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Anaerobic Degradation of Alkanes by Marine Archaea

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Abstract

Alkanes are saturated apolar hydrocarbons that range from their simplest form, methane, to high-molecular-weight compounds. Although alkanes were once considered biologically recalcitrant under anaerobic conditions, microbiological investigations have now identified several microbial taxa that can anaerobically degrade alkanes. Here we review recent discoveries in the anaerobic oxidation of alkanes with a specific focus on archaea that use specific methyl coenzyme M reductases to activate their substrates. Our understanding of the diversity of uncultured alkane-oxidizing archaea has expanded through the use of environmental metagenomics and enrichment cultures of syntrophic methane-, ethane-, propane-, and butane-oxidizing marine archaea with sulfate-reducing bacteria. A recently cultured group of archaea directly couples long-chain alkane degradation with methane formation, expanding the range of substrates used for methanogenesis. This article summarizes the rapidly growing knowledge of the diversity, physiology, and habitat distribution of alkane-degrading archaea.

Contents

1. INTRODUCTION

Alkanes are apolar hydrocarbons consisting entirely of hydrogen atoms and saturated carbon atoms (C_nH_{2n+2}). They form naturally by the thermal decomposition of organic matter (thermogenesis). Methane is also produced as a metabolic product in methanogens (biogenesis). Abiogenic hydrocarbon formation plays a minor role in Earth's carbon cycle (111). Mixtures of natural gas and crude oil produced in deep hot sediments either migrate toward the surface or are trapped beneath impermeable cap rocks. Such hydrocarbon deposits can form commercially relevant oil and gas fields over millions of years. Today, over 31% of the world's energy consumption is fueled by crude oil, with an annual production of approximately 4.5 Gt and approximately 200 Gt remaining for canonical oil production (54). Early oil production research identified geochemical signatures of microbial oil degradation, and research suggests that temperature, water content, and availability of terminal electron acceptors control microbial degradation of oil reservoirs (46).

Broad interest in microbial alkane degradation was further sparked by the role microorganisms play in bioremediation after major oil spills and how this degradation potential is influenced by the physicochemical environment. For instance, the *Exxon Valdez* oil spill in 1989 contaminated the subarctic Pacific with 37,000 tons of oil, and microbial remediation takes decades owing to cold temperatures and is slowed even further where oxygen is absent (8). The *Deepwater Horizon* oil well blowout in 2010 injected 600,000 tons of liquid oil and up to 500,000 tons of gaseous hydrocarbons into the Gulf of Mexico, with severe environmental and economic consequences for the region despite a relatively active microbial community degrading the fine oil droplets (25, 57) and volatile hydrocarbon fractions (24, 75, 99). Natural seepage from submarine oil and gases into the water column accounts for 200,000–10,000,000 tons of oil per year (68, 74, 146). Autochthonous microbial communities quickly oxidize the volatile components as long as oxygen and nutrients are available to support their growth.

The capability of alkane degradation is now recognized across all three domains of life (14, 139, 160). In this context, aerobic alkane-degrading microorganisms are well known (36, 119), and their role across a wide range of marine environments and habitats has been recently assessed (19). Bacteria from at least 30 cultured genera oxidize alkanes aerobically (47). Halophilic archaea

such as *Haloferax*, *Haloarcula*, *Halobacterium*, and *Halococcus* also possess this capability (3, 14, 35, 126). These aerobes activate alkanes using oxygen-dependent methane or alkane monooxygenases and produce methanol or other alcohols as primary intermediates (110). Some bacteria can even produce the required oxygen through the dismutation of nitric oxide (32, 153, 159). In addition to these aerobic alkane degraders, several recent findings suggest that there is also a wide variety of anaerobic microbial alkane degraders with important biogeochemical roles in the marine environment (97).

Microbiologists postulated in the 1940s that alkanes are also consumed in anoxic environments (91, 102, 103), but little was known about the identity and functioning of the involved microorganisms. In the early 1990s, researchers identified bacteria that oxidize alkanes of different chain lengths coupled to sulfate reduction (2, 60, 64, 104, 118) and denitrification (30, 98). Sulfate-reducing microorganisms are of interest to the fossil fuel industry because microbial souring of oil and gas fields from H_2S can occur from the injection of sulfate-rich seawater, fueling active sulfate reduction (116). The coupling of nonmethane alkane degradation to metal reduction in cultured microorganisms has not yet been confirmed, but the corrosion of steel by anaerobic microorganisms is a well-known phenomenon apparently linked to oil-field microbial communities (124, 135). In the absence of sulfate, some alkane-degrading bacteria from the former class *Deltaproteobacteria* form syntrophic consortia with methanogens, resulting in net methanogenesis as a terminal electron–accepting process (56, 125, 155). Most of the anaerobic alkane-degrading bacteria activate their substrate by adding fumarate, catalyzed by alkyl succinate synthases (122). In addition, some bacteria may activate alkanes by hydroxylation (21, 117). Khelifi et al. (58) proposed that the hyperthermophilic archaeon *Archaeoglobus fulgidus* could thrive on hexadecane, for which it acquired the fumarate addition pathway from bacteria by horizontal gene transfer.

The anaerobic oxidation of methane (AOM) and ethane, the two simplest alkanes, has been described exclusively for archaea. Observations of concurrent consumption of methane and sulfate in oxygen-free sulfate-methane transition zones were the first evidence for AOM in marine anoxic sediments (11, 55, 76, 100). Radiotracer experiments by Zehnder & Brock (154) revealed that methanogens convert small amounts of methane to carbon dioxide, suggesting that related organisms perform AOM. On the basis of these experiments and thermodynamic considerations, it was proposed that methanogens or related organisms oxidize methane by reverse methanogenesis and that this process is syntrophically coupled to sulfate reduction in sulfate-reducing bacteria (49). Indeed, the discovery of highly ^{13}C-depleted archaeal lipids in methane-rich sediments suggested a critical role of archaea in AOM (48, 95). 16S rRNA gene analysis identified novel lineages of archaea affiliated with methanogens in methane-rich environments, containing these ^{13}C-depleted lipids, and these organisms were termed anaerobic methane-oxidizing archaea (ANME) (48, 92, 127). Fluorescence in situ hybridization (FISH) with custom oligonucleotide probes designed to target these novel archaeal clades revealed that these ANME form consortia with sulfate-reducing bacteria, consistent with the hypothesis of syntrophic sulfate-coupled AOM (16, 93). Gene-targeted and metagenomics analyses of AOM-active microbial mats, seep sediments, and enrichment cultures have since demonstrated that ANME metabolize methane via the canonical seven-step methanogenesis pathway, but working in the reverse direction, with the enzyme methyl coenzyme M reductase (MCR) catalyzing the activation of methane as methyl coenzyme M (CoM) (42, 65, 83, 113, 138). Genomic reconstructions and fluorescence-based assays targeting the sulfate-reducing bacterial partners of ANME have revealed that the canonical sulfate reduction pathway is localized in the syntrophic partner bacteria (67, 85, 115). Thus, sulfate-dependent AOM requires the syntrophic interaction of ANME and their partner bacteria. In contrast, some ANME perform AOM by transferring the reducing equivalents to alternative electron acceptors

Consortia: physical association of different microbial taxa cooperating for a mutual benefit

AOM: anaerobic oxidation of methane

ANME: anaerobic methane-oxidizing archaea

Methyl coenzyme M reductase (MCR): enzyme that catalyzes the formation of methane from methyl-CoM or the reverse reaction, the activation of methane as methyl-CoM

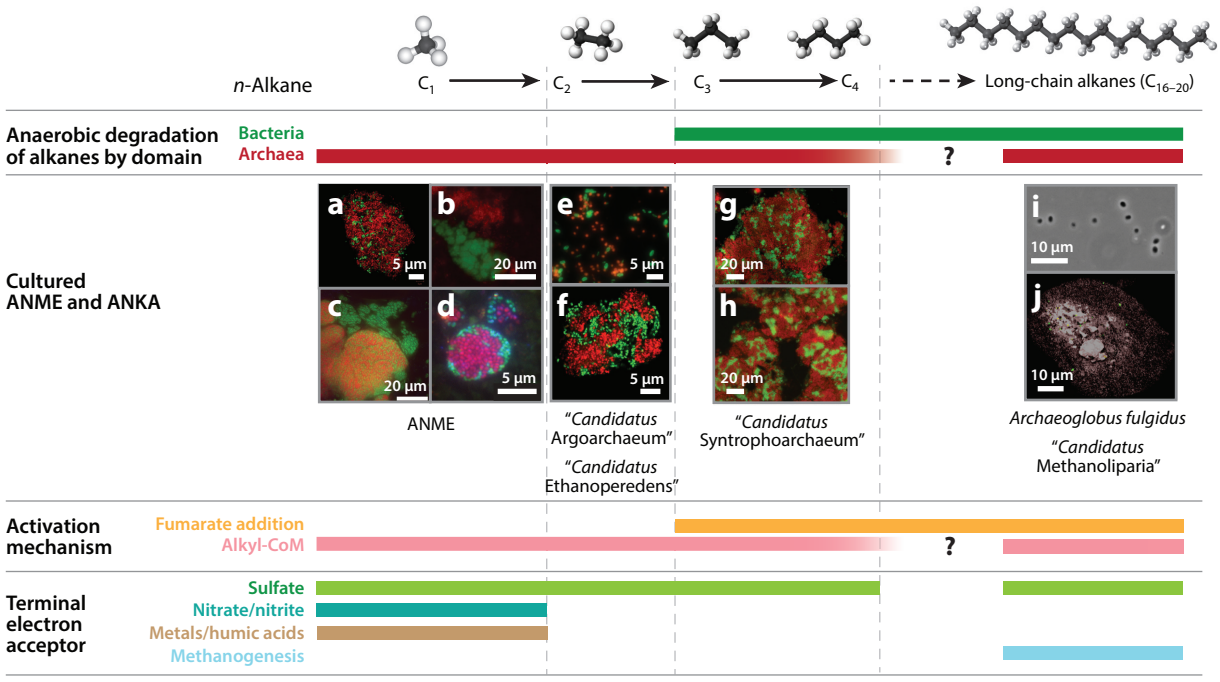


Figure 1

Overview of anaerobic alkane degradation in cultured archaea. So far, only members of the archaea are capable of performing anaerobic methane and ethane degradation, whereas bacteria can only degrade alkanes larger than three carbons. Almost all archaeal alkane degraders use the alkyl-CoM mechanism for alkane activation. Only *Archaeoglobus fulgidus* can activate alkanes using the fumarate addition mechanism, which was likely acquired from a bacterium. Anaerobic alkane degradation is usually coupled to sulfate reduction, but alternative electron acceptors have also been reported. (*a*) Consortia of ANME-1 (*red*) and the partner bacteria "*Candidatus* Desulfofervidus auxilii" (*green*). (*b*) "*Candidatus* Methanoperedens nitroreducens"/ANME-2d (*green*) and "*Candidatus* Methylomirabilis" (*red*). Note that "*Ca*. Methylomirabilis" uses the nitrite for the production of molecular oxygen as the electron acceptor for the activation of methane. (*c*) Consortia of ANME-2c (*red*) and partner bacteria (*green*). (*d*) Consortia of ANME-2 (*red*) and Seep-SRB1a (*green*). (*e*) "*Candidatus* Argoarchaeum" (*red*) and associated bacteria (*green*). (*f*) Consortia of "*Candidatus* Ethanoperedens" (*red*) and "*Candidatus* Desulfofervidales" (*green*). (*g*,*h*) "*Candidatus* Syntrophoarchaeum" (*red*) and "*Ca*. Desulfofervidus" (*green*). (*i*) *Archaeoglobus fulgidus*. (*j*) "*Candidatus* Methanoliparia" cells (*green*) attached to an oil droplet (*gray*). Panel *b* courtesy of Cornelia Welte and Arslan Arshad; panel *e* courtesy of Florin Musat; panel *f* courtesy of Cedric Hahn; and panel *i* courtesy of Cornelia Welte and Julia Kurth. Abbreviations: alkyl-CoM, alkyl coenzyme M; ANKA, anaerobic multicarbon alkane-degrading archaea; ANME, anaerobic methane-oxidizing archaea.

such as nitrate, metal oxides, and humic substances. These ANME seem to not require physical association with syntrophic partners (13, 20, 33, 43, 72, 107).

