



Biogeochemistry and microbial ecology of methane oxidation in anoxic environments: a review

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Abstract

Evidence supporting a key role for anaerobic methane oxidation in the global methane cycle is reviewed. Emphasis is on recent microbiological advances. The driving force for research on this process continues to be the fact that microbial communities intercept and consume methane from anoxic environments, methane that would otherwise enter the atmosphere. Anaerobic methane oxidation is biogeochemically important because methane is a potent greenhouse gas in the atmosphere and is abundant in anoxic environments. Geochemical evidence for this process has been observed in numerous marine sediments along the continental margins, in methane seeps and vents, around methane hydrate deposits, and in anoxic waters. The anaerobic oxidation of methane is performed by at least two phylogenetically distinct groups of archaea, the ANME-1 and ANME-2. These archaea are frequently observed as consortia with sulfate-reducing bacteria, and the metabolism of these consortia presumably involves a syntrophic association based on interspecies electron transfer. The archaeal member of a consortium apparently oxidizes methane and shuttles reduced compounds to the sulfate-reducing bacteria. Despite recent advances in understanding anaerobic methane oxidation, uncertainties still remain regarding the nature and necessity of the syntrophic association, the biochemical pathway of methane oxidation, and the interaction of the process with the local chemical and physical environment. This review will consider the microbial ecology and biogeochemistry of anaerobic methane oxidation with a special emphasis on the interactions between the responsible organisms and their environment.

Introduction

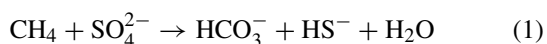
More than 25 years have elapsed since geochemical studies first revealed the anaerobic oxidation of methane (AOM) in anoxic marine sediments and waters (Barnes & Goldberg 1976; Reeburgh 1976; Martens & Berner 1977). Subsequent geochemical, microbiological and biogeochemical studies have contributed to understanding the importance of this process to the global methane (CH₄) cycle. Recent studies employing modern tools of molecular biology and biogeochemistry have provided further insight into the microbial ecology of this process (Hinrichs et al. 1999; Boetius et al. 2000; Orphan et al. 2001b). There now exists compelling evidence that AOM is

performed by a consortium of CH₄-oxidizing archaea and sulfate-reducing bacteria (SRB) in some environments. There is also now compelling evidence that AOM is mediated by more than one species of archaea with the possibility that some archaea oxidize CH₄ without the need for a syntrophic partner bacterium. This review integrates previous studies of AOM and considers the process in the context of the surrounding environment. Particular emphasis is placed on the environmental prevalence of AOM, the nature of the syntrophic association, the chemical and physical interactions between this process and the environment, and the relevance of this process to the subsurface biosphere.

Environmental prevalence and biogeochemistry

Anoxic sediments

Depth distributions of CH₄ concentration in anoxic marine sediments provided the first evidence for AOM (Barnes & Goldberg 1976; Reeburgh 1976; Martens & Berner 1977). Subsequent geochemical studies including radio-tracer studies (Panganiban et al. 1979; Reeburgh 1980; Zehnder & Brock 1980; Devol & Ahmed 1981; Iversen & Blackburn 1981; Devol 1983; Alperin & Reeburgh 1985; Iversen & Jørgensen 1985; Iversen et al. 1987; Ward & al. 1987; Alperin 1989; Ward et al. 1989; Reeburgh et al. 1991; Hoehler et al. 1994; Hansen et al. 1998; Joye et al. 1999; Boetius et al. 2000; Fossing et al. 2000; Jørgensen et al. 2001; Thomsen et al. 2001), stable isotope distributions (DesMarais 1983; Whiticar & Faber 1986; Alperin et al. 1988; Alperin 1989; Reeburgh et al. 1991; Blair & Aller 1995; Whiticar 1996; Martens et al. 1999; Whiticar 1999; Borowski et al. 2000; Oremland & Paull et al. 2000; Valentine & Reeburgh 2000), and the application of diagenetic (advection-reaction-diffusion) models (Barnes & Goldberg 1976; Reeburgh 1976; Martens & Berner 1977; Reeburgh & Heggie 1977; Berner 1980; Devol & Ahmed 1981; Alperin & Reeburgh 1984; Scranton 1988; Alperin 1989; Hoehler et al. 1994; Blair & Aller 1995; Borowski et al. 1996; Borowski et al. 1997; Niewohner et al. 1998; Borowski et al. 1999; Martens et al. 1999; Borowski et al. 2000; Fossing et al. 2000; Jørgensen et al. 2001; Thomsen et al. 2001) provided compelling evidence for this process in marine sediments. Several previous works have reviewed the geochemical evidence supporting AOM (Alperin & Reeburgh 1984; Hoehler et al. 1994; Hoehler & Alperin 1996; Valentine & Reeburgh 2000). The net chemical reaction associated with AOM is given in Equation (1).



