogenases that are abundant in alkane-grown cells. When cells were grown on *n*-pentane in the presence of THF, γ-butyrolactone is generated and consumed and, based on substantial increases in culture density, appears to enhance the growth of the bacterium compared to cells grown on *n*-pentane alone (Lan et al. 2013). Although 14D is extensively consumed, a growth stimulating effect is not observed for cells grown on *n*-pentane in the presence of 14D. This suggests that metabolites generated from 14D oxidation are not further degraded or are degraded at rates too slow to impact growth.

6 Future Research

As this chapter has hopefully demonstrated, there is strong and consistent evidence that a wide variety of simple ether-bonded compounds can be biodegraded by aerobic microbial processes. In many cases, these biodegradation processes involve the activities of non-specific oxygenase enzymes that catalyze oxidative ether cleavage reactions. However, as studies with some compounds such as BCIP (Garbe et al. 2004) and TAA (Schuster et al. 2012) have demonstrated, these may also involve desaturation reactions. The possibility that nonoxidative oxygenase-catalyzed reactions are involved in other ether degradation reactions could reveal interesting results.

Another theme that has emerged from recent research into aerobic ether biodegradation is the concentration of ethers that can be biodegraded by organisms that can grow on ethers and those that can only cometabolically transform these compounds. For example, there is a wealth of information about strains such as *P. dioxanivorans* CB1190 and *Mycobacterium dioxanitrophicus* PH-06 that can grow on 14D. However, the K_s values for the oxidation 14D by these strains have recently been determined to be 145 ± 17 mg L^{-1} and 78 ± 10 mg L^{-1}, respectively (He et al. 2018), and are both far above the μg L^{-1} concentrations of 14D that are frequently encountered in 14D-contaminated environments. A recent kinetic and modeling study has suggested that organisms such as strain CB1190 are unlikely to be effective at biodegrading 14D at concentrations below ~400 μg L^{-1}, whereas cometabolic processes are effective below this concentration (Barajas-Rodriguez and Freedman 2018). Recent studies with pure cultures (Deng et al. 2018b) and field studies (Lippincott et al. 2015; Chu et al. 2018) have also confirmed that propanotrophic bacteria are very effective at degrading 14D to low μg L^{-1} concentrations and have the added capability of simultaneously degrading chlorinated solvent co-contaminants that are frequently detected with 14D in groundwater. There is also growing interest in the role of cometabolism in the natural attenuation of ethers such as 14D. To fully understand this, additional research is needed to define the kinetics of ether biodegradation reactions at low concentrations and physiological studies are needed to determine whether ethers such as 14D have their own independent regulatory effects on the expression of these oxygenases that can biodegrade this compound. Additional research is also needed to determine whether 14D-derived metabolites can contribute to the carbon nutrition of microorganisms that can otherwise not grow on this compound and the interspecies interactions that can be established through metabolite exchange between bacteria that can cometabolically degrade 14D and those that can grow on excreted metabolites generated from this process.

References

Agency for Toxic Substances and Disease Registry (ATSDR) (2017) Toxicological profile for bis(2-chloroethyl)ether (BCEE). https://www.atsdr.cdc.gov/toxprofiles/tp127.pdf

Barajas-Rodriguez FJ, Freedman DL (2018) Aerobic biodegradation kinetics for 1,4-dioxane under metabolic and cometabolic conditions. J Hazard Mater 350:180–188

Bastida F, Rosell M, Franchini AG, Seifert J, Finsterbusch S, Jehmlich N, Jechalke S, von Bergen M, Richnow HH (2010) Elucidating MTBE degradation in a mixed consortium using a multidisciplinary approach. FEMS Microbiol Ecol 73:370–384

Bednar AJ, Kirgan RA, Karn RA, Donovan B, Mohn MF, Sirkis DM (2009) Mobility and sorption of bis-2-chloroethyl ether in an aquifer material. J Hazard Mater 168:1041–1046

Belhaj A, Desnoues N, Elmerich C (2002) Alkane biodegradation in *Pseudomonas aeruginosa* strains isolated from a polluted zone: identification of *alkB* and *alkB*-related genes. Res Microbiol 153:339–344

Bennett P, Hyman M, Smith C, El Mugammar H, Chu M-Y, Nicklelsen M, Aravena R (2018) Enrichment with carbon-13 and deuterium during monooxygenase-mediated biodegradation of 1,4-dioxane. Environ Sci Technol Lett 5:148–153