It is chemically feasible that MCR-like enzymes activate multicarbon compounds (39, 106), but microbes using this mechanism were only recently identified in sediments and enrichment cultures produced from the Guaymas Basin and the Gulf of Mexico (**Figure 1**; for additional sites see **Supplemental Text 1** and **Supplemental Table 1**). The butane degrader "*Candidatus* Syntrophoarchaeum" contains multiple MCRs that are highly divergent from those of methanogens and methanotrophs (71). It uses these enzymes to activate butane (and propane) as CoM-bound alkyl groups, representing the first evidence for the physiological activation of nonmethane alkanes by MCR-like enzymes (71). Later, the ethane oxidizers "*Candidatus* Argoarchaeum" and "*Candidatus* Ethanoperedens" (both of which belong to the GoM-Arc1 clade) were discovered with their own specific MCR types, which exclusively activate ethane (23, 40). Consequently, these

GoM-Arc1: archaeal sister clade to the methanotrophic "*Ca*. Methanoperedens" that includes two ethane-degrading genera, "*Ca*. Argoarchaeum" and "*Ca*. Ethanoperedens"

Supplemental Material >

MCR-like enzymes have been named alkyl coenzyme M reductases (ACRs) (128). Of the cultured anaerobic multicarbon alkane-degrading archaea (ANKA), "*Ca.* Syntrophoarchaeum," "*Ca.* Ethanoperedens," and "*Ca.* Argoarchaeum" completely oxidize the alkyl units to CO_2. Similar to most ANME, these archaea do not encode a reductive pathway but likely transfer the released electrons to their sulfate-reducing partner bacteria (40, 71). Oil reservoirs are often inhabited by microorganisms that thrive on the rich supply of organic compounds. One of the most prominent members of this habitat is the class "*Candidatus* Methanoliparia," which couple alkane degradation to methanogenesis (17, 69). These archaea use ACR for multicarbon activation and MCR for methane formation and apparently do not require syntrophic partners (158). Thus, the discovery of the novel, multicarbon-compound-oxidizing MCRs substantially expands the role of archaea in hydrocarbon metabolism.

Alkyl coenzyme M reductase (ACR): enzyme related to MCR that catalyzes the activation of multicarbon alkanes to alkyl-CoM

Anaerobic multicarbon alkane-degrading archaea (ANKA): include the clade GoM-Arc1, the genus "*Ca.* Syntrophoarchaeum," and the class "*Ca.* Methanoliparia" as cultured groups

Supplemental Material >

2. CULTIVATION OF ALKANE-DEGRADING ARCHAEA

The cultivation of anaerobic alkane-degrading archaea requires strictly anoxic conditions throughout all sample treatments, including a rapid transfer of culture material to anoxic conditions and exclusion of oxygen contaminations in all further steps (70, 145). Because alkane-degrading archaea grow slowly, cultivation usually requires sediments from hydrocarbon-rich cold seeps and hot vents with naturally enriched populations (15). Sites that host such organisms are described in **Supplemental Text 1** and **Supplemental Table 1**. Long-term cultivation under high methane partial pressures resulted in the first actively growing cultures composed of ANME-2 lineage archaea and their partner bacteria (5, 37, 87, 156). They grow with activity-based doubling times of several months, whereas cold-adapted sediment-free enrichments were obtained by continuous cultivation over several years (50, 150). "*Candidatus* Methanoperedens" has been cultured from freshwater samples with diverse electron acceptors (nitrate and iron and manganese oxides) (20, 43, 72, 132). Cultivation from hydrothermal vent sediments from Guaymas Basin has generated sediment-free methanotrophic enrichments of thermophilic ANME-1 with the deep-branching sulfate-reducing bacterium "*Candidatus* Desulfofervidus auxilii" (formerly HotSeep-1), with doubling times of approximately 2 months at 50°C (51). From the same environment, the thermophilic butane oxidizer "*Ca.* Syntrophoarchaeum" and the ethane degrader "*Ca.* Ethanoperedens" were also cultured in consortia with "*Ca.* Desulfofervidus," both with doubling times on the order of weeks (40, 71). In comparison, the establishment of a sediment-free psychrophilic ethane-oxidizing coculture of "*Ca.* Argoarchaeum" and partner bacteria required more than a decade of continuous enrichment due to doubling times of several months (23). A culture of the methanogenic alkane degrader "*Ca.* Methanoliparia" growing on multiple long-chain hydrocarbons was recently established from a sample of terrestrial oil sludge (158). So far, all the abovementioned cultured ANME and ANKA grow in enrichment cultures in which these archaea dominate, but they are not pure and most need partner bacteria. Some of these alkane degraders may never be isolated because they are obligate syntrophs.

3. MORPHOLOGY OF CONSORTIA OF ALKANE-DEGRADING ARCHAEA

Our understanding of the environmental distribution, functioning, and syntrophic partner associations of alkane-oxidizing archaea greatly benefited from the visualization of the microorganisms by microscopy (16, 93). FISH methods have shown the frequent occurrence of densely packed, dual-species consortia of ANME and ANKA with their sulfate-reducing partner bacteria in the environment and in enrichment cultures, consistent with their obligate syntrophic lifestyle (**Figure 1**). Like methanogens, alkane-oxidizing archaea can also be directly visualized by the

strong autofluorescence of their water-soluble electron carrier F_{420} (67). In addition to conventional 16S rRNA-targeted FISH, the application of single-cell mRNA detection by hybridization chain reaction–FISH and antibody staining of key enzymes in these organisms has also yielded information about their metabolic activities in the context of their consortial partners (12, 82, 85). Combinations of transmission electron microscopy and FISH have been used to determine the ultrastructure of different environmental ANME groups and their partner sulfate-reducing bacteria in consortia, with some ANME lineages harboring intracellular polyphosphate inclusions (ANME-2b) while different syntrophic sulfate-reducing bacterial partners contain magnetosome-like chains of greigite, mitochondria-like membrane invaginations, and carbon storage granules (80, 101). Studies applying multi-isotope imaging of ANME consortia using secondary ion mass spectrometry combined with FISH (FISH-SIMS or FISH-nanoSIMS) have provided fundamental insights into the physiology of ANME consortia in environmental samples from cellular $\delta^{13}C$ evidence of methane oxidation (52, 93, 94) and into their role in nitrogen fixation (26, 82). These studies have also supported the direct interspecies electron transfer (DIET) hypothesis as a main syntrophic mechanism between ANME and their sulfate-reducing bacterial partners (79, 107). The use of the electron microscopy stain diaminobenzidine targeting redox-active proteins (e.g., cytochromes) provides additional support for DIET between ANME-2 archaea and their bacterial partners, with dense staining occurring in the extracellular space between cells in consortia, consistent with the predicted location of extracellular multiheme cytochromes (67, 79).

F_{420}: a coenzyme in redox reactions in methanogens and an electron carrier in many anaerobic microorganisms; has characteristically strong UV fluorescence

DIET: direct interspecies electron transfer

Syntrophy: form of cooperation in which microorganisms depend on each other to perform the metabolic activity observed

In nature, ANME-1 cells appear as single cells or in multicellular chains sometimes longer than 100 μm (51, 94) or as thick biofilms and chemoherms (63, 84, 131). Ultrastructural analysis based on electron microscopy revealed that ANME-1 archaea form proteinaceous envelopes, as similarly shown for close methanogenic relatives, giving the cells a cylindrical shape (67, 101, 144). As observed with ANME-2 consortia, the ANME-1 archaea and their partners produce many cytochromes present in the extracellular matrix (67). When growing in syntrophy, the partner bacterium "*Ca.* D. auxilii" also overexpresses pili and cytochrome-encoding genes (144). These genes are underexpressed when "*Ca.* D. auxilii" grows alone using hydrogen gas as energy source. These observations further strengthen evidence of the involvement of pili and cytochromes in interspecies electron transfer (66, 144).

Compared with data from the diverse imaging techniques that have been applied to methanotrophic ANME consortia, morphological and ultrastructural data on the more recently cultured ANKA are more limited. The thermophilic short-chain alkane degraders "*Ca.* Ethanoperedens" and "*Ca.* Syntrophoarchaeum" form consortia with the partner bacterium "*Ca.* Desulfofervidus," as similarly shown for thermophilic ANME-1 (**Figure 1**). Within the consortia, the archaeal and bacterial partners occur in larger monospecies clusters, with greater distances between the syntrophic partners that have to be bridged to allow for DIET (40, 71). In contrast, the cultured psychrophilic ethane oxidizer "*Ca.* Argoarchaeum" forms unstructured aggregates with yet uncharacterized partner bacteria (23). In marine sediments, both GoM-Arc1 and "*Ca.* Syntrophoarchaeum" have also been observed under lower temperatures in structured consortia with partners of the phylum *Desulfobacterota* (40). "*Ca.* Methanoliparia" are the only ANKA in enrichment culture that grow as single cells. They have been visualized from environmental samples associated with oil droplets from the Gulf of Mexico (**Figure 1**) and in cultures derived from an oil field in China (158).

4. PHYLOGENY AND EVOLUTION OF ANME/ANKA AND THEIR *mcr* AND *acr* GENES

Anaerobic alkane metabolism was first detected in ANME (16, 88, 92), a functional group of microorganisms within the phylum *Halobacteriota* (formerly *Euryarchaeota*). ANME are

polyphyletic and are divided in three major lineages: ANME-1, which is a unique order-level clade represented by multiple genera, and ANME-2 and ANME-3, both of which belong to the order *Methanosarcinales*. ANME-2 archaea can be subdivided into four clades. ANME-2a ("*Candidatus* Methanocomedens") and ANME-2b ("*Candidatus* Methanomarinus") are two genera of the same family ("*Candidatus* Methanocomedenaceae"), distinct from the family of ANME-2c ("*Candidatus* Methanogasteraceae") (22, 61). Finally, ANME-2d ("*Ca.* Methanoperedens") is a closely related genus within the family *Methanoperedenaceae*, which is next to the clade GoM-Arc1. Similar to methanogens, ANME genomes have a single MCR, which in this case is used for methane activation rather than for the terminal step in methane formation (**Figure 2**). MCR-based multicarbon alkane metabolism also has been recently detected in novel halobacterotal groups ("*Ca.* Methanoliparia") and in relatives of ANME-1 ("*Ca.* Syntrophoarchaeum") and ANME-2d (GoM-Arc1) (23, 40, 71) (**Figure 2**). In contrast to their genomic relatedness, the MCRs of ANKA are highly divergent from those of methanogens and ANME methanotrophs and therefore are termed ACRs. Based on this difference in the encoded MCR/ACR type, the potential metabolism of ANME/ANKA can be distinguished at the genomic level. Multiple archaeal metagenome-assembled genomes (MAGs) encode novel MCR types. These MAGs belong to the class *Archaeoglobi* (*Halobacterota*), to the phyla "*Candidatus* Hadarchaeota" and *Asgardarchaeota*, and to various archaea from the phylum *Thermoproteota* (17, 18, 34, 53, 81, 109, 141) (for details see **Supplemental Table 1**). The genomic features of some of these organisms suggest a capacity for methane metabolism, and others may be multicarbon alkane-degrading archaea.

The ANME-1 clade, recently renamed "*Candidatus* Methanophagales" (1), and its sister clade *Syntrophoarchaeales* are closely related based on 16S rRNA gene analysis and genome phylogeny (17) (**Figure 2*a***), forming the class *Syntrophoarchaeia* within the phylum *Halobacteriota* according to the Genome Taxonomy Database (GTDB) taxonomy (96). They share characteristic genomic features such as the substitution of the Mer (F_{420}-dependent methylene-H_4MPT reductase) enzyme by MetFV (methylene-H_4F reductase) in the Wood–Ljungdahl/reverse methanogenesis pathway. Both ANME-1 and the order *Syntrophoarchaeales* code for fatty acid oxidation pathways, but only in *Syntrophoarchaeales* does this pathway have an obvious catabolic function (140). Whereas ANME-1 archaea contain a single MCR that is related to those of methanogens, *Syntrophoarchaeales* have four different ACR enzymes, presumably to activate different alkanes.

This difference raises the question, Which organism(s) evolved first, ANME-1 or the related multicarbon alkane metabolizers? Recent phylogenomic analysis supports a basal position for the butane-oxidizing "*Ca.* Syntrophoarchaeum" relative to methanotrophic ANME-1, suggesting that multicarbon metabolism preceded methanotrophy within this clade (142). Notably, the MCR of ANME-1 is related to those of methanogenic members of the classes *Thermoplasmata* (*Methanomassiliicoccaceae*) and *Thermococci* ("*Candidatus* Methanofastidiosa"), archaea that are only distantly related (**Figure 2*a***). This finding indicates that ANME-1 archaea likely acquired their MCR by horizontal gene transfer and lost the ACRs present in a "*Ca.* Syntrophoarchaeum"–like ancestor. This hypothesis is supported by the presumed relic β-oxidation pathway present in ANME-1. This pathway is necessary for multicarbon alkane oxidation but has no apparent function in methanotrophy. Recently, MAGs of additional sister clades related to ANME-1 and *Syntrophoarchaeales*, also referred to as *Alkanophagales* and *Santabarbaraceales*, were identified (28, 142). These MAGs contain between 3 and 6 *acr* gene clusters, which are likely involved in multicarbon alkane activation, further strengthening the hypothesis that the ancestor of the class *Syntrophoarchaeia* was likely an alkane degrader with multiple ACRs.

