Anaerobic microorganisms play key roles in the biogeochemical cycling of methane and non-methane alkanes. To date, there appear to be at least three proposed mechanisms of anaerobic methane oxidation (AOM). The first pathway is mediated by consortia of archael anaerobic methane oxidizers and sulfate-reducing bacteria (SRB) via “reverse methanogenesis” and is catalyzed by a homolog of methyl-coenzyme M reductase (MCR). The second pathway is also mediated by anaerobic methane oxidizers and SRB, wherein the archael members catalyze both methane oxidation and sulfate reduction and zero-valent sulfur is a key intermediate. The third AOM mechanism is a nitrite-dependent, “intra-aerobic” pathway described for the denitrifying bacterium, Candidatus Methylomirabilis oxyfera. It is hypothesized that AOM proceeds via reduction of nitrite to nitric oxide, followed by the conversion of two nitric oxide molecules to dinitrogen and molecular oxygen. The latter can be used to functionalize the methane via a particular methane monooxygenase. With respect to non-methane alkanes, there also appear to be novel mechanisms of activation. The most well-described pathway is the addition of non-methane alkanes across the double bond of fumarate to form alkyl-substituted succinates via the putative glycy1 radical enzyme, alkylsuccinate synthase (also known as methylalkylsuccinate synthase). Other proposed mechanisms include anaerobic hydroxylation via ethylbenzene dehydrogenase-like enzymes and an “intra-aerobic” denitification pathway similar to that described for Methylomirabilis oxyfera.

Keywords: anaerobic, oxidation, alkanes, methane, paraffins

INTRODUCTION

Alkanes are saturated hydrocarbons that are derived from both natural and anthropogenic sources. Due to their apolar C-H bonds, alkanes are considered to be among the least chemically reactive organic compounds. The activation or functionalization of alkanes is initiated via cleavage of a C-H bond. Aerobic microorganisms achieve this step via monooxygenase or dioxygenase enzymes, in which oxygen serves as both the physiological terminal electron acceptor and as a reactant (for review of mechanisms and enzymes see Austin and Groves, 2011). The role of oxygen in the functionalization of alkanes led to the belief for many years that anaerobic microorganisms would be unable to activate and utilize these compounds as growth substrates. However, research during the last 25 years has demonstrated that anaerobic microorganisms have their own novel mechanisms of activating alkanes.

ANAEROBIC OXIDATION OF METHANE

The shortest alkane, methane, is the most environmentally relevant hydrocarbon due to its biological production by methanogenic archaea in freshwater systems, swamps, landfills, marine sediments and seeps, rice fields, and other anaerobic environments, as well as its role as a greenhouse gas (Conrad, 2009). Anaerobic methane oxidation (AOM) is thought to account for the removal of over 300 Tg of methane per year in oceanic systems (Hinrichs and Boetius, 2002; Reeburgh, 2007). Therefore, from the perspective of climate change, AOM serves as a significant greenhouse “sink.” AOM can be coupled to the reduction of sulfate, nitrate and nitrite (for reviews see Knittel and Boetius, 2011; Shen et al., 2012), manganese (Beal et al., 2009), and iron (Beal et al., 2009; Crowe et al., 2011; Sivan et al., 2011; Amos et al., 2012; Zhu et al., 2012). Although the mechanisms of aerobic methane oxidation are well-described (Austin and Groves, 2011), the mechanisms of AOM have been hotly debated.

REVERSE METHANOGENESIS

To date, several studies have revealed that AOM coupled to sulfate reduction is mediated by consortia of archael anaerobic methane oxidizers (ANME-1, ANME-2, and ANME-3) and sulfate-reducing bacteria (SRB) (Knittel and Boetius, 2011). AOM with sulfate as the terminal electron acceptor appears to proceed via “reverse methanogenesis” (Zehnder and Brock, 1979; Hoehler et al., 1994; Hallam et al., 2004) and is catalyzed by a homolog of methyl-coenzyme M reductase (MCR), the Ni-containing enzyme responsible for the last step of methanogenesis (Krüger et al., 2003; Scheller et al., 2010a; Figure 1A). MCR homologues have been found in high concentrations in methanotrophic archaea associated with SRB (Hallam et al., 2003; Krüger et al., 2003; Heller et al., 2008; Mayr et al., 2008; Nünzoura et al., 2008). In a recent study, an ANME-1 MCR complex was isolated from a Black Sea microbial mat, and the X-ray structure was reported (Shima et al., 2012). Compared to the methanogenic MCR, the ANME-1 MCR...
Callaghan Anaerobic oxidation of alkanes

FIGURE 1 | Proposed pathways for the anaerobic oxidation of methane (AOM) via: (A) Reverse methanogenesis (adapted from Scheller et al., 2010a,b); (B) Zero-valent sulfur as a key intermediate in AOM (adapted from Milucka et al., 2012); (C) Nitrite-dependent anaerobic methane oxidation (“intra-aerobic denitrification”; adapted from Ettwig et al., 2010); and (D) Methane addition to fumarate (adapted from Beasley and Nanny, 2012). Enzyme nomenclature: methyl-coenzyme M reductase (MCR); nitrite reductase (NIR); particulate methane monooxygenase (PMO); and the putative thiyl radical of the alkylsuccinate/methylalkylsuccinate synthase-like enzyme (Enz-CysS).