Bock C, Kroppenstedt RM, Diekmann H (1996) Degradation and bioconversion of aliphatic and aromatic hydrocarbons by *Rhodococcus ruber* 219. Appl Microbiol Biotechnol 45:408–410

Bravo AL, Sigala JC, Borgne SL, Morales M (2015) Expression of an alkane hydroxylase (*alkb*) gene and methyl-*tert*-butyl ether co-metabolic oxidation in *Pseudomonas citronellolis*. Biotechnol Lett 37:807–814

Burback BL, Perry JJ (1993) Biodegradation and biotransformation of groundwater pollutant mixtures by *Mycobacterium vaccae*. Appl Environ Microbiol 59:1025–1029

Cappelletti M, Presentato A, Milazzo G, Turner RJ, Fedi S, Frascari D, Zannoni D (2015) Growth of *Rhodococcus* sp. strain BCP1 on gaseous *n*-alkanes: new metabolic insights and transcriptional analysis of two soluble di-iron monooxygenase genes. Front Microbiol 6. https://doi.org/10.3389/fmicb.2015.00393

Chauvaux S, Chevalier F, Le Dantec C, Fayolle F, Miras I, Kunst F, Béguin P (2001) Cloning of a genetically unstable cytochrome P-450 gene cluster involved in degradation of the pollutant ethyl *tert*-butyl ether by *Rhodococcus ruber*. J Bacteriol 183:6551–6557

Chen D-Z, Jin X-J, Chen J, Ye J-X, Jiang N-X, Chen J-M (2016) Intermediates and substrate interaction of 1,4-dioxane degradation by effective metabolizer *Xanthobacter flavus* DT8. Int Biodeterior Biodegrad 106:133–140

Chu M-YJ, Bennett PJ, Dolan ME, Hyman MR, Peacock AD, Bodour A, Andersen RH, Mackay DM, Goltz MN (2018) Concurrent treatment of 1,4-dioxane and chlorinated aliphatics in a groundwater recirculation system via aerobic cometabolism. Groundwater Monit Remediat 38:53–64

Colby J, Stirling DI, Dalton H (1977) The soluble methane mono-oxygenase of *Methylococcus capsulatus* (Bath). Its ability to oxygenate *n*-alkanes, *n*-alkenes, ethers, and alicyclic, aromatic and heterocyclic compounds. Biochem J 165:395–402

Coleman NV, Le NB, Ly MA, Ogawa HE, McCarl V, Wilson NL, Holmes AJ (2012) Hydrocarbon monooxygenase in *Mycobacterium*: recombinant expression of a member of the ammonia monooxygenase superfamily. ISME J 6:171–182

Curry S, Ciuffetti L, Hyman M (1996) Inhibition of growth of a *Graphium* sp. on gaseous *n*-alkanes by gaseous *n*-alkynes and *n*-alkenes. Appl Environ Microbiol 62:2198–2200

de Bont JAM, Mulder EG (1974) Nitrogen fixation and co-oxidation of ethylene by a methane-utilizing bacterium. J Gen Microbiol 83:113–121

Deng D, Li F, Li M (2018a) A novel propane monooxygenase initiating degradation of 1,4-dioxane by *Mycobacterium dioxanotrophicus* PH-06. Environ Sci Technol Lett 5:86–91

Deng D, Li F, Wu C, Li M (2018b) Synchronic biotransformation of 1,4-dioxane and 1,1-dicloroethylene by a Gram-negative propanotroph *Azoarcus* sp. DD4. Environ Sci Technol Lett 5:526–532

Fayolle F, Hernandez G, Roux FL, Vandecasteele J-P (1998) Isolation of two aerobic bacterial strains that degrade efficiently ethyl *t*-butyl ether (ETBE). Biotechnol Lett 20:283–286

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

Ferreira NL, Mathis H, Labbé D, Monot F, Greer CW, Fayolle-Guichard F (2007) *N*-alkane assimilation and *tert*-butyl alcohol (TBA) oxidation capacity in *Mycobacterium austroafricanum* strains. Appl Microbiol Biotechnol 75:909–919

Fisher CR, Childress JJ, Oremland RS, Bidigare RR (1987) The importance of methane and thiosulfate in the metabolism of the bacterial symbionts of two deep-sea mussels. Mar Biol 96:59–71