All ANME-2 described to date are methanotrophic archaea. The ANME-2a, ANME-2b, and ANME-2c subgroups are predominantly marine organisms that commonly oxidize methane syntrophically with sulfate-reducing partners, and members of the ANME-2d ("*Ca.*

Genome Taxonomy Database (GTDB) taxonomy: database based on concatenated protein phylogeny that normalizes taxonomic ranks according to relative evolutionary divergence

Wood–Ljungdahl pathway: microorganisms use the pathway for CO_2 fixation to form acetyl-CoA or to degrade acetyl-CoA units into CO_2, methane, or both; many of this pathway's steps are shared with the methanogenesis pathway; also known as the reductive acetyl-CoA pathway

Supplemental Material >

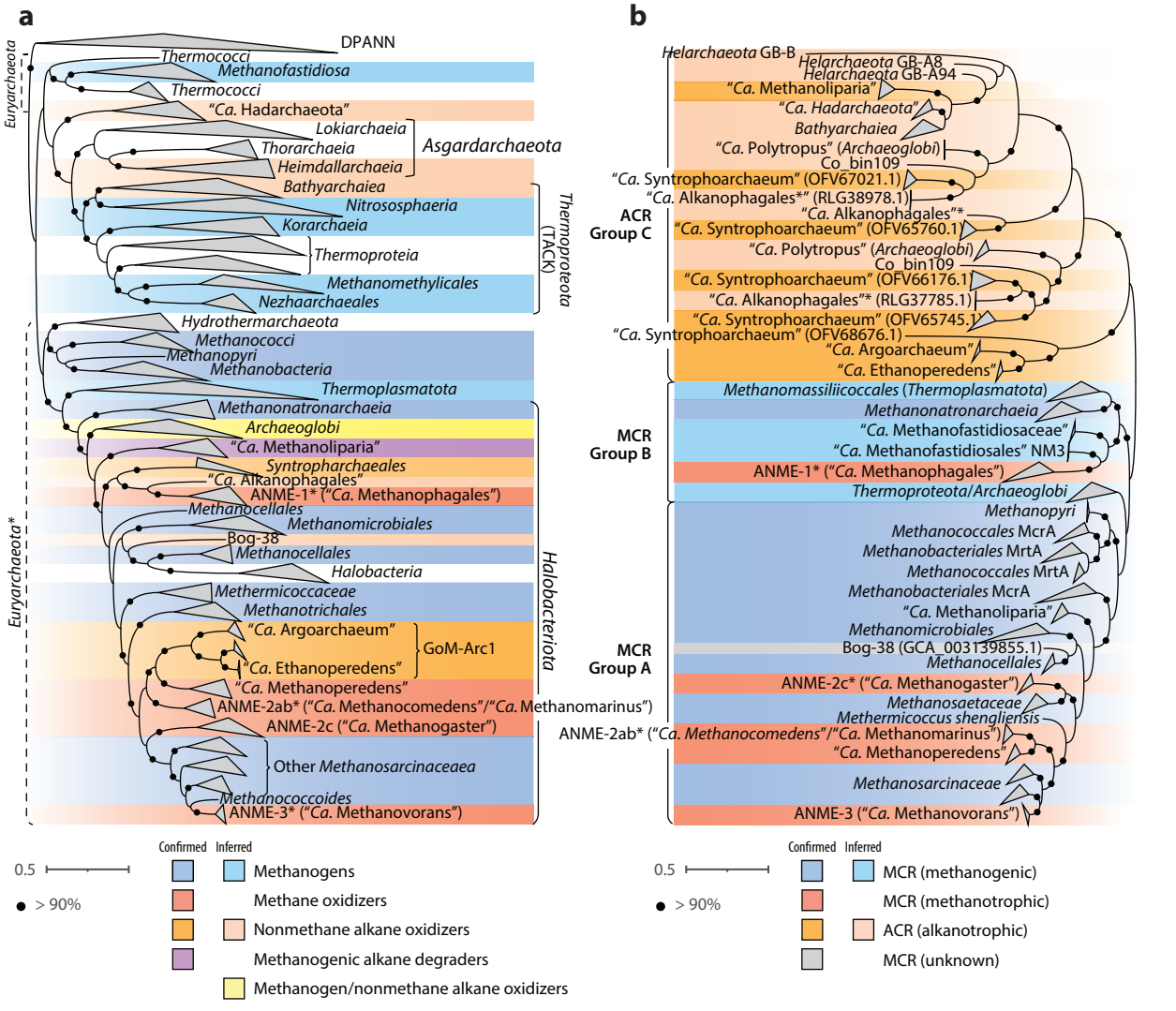


Figure 2

Phylogeny of alkane-degrading archaea and their MCR-A/ACR-A. (*a*) Phylogenomic tree of the domain *Archaea*. Colored backgrounds indicate that at least one member of the clade has the corresponding metabolism (confirmed or inferred from genomic information). Naming is according to GTDB taxonomy; names with an asterisk (*) are former common names. (*b*) Phylogenetic tree of the α subunit of the MCR/ACR protein. Colored backgrounds indicate the reactions catalyzed by the corresponding MCR/ACR (confirmed or inferred by genomic information). The MCR/ACR groups are indicated in the text. In brief, MCR group A consists of canonical methanogens (plus ANME-2 and ANME-3); MCR group B consists of novel uncultured methanogens (plus ANME-1); and ACR group C consists of novel multicarbon alkane-activating enzymes. Abbreviations: ACR, alkyl coenzyme M reductase; ANME, anaerobic methane-oxidizing archaea; DPANN, archaeal superphylum named after *Diapherotrites*, *Parvarchaeota*, "*Candidatus* Aenigmarchaeota," *Nanoarchaeota*, and *Nanohaloarchaeota*; GTDB, Genome Taxonomy Database; MCR, methyl coenzyme M reductase.

Methanoperedens") clade are freshwater nitrate- and metal-reducing archaea (20, 33, 43, 72). The sister clade GoM-Arc1 is frequently detected in hydrocarbon-rich marine environments and is currently represented by two cultured ethane-degrading genera, "*Ca.* Argoarchaeum" (23) and "*Ca.* Ethanoperedens," both of which code for a specific ACR type that is divergent from

the MCR in ANME-2d (40). Most likely, the GoM-Arc1 clade developed from the ANME-2d archaea by evolving or acquiring a divergent ACR type for ethane activation and additional features to metabolize the CoM-bound ethyl groups. However, GoM-Arc1 cannot grow on the electron acceptors used by "*Ca.* Methanoperedens" and, like other marine ANME and the ANKA "*Ca.* Syntrophoarchaeum," form consortia with sulfate-reducing bacteria.

ANME-3 archaea [renamed "*Candidatus* Methanovorans" (22)] compose a genus of yet uncultured, consortia-forming putative methanotrophs that occur in diverse marine environments, including deep-sea whale falls, methane seeps, and mud volcanoes (38, 90, 92). In the Haakon Mosby mud volcano in the Barents Sea, ANME-3 archaea were the dominant anaerobic methanotrophs in association with a novel member of the family *Desulfobulbaceae* (73, 90). ANME-3 archaea are closely related to *Methanococcoides*, with which they share many features such as highly similar *mcr* genes (38, 73). This finding suggests ANME-3 archaea evolved from their methanogenic ancestors relatively late. ANME-3 archaea seem to have some distinct features of ANME organisms, absent in their methanogenic relatives, for instance, the presence of multiheme *c*-type cytochromes and other electron cycling mechanisms. The acquisition of these features was probably crucial for the switch from methanogenesis to syntrophic methane oxidation (22).

Recently, members of the class "*Ca.* Methanoliparia" have been found to degrade multicarbon alkanes (158). "*Ca.* Methanoliparia" are a basal halobacterotal lineage related to *Archaeoglobi* and *Syntrophoarchaeia* and consist of a marine subclade and a terrestrial subclade (17, 69). "*Ca.* Methanoliparia" are unique among ANKA. They contain a highly divergent ACR type for the activation of long-chain alkanes and other alkylated hydrocarbons and an MCR that is used for methane formation. The origin of the divergent ACR is unclear, but multicarbon alkane metabolism coupled to methanogenesis may have been a relatively basic trait of microorganisms developed on early Earth with reduced conditions. The canonical MCR of "*Ca.* Methanoliparia" seems to reflect the genome phylogeny.

MAGs of different archaeal phyla harbor diverse *acr* genes, suggesting that multicarbon alkane metabolism is more widely distributed than previously assumed. Most of these ACRs are similar to the ACR of "*Ca.* Methanoliparia." The only exceptions are the ACRs from *Alkanophagales* and the *Archaeoglobi* strain "*Candidatus* Polytropus marinifundus," which are related to the ACRs from "*Ca.* Syntrophoarchaeum." The polyphyly of these ACRs does not reflect the genome-based phylogeny, which might be caused by convergent evolution (homoplasy of the ACR) or different horizontal gene transfer events between the different ACR-containing archaea. It could be hypothesized that the ACRs in members of "*Ca.* Hadarchaeota" and *Archaeoglobi* formed by convergent evolution from an MCR-containing ancestor, similar to the ACR of "*Ca.* Methanoliparia," since they belong, or are closely related, to the *Halobacterota.* MCR is likely a feature of the last common ancestor of this phylum. Yet horizontal gene transfer of these ACRs cannot be ruled out. In the cases of *Bathyarchaeia* and *Helarchaeota*, it is most likely that the ACRs were acquired through horizontal gene transfer from a halobacterotal organism, as no members of these clades encode for any kind of MCR. Future research should shed light on the origin of this metabolism and how widespread it is among the different archaeal clades.

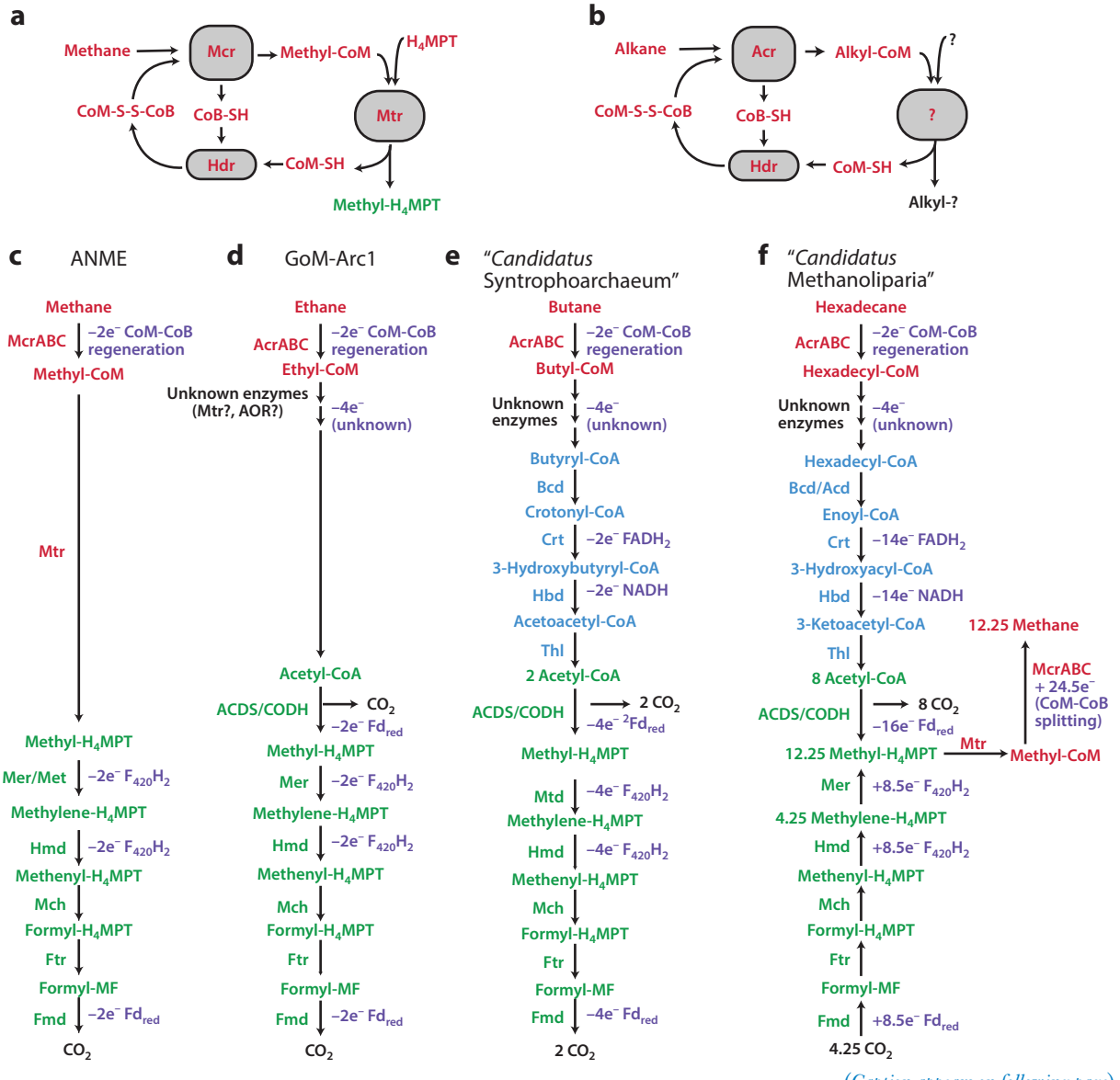
5. BIOCHEMISTRY OF ALKANE DEGRADATION IN ARCHAEA

5.1. Methane Oxidation in ANME

AOM is catalyzed by enzymes known from methanogenesis, yet in methane oxidizers these enzymes operate primarily in the reverse direction, toward the net formation of CO_2 rather than methane. Central to this metabolism is the activation of methane as methyl-CoM by specific MCRs that are highly similar to those of methanogens (105, 130). During AOM, ANME strongly