complex is similar in overall structure and contains coenzyme M and coenzyme B. However, it differs from the methanogenic MCR in that it contains an F430 variant, as well as a cysteine-rich region and an altered post-translational amino acid modification pattern (Shima et al., 2012). Although not clearly elucidated, the role of the cysteine-rich region between the F430 and the protein surface may be to operate as a redox-relay system for electron or $\text{H}^+/\text{e}^-$ transport for the reduction of ANME-1 MCR from the inactive $\text{Ni}^{2+}$ to the active $\text{Ni}^+$ oxidation state (Mayr et al., 2008; Shima et al., 2012), whereas, the altered post-translational amino acid modifications may reflect phylogenetic adaptation to environmental conditions (Shima et al., 2012) based on the distribution of different ANME groups within microbial mats (Krüger et al., 2008).

The “reversibility” of MCR is supported by thermodynamic and kinetic considerations (Thauer, 2011), as well as activity assays with purified MCR from Methanothermobacter marburgensis (Scheller et al., 2010a). However, there are still several hypotheses regarding the reaction intermediates of both methane formation and methane oxidation by MCR. The two main competing theories argue for an organometallic methyl-$\text{Ni}^{III}$F$_{430}$ intermediate (Ermler et al., 1997; Dey et al., 2010) or the formation of a methyl radical and a CoM-S-Ni$^{II}$F$_{430}$ intermediate (Pelmenschikov et al., 2002; Pelmenschikov and Siegbahn, 2003). The latter was challenged in an experimental study which predicted the formation of (σ-alkane)-NiF$_{430}$ and (H)(alkyl)NiF$_{430}$ complexes as intermediates (Scheller et al., 2010b). However, recent active site models of the MCR X-ray crystal structure (PDB code 1HBN) from Methanothermobacter thermoautotrophicus support the theory that methane oxidation proceeds via a methyl radical and a CoM-S-Ni$^{II}$F$_{430}$ intermediate (Chen et al., 2012).
ALTERNATIVE AOM MECHANISMS UNDER SULFATE-REDUCING CONDITIONS

There has been tremendous debate regarding the interspecies intermediates of sulfate-dependent AOM, and several mechanisms have been proposed for consortia of ANME and SRB. One hypothesis proposes the formation of acetic acid and \( \text{H}_2 \) from methane and water by methane-oxidizing archaea, with subsequent utilization of the acetic acid and \( \text{H}_2 \) by SRB (Valentine and Reeburgh, 2000). Another variation of this process was previously proposed as “reverse acetoclastic methanogenesis” in which methane-oxidizing archaea produce acetate from \( \text{CO}_2 \) and \( \text{CH}_4 \), and the acetate is utilized by SRB (Zehnder and Brock, 1980; Hoehler et al., 1994). More recently, a “methylogenic mechanism” was proposed, in which methanethiol (MeSH) serves as an interspecies compound that transfers methane-derived carbon from the methanotroph to the SRB (Moran et al., 2008). With respect to the latter, there is some evidence that methanethiol inhibits AOM (Moran et al., 2008; Meuléas et al., 2010) and sulfite reduction (Meuléas et al., 2010). Therefore, the role of methylsulfides is still unclear. Additionally, several studies have provided experimental evidence and theoretical predictions that compounds such as acetate, hydrogen, formate, methanol, and carbon monoxide are unlikely to serve as interspecies compounds based on their effects on sulfate reduction and AOM, their diffusion distances, and thermodynamic considerations (see Meuléas et al., 2010 and references therein). Finally, extracellular electron transfer between archaea and deltaproteobacteria via nanowires (Reguera et al., 2005; therein). Finally, extracellular electron transfer between archaea and deltaproteobacteria via nanowires (Reguera et al., 2005; therein).

NEW MODEL FOR AOM MECHANISM UNDER SULFATE-REDUCING CONDITIONS: ZERO-VALENT SULFUR AS A KEY INTERMEDIATE

The studies focusing on the identification of AOM intermediates under sulfate-reducing conditions (see above) have mainly been predicated on the hypothesis that the ANME/SRB consortia catalyze AOM via an obligate syntrophic mechanism (for review see Knittel and Boetius, 2009). However, a new study of an enrichment culture obtained from sediments of the Mediterranean mud volcano Isis challenges that paradigm and also provides new insight to potential intermediates that may be transferred from the archaeal members to the SRB (Mäckel et al., 2012). The culture consists of ANME-2 and bacteria belonging to the Desulfococcus/Desulfosarcina (DSS) cluster (Schreiber et al., 2010), and evidence suggests that the ANME-2 assimilators have previously oxidized alcohol and the reduction of sulfate to zero-valent sulfur (S\(^0\)) and possibly to sulfide. Although experimental evidence is not yet available, the reduction of sulfate by ANME-2 is proposed to proceed via a non-canonical enzymatic pathway in which the electron donor may be formaldehyde and formaldehyde reductase. The zero-valent sulfur can react with sulfide to form polysulfides, such as disulfide. The DSS then couple disproportionation of disulfide to sulfide and sulfate (Figure 1B) with autotrophic carbon assimilation. Presumably, the disproportionation reaction in DSS could be catalyzed by enzymes such as sulfate adenylyltransferase (SAT) and adenylylsulfate reductase (APR), which have been shown to be involved in the disproportionation of compounds such as elemental sulfur, thiosulfate, and sulfite (Krämer and Cypionka, 1989). The sulfate generated by the DSS can be partly reused by the ANME-2. Stoichiometrically, no sulfate production occurs. Given these findings, it appears as though the ANME-2 are not necessarily dependent upon the sulfate-reducing partner and may be able to associate with any bacteria capable of scavenging disulfide and coupling it to energy generation and growth. This has important implications with respect to the biogeochemistry of methane oxidation and sulfur cycling as well as studies investigating interspecies compounds that are indicative of an obligate syntrophic AOM mechanism (see above).