Fournier D, Hawari J, Halasz A, Streger SH, McClay KR, Masuda H, Hatzinger PB (2009) Aerobic biodegradation of *N*-nitrosodimethylamine by the propanotroph *Rhodococcus ruber* ENV425. Appl Environ Microbiol 75:5088–5093

François A, Mathis H, Godefroy D, Piveteau P, Fayolle 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 68:2754–2762

François A, Garnier L, Mathis H, Fayolle F, Monot F (2003) Roles of *tert*-butyl formate, *tert*-butyl alcohol and acetone in the regulation of methyl *tert*-butyl ether degradation by *Mycobacterium austroafricanum* IFP 2012. Appl Microbiol Biotechnol 62:256–262

Garbe LA, Moreno-Horn M, Rewicki D, Tressl R, Görisch H (2004) Microbial desaturation of bis (1-chloro-2-propyl) ether into a dichloro vinyl ether. Chembiochem 5:876–878

Garbe LA, Moreno-Horn M, Tressl R, Görsich H (2006) Preferential attack of the (S)-configured ether-linked carbons in bis-(1-chloro-2-propyl) ether by *Rhodococcus* sp. strain DTB. FEMS Microbiol Ecol 55:113–121

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

Garnier PM, Auria R, Augur C, Revah S (2000) Cometabolic biodegradation of methyl *tert*-butyl ether by a soil consortium: effect of components present in gasoline. J Gen Appl Microbiol 46:79–84

Gedalanga PB, Pornwongthing P, Mora R, Chiang S-YD, Baldwin B, Ogles D, Mahendra S (2014) Identification of biomarker genes to predict biodegradation of 1,4-dioxane. Appl Environ Microbiol 80:3209–3218

Gedalanga P, Madison A, Miao YR, Richards T, Hatton J, DiGuiseppi WH, Wilson J, Mahendra S (2016) A multiple lines of evidence framework to evaluate intrinsic biodegradation of 1,4-dioxane. Remediat J 27:93–114

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

Hardison LK, Curry SS, Ciuffetti LM, Hyman MR (1997) Metabolism of diethyl ether and cometabolism of methyl *tert*-butyl ether by a filamentous fungus, a *Graphium* sp. Appl Environ Microbiol 63:3059–3067

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

Hatzinger PB, Banerjee R, Rezes R, Streger SH, McClay K, Schaefer CE (2017) Potential for cometabolic biodegradation of 1,4-dioxane in aquifers with methane or ethane as primary substrates. Biodegradation 28:453–468

Hauck R, Adrian L, Wendelr P, Amidjojo M, Hegemann W, Görisch H (2001) Transformation of 2,2′-dichlorodiisopropyl ether in mixed and pure culture. Appl Microbiol Biotechnol 56:491–495

Hazeu W (1975) Some cultural and physiological aspects of methane-utilizing bacteria. Antonie Van Leeuwenhoek 41:121–134

He Z, YaoY LZ, Ye Y (2014) Dynamic metabolic and transcriptional profiling of *Rhodococcus* sp. strain YYL during the degradation of tetrahydrofuran. Appl Environ Microbiol 80:2656–2664

He Y, Wei K, Si K, Mathieu J, Li M, Alvarez PJJ (2017a) Whole genome sequence of the 1,4-dioxane-degrading bacterium *Mycobacterium dioxanotrophicus* PH-06. Genome Announc 5: e00625-17

He Y, Mathieu J, Yang Y, Yu P, Da Silva MLB, Alvarez PJJ (2017b) 1,4-Dioxane biodegradation by *Mycobacterium dioxanotrophicus* PH-06 is associated with a group 6 soluble di-iron monooxygenase. Environ Sci Technol Lett 4:494–499

He Y, Mathieu J, da Silva MLB, Li M, Alvarez PJJ (2018) 1,4-Dioxane-degrading consortia can be enriched from uncontaminated soils: prevalence of *Mycobacterium* and soluble di-iron monooxygenase genes. Microb Biotechnol 11:189–198

Hernandez-Perez G, Fayolle F, Vandecasteele J-P (2001) Biodegradation of ethyl *t*-butyl ether (ETBE), methyl *t*-butyl ether (MTBE) and t-amyl ether (TAME) by *Gordonia terrae*. Appl Microbiol Biotechnol 55:117–121

Higgins IJ, Hammond RC, Sariaslani FS, Best D, Davies MM, Tryhorn SE, Taylor F (1979) Biotransformation of hydrocarbons and related compounds by whole organism suspensions of methane-grown *Methylosinus trichosporium* OB3b. Biochem Biophys Res Commun 89:671–677