F_{430}: cofactor of MCR; a tetrapyrrole containing nickel with a spectroscopic absorbance peak at 430 nm

overexpress *mcr* (67, 138, 144), and MCR makes up approximately 10% of the extractable proteins from AOM active mats (77). The α subunit of the MCR hosts the catalytic center with the nickel porphinoid coenzyme F_{430} (31, 112). Here methane is activated by a Ni(I) atom and the heterodisulfide CoM-S-S-CoB, forming methyl-CoM and free coenzyme B (CoB) as products (147) (**Figure 3*a***). The F_{430} in ANME-1 is slightly modified by the addition of a methyl thiol group (4, 112). The reason for this modification is currently unknown, but its absence in other ANME suggests that it is not universally required for archaeal oxidation of methane. Both methanogens and methanotrophs have several posttranslational modifications in their MCRs whose function



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Catabolic flow and redox balances in cultured alkane-degrading archaea. Schemes of the activation of (*a*) methane and (*b*) multicarbon alkanes via MCR and ACR and regeneration of the cofactors. Schemes of catabolic reaction in (*c*) ANME, (*d*) the clade GoM-Arc1, (*e*) "*Candidatus* Syntrophoarchaeum," and (*f*) "*Candidatus* Methanoliparia." Enzymes/compounds characteristic of alkane-metabolizing archaea are in red. Enzymes/compounds characteristic of the β-oxidation pathway are in blue. Enzymes/intermediates of the Wood–Ljungdahl pathway are in green. Unknown steps in the pathway are indicated. Electron flow and carriers are depicted in violet. The model does not account for carbon assimilation. Abbreviations: ACDS/CODH, acetyl-CoA decarbonylase/synthase:CO dehydrogenase; ACR, alkyl coenzyme M reductase; ANME, anaerobic methane-oxidizing archaea; AOR, aldehyde ferredoxin oxidoreductase; Bcd/Acd, butanoyl/alkynyl-CoA; CoA, coenzyme A; CoB, coenzyme B; CoM, coenzyme M; Crt, enoyl-CoA hydratase dehydrogenase; Fd_{red}, reduced ferredoxin; Fmd, formylmethanofuran dehydrogenase; Ftr, formylmethanofuran:H_4MPT formyltransferase; H_4MPT, tetrahydromethanopterin; Hbd, 3-hydroxybutyryl-CoA dehydrogenase; Hdr, heterodisulfide reductase; Hmd, 5,10-methenyl-H_4MPT hydrogenase; Mch, methenyl-H_4MPT cyclohydrolase; MCR, methyl coenzyme M reductase; Mer, methylene-H_4MPT reductase; Met, methylenetetrahydrofolate reductase; MF, methanofuran; Mtd, methylene-H_4MPT dehydrogenase; Mtr, methyl-H_4MPT:coenzyme M methyltransferase; Thl, acetyl-CoA acetyltransferase.

has not yet been determined but may play important roles in the kinetics and the stability of the enzyme (31, 112, 136).

The next step in AOM is the exchange of the CoM with the C_1 carrier tetrahydromethanopterin (H_4MPT) (**Figure 3*c***). This reaction is catalyzed by the membrane complex methyl-H_4MPT:coenzyme M methyltransferase (Mtr). This endergonic reaction is enabled by the influx of approximately two sodium ions (78). In three consecutive reactions, ANME further oxidize the H_4MPT-bound methyl group stepwise to methylene, methenyl, and formyl-H_4MPT (42). The enzymes responsible for these steps are methylene-H_4MPT reductase (Mer), methylene-H_4MPT dehydrogenase (Mtd), and a methenyl-H_4MPT cyclohydrolase (Mch), respectively. Together these steps are coupled to the reduction of two F_{420} molecules. Notably, ANME-1 archaea do not code for a canonical Mer, which is assumed to be substituted by methylenetetrahydrofolate reductase (Met) (67, 83, 123). The formyl group is transferred to methanofuran (MF) by formylmethanofuran:H_4MPT formyltransferase (Ftr), and the carbon is oxidized to CO_2 by formylmethanofuran dehydrogenase (Fmd) in an exergonic reaction that produces reduced ferredoxin (Fd_{red}). The reactions of the reverse methanogenesis release eight reducing equivalents (electrons) from methane that reduce the heterodisulfide CoM-S-S-CoB to the thioether CH_3–S-CoM (methyl-CoM) and the thiol coenzyme B (CoB-SH), and that reduce two molecules of F_{420} and one molecule of ferredoxin. All ANME encode homologs of cytoplasmic heterodisulfide reductases (HdrABC) (67). In hydrogenotrophic methanogens, HdrABC form complexes with MvhADG hydrogenase. This complex couples the last step of methanogenesis (the exergonic methyl-CoM reduction) with the first step of methanogenesis (the endergonic reduction of CO_2 to formyl-MF) in a bifurcation reaction (129). In ANME, a complex of HdrABC, FrhB, and MvhD may catalyze a confurcation reaction, the regeneration of ferredoxin and CoM-S-S-CoB coupled to the reduction of two F_{420} units (78, 148). At the membrane, F_{420} is regenerated by the transfer of electrons to quinones by $F_{420}H_2$:quinone oxidoreductase complexes (Fqo) in ANME-1 or presumably to methanophenazine by $F_{420}H_2$:methanophenazine oxidoreductase complexes (Fpo) in ANME-2 and ANME-3, allowing the translocation of approximately one proton per electron couple (22) (**Supplemental Figure 1**). ANME-2 and ANME-3 encode additional membrane-bound HdrDE complexes. This enzyme may regenerate CoM-S-S-CoB coupled to an inflow of two protons (7, 22, 114). Encoded Rnf complexes would allow the regeneration of ferredoxin coupled to proton translocations across the membrane in ANME-2 and ANME-3.

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Ferredoxin: iron–sulfur proteins that mediate electron transfer in various metabolic reactions at low potentials

Bifurcation: metabolic reaction that splits a hydride electron pair into one electron with a more positive reduction potential and one electron with a more negative reduction potential

Confurcation: the reversal of bifurcation; a reaction that combines electrons with different reduction potentials with a hydride electron pair with intermediate reduction potential

5.2. Multicarbon Alkane Degradation in ANKA

Multicarbon alkane-oxidizing archaea contain most enzymes of the methanogenesis and additional pathways to degrade the multicarbon intermediates. They activate the multicarbon alkanes

with phylogenetically and functionally divergent variants of the MCR enzyme, the ACRs, that catalyze a reaction analogous to the biochemical principles of anaerobic activation of methane (128) (see **Figure 3*b***). None of the known ANKA can activate methane with these enzymes. Alkanes such as ethane, butane, and hexadecane react with the heterodisulfide CoM-S-S-CoB, forming alkyl- (ethyl-, butyl-, or hexadecyl-)CoM and free CoB as reaction products. It is still unclear how the active centers of ACRs can accommodate such large substrates. The structure of the ethane-activating ACR of "*Ca.* Ethanoperedens" shows that multiple loop insertions and posttranslational amino acid modifications result in the formation of a hydrophobic tunnel for the substrate. This structural feature may facilitate the reaction kinetics by providing an alternative path toward the enzymes' active center (41). In addition, the nickel porphinoid coenzyme F_{430} is modified by two methylations that likely ensure the correct positioning of the cofactor in an enlarged cavity. As a result, the ACR of "*Ca.* Ethanoperedens" can activate only ethane (40). "*Ca.* Syntrophoarchaeum" possesses four ACRs, yet their substrate specificity is unknown.

The next step in multicarbon alkane degradation is the oxidation of the alkyl groups to an acyl unit and the transfer to the cofactor coenzyme A (CoA). The nature of these transformations and the involved enzymes are largely unknown. The ethane degraders of the GoM-Arc1 code for an Mtr that might catalyze the cleavage of the CoM and the transfer of the ethyl group to a different cofactor (23). The following oxidation reactions may involve aldehyde ferredoxin oxidoreductases, which are present and expressed in multiple copies (40). In the case of ethane degradation, these reactions directly lead to acetyl-CoA, a central metabolite in most archaea (**Figure 3*d***). In the case of longer alkanes, alkyl-CoM is transformed to acyl-CoA intermediates, which are degraded to acetyl-CoA. To break down these intermediates, "*Ca.* Syntrophoarchaeum" and "*Ca.* Methanoliparia" contain a β-oxidation pathway, a fundamental feature in most ANKA to split the fatty acids into acetyl units (**Figure 3*e*,*f***). The β-oxidation pathway consist of four steps, for which ANKA usually have multiple copies of the corresponding enzymes, suggesting a certain substrate specificity (71).

In syntrophic ANKA, the acetyl-CoA is completely oxidized to CO_2 (**Figure 3*d–f***). First, acetate is split into CO_2 and a methyl group by using the acetyl-CoA decarbonylase/synthase:CO dehydrogenase (ACDS/CODH) enzyme. Then, the methyl group is transferred to H_4MPT and oxidized to CO_2 on the methyl branch of the Wood–Ljungdahl pathway, which consists of the same steps as the downstream part of the reverse methanogenesis, with five steps catalyzed by Mer/Met, Hmd, Mch, Ftr, and Fmd. Similar to ANME-1, "*Ca.* Syntrophoarchaeum" likely replaces the Mer enzyme by Met. In both "*Ca.* Syntrophoarchaeum" and GoM-Arc1, the reducing equivalents produced during the alkane oxidation must be transferred to membrane-soluble molecules such as quinones and methanophenazine, from which they will be channeled to the sulfate-reducing partner bacteria as discussed in the next section. The electron cycling mechanisms within ANKA are similar to those of their close ANME relatives. To regenerate CoM-S-S-CoB, GoM-Arc1 archaea have genes for both HdrABC and HdrDE while "*Ca.* Syntrophoarchaeum" harbors genes for several HdrABC complexes (**Supplemental Figure 1**). For the reoxidation of F_{420}, GoM-Arc1 archaea encode Fpo and "*Ca.* Syntrophoarchaeum" encodes Fqo. The oxidation of alkyl-CoM to the original acyl-CoA releases four electrons, yet the involved enzymes are unknown. In the case of "*Ca.* Syntrophoarchaeum," the split of butyl-CoA into two acetyl units releases four electrons per reaction that are reducing the electron carriers NAD^+ and flavin adenine dinucleotide (FAD) from an electron transfer flavoprotein. The genes encoding the electron transfer flavoprotein complex form an operon with an [FeS]-oxidoreductase that is likely involved in further electron cycling. Because the redox potential of $FADH_2$ ($E^{\circ\prime} = -125$ mV) is too high to allow a coupling to sulfate reduction in the partner bacteria ($E^{\circ\prime} = -220$ mV), a

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potential shift of those reducing equivalents is required. The potential shift might be catalyzed in another confurcation reaction involving Fd_{red} or energy-driven reverse electron transfer (71).

5.3. Transfer of Reducing Equivalents to Partner Bacteria in Syntrophic ANME and ANKA

To sustain alkane oxidation, the reducing equivalents released during these reactions must find a sink. For marine anaerobic alkane-oxidizing archaea sulfate is the dominant electron acceptor. Yet all cultured ANME and ANKA appear to lack a dissimilatory sulfate reduction pathway. Instead, sulfate reduction is performed by syntrophic partners, members of the *Desulfobacterota* (formerly *Deltaproteobacteria*) such as those belonging to the SeepSRB1a or the SeepSRB2 cluster or to the thermophilic deep-branching class *Desulfofervidia* (51, 59, 62). Syntrophic alkane-oxidizing archaea often have partner bacteria of the same genus as methane oxidizers. One exception is the ultraslow-growing ethanotroph "*Ca.* Argoarchaeum," which forms unstructured consortia with an unidentified bacterial partner (23) (**Figure 1**).