NITRITE-DEPENDENT ANAEROBIC METHANE OXIDATION (“INTRA-AEROBIC DENITRIFICATION”)

Anaerobic methane oxidation coupled to denitrification through “reverse methanogenesis” was first proposed for a consortium (“Trente”) enriched from Trentekanaal sediment (Netherlands) (Raghoebarsing et al., 2006). The culture consisted of a bacterium belonging to the candidate division, “NC10,” and archaea distant related to Methanosaeta and ANME-II (Raghoebarsing et al., 2006; Etting et al., 2009). Subsequent investigations of the “Trente” culture showed AOM in the absence of the archaea (Etting et al., 2008, 2009), and further characterization of the “Trente” and another culture (“Ooij”) (Etting et al., 2009) revealed microbial populations dominated by the denitrifying bacterium, “Canadellus Methylomirabilis oxyfera” (Etting et al., 2010).

Genome assembly obtained from metagenomic analyses shows that “Methylomirabilis oxyfera” contains genes encoding enzymes for the complete pathway for aerobic methane oxidation, including a particulate monoxygenase (pMMO). The genome also contains several genes for denitrification, but lacks the genes encoding the enzymes for the reduction of nitrous oxide to dinitrogen (nosZDFY), an interesting finding given the observed production of \( N_2 \) in the “Trente” and “Ooij” cultures during AOM (Raghoebarsing et al., 2006; Etting et al., 2008, 2009). “Methylomirabilis oxyfera” also lacks genes encoding homologs of MGR or alkaline-activating glycol radical enzymes (Etting et al., 2010). Isotopic labeling experiments suggest that AOM by “Methylomirabilis oxyfera” proceeds via reduction of nitrite to nitric oxide, followed by the conversion of two nitric oxide molecules to dinitrogen and molecular oxygen. The latter can be used to functionalize the methane via the pMMO (Figure 1C). Based on stoichiometry (\( 3\text{CH}_4 + 8\text{NO}_2^- + 8\text{H}^+ \rightarrow 3\text{CO}_2 + 4\text{N}_2 + 10\text{H}_2\text{O} \)), four molecules of \( \text{O}_2 \) could be generated from eight molecules of nitrite, of which only three are required for methane activation by pMMO (Wu et al., 2011). Based on oxygen uptake and inhibition experiments with cell extracts and UV-visible absorption spectral characteristics and electron spin resonance (ESR) spectroscopy of solubilized membranes, the remaining oxygen might be consumed by a membrane-bound \( \text{h}_o \)-type terminal oxidase (Wu et al., 2011).
METHANE ADDITION TO FUMARATE

