Anaerobic Oxidation of Methane: Progress with an Unknown Process

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Key Words

Archaea, sulfate-reducing bacteria, ANME, microbial consortia, *mcr*A gene

Abstract

Methane is the most abundant hydrocarbon in the atmosphere, and it is an important greenhouse gas, which has so far contributed an estimated 20% of postindustrial global warming. A great deal of biogeochemical research has focused on the causes and effects of the variation in global fluxes of methane throughout earth's history, but the underlying microbial processes and their key agents remain poorly understood. This is a disturbing knowledge gap because 85% of the annual global methane production and about 60% of its consumption are based on microbial processes. Only three key functional groups of microorganisms of limited diversity regulate the fluxes of methane on earth, namely the aerobic methanotrophic bacteria, the methanogenic archaea, and their close relatives, the anaerobic methanotrophic archaea (ANME). The ANME represent special lines of descent within the Euryarchaeota and appear to gain energy exclusively from the anaerobic oxidation of methane (AOM), with sulfate as the final electron acceptor according to the net reaction:

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O.$$

This review summarizes what is known and unknown about AOM on earth and its key catalysts, the ANME clades and their bacterial partners.

Contents

INTRODUCTION

The Significance of AOM in Global Methane Budgets

AOM: anaerobic oxidation of methane

Most methane on earth is produced by methanogenesis, the final step in the fermentation of organic matter, which takes place in rice fields, the guts of animals, soils, wetlands, and landfills, as well as in freshwater and marine sediments. As a simple assumption, about 10–20% of reactive organic material buried in soils and sediments is converted to methane. For the ocean, which covers 70% of the earth's surface, an annual rate of methanogenesis of 85–300 Tg CH_4 $year^{-1}$ has been estimated, of which >90% is consumed by anaerobic oxidation of methane (AOM) (33, 86). This accounts for 7–25% of the total global methane production. AOM efficiently controls the atmospheric methane efflux from the ocean (<2% of the global flux; 86), because almost all the methane produced in ocean sediments is consumed by AOM within the sulfate-penetrated seafloor zones.

Today, certainly the most important control of global atmospheric methane fluxes is anthropogenic land use. However, rapid imbalances could come from the giant, temperature-sensitive clathrate reservoir (hydrate capacitor) of an estimated 10^7 Tg C of methane stored in marine sediments, with a potential to escape microbial consumption when rapidly released by warming (20). An important question with significance to the global carbon cycle and the role of the ocean in climate change is how much of the methane efflux from hydrate reservoirs could be consumed by AOM in marine sediments and which factors control the anaerobic methanotrophs.

History of AOM Research

The first evidence of the removal of methane within anoxic sediments and seawaters came from geochemical observations showing that methane diffuses upward from deeper sediment horizons and disappears in the same zone as sulfate, before any contact with oxygen (4, 57, 85). Zehnder & Brock (119) investigated AOM in vitro by incubating a variety of methanogenic enrichments and found evidence of degradation of methane at a small percentage of methane production. They proposed a cooperation between a methanogen responsible for the activation of methane and a syntrophic partner acting as electron sink. Radioactive tracer incubations

with ^{14}C-labeled methane and ^{35}S-labeled sulfate showed that methane oxidation coincided with increased sulfate reduction (SR) (40). Field observations and experiments led to the hypothesis of a coupled mechanism in which both methanogenic archaea and sulfate-reducing bacteria (SRB) could profit from AOM, despite the generally low thermodynamic energy yield from this reaction (35). But microbiologists still believed that because of its low energy yield, this reaction could not support life: The free energy change of AOM under standard conditions at room temperature is only $\Delta G^{\circ\prime} = -16$ kJ mol^{-1}, which would have to be shared by the two partners involved in AOM. Intrigued by the growing geochemical evidence of AOM as a main process controlling methane emission from the seabed (86), many microbiologists attempted to isolate a reversible methanogen using methane as the sole energy and carbon source for growth (112)—unfortunately in vain.

The first evidence of the existence of anaerobic methanotrophs came from Hinrichs et al. (34), who extracted archaea-specific lipid biomarkers from a hydrate-bearing site on the continental slope off California (Eel River Basin)—a so-called cold seep. The archaeal lipids retrieved from Eel River Basin and from other sites such as eastern Mediterranean mud volcanoes (80) and gas-hydrate-bearing sediments from Hydrate Ridge (23) were known from cultivated methanogens of the order *Methanosarcinales*, but here they were conspicuously depleted in the carbon isotope ^{13}C ($<-100‰$ δ^{13}C versus PeeDee Belemnite), indicating that methane was the carbon source for the organisms that synthesized the lipids. Sequences of the rRNA gene library from Eel River Basin constituted a novel clade related to methanogens that was proposed by the authors to represent the anaerobic methanotrophs (34).

The next step in the discovery of the anaerobic methanotrophic archaea (ANME) was the visual identification of the methanotrophs via microscopy of cells hybridized with fluorochrome-labeled specific oligonucleotide probes (10). Surprisingly, in sediments from Eel River Basin, Hydrate Ridge, and the Black Sea, conspicuous aggregates of archaea and SRB (AOM consortia) were highly abundant, representing >90% of the total microbial community (10, 61, 78). This finding supported the hypothesis (35) that methane could be used as a substrate source via the cooperation of archaea able to activate methane and SRB able to provide an electron sink. The first in vitro experiment utilizing such sediments naturally enriched in ANME then successfully demonstrated AOM coupled to SR (65). The direct proof of anaerobic methanotrophy was provided by ion microprobe mass spectrometry confirming the extreme ^{13}C depletion of the aggregate biomass as predicted from the biomarker extractions (79). Today, all known ANME are related clades of the methanogenic Euryarchaeota (47); however, not a single member of these groups has been obtained in culture yet.

SR: sulfate reduction

SRB: sulfate-reducing bacteria

Mud volcanoes: large geostructures created by gas eruption and subsequent seabed deformation

ANME: anaerobic methanotroph

Consortia: physical associations of different taxa of microorganisms cooperating for a mutual benefit

SMTZ: sulfate-methane transition zone

BIOGEOCHEMISTRY OF AOM

Geochemical Signatures

The main niche for anaerobic methanotrophs on earth is the so-called sulfate-methane transition zone (SMTZ) in the seabed (86 and references therein). SMTZs are found in all anoxic aquatic systems, where the transport of methane from below and sulfate from above provides a niche defined by a minimum yield of energy to the anaerobic methanotrophs. Methane is completely consumed in the SMTZ, which may be found at decimeters to tens of meters below the seafloor, depending on the burial rate of reactive organic matter, the depth of the methane production zone, and the transport velocity of methane and sulfate and their consumption rates. Because AOM leads to a significant increase of inorganic carbon and alkalinity, calcium ions are precipitated according to the net equation

$$CH_4 + SO_4^{--} + Ca^{++} \rightarrow CaCO_3 + H_2S + H_2O. \quad 1.$$

Consequently, some AOM habitats are characterized by a massive deposition of carbonate plates and chimney-like structures (2).

In sediments dominated by diffusive transport, key signatures of AOM can be detected in the typical shape of the concentrations of the educts methane and sulfate, and of the products sulfide and carbonate. The concave distribution of methane and sulfate forming an intersection is most characteristic for anoxic marine sediments dominated by AOM (see figure 1 in Reference 112). In sediments characterized by high advective transport, and especially in those with methane concentrations above its solubility at atmospheric pressure, the methane gradients obtained from ex situ porewater sampling are severely altered because of gas ebullition. Upon retrieval from depth to the ship laboratory, the methane overpressure may destroy any geochemical gradient. For such sediments, the most reliable geochemical signature of AOM processes has been obtained by in situ microprofiling (**Figure 1**).