Hine J, Mookerjee PK (1975) The intrinsic hydrophilic character of organic compounds. Correlations in terms of structural contributions. J Org Chem 40:292–298

Hofrichter M, Ullrich R (2014) Oxidations catalyzed by fungal peroxygenases. Curr Opin Chem Biol 19:116–125

House AJ, Hyman MR (2010) Effects of gasoline components on MTBE and TBA cometabolism by *Mycobacterium austroafricanum* JOB5. Biodegradation 21:525–541

Hristova KR, Schmidt R, Chakicherla AY, Legler TC, Wu J, Chain PS, Scow KM, Kane SR (2007) Comparative transcriptome analysis of *Methylibium petroleiphilum* PM1 exposed to the fuel oxygenates methyl *tert*-butyl ether and ethanol. Appl Environ Microbiol 73:7347–7357

Huang H, Shen D, Li N, Shan D, Shentu J, Zhou Y (2014) Biodegradation of 1,4-dioxane by a novel strain and its biodegradation pathway. Water Air Soil Pollut 225:2135–2146

Hur H-G, Newman LM, Wackett LP, Sadowsky MJ (1997) Toluene-2-monooxygenase-dependent growth of *Burkholderia cepacia* G4/PR1 on diethyl ether. Appl Environ Microbiol 63:1606–1609

Hyman MR (2009) Aerobic cometabolism of ether-bonded compounds. Final report. United State Environmental Protection Agency grant # R823426. https://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/673/report/F

Hyman MR, Page CL, Arp DJ (1994) Oxidation of methyl fluoride and dimethyl ether by ammonia monooxygenase in *Nitrosomonas europaea*. Appl Environ Microbiol 60:3033–3035

Inoue D, Tsunoda T, Sawada K, Yamamoto N, Saito Y, Sei K, Ike M (2016) 1,4-Dioxane degradation potential of members of the genera *Pseudonocardia* and *Rhodococcus*. Biodegradation 27:277–286

Inoue D, Tsunoda T, Yamamoto N, Ike M, Sei K (2018) 1,4-Dioxane degradation characteristics of *Rhodococcus aetherivorans* JCM 14343. Biodegradation 29:301–310

Interstate Technology & Regulatory Council (ITRC) (2005) Overview of groundwater remediation technologies for MTBE and TBA. MTBE-1. Interstate Technology & Regulatory Council, MTBE and Other Fuel Oxygenates Team, Washington, DC

Janssen DB, Gerritse J, Brackman J, Kalk C, Jager D, Witholt B (1988) Purification and characterization of a bacterial dehalogenase with activity toward halogenated alkanes, alcohols, and ethers. Eur J Biochem 171:67–72

Jin X-J, Chen D-Z, Zhu RY, Chen J, Chen J-M (2012) Characteristics of 1,4-dioxane degradation by *Xanthobacter flavus* DT8. Environ Sci 33:1657–1662

Kane SR, Chakicherla AY, Chain PSG, Schmidt R, Shin MW, Legler TC, Scow KM, Larimer FW, Lucas SM, Richardson PM, Hristova KR (2007) Whole-genome analysis of the methyl-*tert*-butyl ether-degrading beta-proteobacterium *Methylibium petroleiphilum* PM1. J Bacteriol 189:1931–1945

Katapodis AG, Wimalesena K, Lee J, May SW (1984) Mechanistic studies on non-heme iron monooxygenase catalysis: epoxidation, aldehyde formation, and demethylation by the ω-hydroxylation system of *Pseudomonas oleovorans*. J Am Chem Soc 110:7928–7935

Kim Y-H, Engesser K-H (2004) Degradation of alkyl ethers, aralkyl ethers, and dibenzyl ether by *Rhodococcus* sp. Strain DEE5151, isolated from diethyl ether-containing enrichment cultures. Appl Environ Microbiol 70:4398–4401

Kim Y-H, Engesser K-H (2005) Inhibition of diethyl ether degradation by *Rhodococcus* sp. strain DEE5151 by glutaraldehyde and ethyl vinyl ether. FEMS Microbiol Lett 243:317–322

Kim Y-H, Engesser K-H, Kim S-J (2007) Physiological, numerical and molecular characterization of alkyl ether-utilizing rhodococci. Environ Microbiol 9:1497–1510