The addition of non-methane alkanes to fumarate (i.e., fumarate addition) is a prevalent anaerobic alkane activation mechanism observed under nitrate- and sulfate-reducing conditions (see below). Although the majority of studies have focused on longer-chain alkanes, a few investigations have revealed that short alkanes, such as propane, are also added to fumarate terminally or subterminally under sulfate-reducing conditions (Kniemeyer et al., 2007; Savage et al., 2010). These findings are supported by field metabolicomic studies of petroleum reservoirs and coalbeds in which low molecular weight gases such as methane are prevalent and low molecular weight alkylsuccinates, including methylsuccinate, have been detected (Duncan et al., 2009; Gieg et al., 2010; Wawrik et al., 2012). Due to the complex mixture of hydrocarbons in oil reservoirs and coalbeds, it is possible that the low molecular weight alkylsuccinates are derived from cometabolic hydrocarbon metabolism, as has been observed for toluene in anaerobic alkane-degrading bacteria (Rabus et al., 2011). However, the detection of methylsuccinate is intriguing nonetheless because it suggests that AOM via fumarate addition may be possible (Figure 1D). Thermodynamically, however, there are several considerations with respect to the formation of the methyl radical and the terminal electron accepting conditions. The formation of a methyl radical (439 kJ mol^(-1)) (Lids, 2003) would be at the expense of the glycyl radical formation (350 kJ mol^(-1)) (Armstrong et al., 1996). The difference in dissociation energies (90 kJ) is considerably larger than that for other alkane substrates (~60 kJ) (Thauer and Shima, 2008), and at first glance, it would not appear that the transition state of the enzyme could overcome such a barrier. A study using quantum chemical calculations to investigate the energetics of methane addition to fumarate (Beasley and Heider, 2010; Meckenstock and Mouttaki, 2011), alkane activation is presumably catalyzed by the glycyl radical enzyme, alkylsuccinate synthase (ASS) (Callaghan et al., 2008b) or methylalkylsuccinate synthase (MAS) (Grundmann et al., 2008), for which the sulfate-reducing strain Denalibacter alkeneovorans AK-01 and the denitrifying strain Aromatoleum HxN1 currently serve as model strains, respectively. Comparison of animas genes to bos genes has revealed several similarities. Both AK-01 and HxN1 contain genes encoding putative glycyl radical activating enzymes, belonging to the radical S-adenosylmethionine (SAM) superfamilies. Similar to BSS activase, which generates a glycyl radical on the catalytic subunit of BSS (Leitner et al., 1998; Krieger et al., 2001; Verfurth et al., 2004), it is thought that the ASS activase is responsible for the generation of the glycyl radical on the catalytic subunit of the alkane-activating enzyme. Subsequent generation of a thyl radical on a conserved cysteine residue (Selmer et al., 2005) would result in the abstraction of an H atom from the hydrocarbon substrate (Heider et al., 1998). In the case of BSS, the reaction results in the stereospecific formation of (R)-1-benzylsuccinate (Beller and Spormann, 1998; Leitner and Heider, 1999), wherein the initially abstracted H atom is transferred to the same face of fumarate (Quao and Marsh, 2005). Unlike benzylsuccinate, however, methylalkylsuccinic acids contain two chiral carbons. Metabolomic investigations of numerous sulfate- and nitrate-reducing cultures utilizing n-alkanes show the formation of two diastereomers of the methylalkylsuccinic acid metabolites (Kropp et al., 2001; Rabus et al., 2001; Wilkes et al., 2002; Cravo-Laureau et al., 2005; Callaghan et al., 2006), and gas chromatography-mass spectrometry (GC-MS) analysis of a sulfate-reducing enrichment culture utilizing ethylcyclopentane resolved up to five peaks corresponding to the requisite ethylcyclopentylsuccinic acids (Rios-Hernandez et al., 2003). Recent stereochemical investigation of ‘Aromatoleum’ HxN1 growing on n-hexane showed only the formation of 2R, 5R and 2S, 5R isomers of 1-methylpentylsuccinate and that the initial step in activation is via abstraction of the pro-5 hydrogen from C5 of hexane, with subsequent addition of the alkyl species intermediates to fumarate on the opposite face from which the hydrogen atom was abstracted (Figure 2A; Jarling et al., 2012). It is also proposed that the initial 1-methylpentylsuccinate isomer is epimerized to...
FIGURE 2 | (A) Proposed pathway and stereochemistry for the anaerobic activation and degradation of hexane via subterminal addition to fumarate (adapted from Jarling et al., 2012); (B) Proposed pathway of activation and degradation of non-methane alkanes via “intra-aerobic denitrification” (adapted from Zedelius et al., 2011); and (C) Proposed pathway of activation and degradation of non-methane alkanes via anaerobic hydroxylation by Desulfococcus oleovorans Hxd3 (Sünwoldt et al., 2012). Enzyme nomenclature: putative thiyl radical of the alkylsuccinate/methylalkylsuccinate synthase-like enzyme (Enz-CysS); alkane monooxygenase (ALK); cytochromes P450 (CYP); and ethylbenzene dehydrogenase (EBDH).

generate a diastereomer that then undergoes the reported carbon-skeleton rearrangement and subsequent decarboxylation (Jarling et al., 2012).

The discoveries of ASS and MAS have enabled the interrogation of hydrocarbon-impacted environments as well as isolates and enrichment cultures (Callaghan et al., 2010; Kolukirik et al., 2011; Andrade et al., 2012; Li et al., 2012; Mbadinga et al., 2012; Wang et al., 2012; Wovirek et al., 2012; Zhou et al., 2012; Cheng et al., 2013). In addition to alkanes of medium chain lengths, ASS/MAS enzymes appear to play a role in the degradation of very short alkanes (e.g., propane) (Kniemeyer et al., 2007; Callaghan et al., 2010; Savage et al., 2010) and recent investigations of anaerobic biodegradation of solid paraffins under methanogenic conditions suggest that microorganisms may also activate paraffin waxes via fumarate addition (Callaghan et al., 2010). With respect to the latter, the detection and expression of several assA genotypes was demonstrated in a methanogenic consortium utilizing octacosane (C28) (Davidova et al., 2011). However, the requisite metabolites have not been identified in this study or in other studies examining alkane activation under methanogenic conditions for which assA genotypes have been detected (Mbadinga et al., 2012; Wang et al., 2012; Zhou et al., 2012; Atkin et al., 2013; Cheng et al., 2013). It has been hypothesized that either the metabolites that result from fumarate addition do not accumulate to detectable levels under the slow-growing conditions and/or that other mechanisms, such as hydroxylation, may be important (Atkin et al., 2013).