At seeps and vents that host various escape routes for gas bubbles and methane-rich fluids, as well as in gas-laden tidal flats (90) and sand beds, a substantial proportion of methane can bypass the microbial methanotrophic filter. For example, at the Haakon Mosby mud volcano more than 60% of the total annual methane flux escapes benthic consumption (70). The methane leak from gas-seeping shelf sands was even >80% of the total methane flux (116). Unfortunately, the number of and total seafloor area of seeps, vents, and gassy shelf sediments, as well as the methane efflux from such sites, remain poorly constrained, leading to

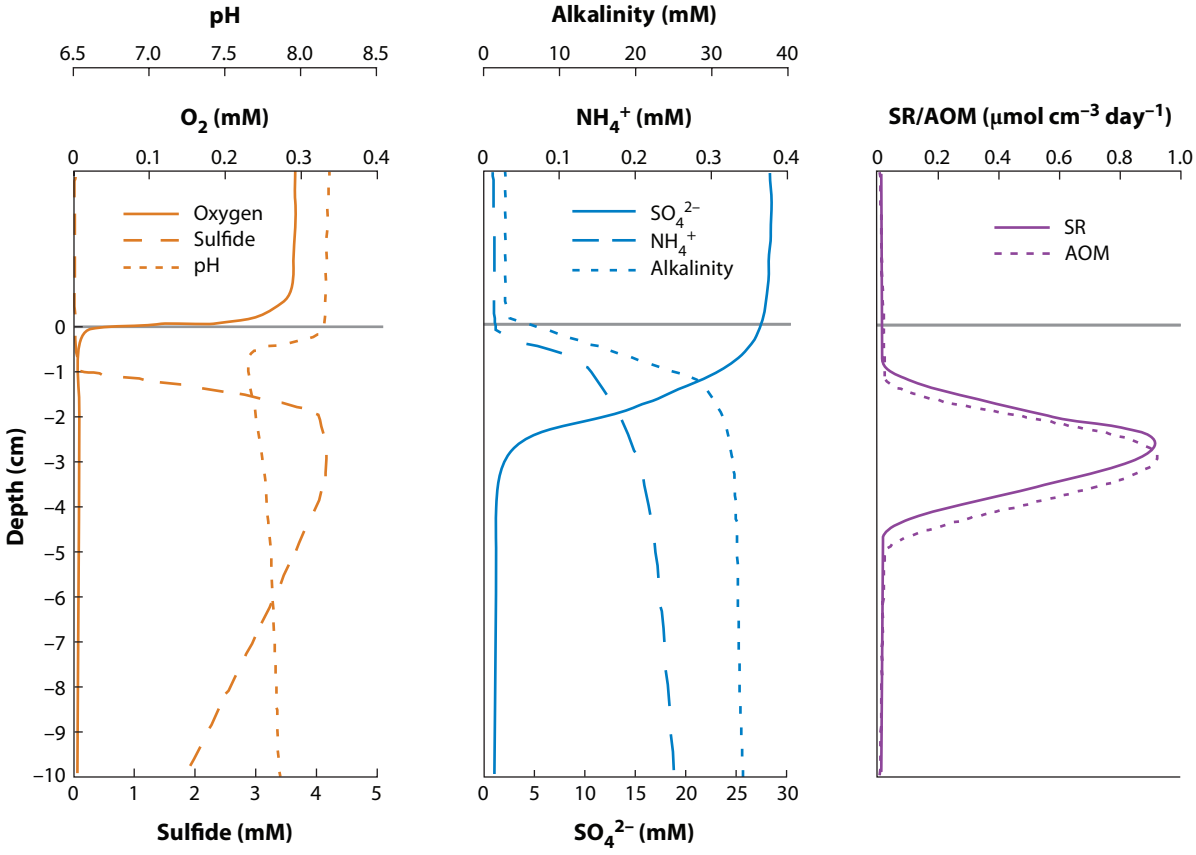

Figure 1

Scheme of biogeochemical gradients in the AOM zone above hydrates (modified after Reference 9). AOM, anaerobic oxidation of methane; SR, sulfate reduction.

considerable uncertainty of the ocean's role in the global methane budget (86).

The AOM process also leaves typical patterns in the stable isotope signatures of carbon, in both the methane and the dissolved inorganic carbonate pools. Methane in the SMTZ becomes enriched in ^{13}C (isotopically heavier) owing to preferential oxidation of the lighter ^{12}C isotope in the source methane. As a result, dissolved inorganic carbonate becomes isotopically lighter in the SMTZ (see figure 1 in Reference 112). For seawater and surface sediments this is reviewed in Reference 86, and for subsurface sediments see Reference 96. Fractionation factors (αCH_4) for the carbon in methane by AOM obtained from field studies on marine and brackish sediments range from 1.009 to 1.024 (3, 86, 117). Recently, an in vitro study using highly enriched ANME consortia found substantial fractionation during anaerobic oxidation of methane with 1.012 to 1.039 for $^{13}CH_4/^{12}CH_4$ and 1.109 to 1.315 for CDH_3/CH_4 (T. Holler, G. Wegener, K. Knittel, A. Boetius, B. Brunner, M.M. Kuypers & F. Widdel, unpublished data). The sulfur fractionation factors for methane-driven SR are not known.

Rates

AOM is limited to anoxic habitats and covers a wide range of rates from a few pmol cm^{-3} day^{-1} in subsurface SMTZ of deep margins, to a few μmol cm^{-3} day^{-1} in surface sediments above gas hydrates. Generally, AOM occurs where methane and sulfate overlap, which may be millimeters to >200 meters below the seafloor. In most settings, methane supply is the limiting factor determining the depth of the SMTZ (96); exceptions are known from cold seeps governed by high upward flow of sulfate-free geofluids (18). To quantify AOM or methane-fueled SR, intact seafloor sediments are incubated with radioactive tracers, i.e., $^{14}CH_4$ and $^{35}SO_4^{2-}$ (107). Characteristically, the highest AOM and SR rates occur directly in the SMTZ (40, 86; see the geochemical model in Reference 15). Methane-laden sediments, such as those located above dissociating gas hydrate or gas chimneys, show the peak in AOM directly below the surface, where the sulfate supply from downward diffusing seawater is highest (18). Typical AOM rates measured in samples from a variety of aquatic ecosystems at in situ temperatures (but at atmospheric pressure) are summarized in **Table 1**. One may speculate that AOM rates are generally higher in situ because of the higher methane pressures at depth. However, first measurements of AOM rates with a novel in situ injection technique show that the effect of sediment retrieval and subsequent release of pressure on AOM rates depends on which factor is limiting, and may result in higher (methane limitation) or lower (sulfate limitation) in situ rates, respectively, with an on average difference of ±50% (A. Boetius, unpublished data).

Gas hydrate: an ice-like solid formed by water crystals encaging large volumes of natural gas

Cold seeps: seabed structures where reduced fluids enriched in methane migrate to the seafloor

Table 1 AOM rates in different aquatic habitats

Habitat	Range of AOM rates (nmol cm^{-3} day^{-1})	References
Black Sea microbial reefs	1,000–10,000	(61, 111)
Seeps with surface hydrates	100–5000	(42, 107)
Mud volcanoes, gas chimneys	10–1500	(70, 73)
Coastal SMTZ	1–50	(45, 81, 109, 116)
Margin SMTZ	0.1–10	(67, 69, 110)
Subsurface SMTZ	0.001–1	(96 and references therein)
Marine anoxic water columns	0.0001–0.01	(86, 94)
Lake water	0.0001–1	C. Schubert, unpublished data
Lake sediments	0.0001–1	C. Schubert, unpublished data

AOM, anaerobic oxidation of methane; SMTZ, sulfate-methane transition zone.