Kim Y-H, Cha C-J, Engesser K-H, Kim S-J (2008) Degradation of various alkyl ethers by alkyl ether-degrading *Actinobacteria* isolated from activated sludge of a mixed wastewater treatment. Chemosphere 73:1442–1447

Kim Y-M, Jeon J-R, Murugesan K, Kim E-J, Chang Y-S (2009) Biodegradation of 1,4-dioxane and transformation of related cyclic compounds by a newly isolated *Mycobacterium* sp. PH-06. Biodegradation 20:511–519

Kinne M, Poraj-Kobielska M, Ralph SA, Ullrich R, Hofrichter M, Hammel KE (2009) Oxidative cleavage of diverse ethers by an extracellular fungal peroxygenase. J Biol Chem 284:29343–29349

Kohlweyer U, Thiemer B, Schräder T, Andreesen JR (2000) Tetrahydrofuran degradation by a newly isolated culture of *Pseudonocardia* sp. strain K1. FEMS Microbiol Lett 186:301–306

Kotani T, Yamamoto T, Yurimoto H, Sakai Y, Kato N (2003) Propane monooxygenase and NAD^+-dependent secondary alcohol dehydrogenase in propane metabolism by *Gordonia* sp. strain TY-5. J Bacteriol 185:7120–7128

Lan RS, Smith CA, Hyman MR (2013) Oxidation of cyclic ethers by alkane-grown *Mycobacterium vaccae* JOB5. Remediat J 23:23–42

Lechner U, Brodkorb D, Geyer R, Hause G, Härtig C, Auling G, Fayolle-Guichard F, Piveteau P, Müller RH, Rohwerder T (2007) *Aquincola tertiaricarbonis* gen. nov., sp. nov., a *tertiary* butyl moiety-degrading bacterium. Int J Syst Evol Microbiol 57:1295–1303

Li M, Mathieu J, Liu Y, van Orden ET, Yang Y, Fiorenza S, Alvarez PJJ (2013) The abundance of tetrahydrofuran/dioxane monooxygenase genes (*thmA*/*dxmA*) and 1,4-dioxane degradation activity are significantly correlated at various impacted sites. Environ Sci Technol Lett 1:122–127

Li S, Wang S, Yan W (2016) Biodegradation of methyl *tert*-butyl ether by co-metabolism with a *Pseudomonas* sp. strain. Int J Environ Res Public Health 13:883

Lippincott D, Streger SH, Schafer CE, Hinkle J, Stormo J, Steffan RJ (2015) Bioaugmentation and propane biosparging for *in situ* biodegradation of 1,4-dioxane. Groundwater Monit Remediat 35:81–92

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

Mahendra S, Alvarez-Cohen L (2005) *Pseudonocardia dioxanivorans* sp. nov., a novel actinomycete that grows on 1,4-dioxane. Int J Syst Evol Microbiol 55:593–598

Mahendra S, Alvarez-Cohen L (2006) Kinetics of 1,4-dioxane biodegradation by monooxygenase-expressing bacteria. Environ Sci Technol 40:5435–5442

Mahendra S, Petzold CJ, Baidoo EE, Keasling JD, Alvarez-Cohen L (2007) Identification of the intermediates of *in vivo* oxidation of 1,4-dioxane by monooxygenase-containing bacteria. Environ Sci Technol 41:7330–7336

Malandain C, Fayolle-Guichard F, Vogel TM (2010) Cytochrome P450-mediated degradation of fuel oxygenates by environmental isolates. FEMS Microbiol Ecol 72:289–296

Masuda H, McClay K, Steffan RJ, Zylstra GJ (2012) Biodegradation of tetrahydrofuran and 1,4-dioxane by soluble diiron monooxygenase in *Pseudonocardia* sp. strain ENV478. J Mol Microbiol Biotechnol 22:312–316

Matsui R, Takagi K, Sakakibara F, Abe T, Shiiba K (2016) Identification and characterization of 1,4-dioxane-degrading microbe separated from surface water by the seawater-charcoal perfusion apparatus. Biodegradation 27:155–163

McClay K, Schaefer CE, Vainberg S, Steffan RJ (2007) Biodegradation of bis(2-chloroethyl) ether by *Xanthobacter* sp. strain ENV481. Appl Environ Microbiol 73:6870–6875

McKelvie JR, Hyman MR, Elsner M, Smith C, Aslett DM, Lacrampe-Couloume G, Sherwood-Lollar B (2009) Isotopic fractionation of methyl *tert*-butyl ether suggests different initial reaction mechanisms during aerobic biodegradation. Environ Sci Technol 43:2793–2799