Marine sediments cover approximately 70% of the Earth's solid surface, and sediments display significant physical and chemical diversity. One important factor pertaining to AOM is the organic content of the sediment. Sediments with high organic content tend to deplete their supply of oxidants closer to the sediment-water interface than sediments with low organic content. Oxidants (O₂, NO₃⁻, Fe(III), Mn(IV), and SO₄²⁻) enter the sediment at the sediment-water interface and are used by microbes for the oxidation of organic material in a thermodynamically-determined

order, with SO₄²⁻ being consumed last. When the supply of oxidants becomes depleted, CO₂ becomes the most powerful oxidant, and decomposition of organic material is coupled to CH₄ production. The depth (zone) in the sediment where sulfate reduction gives way to methanogenesis is referred to as the sulfate to methane transition. This depth also corresponds to the zone of AOM, where CH₄ from depth first encounters SO₄²⁻. Sediments with low organic content tend not to deplete their supply of oxidants, and such sediments do not give way to CH₄ production. Sediments along the world's continents tend to contain significantly more organic material than sediments in the deep ocean because of the high productivity in the overlying surface waters. Thus, methanogenesis (and AOM) is more prevalent in sediments along the continents than in the deep ocean.

Within CH₄-containing sediments there is a diversity of conditions that impact AOM including organic content, CH₄ supply rate, sulfate penetration, temperature, and pressure. In marine sediments that are characterized by diffusion as the dominant mixing process (the majority of sediments), organic content and CH₄ supply significantly impact AOM. For example, the sediments of Skan Bay, AK contain large quantities of organic material, and the CH₄-sulfate transition is located between 25 and 35 cm depth below the seafloor (Alperin 1989). Conversely, sediments of the Blake Ridge have a low organic content, and the CH₄-sulfate transition is located around 20 m below the seafloor in some areas (Borowski et al. 2000). Because of differences in organic content between sediments, and the corresponding CH₄ flux, the process of AOM can be spread out spatially with corresponding changes in the rate.

Several recent advances in understanding AOM have come from studies of CH₄ seeps and vents in geologically active areas (Rusanov et al. 1994; Pimenov et al. 1997; Elvert et al. 1999; Hinrichs et al. 1999; Peckmann et al. 1999; Suess et al. 1999; Thiel et al. 1999; Boetius et al. 2000; Elvert et al. 2000; Hinrichs et al. 2000; Pancost et al. 2000; Pancost et al. 2001; Thiel et al. 2001; Orphan et al. 2001a,b). In such sediments advection is the dominant mixing process, and porewaters are forced through the sediment along cracks and faults by pressure gradients. Porewaters travelling upward are often rich in CH₄ due to the decomposition of organic material below. The combination of high CH₄ concentration and advective flow provides abundant CH₄ for AOM, and leads to very high metabolic rates and dense microbial