Chemosynthetic bivalve and tubeworm: symbiotic invertebrates hosting autotrophic sulfide-oxidizing bacteria in special organs, which provide a carbon and energy source to the animal host

***mcr*A:** gene for alpha subunit of methyl-coenzyme M reductase

Environmental Control

The main factor controlling AOM rates and the growth of AOM consortia is the availability of methane and sulfate (25, 64). So far, no other electron donors or acceptors provide energy to the ANME and their partners. The range of methane and sulfate concentrations in AOM zones covers at least seven orders of magnitude, from 10 nM to 100 mM. When methane builds up in the seabed and porewater gets oversaturated with methane, free gas bubbles form and may precipitate as gas hydrate at high pressures and cold temperatures. As the gas is in equilibrium with the surrounding porewater, the in situ methane concentrations reach tens to 100 mM in the vicinity of hydrate. However, the chemical energy stored in methane is only accessible to the anaerobic methanotrophs in the presence of sulfate. Hence, the highest AOM rates are expected around dissociating gas hydrates that lie within sulfate-penetrated surface sediments (10, 107), or in the top surface sediments of active methane-emitting mud volcanoes characterized by high advective fluxes of methane in the millimolar to molar range m^{-2} day^{-1} (17, 18). In most methane-rich environments sulfate is depleted rapidly because the flux of methane from subsurface sediments often exceeds the flux of sulfate from the bottom waters. Accordingly, the highest densities of ANME populations can be expected where diffusion limitation is overcome; where sulfate gets advected into the seafloor, e.g., in zones of gas ebullition; and below mats of motile sulfide-oxidizing bacteria (70, 91) or burrowing animals such as the chemosynthetic bivalve *Calyptogena* and siboglinid tubeworms, which may actively release sulfate through their roots (13, 70).

Both AOM partner organisms—the ANME and the various SRB associated with them—do not tolerate oxygen. ANME have never been detected in oxygen-penetrated sediments. The exact cause has not been investigated, but it is well known that methanogens are highly intolerant of oxygen because of the redox sensitivity of their enzyme cofactors (41). This is most likely also true for their ANME relatives. Most of the seafloor at continental margins consists of fine-grained clay sediments where oxygen penetration is restricted to a few centimeters to a decimeter. However, at fluid-flow-impacted cold seeps, oxygen penetration is limited to a few millimeters or less because of the upward advection of sulfate-free porewaters (18).

Apparent optima for AOM at different pressure, salinity, temperature, and pH show an adaptation to a relatively wide range of variation in these environmental factors (51, 66). Generally, increased pressure may enhance AOM simply because of the higher availability of methane. In vitro experiments with a variety of naturally enriched seep sediments show that the apparent temperature optimum for AOM is usually found at 5–10°C above the in situ temperature (9). High-resolution in situ pH measurements in marine sediments with high AOM activities indicate only small variations in pH value, between 7.7 and 7.9 in the AOM zone, and no substantial deviation of pH from the habitat appears to be caused by AOM (18). In vitro experiments on the pH optimum of AOM with different ANME populations also showed a maximum within the range of pH 7–8, which is the typical pH range for marine sediments (66). However, ANME populations have also been found in extreme environments: for example, at temperatures of up to 95°C in the hydrothermal sediments of the Guaymas Basin (92, 101); in the CO_2-vented sediments of the Yonaguni Knoll with an in situ pH of probably as low as 4 (36); in alkaline fluids of carbonate chimneys at Lost City hydrothermal field with pH values of 9–11 and temperatures up to 70°C (11); and at elevated salt concentrations (55, 98).

CONSORTIA MEDIATING THE ANAEROBIC OXIDATION OF METHANE

Diversity of ANME Populations

In the last decade, the diversity of ANME populations has been studied intensively. Most investigations were based on the 16S rRNA or *mcr*A gene phylogeny; others used ANME-specific

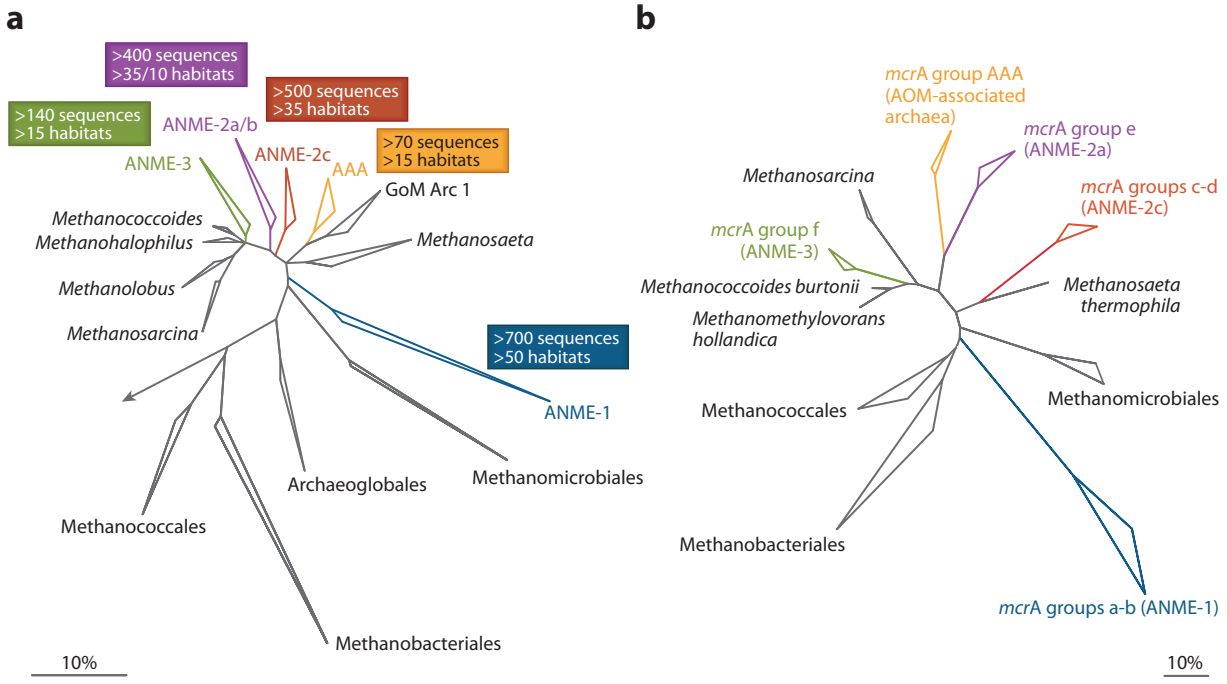

Figure 2

Phylogenetic trees showing the affiliations of (*a*) ANME 16S rRNA gene sequences to selected reference sequences of the domain Archaea. Data in colored boxes give information about the distribution and abundance of sequence retrieval (published and unpublished). Bar, 10% estimated sequence divergence. (*b*) ANME gene sequences coding for the alpha subunit of methyl-coenzyme M-reductase (*mcr*A) to selected sequences of the domain Archaea. *mcr*A tree by courtesy of A. Meyerdierks. Bar, 10% estimated amino acid changes.

lipid biomarkers and their stable carbon isotope signatures for identification.

16S rRNA genes. AOM in the marine environment is mediated by three distinct clusters of Euryarchaeota, namely ANME-1, ANME-2, and ANME-3. The clusters are distantly or closely related to the orders *Methanosarcinales* and *Methanomicrobiales*, which comprise a major part of the cultivated methanogens (**Figure 2*a***). According to their 16S rRNA gene phylogeny ANME groups are not monophyletic, and the phylogenetic distance between the three groups is large, with a sequence similarity of 75–92%. Even intergroup similarity of ANME-2 subgroups -2a, -2b, and -2c is comparably low. Thus, members of ANME-1, ANME-2, and ANME-3 certainly belong to different orders or families that have apparently similar physiological properties, namely the capability to mediate AOM in a wide range of environmental settings.