Meyers AJ (1982) Obligate methylotrophy: evaluation of dimethyl ether as C_1 compound. J Bacteriol 150:966–968

Miller LE, Coutlakis MD, Oremland RS, Ward BB (1993) Selective inhibition of ammonium oxidation and nitrification-linked N_2O formation by methyl fluoride and dimethyl ether. Appl Environ Microbiol 59:2457–2464

Mohr T, Stickney J, Diguiseppi B (2010) Environmental investigation and remediation: 1,4-dioxane and other solvent stabilizers. CRC Press, Boca Raton

Morales M, Nava V, Velásquez E, Razo-Flores E, Revah S (2009) Mineralization of methyl *tert*-butyl ether and other gasoline oxygenates by Pseudomonads using short chain *n*-alkanes as growth source. Biodegradation 20:271–280

Moreno-Horn M, Garbe L-A, Tressl R, Adrian L, Görisch H (2003) Biodegradation of bis(1-chloro-2-propyl) ether via initial ether scission and subsequent dehalogenation by *Rhodococcus* sp. strain DTB. Arch Microbiol 179:234–241

Moreno-Horn M, Garbe L-A, Tressl R, Görisch H (2005) Transient accumulation of γ-butyrolactone during degradation of bis(4-chloro-*n*-butyl) ether by diethyl ether-grown *Rhodococcus* sp. strain DTB. Appl Microbiol Biotechnol 69:335–340

Müller RH, Rohwerder T, Harms H (2007) Carbon conversion efficiency and limits or productive degradation of methyl *tert*-butyl ether and related compounds. Appl Environ Microbiol 73:1783–1791

Müller RH, Rohwerder T, Harms H (2008) Degradation of fuel oxygenates and their main intermediates by *Aquincola tertiaricarbonis* L108. Microbiology 154:1414–1421

Nakamiya K, Hashimoto S, Ito H, Edmonds JS, Morita M (2005) Degradation of 1,4-dioxane and cyclic ethers by an isolated fungus. Appl Environ Microbiol 71:1254–1258

Nakatsu CH, Hristova K, Hanada S, Meng X-Y, Hanson JR, Scow KM, Kamagata Y (2006) *Methylibium petroleiphilum* gen nov., sp. nov., a novel methyl *tert*-butyl ether-degrading methylotroph of the Betaproteobacteria. Int J Syst Evol Microbiol 56:983–989

Newman LM, Wackett LP (1995) Purification and characterization of toluene-2-monooxygenase from *Burkholderia cepacia* G4. Biochemistry 34:14066–14076

Oremland RS, Culbertson CW (1992) Evaluation of methyl fluoride and dimethyl ether as inhibitors of aerobic methane oxidation. Appl Environ Microbiol 58:2983–2992

Parales RE, Adamus JE, White N, May HD (1994) Degradation of 1,4-dioxane by an actinomycete in pure culture. Appl Environ Microbiol 60:4527–4530

Patel RN, Hou CT, Felix A (1976) Inhibition of dimethyl ether and methane oxidation in *Methylococcus capsulatus* and *Methylosinus trichosporium*. J Bacteriol 126:1017–1019

Payne WD, Collette TW (1989) Identification of bis(2-chloroethyl) ether hydrolysis products by direct aqueous injection GC/FT-IR. J High Resolut Chromatogr 12:693–696

Pugazhendi A, Banu JR, Dhavamani J, Yeom IT (2015) Biodegradation of 1,4-dioxane by *Rhodanobacter* AYS5 and the role of additional substrates. Ann Microbiol 65:2210–2208

Redmond MC, Valentine DL, Sessions LA (2010) Identification of novel methane-ethane-, and propane-oxidizing bacteria at marine hydrocarbon seeps by stable isotope probing. Appl Environ Microbiol 76:6412–6422

Ribbons DW (1975) Oxidation of C_1-compounds by particulate fractions from *Methylococcus capsulatus*: distribution and properties of methane-dependent reduced nicotinamide adenine dinucleotide oxidase (methane hydroxylase). J Bacteriol 122:1351–1363

Salazar M, Morales M, Revah S (2012) Biodegradation of methyl tert-butyl ether by cometabolism with hexane in biofilters inoculated with *Pseudomonas aeruginosa*. J Environ Sci Health A Tox Hazard Subst Environ Eng 47:1017–1026