The high similarity in the consortia structure and the partner suggests similar modes to exchange the reducing equivalents. However, the mechanisms underlying this syntrophy are poorly understood. Early studies proposed the transfer of molecular intermediates such as hydrogen, formate, and acetate between ANME and their partners, as observed in other syntrophic interactions of microorganisms (49, 108). In alkane oxidation, however, such chemical intermediates are unlikely because the required low equilibrium concentration in the low or subnanomolar range would kinetically inhibit the process (120) and because the large distances observed between active ANME and sulfate-reducing bacterial cells in syntrophic consortia are inconsistent with molecular diffusion (44, 79). Furthermore, most ANME and their partners lack hydrogenases, suggesting that hydrogen cannot be produced as an intermediate (22, 67, 89, 115, 143, 149). Instead, a direct transfer of reducing equivalents between the partners in the form of electric current is likely. In the DIET hypothesis, electrons from $F_{420}H_2$ and Fd_{red} are transported out of the cell by membrane-bound carriers such as menaquinone (ANME-1, "*Ca.* Syntrophoarchaeum") or methanophenazine (ANME-2, ANME-3, GoM-Arc1) and membrane-spanning and extracellular multiheme cytochromes (22, 67, 79, 115, 144). The electrons are received by the sulfate-reducing partner bacteria via additional extracellular and membrane-spanning cytochromes (67, 115, 144). The partner bacterium "*Ca.* Desulfofervidus" expresses large amounts of pili and cytochrome-encoding genes. These proteins were hypothesized to form nanowire-like structures similar to that described for *Geobacter* (67, 144, 137). Consortia of thermophilic multicarbon alkane-oxidizing microorganisms show similar structures and gene expression patterns, suggesting analogous electron transfer mechanisms (40, 69). Recently, a mixed model combining DIET and chemical diffusion of low-mass intermediates (i.e., formate) in ANME–bacteria syntrophy was proposed based on energetic considerations (44).

5.4. Alternative Electron Sinks of ANME

AOM can be coupled to various additional electron acceptors, including nitrate (NO_3^-), oxidized metal species such as Fe(III) or Mn(IV), and humic substances like anthraquinone-2,6-disulfonate (AQDS) (**Table 1**). Under standard conditions, these compounds allow energy yields even higher than those for sulfate reduction. In marine environments, these compounds are rare or poorly accessible, but they may play a vital role in freshwater habitats (20, 72, 133).

The cultured methane oxidizers of the ANME-2d clade do not couple AOM to sulfate reduction. Instead, they have acquired other electron-sinking mechanisms. "*Candidatus*

Table 1 Coupling of methane oxidation to different electron acceptors

Reaction	Organisms	Enrichment source/status	Reference(s)
$CH_4 + 4NO^-_3 \rightarrow 4NO^-_2 + 2H_2O$ $\Delta G^{0'} = -503$ kJ mol^{-1} CH_4	"*Candidatus* Methanoperedens"	Multiple stable cultures (enriched)	43
$CH_4 + 8Fe^{3+} + 2H_2O \rightarrow CO_2 + 8Fe^{2+} + 8H^+$ $\Delta G^{0'} = -454$ kJ mol^{-1} CH_4	"*Ca.* Methanoperedens," additional evidence in ANME-2	Enrichment in bioreactor; culture not available	6, 13, 20, 33, 107
$CH_4 + 4MnO_2 + 7H^+ \rightarrow HCO^-_3 + 4Mn^{2+} + 5H_2O$ $\Delta G^{0'} = -383$ kJ mol^{-1} CH_4	"*Ca.* Methanoperedens," additional evidence in ANME-2	Enrichment in bioreactor; culture not available	13, 72
$CH_4 + 4AQDS + 3H_2O \rightarrow HCO^-_3 + 4AQH_2DS + H^+$ $\Delta G^{0'} = -41$ kJ mol^{-1} CH_4	ANME-2	Short-term experiments; no cultures	9, 107, 149

Methanoperedens nitroreducens" directly couples AOM to the reduction of nitrate to nitrite using membrane-bound nitrate reductases. This metabolism does not strictly require a syntrophic relationship, but "*Ca.* M. nitroreducens" usually appears associated with nitrite-reducing ("*Candidatus* Methylomirabilis oxyfera") or anammox (i.e., *Kuenenia*) bacteria (43). "*Candidatus* Methanoperedens ferrireducens" and "*Candidatus* Methanoperedens manganicus" couple AOM to iron and manganese reduction (20, 72). These metal-reducing ANME code for a large number of different, highly expressed cytochromes, including 4-heme cytochromes and others with higher heme numbers and S-layer domains that apparently allow electron transfer to metal oxides. In marine habitats, ANME-2d archaea are rare. Short-term incubation experiments suggest that some marine ANME-2 strains can couple AOM to metal reduction (6, 13, 33, 107). Other experiments suggest that ANME-2 can transfer electrons to organic compounds such as the humic acids or their synthetic analogs (i.e., AQDS) (9, 107, 134, 149). Albeit thermodynamically feasible, similar redox couplings in ANKA have not been documented.

5.5. Methanogenic Multicarbon-Compound Degradation by "*Candidatus* Methanoliparia"

"*Ca.* Methanoliparia" thrive as single or aggregated cells without apparent association with partner bacteria, forming methane and CO_2 as metabolic products (158). "*Ca.* Methanoliparia" encode a single ACR (17, 69) that apparently activates various long-chain alkanes and other alkyl-substituted compounds according to recent cultivation experiments (158). Genomic evidence suggests that "*Ca.* Methanoliparia" transform these compounds via free alcohols to CoA-bound acyl units. Similar to other ANKA, "*Ca.* Methanoliparia" break these compounds via β-oxidation into several acetyl-CoA units. As shown by incubation experiments, "*Ca.* Methanoliparia" can also activate and degrade alkyl-substituted aromatic compounds. β-Oxidation of the alkyl chains spares benzene rings that are opened by an encoded benzoyl-CoA reductase (69, 158). The acetyl-CoA is further split into CO_2 and H_4MPT-bound methyl groups using the ACDS/CODH complex of the Wood–Ljungdahl pathway. These oxidative reactions release reducing equivalents in the form of reduced electron carriers, including NADH, $FADH_2$, and Fd_{red} (**Figure 3*f***; **Supplemental Figure 2**). In contrast to syntrophic ANKA that transfer the electrons toward partner bacteria, "*Ca.* Methanoliparia" regenerate these electron carriers by the formation of methane. Notably, "*Ca.* Methanoliparia" encode an Mtr that transfers the methyl group from H_4MPT to CoM, and a second and canonical MCR catalyzes the reduction of methyl-CoM to methane. Using the C_1 branch of the Wood–Ljungdahl pathway, "*Ca.* Methanoliparia" deplete the surplus of reducing equivalents generated during hydrocarbon degradation by reducing additional CO_2 into methane

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(**Figure 3*f***). "*Ca.* Methanoliparia" likely translocate sodium ions at the membrane via Rnf (use of the potential difference between Fd_{red} and NADH; translocation of one to two sodium ions) and via Mtr (transfer of methyl groups from H_4MPT to CoM; translocation of two sodium ions) (**Supplemental Figure 2**).

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6. FUNCTION OF UNCULTURED MCR-CONTAINING ARCHAEA

The increasingly diverse MCR-like enzymes can be sorted into three major groups (**Figure 2*b***): (*a*) the canonical MCR types of the cultured methanogens and methanotrophs (formerly *Euryarchaeota*); (*b*) the MCRs found in MAGs of various uncultured archaea, mostly from the *Thermoproteota* (formerly the TACK groups *Verstraetearchaeota*, *Nezhaarchaeota*, and *Korarchaeota*), that most likely perform methanogenesis (plus the methanotrophic ANME-1 MCRs); and (*c*) the group of multicarbon alkane-activating ACRs. The last group can be subdivided into ACRs that are similar to the ethane-activating GoM-Arc1: the "*Ca.* Syntrophoarchaeum" group that also includes sequences of uncultured *Alkanophagales* (142) and *Archaeoglobi* (18) and the "*Ca.* Methanoliparia" ACR type that activates long-chain alkanes and clusters with other ACRs found in MAGs of uncultured organisms from diverse archaeal groups such as *Bathyarchaeia*, "*Ca.* Hadarchaeota," and *Helarchaeales*. Here we speculate on the metabolism of these uncultured MCR-/ACR-containing archaea. Substantial cultivation efforts and physiological investigations are required to validate these hypotheses.

The cultured *Archaeoglobi* are obligate thermophilic sulfate reducers. As relatives of the methanogens, they contain a Wood–Ljungdahl pathway but do not code for MCR and MTR. In contrast, the *Archaeoglobi* MAG WYZ-LMO2 retrieved from Obsidian Pool, a hot spring in Yellowstone National Park, contains *mcr* and *mtr* genes, lacks the sulfate reduction genes, and is hypothesized to be a methanogen (141). Other *Archaeoglobi* MAGs retrieved from Obsidian Pool contain the complete methanogenesis pathway and a dissimilatory sulfate reduction pathway. These organisms potentially perform sulfate-dependent AOM without a syntrophic partner (141). "*Ca.* P. marinifundus" is a basal *Archaeoglobi* lineage described from a MAG recovered from deep subsurface fluids from the Juan de Fuca Ridge hydrothermal vent system (18). This MAG contained two *acr* genes that are closely related to those of "*Ca.* Syntrophoarchaeum." Hence, "*Ca.* P. marinifundus" may be capable of degrading short-chain alkanes. This uncultured archaeon lacks a canonical sulfate reduction pathway but harbors genes that may enable the utilization of nitrate, iron, and sulfur compounds as electron sinks.

Alkanophagales were originally described as a distinct clade of ANME-1 MAGs containing *acr* genes from the Guaymas Basin (27). A few years later, this group was renamed *Alkanophagales*, representing a basal clade sitting between the *Syntrophoarchaeales* and ANME-1 that is hypothesized to perform multicarbon alkane metabolism. They also contain a β-oxidation pathway and a Wood–Ljungdahl pathway (142). The study of their phylogenomic position will help resolve the evolution of methanotrophy and alkanotrophy within the *Syntrophoarchaeia* clade. A recent study of a Scotian Basin cold seep described a novel ACR-containing MAG (Co_bin109) (29). This MAG is affiliated with a still unnamed halobacterotal class, closely related to the methanogenic *Methanocellales* (**Figure 2*a***), and contains two *acr* genes related to those of "*Ca.* Syntrophoarchaeum." The Co_bin109 MAG also encodes all known elements for archaeal alkane degradation, including the β-oxidation and Wood–Ljungdahl pathways. These ACR-containing lineages further expand the role of *Halobacteriota* in anaerobic alkane oxidation.

Bathyarchaeia represent a diverse group of uncultured, likely anaerobic microorganisms that may ferment different organic substrates (151) or perform acetogenesis (45). MAGs of *Bathyarchaeia* retrieved from a coalbed contained the first described *acr* operon of the "*Ca.*

Methanoliparia" type, yet due to the lack of cultures and lack of knowledge of the expanded substrate use of the MCR protein family at the time, these archaea were hypothesized to be either methanogens or methanotrophs (34). Notably these MAGs lacked genes coding for fatty acid degradation (34) and for cofactor F_{430} synthesis (17) that should be essential for long-chain alkane oxidizers. Hence, the role of these archaea in alkane oxidation is unclear. "*Ca.* Hadarchaeota" are widespread subsurface microorganisms without cultured representatives (10, 86). Phylogenomic analyses place them as a lineage within or next to the former *Euryarchaeota* phylum (10, 53, 96) or as a separate group in the former TACK superphylum (1). Metagenomics analyses suggest that most "*Ca*. Hadarchaeota" ferment sugars (86) or grow by oxidizing carbon monoxide and hydrogen (10). Some MAGs of "*Ca*. Hadarchaeota" retrieved from alkane-rich heated sediments encode one or two ACRs similar to those of "*Ca.* Methanoliparia," along with a complete fatty acid degradation pathway and the Wood–Ljungdahl pathway (141). Hence, these organisms may be alkane oxidizers. The ACR-containing MAGs of the *Helarchaeales* were retrieved from hydrocarbon-rich hydrothermal vents in the Guaymas Basin (109) and from organic-rich sediments off the Costa Rica Margin (157). These MAGs contain two or three *acr* gene sets that are related to those of "*Ca.* Methanoliparia"; hence, *Helarchaeales* may also be involved in long-chain alkane oxidation in the environment. Notably, this group lacks cytochromes but contains genes for hydrogenases and formate dehydrogenases. Therefore, hydrogen and formate have been proposed as possible intermediates in a syntrophic interaction between these *Helarchaeales* and specific sulfate-reducing bacteria, which seem to co-occur in the same environments according to genomic abundances (157). *Helarchaeales* belong to the *Asgardarchaeota* superphylum, which may include the last common ancestor of eukaryotes (152). Therefore, a recent model suggested that the hypothetical last common ancestor of eukaryotes might have evolved from a syntrophic asgardarchaeum capable of alkane degradation and dependent on partner bacteria, similar to some ANME and ANKA (121).