A novel clade closely related to ANME-2 has been described as the fourth ANME-2 subgroup, ANME-2d (58, 62). The same group was found by Lloyd et al. (55) and called GoM Arc I because this clade is not monophyletic with the other ANME-2 subgroups and it has not yet been proven to mediate AOM or form consortia with SRB. Hence its role in methane biogeochemistry remains unclear.

Furthermore, 16S rRNA genes of another new clade of archaea closely related to ANME-2 archaea have been retrieved from a bioreactor that couples AOM to denitrification (84). The archaea in the original inoculate from a Dutch canal were later lost from the bioreactor biomass (24). This new group also comprises sequences from other limnic sediments, the meromictic freshwater Lake Cadagno

AAA: AOM-associated archaea

IPL: intact polar lipid

Supplemental Material

(Switzerland) (C.J. Schubert, F. Vazquez, T. Lösekann, K. Knittel & A. Boetius, unpublished data) and Lake Michigan (100), but also sequences from marine habitats such as an extinct Antarctic methane seep (H. Niemann, D. Fischer, D. Graffe, K. Knittel, A. Montiel, et al., unpublished data), deep-sea sulfide chimneys (93), and subsurface sediments (37), as well as diverse soils and aquifers (**Supplemental Table 1;** follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). We name this clade AOM-associated archaea (AAA) (**Figure 2*a***) as long as their physiology remains unknown. Their sequences are most similar to those of the ANME-2 branch, but they are not monophyletic with any of the ANME-2 subclusters. On the basis of *mcr*A phylogeny, AAA from the Nijmegen denitrifying bioreactor (24) are most closely related to environmental sequences from the Pearl River estuary (ACJ11604, L.J. Jiang, database entry) and to enrichments from Tibetan wetlands, which are versatile methanogens (see Reference 24 and references therein). *Methanosarcinales* and the *mcr*A e cluster (ANME-2a) are only distantly related to clade AAA *mcr*A (**Figure 2*b***). Experiments with the bioreactor biomass in its early phase have shown that these archaea are characterized by a highly depleted carbon isotopic signature of their lipids of −40‰ compared with the methane source, and that they assimilate ^{13}C-labeled methane (24). The depletion of the lipids and the incorporation of ^{13}C are consistent with a methanotrophic lifestyle. However, the final proof for AOM by members of this novel clade is still lacking and other biogeochemical functions cannot be excluded.

Specific lipid biomarkers. Specific lipid biomarkers and their stable carbon isotope signatures are widely used for the identification of ANME populations in natural environments. Here, taxonomic information can be linked with function (^{13}C signatures indicate methane assimilation) and the obtained lipid profiles serve as community fingerprints and relative indicators of ANME biomasses. These lipid biomarkers also serve to detect AOM hotspots in the fossil record (105).

Recent reviews summarize work on archaeal biomarkers and their typical carbon isotope signature (68, 86). Briefly, all specific archaeal lipid biomarkers show a substantial depletion of −40 to −70‰ compared to the stable carbon isotope ratio of the source methane.

Different ANME groups produce specific ratios of strongly ^{13}C-depleted membrane lipids, including isoprenoidal dialkyl glycerol diethers (archaeol, hydroxyarcheol), as well as tetramethylhexadecane (crocetane) and pentamethylicosenes. When ANME enrichments are amended with ^{13}C-methane, these lipids—foremost archaeol and hydroxyarchaeol—are labeled with ^{13}C, indicating growth of ANME organisms (8, 84, 115). Glycerol dialkyl glycerol tetraethers (GDGTs) were also used as an indicator of ANME distribution (92), but the ANME GDGTs appear to show some overlap with those of benthic Crenarchaeota, which often share the same niche in the seabed (47). The sulfate-reducing partner in AOM consortia also produce characteristic ^{13}C-depleted fatty acids such as $C_{16:1\omega5}$, cy-$C_{17:0\omega5,6}$, and $C_{17:1\omega6}$ (68). However, their lipids are usually less depleted than the archaeal lipids, and their natural isotope signature as well as that from ^{13}C-tracer assimilation is best explained by autotrophic growth on methane-derived dissolved inorganic carbon (115).

Recently, the biomarker approach was extended to intact polar lipids (IPLs), which are of higher taxonomic specificity (orders or higher) and property to select for living cells (5, 88). Characteristic IPL molecular fingerprints have been reported for each specific ANME type: The IPLs of ANME-1 archaea are strongly dominated by diglycosidic GDGT (2-Gly-GDGT) derivates, while no polar derivative of hydroxyarchaeol was detected. Thus, with respect to IPL composition ANME-1 are distinct from all other major families of methanogens that produce significant amounts of archaeol (88). IPLs of ANME-2 and ANME-3 are dominated by phosphate-based polar derivatives of archaeol and hydroxyarchaeol, which is

consistent with their phylogenetic affiliations with the order *Methanosarcinales* (88).

Structure and Morphology

ANME-1 cells have a typical rectangular morphology and are autofluorescent under UV light, a feature typical of methanogenic archaea containing coenzyme F_{420}. ANME-1 archaea most often occur as single cells (**Figure 3*a***) or in chains of two to four cells. However, they have also been observed in extremely long multicellular chains exceeding 100 μm in length (87). Transmission electron microscopy analyses revealed external sheaths of ANME-1 cells that seem to consist of a resistant biopolymer. Some ANME-1 cells contain structures that resemble thick stacks of intracytoplasmatic membranes,