Sales CM, Mahendra S, Grostern A, Parales R, Goodwin LA, Woyke T, Nolan M, Lapidus A, Chertkov O, Ovchinnikova G, Sczyrba A, Alvarez-Cohen L (2011) Genome sequence of the 1,4-dioxane-degrading *Pseudonocardia dioxanivorans* strain CB1190. J Bacteriol 193:4549–4550

Sales CM, Grostern A, Parales JV, Parales RE, Alvarez-Cohen L (2013) Oxidation of cyclic ethers 1,4-dioxane and tetrahydrofuran by a monooxygenase in two *Pseudonocardia* species. Appl Environ Microbiol 72:7702–7708

Sayavedra-Soto LA, Hamamura N, Liu CW, Kimbrel JA, Chang JH, Arp DJ (2011) The membrane-associated monooxygenase in the butane-oxidizing Gram-positive bacterium *Nocardiodes* sp. strain CF8 is a novel member of the AMO/PMO family. Environ Microbiol Rep 3:390–396

Schmidt R, Battaglia V, Scow K, Kane S, Hristova KR (2008) Involvement of a novel enzyme, MdpA, in methyl-*tert*-butyl ether degradation in *Methylibium petroleiphilum* PM1. Appl Environ Microbiol 74:6631–6638

Schuster J, Schäfer F, Hübler N, Brandt A, Rosell M, Härtig C, Harms H, Müller RH, Rohwerder T (2012) Bacterial degradation of *tert*-amyl alcohol proceeds *via* hemiterpene 2-methyl-3-buten-2-ol by employing the *tertiary* alcohol desaturase function of the Rieske nonheme mononuclear iron oxygenase MdpJ. J Bacteriol 194:972–981

Schuster J, Purswani J, Breuer U, Pozo C, Harms H, Müller RH, Rohwerder T (2013) Constitutive expression of the cytochrome P450 *ethABCD* monooxygenase system enables degradation of synthetic dialkyl ethers in *Aquincola tertiaricarbonis* L108. Appl Environ Microbiol 79:2321–2327

Sei K, Miyagaki K, Kakinoki T, Fukugasako K, Inoue D, Ike M (2013a) Isolation and characterization of bacterial strains that have high ability to degrade 1,4-dioxane as a sole carbon and energy source. Biodegradation 24:665–674

Sei K, Oyama M, Kakinoki T, Inoue D, Ike M (2013b) Isolation and characterization of tetrahydrofuran-degrading bacteria for 1,4-dioxane-containing wastewater treatment by cometabolic degradation. J Water Environ Technol 11:11–19

Semelsberger TA, Borup RL, Greene HL (2006) Dimethyl ether (DME) as an alternative fuel. J Power Sources 156:497–511

Sharp JO, Sales CM, LeBlanc JC, Liu J, Wood TK, Eltis LD, Mohn WW, Alvarez-Cohen L (2007) An inducible propane monooxygenase is responsible for *n*-nitrosodimethylamione degradation by *Rhodococcus* sp. strain RHA1. Appl Environ Microbiol 73:6930–6938

Sharp JO, Sales CM, Alvarez-Cohen L (2010) Functional characterization of propane-enhanced *N*-nitrosodimethylamine degradation by two actinomycetales. Biotechnol Bioeng 107:924–932

Shields MS, Reagin MJ (1992) Selection of a *Pseudomonas cepacia* strain constitutive for the degradation of trichloroethylene. Appl Environ Microbiol 58:3977–3983

Skinner KM, Martinez-Prado A, Hyman MR, Williamson KJ, Ciuffetti LM (2008) Pathway, inhibition and regulation of methyl *tertiary* butyl ether oxidation in a filamentous fungus, *Graphium* sp. Appl Microbiol Biotechnol 77:1359–1365

Skinner K, Cuifetti L, Hyman M (2009) Metabolism and cometabolism of cyclic ethers by a filamentous fungus, a *Graphium* sp. Appl Environ Microbiol 75:5514–5522

Smith CA, Hyman MR (2004) Oxidation of methyl *tert*-butyl ether by alkane hydroxylase in dicyclopropylketone-induced and n-octane-grown *Pseudomonas putida* GPo1. Appl Environ Microbiol 70:4544–4550

Smith CA, Hyman MR (2010) Oxidation of gasoline oxygenates by closely related non-haem-iron alkane hydroxylases in *Pseudomonas mendocina* KR1 and other n-octane-utilizing *Pseudomonas* strains. Environ Microbiol Rep 2:426–432