SUMMARY POINTS

1. Anoxic gas- and oil-rich marine subsurface sediments harbor abundant archaea that thrive on the oxidation of methane [anaerobic methane-oxidizing archaea (ANME)], ethane (GoM-Arc1), propane and butane ("*Candidatus* Syntrophoarchaeum"), and long-chain hydrocarbons ("*Candidatus* Methanoliparia").
2. Syntrophic methane-oxidizing archaea of the ANME-1, ANME-2, and ANME-3 clades are globally distributed in gas-rich marine environments. Their habitats overlap with sulfate-dependent anaerobic multicarbon alkane-degrading archaea (ANKA) when multicarbon alkanes are available. "*Candidatus* Methanoperedens" inhabits iron- and nitrate-rich niches in freshwater environments. "*Ca.* Methanoliparia" thrive in sulfate-depleted oil-rich subsurface habitats.
3. Diverse ANME and ANKA representatives have been cultured with the use of specific alkanes as substrates. Their growth is slow, however, with generation times of weeks (ANKA) to months (ANME), and no pure isolates are available thus far.
4. The sulfate-dependent alkane-degrading archaea form consortia with specific sulfate-reducing partner bacteria, some of which can grow with both ANME and ANKA. The methanogenic "*Ca*. Methanoliparia" are capable of independent growth.
5. Methyl coenzyme M reductase (MCR)-/alkyl coenzyme M reductase (ACR)-based alkane metabolism is widespread across the *Halobacterota*. In most instances, MCR-based

phylogeny follows genome phylogeny. By contrast, *acr* genes have likely been horizontally transferred across multiple archaeal phyla, and their divergence reflects the evolutionary selection pressure of the different substrates.

6. ANME and ANKA use specific types of MCRs and ACRs to activate their substrates and do not compete for the same substrates. ANME reverse the methanogenesis pathway for complete methane oxidation. Syntrophic ANKA combine the methanogenesis pathway with the Wood–Ljungdahl pathway (and the β-oxidation pathway for alkanes with three or more carbon atoms) for the degradation of multicarbon alkanes. "*Ca.* Methanoliparia" contain both ACR and MCR to couple long-chain alkane degradation to methanogenesis.
7. Metagenome-assembled genomes of various uncultured archaea encode ACRs, suggesting an uncharted potential of alkane metabolism in several archaeal phyla and new cultivation targets.

FUTURE ISSUES

1. What is the environmental role of anaerobic alkane-degrading archaea compared to that of bacteria with similar capabilities?
2. What are the physicochemical limits of those anaerobic alkane degraders in regard to temperature, salinity, and pH?
3. What is the substrate range for alkane-oxidizing archaea, and which substrate MCR-like enzymes can be activated by which biochemical mechanisms?
4. What are the electron acceptors of alkane-degrading archaea?
5. How is the interspecies electron exchange between archaea and bacteria organized, and do multiple mechanisms exist?
6. How and when did MCR-containing archaea, in particular those with multiple and highly divergent MCR types, evolve? How widely is MCR-based metabolism distributed in archaea?
7. What are the mechanisms and intermediates in the transformation of coenzyme M–bound alkyl units into coenzyme A–bound acyl groups?

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LITERATURE CITED

1. Adam PS, Borrel G, Brochier-Armanet C, Gribaldo S. 2017. The growing tree of Archaea: new perspectives on their diversity, evolution and ecology. *ISME J.* 11:2407–25
2. Aeckersberg F, Bak F, Widdel F. 1991. Anaerobic oxidation of saturated hydrocarbons to CO_2 by a new type of sulfate-reducing bacterium. *Arch. Microbiol.* 156:5–14
3. Al-Mailem DM, Sorkhoh NA, Al-Awadhi H, Eliyas M, Radwan SS. 2010. Biodegradation of crude oil and pure hydrocarbons by extreme halophilic archaea from hypersaline coasts of the Arabian Gulf. *Extremophiles* 14:321–28
4. Allen KD, Wegener G, White RH. 2014. Discovery of multiple modified F_{430} coenzymes in methanogens and anaerobic methanotrophic archaea suggests possible new roles for F_{430} in nature. *Appl. Environ. Microbiol.* 80:6403–12
5. Aoki M, Ehara M, Saito Y, Yoshioka H, Miyazaki M, et al. 2014. A long-term cultivation of an anaerobic methane-oxidizing microbial community from deep-sea methane-seep sediment using a continuous-flow bioreactor. *PLOS ONE* 9:e105356
6. Aromokeye DA, Kulkarni AC, Elvert M, Wegener G, Henkel S, et al. 2019. Rates and microbial players of iron-driven anaerobic oxidation of methane in methanic marine sediments. *Front. Microbiol.* 10:3041
7. Arshad A, Speth DR, de Graaf RM, Op den Camp HJM, Jetten MSM, Welte CU. 2015. A metagenomics-based metabolic model of nitrate-dependent anaerobic oxidation of methane by *Methanoperedens*-like archaea. *Front. Microbiol.* 6:1423
8. Atlas R, Bragg J. 2009. Bioremediation of marine oil spills: when and when not–the *Exxon Valdez* experience. *Microb. Biotechnol.* 2:213–21
9. Bai YN, Wang XN, Wu J, Lu YZ, Fu L, et al. 2019. Humic substances as electron acceptors for anaerobic oxidation of methane driven by ANME-2d. *Water Res.* 164:114935
10. Baker BJ, Saw JH, Lind AE, Lazar CS, Hinrichs K-U, et al. 2016. Genomic inference of the metabolism of cosmopolitan subsurface Archaea, Hadesarchaea. *Nat. Microbiol.* 1:16002
11. Barnes RO, Goldberg ED. 1976. Methane production and consumption in anoxic marine-sediments. *Geology* 4:297–300
12. Basen M, Krüger M, Milucka J, Kuever J, Kahnt J, et al. 2011. Bacterial enzymes for dissimilatory sulfate reduction in a marine microbial mat (Black Sea) mediating anaerobic oxidation of methane. *Environ. Microbiol.* 13:1370–79
13. Beal EJ, House CH, Orphan VJ. 2009. Manganese- and iron-dependent marine methane oxidation. *Science* 325:184–87
14. Bertrand JC, Almallah M, Acquaviva M, Mille G. 1990. Biodegradation of hydrocarbons by an extremely halophilic archaebacterium. *Lett. Appl. Microbiol.* 11:260–63
15. Boetius A, Holler T, Knittel K, Felden J, Wenzhöfer F. 2009. The seabed as natural laboratory: lessons from uncultivated methanotrophs. In *Uncultivated Microorganisms*, ed. SS Epstein, pp. 293–316. Berlin: Springer
16. Boetius A, Ravenschlag K, Schubert CJ, Rickert D, Widdel F, et al. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407:623–26
17. **Borrel G, Adam PS, McKay LJ, Chen L-X, Sierra-García IN, et al. 2019. Wide diversity of methane and short-chain alkane metabolisms in uncultured archaea. *Nat. Microbiol.* 4:603–13**
18. Boyd JA, Jungbluth SP, Leu AO, Evans PN, Woodcroft BJ, et al. 2019. Divergent methyl-coenzyme M reductase genes in a deep-subseafloor Archaeoglobi. *ISME J.* 13:1269–79

17. First suggestion of ACR/MCR-based methanogenic hydrocarbon degradation in a single archaeon.

19. Brzeszcz J, Kaszycki P. 2018. Aerobic bacteria degrading both *n*-alkanes and aromatic hydrocarbons: an undervalued strategy for metabolic diversity and flexibility. *Biodegradation* 29:359–407
20. Cai C, Leu AO, Xie G-J, Guo J, Feng Y, et al. 2018. A methanotrophic archaeon couples anaerobic oxidation of methane to Fe(III) reduction. *ISME J.* 12:1929–39
21. Callaghan AV. 2013. Metabolomic investigations of anaerobic hydrocarbon-impacted environments. *Curr. Opin. Biotechnol.* 24:506–15
22. Chadwick GL, Skennerton CT, Laso-Pérez R, Leu AO, Speth DR, et al. 2021. Comparative genomics reveals electron transfer and syntrophic mechanisms differentiating methanotrophic and methanogenic archaea. *PLOS Biol.* 20:e3001508
23. Chen SC, Musat N, Lechtenfeld OJ, Paschke H, Schmidt M, et al. 2019. Anaerobic oxidation of ethane by archaea from a marine hydrocarbon seep. *Nature* 568:108–11
24. Crespo-Medina M, Meile C, Hunter K, Diercks A, Asper V, et al. 2014. The rise and fall of methanotrophy following a deepwater oil-well blowout. *Nat. Geosci.* 7:423–27
25. Crone TJ, Tolstoy M. 2010. Magnitude of the 2010 Gulf of Mexico oil leak. *Science* 330:634
26. Dekas AE, Poretsky RS, Orphan VJ. 2009. Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia. *Science* 326:422–26
27. Dombrowski N, Seitz KW, Teske AP, Baker BJ. 2017. Genomic insights into potential interdependencies in microbial hydrocarbon and nutrient cycling in hydrothermal sediments. *Microbiome* 5:106
28. Dombrowski N, Teske AP, Baker BJ. 2018. Expansive microbial metabolic versatility and biodiversity in dynamic Guaymas Basin hydrothermal sediments. *Nat. Commun.* 9:4999
29. Dong X, Rattray JE, Campbell DC, Webb J, Chakraborty A, et al. 2020. Thermogenic hydrocarbon biodegradation by diverse depth-stratified microbial populations at a Scotian Basin cold seep. *Nat. Commun.* 11:5825
30. Ehrenreich P, Behrends A, Harder J, Widdel F. 2000. Anaerobic oxidation of alkanes by newly isolated denitrifying bacteria. *Arch. Microbiol.* 173:58–64
31. Ermler U, Grabarse W, Shima S, Goubeaud M, Thauer RK. 1997. Crystal structure of methyl-coenzyme M reductase: the key enzyme of biological methane formation. *Science* 278:1457–62
32. Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, et al. 2010. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature* 464:543–48
33. Ettwig KF, Zhu B, Speth D, Keltjens JT, Jetten MSM, Kartal B. 2016. Archaea catalyze iron-dependent anaerobic oxidation of methane. *PNAS* 113:12792–96
34. Evans PN, Parks DH, Chadwick GL, Robbins SJ, Orphan VJ, et al. 2015. Methane metabolism in the archaeal phylum *Bathyarchaeota* revealed by genome-centric metagenomics. *Science* 350:434–38
35. Fathepure BZ. 2014. Recent studies in microbial degradation of petroleum hydrocarbons in hypersaline environments. *Front. Microbiol.* 5:173
36. Foster JW. 1962. Hydrocarbons as substrates for microorganisms. *Antonie Van Leeuwenhoek J. Microbiol. Serol.* 28:241–74
37. Girguis PR, Orphan VJ, Hallam SJ, DeLong EF. 2003. Growth and methane oxidation rates of anaerobic methanotrophic archaea in a continuous-flow bioreactor. *Appl. Environ. Microbiol.* 69:5472–82
38. Goffredi SK, Wilpiszeski R, Lee R, Orphan VJ. 2008. Temporal evolution of methane cycling and phylogenetic diversity of archaea in sediments from a deep-sea whale-fall in Monterey Canyon, California. *ISME J.* 2:204–20
39. Gunsalus RP, Romesser JA, Wolfe RS. 1978. Preparation of coenzyme M analogs and their activity in the methyl coenzyme M reductase system of *Methanobacterium thermoautotrophicum*. *Biochemistry* 17:2374–77
40. Hahn CJ, Laso-Pérez R, Vulcano F, Vaziourakis K-M, Stokke R, et al. 2020. "*Candidatus* Ethanoperedens," a thermophilic genus of archaea mediating the anaerobic oxidation of ethane. *mBio* 11:e00600-20
41. Hahn CJ, Lemaire ON, Kahnt J, Engilberge S, Wegener G, Wagner T. 2021. Crystal structure of a key enzyme for anaerobic ethane activation. *Science* 373:118–21
42. Hallam SJ, Putnam N, Preston CM, Detter JC, Rokhsar D, et al. 2004. Reverse methanogenesis: testing the hypothesis with environmental genomics. *Science* 305:1457–62

23. First cultivation of an ethane-oxidizing archaeon.

27. Insights into the diversity of alkane-oxidizing archaea, including the first GoM-Arc1 genome, of the Guaymas Basin.

34. First detection of noncanonical MCR genes by metagenomics.

41. First structural elucidation of ethane-activating ACR by native crystallization.

43. Haroon MF, Hu S, Shi Y, Imelfort M, Keller J, et al. 2013. Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. *Nature* 500:567–70

43. First cultivation of "*Candidatus* Methanoperedens," an archaeon that couples methane oxidation to nitrate reduction.