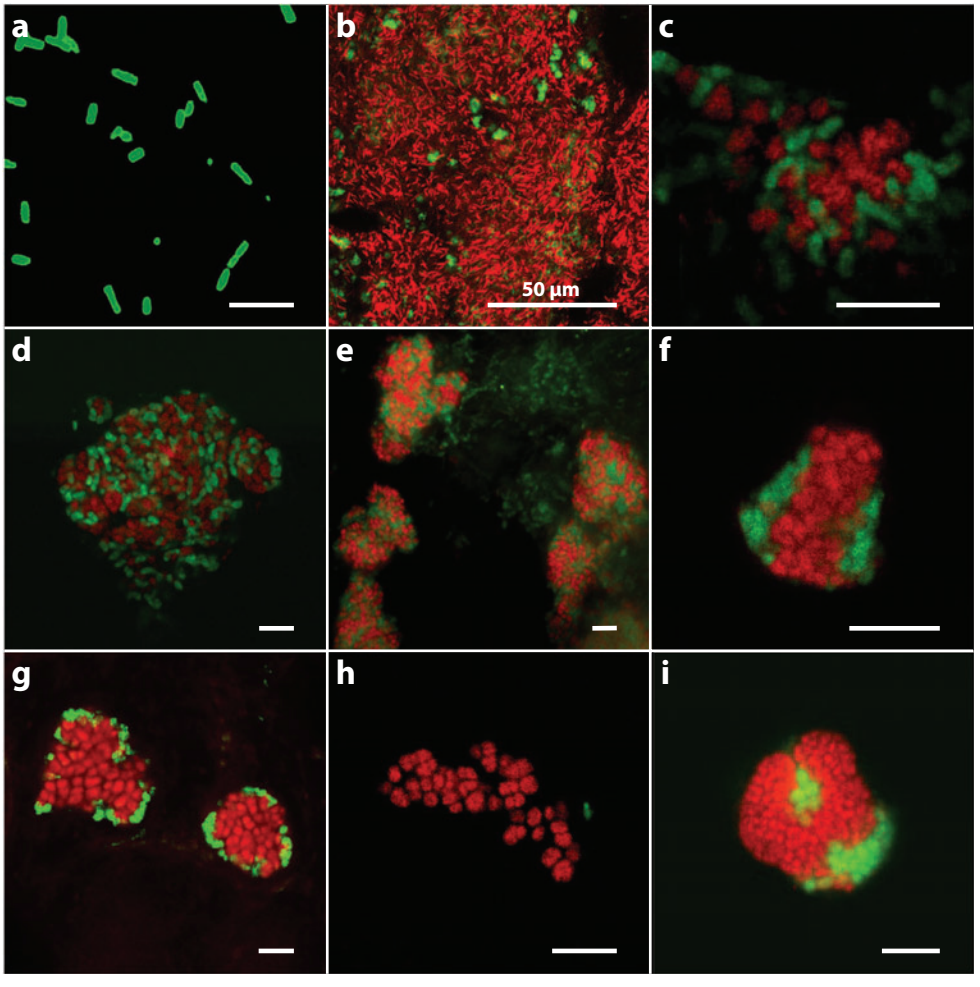

Figure 3

Epifluorescence micrographs of different ANME single cells and aggregates visualized by FISH or CARD-FISH. (*a*) Single ANME-1 cells living in a microbial mat from the Black Sea. (*b*) Mat-type consortia formed by ANME-1 (*red*) and DSS cells (*green*). (*c–e*) Mixed-type consortia of ANME-2a (*red*) and DSS (*green*) cells observed in different seep sediments. (*f, g*) Shell-type consortia of ANME-2c (*red*) and DSS (*green*) cells and (*h*) single ANME-2c cells observed in different seep sediments. (*i*) ANME-3/*Desulfobulbus* consortia. Unless otherwise indicated, scale bar is 5 μm. Abbreviations: ANME, anaerobic methanotrophic archaea; CARD, catalyzed reporter deposition; DSS, *Desulfosarcina*; FISH, fluorescence in situ hybridization.

similar to those found in type I and type X aerobic methanotrophic Gammaproteobacteria. The membranes might have a similar function in the ANME-1 cells, because common genes coding for C_1-transfer enzymes exist among methylotrophic bacteria and methanogenic archaea (12). In the Black Sea, mat-type associations of ANME-1 archaea and SRB (**Figure 3*b***) have been reported (47, 61, 87, 111). The 3D structure of mat-type consortia is visualized in **Supplemental Movie 1**.

Supplemental Material

Also in the Black Sea mats and associated with other cold seep ecosystems, diverse forms of associations between ANME-2 and their partner bacteria have been microscopically identified by fluorescence in situ hybridization (FISH) (**Figure 3**). Most often, coccoid ANME-2 archaea form consortia with SRB. In addition to the differences in phylogenetic origin, the two subpopulations ANME-2a and ANME-2c could be distinguished from each other by their aggregate morphology. In general, ANME-2a/*Desulfosarcina* (DSS) aggregates represent the mixed-type. Here, archaea and SRB are completely mixed and the aggregates are not always spherical (**Figure 3*c–e***) (**Supplemental Movie 2**). Typical ANME-2c/DSS aggregates represent the well-known shell-type with an inner core of ANME-2, which is partially or fully surrounded by an outer shell of SRB (**Figure 3*f*,*g***) (47) (**Supplemental Movie 3**). ANME-2 populations grow by an increase in the number and size of the consortia (64). Starting from a few archaea and SRB cells, consortia seem to develop from small to big consortia of up to 100,000 cells (64). Larger consortia separate evenly into two aggregates or unevenly into more aggregates when SRB grow into the archaeal core. In situ the average diameter of consortia is 3–5 μm, with the largest detected consortium of >20 μm. Even much larger aggregates of >50 μm in diameter have been observed in enrichment cultures (64; T. Holler, unpublished data). After reaching a specific size, consortia appear to burst, releasing single cells into the environment. In addition to ANME-2 associated with SRB, aggregated ANME-2 without any partner were also reported as single ANME-2 cells (**Figure 3*b***) (79, 111).

ANME-3 archaea form shell-type aggregates with *Desulfobulbus*-related bacteria as a sulfate-reducing partner (**Figure 3*i***) (56, 70); however, only a few bacteria are associated. At some sites, ANME-3 even occur solely as single cells (73).

Bacterial Partners of ANME

ANME-1 and ANME-2 archaea are usually associated with SRB of the *Desulfosarcina/Desulfococcus* (DSS; SEEP-SRB I) branch of the Deltaproteobacteria (10, 46, 47, 61, 77, 79) (**Supplemental Figure 1**). These SRB are physically attached to ANME-2 archaea, forming cell aggregates covered by a thick organic matrix. The morphology of the DSS cells varies from cocci (mostly associated with ANME-2c cells) to rod-shaped to vibrioform (mostly associated with ANME-2a cells) (J. Arnds, unpublished data), suggesting that they might belong to different species. Intracellular storage inclusions of polyhydroxyalkanoates and iron sulfides (greigite) precipitates have been identified in DSS cells growing in close association with ANME-2 in Black Sea microbial mats (87). Thus, iron cycling has been suggested to be involved in the metabolisms of the microbial population.

ANME-3 archaea are, if at all, associated with SRB of the *Desulfobulbus* branch (see **Supplemental Figure 1**) but have also been detected together with DSS in shallow subsurface gas-hydrate-bearing sediments (T. Lösekann, unpublished data). In Eel River Basin sediments, nearly equal numbers of ANME-2c/*Desulfobulbus* consortia and ANME-2c/DSS have been identified (82), indicating a versatility in bacterial partnership and AOM syntrophy.

There is rising evidence that the diversity of bacteria associated with ANME is not restricted to SRB. The analysis of ANME-2c consortia captured by whole-cell magneto-FISH showed a diversity of bacterial partners of ANME-2 far beyond the Deltaproteobacteria (82). Alphaproteobacteria related to *Sphingomonas* spp.

and Betaproteobacteria related to *Burkholderia* spp. (**Supplemental Figure 1**) have been identified microscopically as the dominant or sole bacterial partner associated with ANME-2c, although these associations were rare compared with those with Deltaproteobacteria. Denitrification is suggested as one possible metabolic strategy conferred by the Betaproteobacteria.

DISTRIBUTION AND HABITATS

Since their discovery in the late 1990s, the distribution of ANME organisms has been studied intensively, mainly based on 16S rRNA gene phylogeny. More than 1800 published and unpublished 16S rRNA gene sequences are now available from more than 50 different marine methane seeps, vents, and SMTZs differing, for example, in temperature, methane flux, salinity, and pH. Furthermore, presence of ANME organisms has also been reported for anoxic marine water columns and for diverse continental habitats: limnic water columns and sediments, soils, and aquifers (see **Supplemental Table 1** for detailed information and references). A comparison of these studies indicates a global distribution of ANME-1 and ANME-2 archaea, whereas the presence of ANME-3 so far appears to be mainly restricted to submarine mud volcanoes and was only sporadically found in other types of seep sediments.

Most AOM zones host several clades of ANME; however, usually only one group makes up most of the ANME biomass per habitat (47), as quantified by FISH or catalyzed reporter deposition-FISH (CARD-FISH). Commonly used probes and hybridization conditions for the detection of ANME are shown in **Supplemental Table 2**.

Within the AOM zones, ANME populations of as few as $<10^6$ cells cm^{-3} (subsurface SMTZ) to as many as $>10^{10}$ cells cm^{-3} (surface of cold seeps) dominate biogeochemical fluxes such as sulfate consumption, sulfide and dissolved inorganic carbon production, and carbonate precipitation. Subsurface SMTZ habitats are an especially good example of harboring rare microorganisms mediating important biogeochemical functions in the environment (6, 54, 89).