INTRA-HYDROXYLATION

An “intra-aerobic” pathway of alkane oxidation similar to that of ‘Methylomirabilis oxyfera’ has also been proposed to be involved in the functionalization of n-hexadecane by the Gammaproteobacterium Hdn1 (Figure 2B; Zedelius et al., 2011). Unlike ‘Methylomirabilis oxyfera’, Hdn1 contains genes for the complete denitrification pathway. The genome of strain Hdn1 does not contain genes that encode an alkane-activating glycyl radical enzyme, but does contain genes that may encode a di-iron monooxygenase, a P450-type monooxygenase and a putative third type of monooxygenase (Zedelius et al., 2011). Consistent with the absence of an alkane-activating glycyl radical enzyme,
alkyl-substituted succinates were not detected via metabolite profiling, but 1-hexadecanol was detected when the anaerobic culture was exposed to air. Based on these findings and growth experiments, the authors proposed a mechanism in which NO$_3^-$, NO or an unknown product of NO$_2^-$ reduction may be required for alkane activation. Specifically, the dissipation of NO$_2^-$ (ΔG°$^{\text{red}}$ = 55.2 kJ mol$^{-1}$) or NO (ΔG°$^{\text{red}}$ = 173.1 kJ mol$^{-1}$) would provide O$_2^-$, which could then be used to hydroxylate the alkane via the putative monoxygenases. Alternatively, N-O species could behave as strongly oxidizing electron acceptors to generate a reactive state of a factor or enzyme site that is involved in the activation of the alkane, or an N-O species may be directly involved in the activation of the alkane (Zeidelis et al., 2011).

**ANAEROBIC HYDROXYLATION FOLLOWED BY CARBOXYLATION**

Finally, there appears to be at least one additional pathway of anaerobic alkane oxidation. Early studies of the sulfate-reducing bacterium Desulfococcus oleovorans Hxd3 demonstrated that incubation with an alkane with an odd number of C atoms yielded predominantly fatty acids with an even number of C atoms, and vice versa (Aecskersberg et al., 1998; So et al., 2003). This is in contrast with what is observed in microorganisms that activate alkanes via fumarate addition (So and Young, 1999). Stable isotope studies of strain Hxd3 incubated with NaH$^{13}$CO$_3$ and [1,2-13C]hexadecane were indicative of incorporation of carbon derived from bicarbonate at C3 and elimination of the C1 and C2 carbon atoms (So et al., 2003). Subsequently, evidence of this pathway was also observed in nitrate- and sulfate-reducing enrichment cultures utilizing n-hexadecane (Callaghan et al., 2006, 2009). However, although it was hypothesized that carboxylation at C3 may be the first step in degradation, the corresponding carboxylated intermediate, 2-ethylpentadecanoic acid, was not detected in any of the above studies. Under standard conditions, the direct carboxylation of an alkane is an endergonic process (ΔG°$^{\text{red}}$ = +28 kJ mol$^{-1}$) (Thauer and Shima, 2008). Thus, carboxylation as the first step in alkane degradation in Hxd3 has been debated for almost 10 years.

With the continual evolution of sequencing technologies, however, genome-enabled analyses are yielding new hypotheses. Consistent with the above experimental observations, the genome of Hxd3 does not contain genes encoding an alkane-activating glycol radical enzyme. Interestingly, the genome contains genes that encode an ethylbenzene dehydrogenase-like complex (Callaghan et al., 2008a), similar to that found in denitrifying strains Aromatoleum aromaticum EHN1 (Knemeyer and Heider, 2001) and Anaerobacillus sp. strain EB1 (Johnson and Spormann, 1999; Johnson et al., 2001). Ethylbenzene dehydrogenase is a molybdenum cofactor-containing enzyme of the dimethylsulfoxide reductase family that catalyzes the anaerobic hydroxylation of ethylbenzene (Ball et al., 1996; Rabus and Heider, 1998). Based on these findings, it is possible that the activation of alkanes by Hxd3 may also occur via anaerobic hydroxylation. Analogous to the steps involved in the anaerobic hydroxylation of ethylbenzene, hydroxylation of the alkane at C2 would produce a secondary alcohol that could be further oxidized to a ketone, similar to the formation of acetophenone during ethylbenzene degradation (Figure 2C; Ball et al., 1996; Rabus and Heider, 1998). Subsequent transformation of the ketone may involve carboxylation at C3, with elimination of the C1 and C2 carbons, to produce a fatty acid that is one carbon shorter than the parent alkane. The latter would be consistent with previous studies (So and Young, 1999; Callaghan et al., 2006, 2009). Proteomic investigations of alkane-degrading cells of Hxd3 implicate the putative ethylbenzene dehydrogenase (Simwoldi et al., 2012). Further study is needed to elucidate the transformation reactions that would ultimately result in the formation of the observed fatty acids.

**CONCLUSION**

The anaerobic oxidation of alkanes plays an important role in the biogeochemical cycling of methane and the bioremediation of hydrocarbon-impacted environments. As we look to the future, advances in next-generation sequencing and annotation will facilitate genome-enabled transcriptomic and proteomic investigations of anaerobic alkane oxidation. The complete genome sequences of several model alkane utilizers are now publicly available and include: Desulfatibacillum alkenivorans Hxd3, ‘Gandidaudia Methylaminobacter. oxyfera’ and the Gammaproteobacterium, strain HgN1. Future work will rely on these model organisms for the purification and characterization of relevant enzymes.