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

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 C5-C8 *n*-alkanes. Appl Environ Microbiol 69:7385–7394

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

Stirling DI, Dalton H (1980) Oxidation of dimethyl ether, methylformate and bromomethane by *Methylococcus capsulatus* (Bath). J Gen Microbiol 116:277–283

Tajima T, Hayashida N, Matsumura R, Omura A, Nakashimada Y, Kato J (2012) Isolation and characterization of tetrahydrofuran-degrading *Rhodococcus aetherivorans* strain M8. Process Biochem 47:1665–1669

Thiemer B, Andressen JR, Schräder T (2003) Cloning and characterization of a gene cluster involved in tetrahydrofuran degradation in *Pseudonocardia* sp. strain K1. Arch Microbiol 179:266–277

Trippe KM, Wolpert TJ, Hyman MR, Ciuffetti LM (2014) RNAi silencing of a cytochrome P450 monooxygenase disrupts the ability of the filamentous fungus, *Graphium* sp., to grow on short-chain gaseous alkanes and ethers. Biodegradation 25:137–151

Tupa PR, Masuda H (2018a) Genomic analysis of propane metabolism in methyl *tert*-butyl ether-degrading *Mycobacterium* sp. strain ENV421. J Genomics 6:24–29

Tupa PR, Masuda H (2018b) Draft genome sequence of a propanotroph, *Rhodococcus* sp. strain ENV425, capable of degrading methyl *tert*-butyl ether and *N*-nitrosodimethylamine. Genome Announc 6:1–2

Tupa PR, Masuda H (2018c) Comparative proteomic analysis of propane metabolism in *Mycobacterium* sp. strain ENV421 and *Rhodococcus* sp. strain ENV425. J Mol Microbiol Biotechnol 28:107–115

U.S. Environmental Protection Agency (2002) Bis(chloroethyl)ether (BCEE); CASRN 111-44-4. Integrated Risk Information System

U.S. Environmental Protection Agency (2016) 1,4-Dioxane (CASRN 123–91-1) – Report on carcinogens, 14th edn

U.S. Environmental Protection Agency (2017) Technical fact sheet – 1,4- dioxane (EPA 505-F-17-011)

U.S. Environmental Protection Agency (2014) Priority Pollutant list. https://www.epa.gov/sites/production/files/2015-09/documents/priority-pollutant-list-epa.pdf

Vainberg S, McClay K, Masuda H, Root D, Condee C, Zylstra GJ, Steffan RJ (2006) Biodegradation of ether pollutants by *Pseudonocardia* sp. ENV478. Appl Environ Microbiol 72:5218–5224

van Beilen JB, Kingma J, Witholt B (1994) Substrate specificity of the alkane hydroxylase system in *Pseudomonas oleovorans* GPo1. Enzym Microb Technol 16:904–911

van den Wijngaard AJ, Prins J, Smal AJAC, Janssen D (1993) Degradation of 2-chloroethyl vinyl ether by *Ancyclobacter aquaticus* AD25 and AD27. Appl Environ Microbiol 59:2777–2783

Vomberg A, Klinner U (2000) Distribution of *alkB* genes within *n*-alkane-degrading bacteria. J Appl Microbiol 89:339–348

White G, Russell N, Tidswell E (1996) Bacterial scission of ether bonds. Microbiol Rev 60:216–232

Wilkinson JF (1971) Hydrocarbons as a source of single cell protein. Symp Soc Gen Microbiol 21:15–46

Wilkinson JF (1975) Physiological studies of bacteria grown on methane. In: Microbial growth on C_1 compounds. The Society of Fermentation Technology, Tokyo, pp 45–58

Yao Y, Lu Z, Zhu F, Min H (2013) Evidence for horizontal gene transfer of a tetrahydrofuran monooxygenase: cloning and analysis of a gene cluster for tetrahydrofuran degradation in *Rhodococcus* sp. YYL. Afr J Microbiol Res 7:1809–1818

Yamamoto N, Saito Y, Inoue D Sei K, Ike M (2018) Characterization of a newly isolated Pseudonocardia sp. N23 with high 1,4-dioxane-degrading ability. J Biosci Bioeng 125:552–558

Zhou Y, Huang H, Shen D (2016) Multi-substrate biodegradation interaction of 1,4-dioxane and BTEX mixtures by *Acinetobacter baumannii* DD1. Biodegradation 27:37–46