Supplemental Material

Cold Seep Ecosystems

Cold seep ecosystems from Eel River Basin (34, 77), Hydrate Ridge (10, 46, 47), the Black Sea (7, 61, 87, 108, 111), Gulf of Mexico (55, 58, 62, 74), the Tommeliten and Gullfaks area in the North Sea (116), and mud volcanoes from the Mediterranean Sea (73) and the Barents Sea (56, 70) have been intensively studied, and dense ANME populations of $>10^{10}$ cells cm^{-3} have been reported for these systems. With the exception of the unique ecosystem in the Black Sea, most of the studies indicate a dominance of ANME-2 or ANME-3 in near-surface sediments (top 10 cm). A prominent example is the Hydrate Ridge, with hot spots of ANME-2 (up to 10^8 aggregates cm^{-3}) just above surficial gas hydrates. ANME-2 subgroups revealed different preferences for either *Beggiatoa* (ANME-2a) or *Calyptogena* (ANME-2c) fields (47), indicating that different environmental conditions select for different ANME groups.

Thus far, two seep systems are known where ANME-1 dominate: (*a*) sediments overlaying a methane-rich brine pool in the Gulf of Mexico (ANME-1b) (55) and (*b*) microbial mats from Black Sea cold seeps (ANME-1a, ANME-1b) (47). In the Black Sea, at depths of >180 m, giant reef-like structures composed of porous carbonates and microbial mats growing vertically or horizontally are found on the seafloor (49, 99, 108). These mats mediate AOM and consist mainly of densely aggregated ANME-1 cells and SRB (8, 47, 61, 87, 108, 111). Treude et al. (111) combined radiotracer incubations with SIMS and CARD-FISH to locate hot spots of methanotrophy, which were found close to the mat surface associated with dense associations of microcolonies of ANME-1 archaea and DSS. Generally, the Black Sea mats are heterogeneous and also provide niches for ANME-2. The black nodules from the top of the reef, especially, seem to be dominated by ANME-2, as shown by specific ^{13}C-depleted lipids and FISH (8, 49).

Deep biosphere: the part of the seabed below 1 m, including deeply buried sediments and oceanic crusts, not populated by animals but hosts diverse prokaryotic life

SMTZs

In diffusive seabed systems, the distribution of ANME is restricted to the SMTZ because it is the only place where both methane and sulfate are available. The ANME populations and their sulfate-reducing partner bacteria are the same as those at cold seeps; however, population densities of ANME are remarkably low, with $<10^6$ cells cm^{-3} (69), and associated AOM rates are below 10 nmol cm^{-3} day^{-1}. Most likely, the SMTZ-ANME archaea represent the seed populations for cold seep communities. It remains unknown whether these ANME possess special physiological adaptations to their energetically less favorable habitat compared with ANME at cold seeps. Both ANME-1 and ANME-2 archaea occur in SMTZ sediments: Those of Eckernförde Bay (German Baltic) and of a tidal sand flat of the Wadden Sea (Germany) (39) were dominated by ANME-2 archaea (109), while ANME-1 were dominant, for example, in the deep SMTZ of the Tommeliten seep area (69) and in Santa Barbara Basin (31).

Hydrothermal Vents

High methane fluxes are found at hydrothermal vents of mid-ocean ridges; nevertheless, these ecosystems do not offer many niches for ANME communities. The seafloor consists of basalts and lacks a sediment cover; hence the niches for ANME-related organisms are limited to small anoxic zones within vent chimneys and rocks. Furthermore, the fluids of most hydrothermal vents are sulfate free. The seawater, which could provide sulfate, is oxygen rich and toxic to ANME organisms. Sedimentary hydrothermal systems such as the Guaymas Basin may offer suitable habitats within the surface seafloor (92, 101), although the temperature optimum for the Guaymas ANME populations remains unknown (43). A special habitat for ANME with a temperature ranging from <40 to 90°C and a pH between 9 and 11 was found at the Lost City hydrothermal field (11, 44). ANMEs have also been detected in CO_2-vented sediments of the Yonaguni Knoll hydrothermal field, southern Okinawa Trough, with an in situ pH of probably as low as 4 (36).

Deep Biosphere

For the past five years, archaeal 16S rRNA gene libraries from deep subsurface sediments have failed to retrieve ANME- or methanogen-related sequences, possibly because of methodological problems such as primer specificity and detection limits (5, 37, 97, 102, 114). Even in an extensive study by Inagaki et al. (37), sequencing of several thousands of clones obtained from methane hydrate sites from Peru and Cascadia Margin did not result in the retrieval of ANME sequences. Instead, archaea of the marine benthic group B (or deep sea archaeal group) and the miscellaneous crenarchaeotal group were consistently the dominant phylotypes. The study by Biddle et al. (5) supported these findings and suggested, on the basis of stable isotopic compositions and intact archaeal lipids, that these archaea assimilate organic carbon other than methane. However, recently ANME clades have been detected for the first time in some methane-enriched sediments 23 (71) and >1000 m below the seafloor (89). Furthermore, Lever (54) retrieved ANME *mcr*A sequences and Biddle et al. (6) retrieved ANME 16S rRNA gene sequences from SMTZ in subsurface sediments off Peru.

Marine Water Column

The Black Sea is the world's largest surface water reservoir of dissolved methane (94). Methane concentrations are as high as 12 μM and AOM rates have been measured in the range of 1–2 nM day^{-1} (86). Single ANME-1 and ANME-2 archaea are suggested to be responsible for pelagic AOM in the Black Sea (94), each accounting for 3–4% of total cells (21). Similar to findings for sediments, one ANME group dominates, that is, below approximately 600 m water depth ANME-1 archaea and above 600 m ANME-2 archaea (94).

ANME-1 sequences have also been found in eastern Mediterranean hypersaline brine (14).

Terrestrial Habitats

AOM in nonmarine systems has been reported from terrestrial mud volcanoes located in the Carpathian Mountains (Romania) (1). Here, thermal alteration of sedimentary organic compounds leads to the release of methane and higher hydrocarbons into the environment. ANME-2a archaea are responsible for AOM activity in the mud volcano field. Additionally, AOM has been reported from landfills (27) and from the anoxic water body of the eutrophic freshwater Lake Plußsee (northern Germany) (22) in which low in situ numbers (<1%) of single ANME-1 and ANME-2 archaea were detected. ANME sequences have also been repeatedly reported from diverse soils, aquifers, and oilfield production waters (for detailed information see **Supplemental Table 1**). 16S rRNA gene sequences related to other ANME clades (**Figure 2**) were detected in the Twente Canal (the Netherlands), but their role in methane biogeochemistry remains unclear (24, 84).

PHYSIOLOGY OF AOM CONSORTIA

Stoichiometry

The consumption of methane and simultaneous formation of sulfide from sulfate at a molar ratio of approximately 1:1 were shown by geochemical modeling, radioisotope incubations, and enrichment experiments, and this ratio is in accordance with the stoichiometric equation of AOM. This ratio as well as the lack of sulfide production in control experiments without methane show that endogenous substrates other than methane are negligible as electron donors. Exceptions are AOM enrichments from oily sediments, which show a high background of sulfide production owing to the presence of diverse communities of SRB using other hydrocarbons as electron donors (73, 76 and references therein).

The stoichiometry of AOM also indicates that almost all methane is used for SR and little is used for cell carbon assimilation. This is in contrast to aerobic methanotrophs, in which up to 60% of the methane carbon is channeled into biosynthesis (52).