**ACKNOWLEDGMENTS**

The author would like to thank Rachel N. Austin, Johann Heider, Jana Milucka, and Mark A. Nanny for editorial comments and suggestions. The preparation of this review was funded by a National Science Foundation grant (MCB-0921265).

**REFERENCES**

Aecskersberg, E., Rainey, F. A., and Widdel, F. (1998). Growth, natural relationships, cellular fatty acids and metabolic adaptation of sulfate-reducing bacteria that utilize long-chain alkanes under anoxic conditions. Arch. Microbiol. 170, 363–369.

Aiken, C. M., Jones, D. M., Maguire, M. J., Gray, N. D., Sherry, A., Bowler, R. J. I., et al. (2013). Evidence that crude oil alkane activation proceeds by different mechanisms under sulfate-reducing and methanogenic conditions. Geomicrobiol. J. 30, 162–174.

Amon, R. T., Rekim, B. A., Costaridis, I. M., Vorytk, M. A., Kirdinnin, J. D., Jones, E. J., et al. (2012). Evidence for iron-mediated anaerobic methane oxidation in a crude oil-contaminated aquifer. Geobiology 10, 506–517.

Andrade, L. L., Leite, D., Ferreira, E., Andrade, L. L., Leite, D., Ferreira, E., et al. (2013). Microbial diversity and anaerobic hydrocarbon degradation potential in an oil-contaminated mangrove sediment. BMC Microbiol. 13:106. doi: 10.1186/1471-2180-13-106

Armstrong, D. A., Yu, D., and Raink, A. (1996). Oxidative damage to the gshf alpha-carbon site in protein, an ab initio study of the C-H bond dissociation energy and the reduction potential of the C-centered radical. Can. J. Chem. 74, 1192–1199.

Austin, R. N., and Groves, J. T. (2011). Alkane-oxidizing metalloenzymes in the carbon cycle. Metallomics 3, 775–785.

Ball, H. A., Johnson, H. A., Rehner, M., and Spormann, A. M. (1996). Initial reactions in anaerobic ethylbenzene oxidation by a denitrifying bacterium, strain EB1. J. Bacteriol. 178, 5755–5761.

Beal, E. J., House, C. H., and Orphan, V. J. (2009). Manganese- and iron-dependent methane oxidation. Science 325, 148–147.

Bearday, K. K., and Nanny, M. A. (2012). Potential energy surface for anaerobic
Callaghan, A. V., Tierney, M., Phelps, C., Gieg, L. M., Kropp, K. G., Suflita, J. M., and Siegel, M. (2012). Anaerobic oxidation of alkanes and aerobically oxidized alcohols. *Environ. Sci. Technol.* 46, 8248–8252.

Biller, H. R., and Spormann, A. M. (1998). Analysis of the novel benzoate-inducible synthesis reaction for anaerobic toluene degradation based on structural studies of the product. *J. Bacteriol.* 180, 5844–5857.

Boll, M., and Heider, J. (2010). Anaerobic degradation of hydrocarbon mechanisms of C4H4 bond activation in the absence of oxygen,” in Handbook of Hydrocarbon and Lipid Microbiology, eds K. N. Timmis, T. K. Kimmich, J. B. Barbeary, and V. De Lorenzo (Berlin Heidelberg: Springer), 1917–2001.

Callaghan, A. V., Austin, R. N., Groen, J. T., Kukor, J. J., Rabad, R., Widdel, F., et al. (2004a). “The complete genome sequence of Desulfococcus elizabethae Hdf1, a sulfate-reducing bacterium,” in American Society for Microbiology 108th General Meeting (Boston: Amer Society for Microbiology).

Callaghan, A. V., Wosiek, R., Ni Chualalann, S. M., Young, L. Y., and Zietz, G. J. (2008). Anaerobic alkanodegraving strain AK-81 contains two alkylbenzene synthase genes. *Biochim. Biophys. Acta* 1789, 162–148.

Callaghan, A. V., Davideva, I. A., Sonneveld, M., Parisi, G., Groen, J. T., Saffeta, J. M., et al. (2010). Diversity of benzyl and alkylbenzene synthase genes in hydrocarbon-impaired environments and enrichment culture. *Environ. Sci. Technol.* 44, 7287–7294.

Callaghan, A. V., Gie, L. M., Kopp, K. G., Saffeta, J. M., and Young, L. Y. (2008). Comparison of mechanisms of alkanes of alkanes and of a bacterial consortium. *Appl. Environ. Microbiol.* 72, 4274–4282.

Callaghan, A. V., Tierney, M., Phillips, C. D., and Young, L. Y. (2009). Anaerobic biodegradation of hydrocarbons by a nitrate-reducing consortium. *Appl. Environ. Microbiol.* 75, 1339–1344.

Chen, L. S., Blomberg, M. R., and Siegel, M. (2012). How is methane formed and emitted reversibly when catalyzed by Ni-containing methyl-coenzyme M reductase? *Chemistry 8*, 6928–6935.

Chen, L. S., Bai, L., Li, Q., Zhang, H., and Liu, Y. (2013). Enrichment and dynamics of novel symbioses in a methanogenic headcanoe-degraving culture from a Chinese oilfield. *FEBS Microbiol. Ecol.* 83, 777–796.