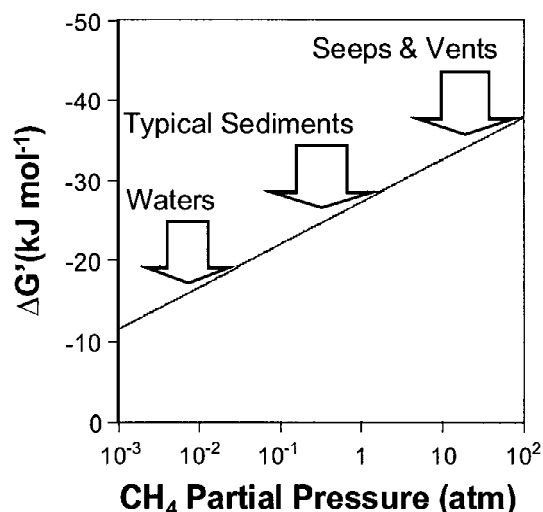


Figure 1. The influence of CH_4 partial pressure (atm) on the Gibbs Free Energy yield (kJ mol^{-1}) for AOM (Equation (1)). Calculations assume the following conditions: Temperature 4°C ; pH 7.2; HCO_3^- 20 mM; HS^- 2 mM; SO_4^{2-} 10 mM. Methane levels typical of anoxic waters, diffusion-dominated sediments, and seeps/vents are given for reference.

communities (Suess et al. 1999; Boetius et al. 2000; Tryon & Brown 2001). The rates of AOM in such high- CH_4 environments can be orders of magnitude higher than in typical diffusion-dominated sediments. However, vents and seeps are not widely distributed, and the rapid rates are not representative of AOM in most marine sediments.

Some anoxic sediments contain CH_4 hydrates, which are solid non-stoichiometric compounds formed from CH_4 and water under low temperature and high pressure. Hydrates occur naturally in some high- CH_4 environments including tectonically active continental margin sediments, as well as in sediments or permafrost areas overlying oil and gas deposits. The anaerobic oxidation of CH_4 occurs in the vicinity of hydrate deposits. Evidence indicates that CH_4 dissolved in pore fluids around the hydrates seems to drive AOM, though hydrates themselves do not harbor many microbes (Lanoil et al. 2001). Factors such as the presence of oxygen (in the case of surficial hydrates), the availability of sulfate, physical interactions between microbes and hydrates, the presence of non- CH_4 hydrocarbons, hydrate stability, and heterogeneity within the hydrate/sediment system further complicates our understanding of AOM in hydrate-bearing environments; these topics are ripe for future investigations.

Anoxic waters

Modeling and tracer experiments indicate that AOM occurs in a variety of anoxic, sulfate-containing waters including the Black Sea, Cariaco Basin, Mono Lake, CA, and Big Soda Lake, NV (Panganiban et al. 1979; Oremland & DesMarais 1983; Iversen et al. 1987; Oremland et al. 1987; Ward et al. 1987; Scranton 1988; Ward et al. 1989; Reeburgh et al. 1991; Joye et al. 1999). In these environments AOM is an important sink for CH_4 , though the rates of AOM vary depending on environmental conditions. Less is known about AOM in anoxic waters than is known about AOM in sediments. One major difference between AOM in the water column compared to sediments is that the CH_4 concentration is generally much lower in anoxic waters, and the sulfate concentration is generally higher. The Gibbs Free Energy available to perform AOM in anoxic waters is poor compared to typical sediments (Figure 1), and it is not clear that organisms in the anoxic waters can utilize CH_4 as their sole carbon and energy source. However, recent observations of isotopically-depleted lipid biomarkers in the anoxic waters of the Black Sea indicate that archaea in such environments may be capable of assimilating CH_4 (Schouten et al. 2001).

AOM in other environments

Available evidence indicates that AOM is coupled to sulfate reduction. Theoretically, other terminal electron acceptors (NO_3^- , Fe(III), Mn(IV)), could also act to oxidize CH_4 under anoxic conditions. Such reactions would provide a greater free energy yield than sulfate-dependent CH_4 oxidation. Furthermore, CH_4 does come into contact with oxidants including abundant Fe(III) in natural environments including peat bogs (Smemo & Yavitt 2000). Despite theoretical arguments for the existence of AOM coupled to alternative electron acceptors, no compelling evidence has been presented.