It has been speculated that not only methanogens but also methanotrophs reverse their energy-generating pathway depending on environmental controls (74, 75, 111). Methanogens oxidize up to 1% of their methane production, even up to 10% in sludge enrichments (30, 119); but no methanogen has ever been found that gains energy by AOM. Likewise, various experiments with field samples have indicated that AOM consortia can produce methane (<10% of methane oxidation), just as their methanogenic relatives can oxidize a small percentage of the methane produced (74, 111, 119).

Supplemental Material

AOM could theoretically also be coupled to electron acceptors such as NO_3^-, Fe(III), and Mn(IV). For a long time, no ANME enrichment that uses electron acceptors other than sulfate could be obtained. However, Raghoebarsing et al. (84) demonstrated AOM coupled to denitrification of nitrite in a bacterial enrichment culture according to

$$3\,CH_4 + 8\,NO_2^- + 8\,H^+ \rightarrow 3\,CO_2 + 4\,N_2 + 10\,H_2O \quad (\Delta G^{\circ\prime} = -928\ \text{kJ mol}^{-1}\ CH_4). \qquad 2.$$

The bioreactor biomass is dominated by bacteria of the NC10 clade, a deep-branching diverse phylogenetic group (24, 84).

Intermediates

The association of ANME with a sulfate-reducing partner is most commonly interpreted as an obligate syntrophic interaction in which the methanotrophic archaeon activates and metabolizes methane, leading to an intermediate that is scavenged as an electron donor by the

sulfate-reducing partner (35, 65, 112). So far, all information generated from biogeochemical data including natural and experimental isotope labeling, tracer measurements, enrichment experiments, and metagenomic and proteomic analyses does not falsify this hypothesis. However, the intermediate(s) channeled from methane oxidation into SR is still unknown.

In vitro feeding studies with the conventional methanogenic substrates, i.e., H_2, formate, acetate, or methanol, in the absence of methane suggested that none of these compounds is an intermediate during AOM (65, 118). Hence, the transfer of reducing equivalents from methane utilization into SR probably does not occur via an intermediate that is a typical methanogenic growth substrate. Recently, Moran et al. (63) suggested methanethiol as a potential intermediate. However, incubation experiments with highly enriched anaerobic methane-oxidizing cultures dominated by ANME-2 did not provide evidence of methylsulfides as an intermediate substrate for SR (T. Holler, C. Deusner & M. Basen, unpublished data). A syntrophic interaction could also occur by a transfer of reducing equivalents via electron shuttles (118). In this case—as for a hydrogen intermediate—the sulfate-reducing bacterium would assimilate methane-derived CO_2 in an autotrophic mode of growth. However, the addition of a variety of electron-capturing shuttles such as phenazines and humic acids did not lead to decoupling of the ANME from the SRB (66). It has also been proposed that permanent structures called nanowires can be established between bacterial cells (26), but this model is not favored by the finding that some ANME cells are not closely associated with bacterial partners (47, 79). Other hypotheses are that both methane oxidation as well as SR may take place in the archaeal cells, or that growth of the sulfate-reducing partner could be explained by scavenging and utilizing a certain amount of reduced, so far unknown metabolites, i.e., as a kind of metabolic parasitism or commensalism. However, stable carbon isotope labeling experiments have shown that growth of methanotrophic archaea and depends their partner bacteria is coupled to and depends on the presence of methane and that the SRB partner appears to grow autotrophically (115).

Kinetics and Energy Yield

Enrichments of AOM consortia show specific SR rates of 1–20 mmol day^{-1} g^{-1} cell dry mass of SRB (up to a few fmol $cell^{-1}$ day^{-1}), which is in the lower range of specific rates observed with various pure cultures of SRB (83). Kinetic constrains of AOM, consortia morphology, and ANME growth have been recently discussed by Nauhaus et al. (64), Dale et al. (16), and Orcutt & Meile (75). The free energy change of AOM under standard conditions is $\Delta G^{\circ\prime} = -16.67$ kJ mol^{-1}. For AOM under various in situ conditions, a free energy change between -10 kJ mol^{-1} and -40 kJ mol^{-1} was estimated, depending on the concentrations of substrates and products in their depth profiles. The energy yield is increased by high methane fluxes. Nauhaus et al. (65) found a four- to fivefold stimulation of the SR rate by an increase of the methane pressure from 0.1 to 1.1 MPa, indicating half saturation constants of AOM for methane in the range of 10 mM. The apparent kinetics for sulfate remain unknown, but first experiments show a significant decrease in AOM when sulfate concentrations drop below 0.5 mM (H. Löbner, K.E. Luley, T. Treude, A. Boetius & C.R. Fisher, unpublished data).

Growth Parameters

Little is known about the biochemical coupling of growth to anaerobic microbial processes with low energy yields such as AOM. Results so far suggest that anaerobic methanotrophs grow slowly and have lower growth yields than, for instance, SRB growing on conventional substrates such as acetate or lactate (which assimilate ~10% of their carbon substrate).

In AOM, only 1% of the totally consumed methane is directly channeled into biosynthesis, whereas 99% is oxidized to CO_2. Nevertheless, more than 2% of methane-derived carbon can

appear in the biomass because a significant proportion of the assimilated CO_2 may be derived from methane through its complete oxidation (64, 115). Assimilation of CO_2 (one possible biosynthetic reaction is acetyl-CoA synthesis by carbon monoxide dehydrogenase) may thus make indirect use of methane carbon for biosynthesis. When methane is the sole organic compound for cell synthesis, formation of the less-reduced cell mass of the bulk formula $C_4H_8O_2N$ (113) requires a biosynthetic net oxidation, which can be achieved by incorporation of CO_2 as the oxidized form of carbon. Nauhaus et al. (64) formulated the assimilation of methane as

$$17\,CH_4 + 15\,CO_2 + 8\,NH_4^+ \rightarrow 8\,C_4H_8O_2N + 14\,H_2O + 8\,H^+. \quad 3.$$

In this study, ANME doubling time was approximately 7 months and the molar growth yield of AOM was 0.6 g cell dry weight (mol CH_4 oxidized)$^{-1}$. The slow growth is due to the bioenergetic limitations caused by the minimal energy yield of AOM (64). The slow growth rate has interesting results for the control of methane fluxes in dynamic submarine habitats such as at mud volcanoes or above dissociating hydrate reservoirs. For example, it was modeled that, even after a strong increase in methane availability, subsequent population growth would only occur with a lag phase of >60 years (17).

FUNCTIONAL GENES, GENOMICS, AND PROTEOMICS

Is AOM Based on Reverse Methanogenesis?

The close phylogenetic relationship between methanotrophic and methanogenic archaea and the biogeochemical link between both pathways in the carbon cycle suggest a coevolution of their biochemistry. First investigations of AOM microbiology were based on the hypothesis (35, 119) that the initial step in methane oxidation is essentially a reversal of the terminal reaction in methanogenesis, i.e., the reduction of methyl-coenzyme M (CoM-S-CH_3) with coenzyme B (H-S-CoB), yielding methane and the heterodisulfide (CoM-S-S-CoB) (103). In methanogens, this reaction is catalyzed by methyl-coenzyme M reductase (MCR), a 300-kDa enzyme composed of three subunits ($\alpha_2\beta_2\gamma_2$) and two tightly but noncovalently bound molecules of a nickel porphinoid, cofactor F_{430}, with a molecular mass of 905 Da.

The first evidence of the existence of MCR in sediments from AOM zones or enrichment cultures was given by the identification of novel *mcr*A genes that could be assigned to ANME-1 and ANME-2 archaea (28). A biochemical proof for the existence of MCR in ANME was provided by Krüger et al. (50), who extracted a prominent nickel compound from AOM-mediating microbial mats displaying the same absorption spectrum as the authentic cofactor F_{430} of the MCR, but with a higher molecular mass (951 Da). The nickel compound is part of an abundant protein (Ni-protein I) that is present in concentrations of up to 7% of the total extracted mat proteins and has not been found in any methanogenic archaea investigated so far (95). The structure of the modified F_{430} cofactor has recently been elucidated to be 172-methylthio-F_{430} (59).