44. He X, Chadwick G, Kempes C, Shi Y, McGlynn S, et al. 2019. Microbial interactions in the anaerobic oxidation of methane: model simulations constrained by process rates and activity patterns. *Environ. Microbiol.* 21:631–47
45. He Y, Li M, Perumal V, Feng X, Fang J, et al. 2016. Genomic and enzymatic evidence for acetogenesis among multiple lineages of the archaeal phylum Bathyarchaeota widespread in marine sediments. *Nat. Microbiol.* 1:16035
46. Head IM, Jones DM, Larter SR. 2003. Biological activity in the deep subsurface and the origin of heavy oil. *Nature* 426:344–52
47. Head IM, Jones DM, Roling WFM. 2006. Marine microorganisms make a meal of oil. *Nat. Rev. Microbiol.* 4:173–82
48. Hinrichs K-U, Hayes JM, Sylva SP, Brewer PG, DeLong EF. 1999. Methane-consuming archaebacteria in marine sediments. *Nature* 398:802–5
49. Hoehler TM, Alperin MJ, Albert DB, Martens CS. 1994. Field and laboratory studies of methane oxidation in an anoxic marine sediment: evidence for a methanogen-sulfate reducer consortium. *Glob. Biogeochem. Cycles* 8:451–63
50. Holler T, Wegener G, Knittel K, Boetius A, Brunner B, et al. 2009. Substantial $^{13}C/^{12}C$ and D/H fractionation during anaerobic oxidation of methane by marine consortia enriched in vitro. *Environ. Microbiol. Rep.* 1:370–76
51. Holler T, Widdel F, Knittel K, Amann R, Kellermann MY, et al. 2011. Thermophilic anaerobic oxidation of methane by marine microbial consortia. *ISME J.* 5:1946–56
52. House CH, Orphan VJ, Turk KA, Thomas B, Pernthaler A, et al. 2009. Extensive carbon isotopic heterogeneity among methane seep microbiota. *Environ. Microbiol.* 11:2207–15
53. Hua Z-S, Wang Y-L, Evans PN, Qu Y-N, Goh KM, et al. 2019. Insights into the ecological roles and evolution of methyl-coenzyme M reductase-containing hot spring Archaea. *Nat. Commun.* 10:4574
54. IEA (Int. Energy Agency). 2021. *World Energy Balances: Overview*. Paris: IEA
55. Iversen N, Jørgensen BB. 1985. Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark). *Limnol. Oceanogr.* 30:944–55
56. Ji J-H, Zhou L, Mbadinga SM, Irfan M, Liu Y-F, et al. 2020. Methanogenic biodegradation of C_9 to C_{12} *n*-alkanes initiated by *Smithella* via fumarate addition mechanism. *AMB Express* 10:23
57. Joye SB, MacDonald IR, Leifer I, Asper V. 2011. Magnitude and oxidation potential of hydrocarbon gases released from the BP oil well blowout. *Nat. Geosci.* 4:160–64
58. Khelifi N, Amin Ali O, Roche P, Grossi V, Brochier-Armanet C, et al. 2014. Anaerobic oxidation of long-chain *n*-alkanes by the hyperthermophilic sulfate-reducing archaeon, *Archaeoglobus fulgidus*. *ISME J.* 8:2153–66
59. Kleindienst S, Ramette A, Amann R, Knittel K. 2012. Distribution and in situ abundance of sulfate-reducing bacteria in diverse marine hydrocarbon seep sediments. *Environ. Microbiol.* 14:2689–710
60. Kniemeyer O, Musat F, Sievert SM, Knittel K, Wilkes H, et al. 2007. Anaerobic oxidation of short-chain hydrocarbons by marine sulphate-reducing bacteria. *Nature* 449:898–901
61. Knittel K, Boetius A. 2009. Anaerobic oxidation of methane: progress with an unknown process. *Annu. Rev. Microbiol.* 63:311–34
62. Knittel K, Boetius A, Lemke A, Eilers H, Lochte K, et al. 2003. Activity, distribution, and diversity of sulfate reducers and other bacteria in sediments above gas hydrate (Cascadia margin, Oregon). *Geomicrobiol. J.* 20:269–94
63. Knittel K, Loesekann T, Boetius A, Kort R, Amann R. 2005. Diversity and distribution of methanotrophic archaea at cold seeps. *Appl. Environ. Microbiol.* 71:467–79
64. Kropp KG, Davidova IA, Suflita JM. 2000. Anaerobic oxidation of *n*-dodecane by an addition reaction in a sulfate-reducing bacterial enrichment culture. *Appl. Environ. Microbiol.* 66:5393–98
65. Krüger M, Meyerdieks A, Glöckner FO, Amann R, Widdel F, et al. 2003. A conspicuous nickel protein in microbial mats that oxidize methane anaerobically. *Nature* 426:878–81

66. Krukenberg V, Harding K, Richter M, Glöckner FO, Berg J, et al. 2016. *Candidatus* Desulfofervidus auxilii, a hydrogenotrophic sulfate-reducing bacterium of the HotSeep-1 cluster involved in the thermophilic anaerobic oxidation of methane. *Environ. Microbiol.* 18:3073–91
67. Krukenberg V, Riedel D, Gruber-Vodicka HR, Buttigieg PL, Tegetmeyer HE, et al. 2018. Gene expression and ultrastructure of meso-and thermophilic methanotrophic consortia. *Environ. Microbiol.* 20:1651–66
68. Kvenvolden KA, Cooper CK. 2003. Natural seepage of crude oil into the marine environment. *Geo-Mar. Lett.* 23:140–46
69. Laso-Pérez R, Hahn C, van Vliet DM, Tegetmeyer HE, Schubotz F, et al. 2019. Anaerobic degradation of non-methane alkanes by "*Candidatus* Methanoliparia" in hydrocarbon seeps of the Gulf of Mexico. *mBio* 10:e01814-19
70. Laso-Pérez R, Krukenberg V, Musat F, Wegener G. 2018. Establishing anaerobic hydrocarbon-degrading enrichment cultures of microorganisms under strictly anoxic conditions. *Nat. Protoc.* 13:1310
71. Laso-Pérez R, Wegener G, Knittel K, Widdel F, Harding KJ, et al. 2016. Thermophilic archaea activate butane via alkyl-coenzyme M formation. *Nature* 539:396–401
72. Leu AO, Cai C, McIlroy SJ, Southam G, Orphan VJ, et al. 2020. Anaerobic methane oxidation coupled to manganese reduction by members of the *Methanoperedenaceae*. *ISME J.* 14:1030–41
73. Loesekann T, Knittel K, Nadalig T, Fuchs B, Niemann H, et al. 2007. Diversity and abundance of aerobic and anaerobic methane oxidizers at the Haakon Mosby Mud Volcano, Barents Sea. *Appl. Environ. Microbiol.* 73:3348–62
74. MacDonald I, Guinasso N, Ackleson S, Amos J, Duckworth R, et al. 1993. Natural oil slicks in the Gulf of Mexico visible from space. *J. Geophys. Res. Oceans* 98:16351–64
75. Mackelprang R, Mason OU. 2016. Marine microbiology: community clean up. *Nat. Microbiol.* 1:16102
76. Martens CS, Berner RA. 1974. Methane production in the interstitial waters of sulfate-depleted marine sediments. *Science* 185:1167–69
77. Mayr S, Latkoczy C, Krüger M, Günther D, Shima S, et al. 2008. Structure of an F_{430} variant from archaea associated with anaerobic oxidation of methane. *J. Am. Chem. Soc.* 130:10758–67
78. McGlynn SE. 2017. Energy metabolism during anaerobic methane oxidation in ANME archaea. *Microbes Environ.* 32:5–13
79. McGlynn SE, Chadwick GL, Kempes CP, Orphan VJ. 2015. Single cell activity reveals direct electron transfer in methanotrophic consortia. *Nature* 526:531–35
80. McGlynn SE, Chadwick GL, O'Neill A, Mackey M, Thor A, et al. 2018. Subgroup characteristics of marine methane-oxidizing ANME-2 archaea and their syntrophic partners as revealed by integrated multimodal analytical microscopy. *Appl. Environ. Microbiol.* 84:e00399-18
81. McKay LJ, Dlakić M, Fields MW, Delmont TO, Eren AM, et al. 2019. Co-occurring genomic capacity for anaerobic methane and dissimilatory sulfur metabolisms discovered in the Korarchaeota. *Nat. Microbiol.* 4:614–22
82. Metcalfe KS, Murali R, Mullin SW, Connon SA, Orphan VJ. 2021. Experimentally-validated correlation analysis reveals new anaerobic methane oxidation partnerships with consortium-level heterogeneity in diazotrophy. *ISME J.* 15:377–96
83. Meyerdierks A, Kube M, Kostadinov I, Teeling H, Glockner FO, et al. 2010. Metagenome and mRNA expression analyses of anaerobic methanotrophic archaea of the ANME-1 group. *Environ. Microbiol.* 12:422–39
84. Michaelis W, Seifert R, Nauhaus K, Treude T, Thiel V, et al. 2002. Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. *Science* 297:1013–15
85. Milucka J, Widdel F, Shima S. 2013. Immunological detection of enzymes for sulfate reduction in anaerobic methane-oxidizing consortia. *Environ. Microbiol.* 15:1561–71
86. Mwirichia R, Alam I, Rashid M, Vinu M, Ba-Alawi W, et al. 2016. Metabolic traits of an uncultured archaeal lineage—MSBL1—from brine pools of the Red Sea. *Sci. Rep.* 6:19181
87. Nauhaus K, Albrecht M, Elvert M, Boetius A, Widdel F. 2007. In vitro cell growth of marine archaeal-bacterial consortia during anaerobic oxidation of methane with sulfate. *Environ. Microbiol.* 9:187–96

71. First description of an archaeon that uses MCR for the activation of multicarbon compounds.

79. First demonstration of direct extracellular electron transfer in AOM by ANME-2 archaea (see also Reference 144).

88. Nauhaus K, Boetius A, Krüger M, Widdel F. 2002. In vitro demonstration of anaerobic oxidation of methane coupled to sulphate reduction in sediments from a marine gas hydrate area. *Environ. Microbiol.* 4:296–305
89. Nauhaus K, Treude T, Boetius A, Krüger M. 2005. Environmental regulation of the anaerobic oxidation of methane: a comparison of ANME-I and ANME-II communities. *Environ. Microbiol.* 7:98–106
90. Niemann H, Losekann T, de Beer D, Elvert M, Nadalig T, et al. 2006. Novel microbial communities of the Haakon Mosby mud volcano and their role as a methane sink. *Nature* 443:854–58
91. Novelli GD, Zobell CE. 1944. Assimilation of petroleum hydrocarbons by sulfate-reducing bacteria. *J. Bacteriol.* 47:447–48
92. Orphan VJ, Hinrichs K-U, Ussler W 3rd, Paull CK, Taylor LT, et al. 2001. Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. *Appl. Environ. Microbiol.* 67:1922–34
93. Orphan VJ, House CH, Hinrichs K-U, McKeegan KD, DeLong EF. 2001. Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science* 293:484–87
94. Orphan VJ, House CH, Hinrichs K-U, McKeegan KD, DeLong EF. 2002. Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. *PNAS* 99:7663–68
95. Pancost RD, Sinninghe Damsté JS, de Lint S, van der Maarel MJEC, Gottschal JC. 2000. Biomarker evidence for widespread anaerobic methane oxidation in Mediterranean sediments by a consortium of methanogenic archaea and bacteria. *Appl. Environ. Microbiol.* 66:1126–32
96. Parks DH, Chuvochina M, Chaumeil P-A, Rinke C, Mussig AJ, Hugenholtz P. 2020. A complete domain-to-species taxonomy for Bacteria and Archaea. *Nat. Biotechnol.* 38:1079–86
97. Rabus R, Boll M, Golding B, Wilkes H. 2016. Anaerobic degradation of *p*-alkylated benzoates and toluenes. *J. Mol. Microbiol. Biotechnol.* 26:63–75
98. Rabus R, Wilkes H, Behrends A, Armstroff A, Fischer T, et al. 2001. Anaerobic initial reaction of *n*-alkanes in a denitrifying bacterium: evidence for (1-methylpentyl) succinate as initial product and for involvement of an organic radical in *n*-hexane metabolism. *J. Bacteriol.* 183:1707–15
99. Redmond MC, Valentine DL. 2012. Natural gas and temperature structured a microbial community response to the *Deepwater Horizon* oil spill. *PNAS* 109:20292–97
100. Reeburgh WS. 1976. Methane consumption in Cariaco trench waters and sediments. *Earth Planet. Sci. Lett.* 28:337–44
101. Reitner J, Peckmann J, Blumenberg M, Michaelis W, Reimer A, Thiel V. 2005. Concretionary methane-seep carbonates and associated microbial communities in Black Sea sediments. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 227:18–30
102. Rona PA, Bougault H, Charlou JL, Appriou P, Nelsen TA, et al. 1992. Hydrothermal circulation, serpentinization, and degassing at a Rift-Valley fracture-zone intersection: Mid-Atlantic Ridge near 15°N, 45°W. *Geology* 20:783–86
103. Rosenfeld WD. 1947. Anaerobic oxidation of hydrocarbons by sulfate-reducing bacteria. *J. Bacteriol.* 54:664–65
104. Rueter P, Rabus R, Wilkest H, Aeckersberg F, Rainey FA, et al. 1994. Anaerobic oxidation of hydrocarbons in crude oil by new types of sulphate-reducing bacteria. *Nature* 372:455–58
105. Scheller S, Goenrich M, Boecher R, Thauer RK, Jaun B. 2010. The key nickel enzyme of methanogenesis catalyses the anaerobic oxidation of methane. *Nature* 465:606–8
106. Scheller S, Goenrich M, Thauer RK, Jaun B. 2013. Methyl-coenzyme M reductase from methanogenic archaea: isotope effects on label exchange and ethane formation with the homologous substrate ethyl-coenzyme M. *J. Am. Chem. Soc.* 135:14985–95
107. Scheller S, Yu H, Chadwick GL, McGlynn SE, Orphan VJ. 2016. Artificial electron acceptors decouple archaeal methane oxidation from sulfate reduction. *Science* 351:703–7
108. Schink B. 1997. Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol. Mol. Biol. Rev.* 61:262–80
109. Seitz KW, Dombrowski N, Eme L, Spang A, Lombard J, et al. 2019. Asgard archaea capable of anaerobic hydrocarbon cycling. *Nat. Commun.* 10:1822
110. Shao Z, Wang W. 2013. Enzymes and genes involved in aerobic alkane degradation. *Front. Microbiol.* 4:116

107. First demonstration of physiological decoupling of syntrophic AOM by ANME from sulfate reduction using AQDS and iron oxides.