Chen, S. L., Blomberg, M. R., and Siegel, M. (2003). The global methane cycle: recent advances in understanding the microbial processes involved. *Environ. Microbiol. Rep.* 1, 285–292.

Chen, S. L., Groen, J. T., and Siegel, M. (2009). Anaerobic methane oxidation by a sulfate-reducing bacterium, Desulfocococcaceae alkivater strain VSC2013. *Appl. Environ. Microbiol.* 75, 3489–3497.

Crowe, S. A., Katera, S., Ledl, K., Stern, A., Magh, C., Nanny, D., et al. (2010). The methaneme cycle in fermentative Lake Mato. *Glob Biochem.* 9, 61–78.

Davidova, I. A., Callaghan, A. V., Duncan, K. E., Sonneveld, M., Rini, B., Wosiek, R., et al. (2011). “Long-chain paraffin metabolism by a methanogenic bacterial consortium enriched from marine sediments,” in 6th International Symposium of Sulphide Microbiology, (Garmisch-Partenkirchen: International Symposium of Subourmet Microbiology).

Davidova, I. A., Gieg, L. M., Nanny, M., Kopp, K. G., and Saffeta, J. M. (2005). Stable isotope studies of n-alkanes metabolism by a sulfate-reducing bacterial enrichment culture. *Appl. Environ. Microbiol.* 71, 6929–6942.

Dey, M., Li, X. H., Kaus, R. C., and Rappe, M. A. (2010). Detection of organometallic and radical intermediates in the catalytic mechanism of methyl-coenzyme M reductase using the natural substrate methyl-hydrogen formic acid, *Science* 330, 1099–1101.

Dey, M., Gieg, L. M., Groen, J. T., Parsons, V. A., Tanner, R. S., Tring, S. G., and Spormann, A. M. (2009). Bioenergetic thermophere metabolisms in Aliakno North Slope oil fields. *Environ. Sci. Technol.* 43, 7972–7977.

Ettwig, K. F., Butler, M. K., Le Paslier, D., Schoonen, K. T., Kahnt, J., Medema, H., van De Stocken, C. M., Detter, J. C., Holman, D., Richardson, P. M., and Delong, E. F. (2003). Identification of methyl coenzyme M reductase A (mcrA) genes associated with methane-oxidizing archaea. *Appl. Environ. Microbiol.* 69, 5483–5491.

Hallam, S. J., Gurgis, P. R., Preston, C. M., Richardson, P. M., and Delong, E. F. (2003). Identification of methyl coenzyme M reductase A (mcrA) genes associated with methane-oxidizing archaea. *Appl. Environ. Microbiol.* 69, 5483–5491.

Hallam, S. J., Putnam, N., Preston, C. M., Detter, J. C., Holman, D., Richardson, P. M., et al. (2004). Reverse methanogeneus testing the hypothesis with environmental genomics. *Science* 305, 1457–1462.

Heider, J., Spormann, A. M., Beller, H., and Widdel, F. (1998). Anaerobic bacterial metabolism of hydrocarbon, *FEBS Microbiol. Ecol.* 22, 459–473.

Hilli, C., Hopper, M., and Putnam, N. (2000). Immunological localization of coenzyme M reductase in anaerobic methane-oxidizing archaea of ANME-1 and ANME-2 type. *Proc. Natl. Acad. Sci. U.S.A.* 97, 8685–8688.

Hronick, K. U., and Boutier, A. (2002). “The anaerobic oxidation of methane: new insights in microbial ecology and biogeochemistry,” in Ocean Mergen Systems, eds G. Weber, D. Billet, D. Hauben, H. Jörgensen, M. Schnizer, and T. Van Weerden (Berlin: Heidelberg: Springer-Verlag), 457–477.

Hoehler, T. M., Alperin, M. J., Albert, D. B., and Martens, C. S. (1994). Field and laboratory studies of methane oxidation in an anoxic marine sediment – evidence for a methanotroph-sulfite reducer consortium. *Glob. Biogeochem. Cycle.* 451–463.

Kukor, J. J., Solarik, M., Luhm, S., Buckel, W., Rabad, R., et al. (2012). Synchrotron investigations reveal the mechanism of the bacterial actvation of 2-alkanes without oxygen. *Angew. Chem. Int. Ed. Engl.* 51, 13548–13558.

Johnson, H. A., Bohnet, D. A., and Spormann, A. M. (2001). Isolation and characterization of anaerobic ethylbenzoate dehydrogenase, a novel Mo-Co-beta enzymes. *J. Bacteriol.* 183, 4556–4562.

Johnson, H. A., and Spormann, A. M. (1999). In vitro studies on the initial reactions of anaerobic ethylbenzene mineralization. *J. Bacteriol.* 181, 5662–5668.

Kniewezer, O., and Heider, J. (2001). Ethylbenzoate dehydrogenase, a novel hydrocarbon-oxidizing molybdenum-sulfite/sulfate enzyme. *J. Biol. Chem.* 276, 21381–21386.

Kniewezer, O., Blaut, F., Neveu, S. M., Kortel, K., Wilke, H., Blumenberg, M., et al. (2007). Anaerobic oxidation of short chain hydrocarbons by marine sulfate-reducing bacteria. *Nature* 450, 898–903.