In addition, a minor fraction of extracted proteins contained Ni-protein II with an unmodified cofactor (50). Ni-protein I could be assigned to ANME-1 archaea, while ANME-2 archaea are the source of Ni-protein II, which appears to contain the same variant of F_{430} that is in methanogens (104; A. Meyerdierks, S. Shima, J. Kahnt & M. Krüger, unpublished data). This suggests that Ni-protein I and Ni-protein II may catalyze the first step of AOM and that the modification of F_{430} is not a mandatory requirement for this catalytic ability (59). Recently, Heller et al. (32) visualized highly expressed MCR in ANME-1 and ANME-2 cells by immunogold-labeling of microbial mats with a specific antibody. For a detailed review of AOM biochemistry see Reference 104.

Surveys of metagenomic libraries revealed the presence of nearly all genes typically associated with methanogenesis in ANME-1, and to a lesser extent in ANME-2 cells (29). The F_{420}-dependent N^5, N^{10}-methenyltetrahydromethanopterin (methylene-H4MPT) reductase (*mer*) was detected in the whole-genome shotgun library from Eel River Basin sediments but could not be clearly assigned to ANME (29, 82). Furthermore, Hallam et al. (29) identified proteins, such as the F_{420}-dependent quinone oxidoreductase (*fqo*), numerous iron-sulfur cluster proteins, and electron input modules encoded by the F_{420}-reducing hydrogenase subunit B (*FrhB*), among the ANME-1 sequences and suggested that "unfavorable thermodynamics of methane activation in AOM might be overcome by metabolic coupling to the energy conservation reactions driven by the F_{420}-dependent respiratory chain" (29).

*mcr*A Phylogeny

*mcr*A has evolved as the key marker gene for studying the diversity of methanotrophic and methanogenenic archaea. Investigations have revealed a remarkably high phylogenetic diversity within *mcr*A among ANME archaea (1, 19, 28, 31, 36, 38, 44, 48, 55, 56, 72), which have been grouped into four subclusters (28, 56): group a-b (ANME-1), group c-d (ANME-2c), group e (ANME-2a), and group f (ANME-3) (**Figure 2*b***). Sequences from AAA archaea from freshwater canal sediment enrichments were distantly but most closely related (70–75% identity) to *mcr*A group e (24). These groups are distinct from those formed by methanogens. ANME *mcr*A gene phylogeny appears to be partially phylogenetically congruent to the 16S rRNA gene (**Figure 2*a***). Quantification of specific ANME groups based on their *mcr*A gene abundance is now an alternative method to 16S rRNA–based FISH to study distributional patterns of anaerobic methanotrophs in AOM zones (72).

Genes Involved in Dissimilatory Sulfate Reduction

ANME archaea occur as single cells or as monospecific aggregations (47, 56, 78, 79, 109), supporting the hypothesis that some ANME groups might mediate AOM alone, without any bacterial partner. ANME-1 archaea especially seem to be less dependent on the activity of a closely associated bacterial partner (47, 79). Two archaeal lineages are yet known to be capable of dissimilatory SR: euryarchaeotal *Archaeoglobus* spp. and crenarchaeotal *Caldivirga/Pyrobaculum* spp. (60 and references therein). However, analysis of fosmid libraries from Black Sea microbial mats (A. Meyerdierks, personal communication) or cold seeps from Eel River Basin (29, 82) did not indicate the presence of respective key genes, i.e., the dissimilatory adenosine-59-phosphosulfate (APS) reductase (*apr*), which converts APS to AMP and sulfite, or the sulfite reductase (*dsr*), which finally reduces sulfite to sulfide, in ANME genomes. Unfortunately, there is also no completed study addressing the genomic potential of DSS in the AOM consortia. Protein extractions from intact methanotrophic Black Sea mats also yielded substantial fractions of enzymes relevant to dissimilatory sulfur pathways. However, these enzymes are most likely of bacterial origin (M. Basen, T. Holler, M. Krüger, A. Meyerdierks, R. Rabus & S. Shima, unpublished data).

Only four field studies have been conducted that investigate the diversity of *apr* and/or *dsr* in SMTZs and seeps (31, 53, 55, 106). *dsrAB* clone sequences affiliated with the DSS group have been found in all studies, yet they cannot be assigned to AOM syntrophic DSS due to the abundance and ubiquitous presence of free-living DSS in marine sediments. Thomsen et al. (106) found high numbers of ANME-1 16S rRNA gene sequences along with a deep-branching cluster of *dsrAB* sequences specifically associated with the SMTZ in Aarhus Bay (Denmark) sediments. These genes might derive from novel SRB but might also be of archaeal origin.

SUMMARY POINTS

1. AOM efficiently controls the atmospheric methane efflux from the ocean and covers a wide range of rates, from a few pmol cm^{-3} day^{-1} in anoxic seawater, or subsurface sediments of deep margins, to a few μmol cm^{-3} day^{-1} at the seafloor above gas hydrates.
2. In diffusive seabed systems, the entire subsurface methane flux is consumed by methanotrophic archaea where methane and sulfate intersect (SMTZ).
3. At cold seeps and other gas-laden sediments, such as intertidal flats, the seabed may leak a substantial fraction of the methane to the hydrosphere owing to rapid advective transport of gas, or limitation in the availability of sulfate as an electron acceptor. These holes in the microbial methane filter and their contribution to global atmospheric methane fluxes remain poorly quantified.
4. Methanotrophic archaea of the ANME-1, ANME-2, and ANME3 clades are cosmopolitan and ubiquitous in all methane environments on earth. Closely related gene sequences are found in subsurface and surface sediments, continental and marine settings, or benthic and pelagic habitats.
5. Various subgroups of the ANME clades co-occur at most seep sites; however, microscopic analysis of their distribution has revealed the dominance of certain types within microniches in the environments, indicating an effect of environmental conditions on distribution and competition.
6. Diverse forms of associations between the different ANME subgroups and various partner bacteria have been microscopically identified by FISH, and ANME cells have also been detected without a bacterial partner attached. However, the most common form of occurrence in methane seeps, and in active, growing enrichment cultures, is the shell-type consortium with SRB.
7. Growth of the AOM consortia is slow, with generation times of months to years, owing to the low energy yield of the reaction, and only 1% of the totally consumed methane is directly channeled into biosynthesis.
8. The biochemical function of AOM remains unknown. So far, only one hypothesis explains the combined results of field observations and enrichment experiments, and metagenomic and proteomic studies: Methanotrophic archaea using the key enzymes of methanogenesis in reverse for methane oxidation provide electrons to an autotrophic sulfate-reducing partner in syntrophic cooperation.

FUTURE ISSUES

1. What is the areal extent of gas leaks from the seabed and how much subsurface methane can pass the microbial filter?
2. How will global climate change, with regard to the expected increase in temperature and sea level, affect the stability of gas hydrate reservoirs and the efficiency of microbial methane consumption?

3. What are the limits for the distribution of ANME with regard to temperature, pH, salinity, and energy availability?
4. How is growth of the ANME and the partner bacteria coupled to the energy yield of AOM? Which other factors control growth rates of the different ANME groups?
5. What are the key enzymes and cofactors in AOM and methane-driven SR, and how are they related to those in methanogenesis and organoclastic SR?
6. What is the role of the bacterial consortia partner(s)? Is it responsible for SR, and if so, what type of intermediate reducing equivalents is it receiving?
7. Can other electron acceptors fuel AOM, and what is the role of such alternative processes in the environment?
8. Which came first, AOM or methanogenesis?

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