111. Sherwood Lollar B, Westgate TD, Ward JA, Slater GF, Lacrampe-Couloume G. 2002. Abiogenic formation of alkanes in the Earth's crust as a minor source for global hydrocarbon reservoirs. *Nature* 416:522–24
112. Shima S, Krueger M, Weinert T, Demmer U, Kahnt J, et al. 2012. Structure of a methyl-coenzyme M reductase from Black Sea mats that oxidize methane anaerobically. *Nature* 481:98–101
113. Shima S, Thauer RK. 2005. Methyl-coenzyme M reductase and the anaerobic oxidation of methane in methanotrophic Archaea. *Curr. Opin. Microbiol.* 8:643–48
114. Simon J, van Spanning RJ, Richardson DJ. 2008. The organisation of proton motive and non-proton motive redox loops in prokaryotic respiratory systems. *Biochim. Biophys. Acta Bioenerg.* 1777:1480–90
115. Skennerton CT, Chourey K, Iyer R, Hettich RL, Tyson GW, Orphan VJ. 2017. Methane-fueled syntrophy through extracellular electron transfer: uncovering the genomic traits conserved within diverse bacterial partners of anaerobic methanotrophic archaea. *mBio* 8:e00530-17
116. Skovhus TL, Lee JS, Little BJ. 2017. Predominant MIC mechanisms in the oil and gas industry. In *Microbiologically Influenced Corrosion in the Upstream Oil and Gas Industry*, ed. TL Skovhus, D Enning, JS Lee, pp. 75–85. Boca Raton, FL: CRC Press
117. So CM, Phelps CD, Young L. 2003. Anaerobic transformation of alkanes to fatty acids by a sulfate-reducing bacterium, strain Hxd3. *Appl. Environ. Microbiol.* 69:3892–900
118. So CM, Young LY. 1999. Initial reactions in anaerobic alkane degradation by a sulfate reducer, strain AK-01. *Appl. Environ. Microbiol.* 65:5532–40
119. Söhngen NL. 1906. Über Bakterien, welche Methan als Kohlenstoffnahrung und Energieqeuelle gebrauchen. *Zbl. Bakteriol. II Nat.* 15:513–17
120. Sørensen K, Finster K, Ramsing N. 2001. Thermodynamic and kinetic requirements in anaerobic methane oxidizing consortia exclude hydrogen, acetate, and methanol as possible electron shuttles. *Microb. Ecol.* 42:1–10
121. Spang A, Stairs CW, Dombrowski N, Eme L, Lombard J, et al. 2019. Proposal of the reverse flow model for the origin of the eukaryotic cell based on comparative analyses of Asgard archaeal metabolism. *Nat. Microbiol.* 4:1138–48
122. Spormann AM, Widdel F. 2000. Metabolism of alkylbenzenes, alkanes, and other hydrocarbons in anaerobic bacteria. *Biodegradation* 11:85–105
123. Stokke R, Roalkvam I, Lanzen A, Haflidason H, Steen IH. 2012. Integrated metagenomic and metaproteomic analyses of an ANME-1-dominated community in marine cold seep sediments. *Environ. Microbiol.* 14:1333–46
124. Tamisier M, Schmidt M, Vogt C, Kümmel S, Stryhanyuk H, et al. 2022. Iron corrosion by methanogenic archaea characterized by stable isotope effects and crust mineralogy. *Environ. Microbiol.* 24(2):583–95
125. Tan B, Fowler SJ, Laban NA, Dong X, Sensen CW, et al. 2015. Comparative analysis of metagenomes from three methanogenic hydrocarbon-degrading enrichment cultures with 41 environmental samples. *ISME J.* 9(9):2028–45
126. Tapilatu YH, Grossi V, Acquaviva M, Militon C, Bertrand J-C, Cuny P. 2010. Isolation of hydrocarbon-degrading extremely halophilic archaea from an uncontaminated hypersaline pond (Camargue, France). *Extremophiles* 14:225–31
127. Teske A, Hinrichs K-U, Edgcomb V, de Vera Gomez A, Kysela D, et al. 2002. Microbial diversity of hydrothermal sediments in the Guaymas Basin: evidence for anaerobic methanotrophic communities. *Appl. Environ. Microbiol.* 68:1994–2007
128. Thauer RK. 2019. Methyl (alkyl)-coenzyme M reductases: nickel F-430-containing enzymes involved in anaerobic methane formation and in anaerobic oxidation of methane or of short chain alkanes. *Biochemistry* 58:5198–220
129. Thauer RK, Kaster A-K, Seedorf H, Buckel W, Hedderich R. 2008. Methanogenic archaea: ecologically relevant differences in energy conservation. *Nat. Rev. Microbiol.* 6:579–91
130. Timmers PHA, Welte CU, Koehorst JJ, Plugge CM, Jetten MSM, Stams AJM. 2017. Reverse methanogenesis and respiration in methanotrophic archaea. *Archaea* 2017:1654237
131. Treude T, Orphan V, Knittel K, Gieseke A, House CH, Boetius A. 2007. Consumption of methane and CO_2 by methanotrophic microbial mats from gas seeps of the anoxic Black Sea. *Appl. Environ. Microbiol.* 73:2271–83

132. Vaksmaa A, Guerrero-Cruz S, van Alen TA, Cremers G, Ettwig KF, et al. 2017. Enrichment of anaerobic nitrate-dependent methanotrophic '*Candidatus* Methanoperedens nitroreducens' archaea from an Italian paddy field soil. *Appl. Microbiol. Biotechnol.* 101:7075–84
133. Vaksmaa A, Lüke C, Van Alen T, Valè G, Lupotto E, et al. 2016. Distribution and activity of the anaerobic methanotrophic community in a nitrogen-fertilized Italian paddy soil. *FEMS Microbiol. Ecol.* 92(12):fiw181
134. Valenzuela EI, Prieto-Davó A, López-Lozano NE, Hernández-Eligio A, Vega-Alvarado L, et al. 2017. Anaerobic methane oxidation driven by microbial reduction of natural organic matter in a tropical wetland. *Appl. Environ. Microbiol.* 83:e00645-17
135. Vigneron A, Head IM, Tsesmetzis N. 2018. Damage to offshore production facilities by corrosive microbial biofilms. *Appl. Microbiol. Biotechnol.* 102:2525–33
136. Wagner T, Wegner C-E, Kahnt J, Ermler U, Shima S. 2017. Phylogenetic and structural comparisons of the three types of methyl coenzyme M reductase from *Methanococcales* and *Methanobacteriales*. *J. Bacteriol.* 199(16):e00197-17
137. Wang F, Gu Y, O'Brien JP, Sophia MY, Yalcin SE, et al. 2019. Structure of microbial nanowires reveals stacked hemes that transport electrons over micrometers. *Cell* 177(2):361–69.e310
138. Wang F-P, Zhang Y, Chen Y, He Y, Qi J, et al. 2014. Methanotrophic archaea possessing diverging methane-oxidizing and electron-transporting pathways. *ISME J.* 8:1069–78
139. Wang L, Wang W, Lai Q, Shao Z. 2010. Gene diversity of CYP153A and AlkB alkane hydroxylases in oil-degrading bacteria isolated from the Atlantic Ocean. *Environ. Microbiol.* 12:1230–42
140. Wang Y, Feng X, Natarajan VP, Xiao X, Wang F. 2019. Diverse anaerobic methane- and multicarbon alkane-metabolizing archaea coexist and show activity in Guaymas Basin hydrothermal sediment. *Environ. Microbiol.* 21:1344–55
141. Wang Y, Wegener G, Hou J, Wang F, Xiao X. 2019. Expanding anaerobic alkane metabolism in the domain of Archaea. *Nat. Microbiol.* 4:595–602
142. Wang Y, Wegener G, Williams TA, Xie R, Hou J, et al. 2021. A methylotrophic origin of methanogenesis and early divergence of anaerobic multicarbon alkane metabolism. *Sci. Adv.* 7:eabj1453
143. Wegener G, Krukenberg V, Ruff SE, Kellermann MY, Knittel K. 2016. Metabolic capabilities of microorganisms involved in and associated with the anaerobic oxidation of methane. *Front. Microbiol.* 7:46
144. Wegener KV, Riedel D, Tegetmeyer HE, Boetius A. 2015. Intercellular wiring enables electron transfer between methanotrophic archaea and bacteria. *Nature* 526:587–90
145. Widdel F, Bak F. 1992. Gram-negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes*, ed. A Balows, HG Trüper, M Dworkin, W Harder, KH Schleifer, pp. 3352–78. Berlin: Springer
146. Wilson R, Monaghan P, Osanik A, Price L, Rogers M. 1974. Natural marine oil seepage. *Science* 184:857–65
147. Wongnate T, Sliwa D, Ginovska B, Smith D, Wolf MW, et al. 2016. The radical mechanism of biological methane synthesis by methyl-coenzyme M reductase. *Science* 352:953–58
148. Yan Z, Ferry JG. 2018. Electron bifurcation and confurcation in methanogenesis and reverse methanogenesis. *Front. Microbiol.* 9:1322
149. Yu H, Skennerton CT, Chadwick GL, Leu AO, Aoki M, et al. 2022. Sulfate differentially stimulates but is not respired by diverse anaerobic methanotrophic archaea. *ISME J.* 16:168–77
150. Yu H, Speth D, Connon S, Goudeau D, Malmstrom R, et al. 2022. Community structure and microbial associations in sediment-free methanotrophic enrichment cultures from a marine methane seep. *Appl. Environ. Microbiol.* 88: e0210921
151. Yu T, Wu W, Liang W, Lever MA, Hinrichs K-U, Wang F. 2018. Growth of sedimentary *Bathyarchaeota* on lignin as an energy source. *PNAS* 115:6022–27
152. Zaremba-Niedzwiedzka K, Caceres EF, Saw JH, Bäckström D, Juzokaite L, et al. 2017. Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature* 541:353–58
153. Zedelius J, Rabus R, Grundmann O, Werner I, Brodkorb D, 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.* 3:125–35
154. Zehnder AJ, Brock TD. 1979. Methane formation and methane oxidation by methanogenic bacteria. *J. Bacteriol.* 137:420–32

144. First demonstration of direct interspecies electron transfer in AOM by thermophilic ANME-1 (see also Reference 79).

155. Zengler K, Richnow HH, Rossello-Mora R, Michaelis W, Widdel F. 1999. Methane formation from long-chain alkanes by anaerobic microorganisms. *Nature* 401:266–69
156. Zhang Y, Maignien L, Zhao X, Wang F, Boon N. 2011. Enrichment of a microbial community performing anaerobic oxidation of methane in a continuous high-pressure bioreactor. *BMC Microbiol.* 11:137
157. Zhao R, Biddle JF. 2021. Helarchaeota and co-occurring sulfate-reducing bacteria in subseafloor sediments from the Costa Rica Margin. *ISME Commun.* 1:25
158. Zhou Z, Zhang C-j, Liu P-f, Fu L, Laso-Pérez R, et al. 2022. Non-syntrophic methanogenic hydrocarbon degradation by an archaeal species. *Nature* 601:257–62
159. Zhu B, Bradford L, Huang S, Szalay A, Leix C, et al. 2017. Unexpected diversity and high abundance of putative nitric oxide dismutase (Nod) genes in contaminated aquifers and wastewater treatment systems. *Appl. Environ. Microbiol.* 83:e02750-16
160. Zobell CE. 1946. Action of microorganisms on hydrocarbons. *Bacteriol. Rev.* 10:1–49

158. Pioneering cultivation of an archaeon that couples alkane oxidation to methanogenesis without requiring syntrophic partners.

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Errata

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