Relevance to the global CH_4 cycle

In most CH_4 -containing marine sediments, AOM occurs at the base of the sulfate reducing zone. Methane travelling upward meets the sulfate travelling downward and AOM ensues. Nearly all of the CH_4 is oxidized in this situation, inhibiting CH_4 transport upward from the subsurface. Assuming that AOM consumes all CH_4 produced in marine sediments, and that the system is near steady state (i.e., the CH_4 reser-

voir in marine sediments remains constant over time), the net production rate of CH₄ in marine sediments is approximately equal to the rate of AOM plus the rate of methane escape into the water column. The net rate of AOM in marine sediments has been estimated from 70 Tg of CH₄ per year (Reeburgh et al. 1993; Reeburgh 1996) to 300 Tg of CH₄ per year (Hinrichs & Boetius 2002). By including the 5–20 Tg CH₄ per year that escapes the sediments, the net rate of CH₄ production in marine sediments can be estimated between 75 and 320 Tg of CH₄ per year. The discrepancy in estimates presented by Reeburgh et al. (1991, 70 Tg CH₄ per year) and Hinrichs & Boetius (2002, 300 Tg CH₄ per year) arises because of the AOM rates and spatial extents used in their calculations. Both estimates rely on averaging previously published rate calculations to estimate global AOM rates. One potential bias in both calculations comes from the choice of representative environments used in the rate calculations. The primary reason many of these environments have been studied is precisely because they exhibit high rates of AOM, much higher than surrounding areas. Both estimates assume that these environments are representative of the entire depth interval worldwide, which is likely an overestimation of the average rate. One further complication is that estimates made by Reeburgh (1991) consider continental shelf sediments, but not deeper sediments along the margins. This likely leads to an underestimation in the spatial extent where AOM occurs. Hinrichs & Boetius (2002) consider a larger depth interval, though they assume high rates of AOM. Their estimate (300 Tg CH₄ per year) is likely an upper estimate of the global rate of AOM. Reeburgh et al. (1991) also use high rates for calculations, though they consider only shelf sediments. The extent to which these factors offset is not clear, and their estimate (70 Tg CH₄ per year) is still viable. These estimates for net CH₄ production/oxidation in marine sediments may prove useful in considering rates of organic matter remineralization and in calculating the magnitude of the marine CH₄ reservoir. Experimental and modeling studies are needed to provide further constraints on these values, and such studies should account for sampling biases.

Syntrophic associations

Phylogenetic, isotopic, and visual evidence clearly indicate that a consortium of archaea and bacteria oxidize CH₄ in some seep environments (Hoehler &

Alperin 1996; Hinrichs et al. 1999; Boetius et al. 2000; Valentine & Reeburgh 2000; Orphan et al. 2001b). The close physical association of ANME-2 archaea and bacteria indicates a syntrophic association (Valentine 2001). However, there is only indirect evidence pertaining to the nature of the syntrophic coupling, and limited evidence indicates that ANME-1 archaea may oxidize CH₄ without a tightly coupled syntrophic partner (Orphan 2002). Relevant evidence can be considered in five distinct categories: phylogenetic inferences, pure culture studies, mesocosm studies, observational studies, and theoretical studies. Each of these categories is considered below.

Phylogenetic inferences

Culture-independent identification of microbes provides a powerful tool to identify organisms in the natural environment, but is of only limited use in determining microbial activity. In the case of AOM, 16SrDNA sequencing has revealed the phylogenetic placement of both archaea and bacteria involved in the process (Hinrichs et al. 1999; Boetius et al. 2000; Hinrichs et al. 2000; Thomsen et al. 2001; Orphan et al. 2001a,b). The ANME-2 archaea are closely related to the *Methanosarcinales*, a group of largely methylotrophic methanogens, including all known acetoclastic methanogens. A few species within the *Methanosarcinales* are capable of performing H₂/CO₂ methanogenesis, though none are capable of methanogenesis using formate. The *Methanosarcinales* have the broadest substrate range among known methanogenic orders, and many species run an oxidative metabolism (from methyl to CO₂) during the dismutation of methylated compounds. Based on information about the *Methanosarcinales* it is possible to infer details about AOM. For example, the archaea involved in the CH₄-oxidizing consortium are most likely the CH₄ oxidizers, as they fall within a group that exclusively metabolizes CH₄. It is also likely that the organisms employ many of the oxidative steps used in methylotrophic methanogenesis, and that they produce reduced intermediates such as H₂, acetate, or other methylated compounds (Zehnder & Brock 1980; Hoehler et al. 1994; Hoehler & Alperin 1996; Valentine & Reeburgh 2000). The bacteria involved in the CH₄-oxidizing consortium fall within the *Desulfosarcinal/Desulfococcus*, both groups of sulfate-reducing bacteria. These groups of sulfate-reducing bacteria are generally complete oxidizers of organic acids, and are often involved in hydrocarbon degrada-