Krittmann, B., and Boutier, A. (2009). Anaerobic oxidation of methane in an Alaskan oilfields. *Amer Soci. Microbiol. 35*, 315–354.

Krittmann, B., and Boutier, A. (2011). “Anaerobic methane degradation in sulfate/sulfite/sulfate,” in Encyclopedia of Geobiology, eds V. Thiel and J. Reimer (Berlin: Springer).

Krollick, M., Ince, O., and Ince, B. R. (2011). Incremental response to anaerobic hydrocarbon degradation activity of halb-bay sediments via nutrient amendment. *Microb. Ecol.* 61, 873–884.

Kramar, M., and Cyponczyk, H. (1999). Sulfate formation via Asp sulfohydroxylase in desulfating-coproportionating and sulfite-coproportionating bacteria. *Arch. Microbiol.* 1, 252–257.

Kriepal, C. J., Blochwey, W., Albrecht, S. P., L., and Spormann, A. M. (2011). Aattle active organisms rular in anaerobic benzoate synthase of Anaerococcus sp strain T J. *J. Biol. Chem.* 286, 6224–6227.

Kukor, J. J., Davideva, I. A., and Saffeta, J. M. (2000). Anaerobic oxidation of 2-alkanes by an addition reaction in a sulfate-reducing bacterial enrichment culture. *Appl. }
Environ. Microbiol. 66, 5593–5598.

Krieg, M., Blumenberg, M., Kasten, S., Wudlaf, A., Kanal, K. L., Klock, J. H., et al. (2008). A novel, multi-layered methanotrophic microbial mat system growing on the sediment of the Black Sea. Environ. Microbiol. 10, 1934–1947.

Krieg, M., Meyerriek, A., Glöck

Handbook of Lide, D. R. (ed.). (2003).

Meckenstock, R. U., and Mouttaki, Krüger, M., Blumenberg, M., Kasten, S., et al. (2008). A novel, multi-layered methanotrophic microbial mat system growing on the sediment of the Black Sea. Environ. Microbiol. 10, 1934–1947.

Frontiers in Microbiology

Callaghan Anaerobic oxidation of alkanes

water amended with

3265–3271.

Chemistry and Physics

incubated under nitrate-, sulfate-

in anaerobic oxidation of

methane and sulfate reduction by

substrates on anaerobic oxidation of

bic initial reaction of

anaerobic methane oxidation pro-

over 500 days.

Geomicrobiol. J.

29, 447–459.

Microbiol.

24, 1222–1239.

Front. Microbiol.

5, 198–208.

Microbiol.

48, 1034–1043.

Microb. Ecol.

12, 43–53.

Front. Microbiol.

3, 269. doi:

Front. Microbiol.

8

Shima, S., Kruger, M., Weintz, T., Demmeu, U., Kuhnt, J., Thauer, R. K., et al. (2012). Structure of a metalfree-8

Brian L. Callaghan

Anaerobic oxidation of alkanes
Callaghan Anaerobic oxidation of alkanes

FEMS Microbiol. Ecol. 81, 26–42.

Wilkes, H., Kühner, S., Bolm, C., Fischer, T., Claessen, A., Wildt, F., et al. (2003). Formation of n-alkane- and cycloalkane-derived organic acids during anaerobic growth of a denitrifying bacterium with crude oil. Org. Geochem. 34, 1323–1333.

Wilkes, H., Babu, R., Fischer, T., Amontoff, A., Rahmuth, A., and Wildt, F. (2002). Anaerobic degradation of n-hexane in a denitrifying bacterium: further degradation of the initial intermediate (1-methylpentyl)succinate via C-skeleton rearrangement. Arch. Microbiol. 177, 235–243.

Wu, M. L., De Vries, S., Van Alen, T. A., Butler, M. K., Den Camp, H. J. M. O., Keltjens, J. T., et al. (2011). Physiological role of the respiratory quinol oxidase in the anaerobic nitrate-reducing methanotroph Candidatus Methyloveloxiobacter oxyfera. Microbiology 157, 420–432.

Zedelius, J., Rabus, R., Grundmann, O., Wetzer, L., Brodkorb, D., Schreiber, F., et al. (2011). Alkane degradation under anoxic conditions by a nitrate-reducing bacterium with possible involvement of the electron acceptor in substrate activation. Environ. Microbiol. Rep. 3, 125–133.

Zehnder, A. J. B., and Brock, T. D. (1979). Methane formation and methane oxidation by methanogenic bacteria. J. Bacteriol. 137, 420–432.

Zehnder, A. J. B., and Brock, T. D. (1980). Anaerobic methane oxidation – occurrence and ecology. Appl. Environ. Microbiol. 38, 194–204.

Zhou, L., Li, K. P., Mlwalinga, S. M., Yang, S. Z., Gu, J. D., and Mu, B. Z. (2012). Analyses of n-alkanes degrading community dynamics of a high-temperature methanogenic consortium enriched from production water of a petroleum reservoir by a combination of molecular techniques. Environ. Microbiol. 21, 1680–1693.

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 December 2012; paper pending published: 31 January 2013; accepted: 31 March 2013; published online: 14 May 2013.

Copyright © 2013 Callaghan. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.