tion. Although less is known about these bacteria than the archaea, it is possible to infer that bacteria are directly reducing sulfate, and receiving some sort of reduced intermediate from the archaea.

Pure culture studies

No organisms have been isolated capable of performing AOM in a manner consistent with environmental observations. However, there have been several pure culture studies that provide insight into the process. Early studies by Zehnder & Brock (1979, 1980) showed that methanogens were capable of oxidizing CH₄ to CO₂, but the rate of oxidation was only a fraction of the simultaneous CH₄-production rate. These studies showed that methanogens are capable of activating the CH₄ molecule. Studies of stable (¹³C) isotope fractionation during methanogenesis (Summons et al. 1998) have shown that some methylotrophic methanogens produce lipids that are highly isotopically depleted compared to both substrate and product, aiding in the interpretation of lipid biomarker ¹³C. Studies performed in our laboratory have shown that low H₂ is not a simple trigger to reverse methanogenesis, indicating that reverse methanogenesis is not a general ability of methanogens (Valentine et al. 2000).

Mesocosm studies

The term mesocosm study is used here to include all studies in which natural samples were collected and manipulated to measure biological activity. Examples include radiotracer additions, inhibition studies, and incubations. Rate studies employing the addition of radioactive tracers provided evidence that CH₄ oxidation was coupled to sulfate reduction (Devol & Ahmed 1981; Devol 1983); inhibition studies employing bromoethanesulfonic acid (BES – a specific inhibitor of methanogens) further linked methanogens and sulfate-reducing bacteria to the process (Alperin & Reeburgh 1985; Hoehler et al. 1994; Hansen et al. 1998); incubation studies have provided further evidence linking methanogens and sulfate-reducing bacteria to AOM (Lidstrom 1983; Hoehler et al. 1994).

Recent mesocosm studies performed by Nauhaus et al. (2002) provide additional evidence for the link between CH₄ oxidation and sulfate reduction, as well as insights into the microbiology of the process. These studies utilized sediment samples taken from CH₄ seeps at Hydrate Ridge, which contained a high abundance (~10¹⁰ cells per gram of dry sediment) of the

(ANME-2, *Desulfosarcina/Desulfococcus*) AOM consortia. Sulfate reduction was observed to be tightly coupled to CH₄ oxidation in all incubations. A broad temperature optimum was observed from 4 to 16 °C, with significantly lower metabolic rates at temperatures greater than 20 °C. This temperature behavior indicates either that one or both of the organisms involved are adapted to low temperature, or that the syntrophic association only functions at low temperature. This work is also the first to clearly demonstrate the predicted influence of CH₄ concentration on rates of AOM and sulfate reduction. A ten-fold increase in CH₄ concentration led to a 3–4 fold increase in the rate of sulfate reduction. The authors also amended incubations with potential intermediates including hydrogen, formate, acetate and methanol, and observed that none of these compounds inhibited or facilitated sulfate reduction. These results can be used to argue against any of these compounds as important intermediates in AOM, though Nauhaus et al. (2002) stop short of making this argument.

Observational studies

Observational studies include the variety of physical, geochemical, biogeochemical, and microbiological studies which attempt to quantify or observe the natural condition within the environment. Examples include pore water chemical distributions, isotopic abundance of biomarkers, and visual (microscopic) analysis of microbial communities. The similarities in sediment pore water chemical distributions across a variety of marine sediments have led to the general consensus that CH₄ oxidation is dependent on sulfate. Because CH₄ is depleted in ¹³C relative to other compounds, natural isotopic abundance can be used to track CH₄-derived carbon. Observations of ¹³C-depleted archaeal and bacterial lipid biomarkers provide evidence for the assimilation of CH₄-derived carbon by the organisms producing the lipids (Elvert et al. 1999, 2000; Hinrichs et al. 1999, 2000; Peckmann et al. 1999; Thiel et al. 1999, 2001; Boetius et al. 2000; Pancost et al. 2000, 2001; Bian et al. 2001; Orphan et al. 2001a,b). Consistent isotopic differences between ¹³C-depleted bacterial and archaeal lipids has been interpreted to indicate that archaea are directly oxidizing CH₄, and that bacteria are acting as syntrophic partners. Microscopic studies employing fluorescence in situ hybridization (FISH), coupled to isotopic analysis of individual microbial aggregates (using secondary ion mass spectrometry, or SIMS),

Table 1. Potential reactions performed by archaea and bacteria involved in AOM

Previously proposed reaction mechanisms for the CH ₄ -consuming archaea	
$\text{CH}_4 + 2\text{H}_2\text{O} \rightarrow \text{CO}_2 + 4\text{H}_2$	(2)
$\text{CH}_4 + 4\text{HCO}_3^- + 2\text{H}^+ \rightarrow \text{CO}_2 + 4\text{HCOOH} + 2\text{OH}^-$	(3)
$\text{CH}_4 + \text{CO}_2 \rightarrow \text{CH}_3\text{COOH}$	(4)
$2\text{CH}_4 + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + 4\text{H}_2$	(5)
Associated reactions catalyzed by SRB (syntrophic partners)	
$\text{SO}_4^{2-} + 4\text{HCOOH} \rightarrow \text{S}^{2-} + 4\text{CO}_2 + 4\text{H}_2\text{O}$	(6)
$\text{SO}_4^{2-} + 4\text{H}_2 \rightarrow \text{S}^{2-} + 4\text{H}_2\text{O}$	(7)
$\text{SO}_4^{2-} + \text{CH}_3\text{COOH} \rightarrow 2\text{HCO}_3^- + \text{H}_2\text{S}$	(8)

have provided compelling evidence that a consortium of CH₄-oxidizing archaea and sulfate-reducing bacteria oxidize CH₄ in seep environments (Orphan et al. 2001b). The combination of FISH with SIMS provides the first direct evidence for CH₄ metabolism by a consortium of archaea and bacteria, and confirms previous observations linking phylogenetic data and lipid isotope abundance to the archaea and the bacteria involved.

In a more recent study Orphan et al. (2002) applied the FISH-SIMS approach to probe other archaea and bacteria involved in AOM. The primary result of this work is the discovery that ANME-1 archaea are isotopically depleted in ¹³C and are thus likely involved as active partners in AOM. Another important observation from this work is that the ANME-1 archaea are not usually found as a close consortium with bacteria, though they are often found in a loose association. In addition to enhancing our knowledge of the archaea involved in AOM, the Orphan et al. (2002) study also indicates additional diversity among the bacteria associated with AOM. Bacterial groups distinct from the *Desulfosarcina*-related organisms (Boetius et al. 2000) were found in close associations with ANME-2 archaea. These results provide clear evidence for the biological complexity of AOM, and indicate that spatially-constrained consortia are not required for AOM. Future research on these topics is likely to reveal even greater diversity in AOM communities than presented here.

Theoretical studies

Theoretical considerations of the syntrophic association driving AOM have focussed on the bioenerget-

ics of the process (Zehnder & Brock 1979; Zehnder & Brock 1980; Hoehler et al. 1994; Harder 1997; Valentine & Reeburgh 2000). Proposed mechanisms presume that the oxidation of CH₄ by an archaea is coupled to the generation of reduced intermediates that are subsequently oxidized by SRB. Zehnder & Brock (1979) proposed that a reversal of H₂/CO₂ methanogenesis could lead to the net oxidation of CH₄ under appropriate environmental conditions (Table 1, Equation (2)). Hoehler et al. (1994) provided evidence supporting this hypothesis, and further calculated the bioenergetic constraints on the process. Studies in our lab indicate that acetate production from two CH₄ molecules (Table 1, Equation (5)) is also consistent with available evidence, and could provide greater Gibbs Free Energy yields for the organisms involved (Valentine & Reeburgh 2000). A direct reversal of aceticlastic methanogenesis has also been considered (Table 1, Equation (4)), but the possibility discarded because of unfavorable kinetics and thermodynamics (Hoehler et al. 1994; Valentine & Reeburgh 2000). However, given the more favorable thermodynamic conditions found in high-CH₄ environments (Figure 1), the phylogenetic placement of the ANME-2 archaea (Hinrichs et al. 1999), the possibility of multiple mechanisms, and favorable kinetics (Sørensen et al. 2001), interspecies acetate transfer (Table 1, Equation (4)) remains a viable mechanism for AOM in high-CH₄ environments. Other mechanisms such as interspecies formate transfer (Table 1, Equation (3)) are also possible, though less likely than those considered above. The two most viable mechanisms (Table 1, Equations (2) and (5)) both represent novel catabolism in the CH₄ fixation step, but are otherwise feasible with known enzymatic pathways found within the *Methanosarcinales*. The metabolism of the syntrophic sulfate reducer (Table 1, Equations (6)–(8)) need not be different from standard metabolism for any of these potential mechanisms.

A recent study by Sørensen et al. (2001) couples thermodynamic considerations into a kinetic model of interspecies electron transfer. By considering the impact of intercellular chemical gradients and diffusion on the Gibbs Free Energy yield of catabolism, Sørensen et al. (2001) conclude that it is not possible for a methane-oxidizing consortium to be based on interspecies transfer of H₂, acetate, or methanol. This study also concludes that formate is a possible shuttle, though revised calculations at environmentally-relevant temperatures indicate it is not. This work does not consider alternative mechan-

isms including the simultaneous transfer of hydrogen and acetate (Valentine & Reeburgh 2000). The conclusions reached by Sørensen et al. (2001) assume low CH₄ and low sulfate concentrations, as are found at the sulfate to methane transition in many sediments. The generalization of these results to methane seep and vent areas may not be valid as the Gibbs Free Energy available in these settings is much higher (Figure 1) than for the conditions assumed by Sørensen et al. (2001). Calculations assuming high-CH₄ conditions indicate that interspecies acetate transfer is favorable, while transfer of hydrogen, formate, or methanol are not favorable.

Environmental controls

Observations indicate that the process of AOM interacts extensively with the local physical and chemical environment. The responsible communities can be controlled by environmental factors, and the process itself can alter the local physical and chemical environment. Factors such as temperature, CH₄ supply, sulfate supply, sediment organic content, sediment porosity, and sediment mineralogy all affect AOM, and lead to complications in studying this process.

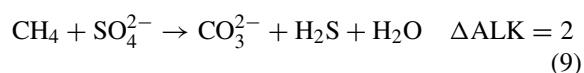
Organic rich coastal sediments

Seasonal changes tend to impact AOM in shallow marine environments, but are unlikely to impact deep environments as conditions there tend to remain relatively constant. In some cases, seasonal changes in climate significantly alter the rates of sulfate reduction and methanogenesis, and also change the depth of the CH₄-sulfate transition. For example, shallow sediments of Cape Lookout Bight, NC change from net CH₄ consumption in the winter to net CH₄ production in the summer (Hoehler et al. 1994). The process of AOM influences the sediments primarily through impacts on pore water chemical distributions and remineralization rates.

CH₄ seeps and hydrate-bearing sediments

The chemical reaction associated with AOM involves consumption of sulfate and CH₄ to produce carbonate and hydrogen sulfide (Equation (9)). This chemical change has the net effect of increasing the alkalinity of the porewater, which facilitates the precipitation of carbonate minerals. Massive authigenic carbonate

deposits are frequently associated with marine CH₄ seeps, and are found to be depleted in the heavy isotope of carbon, ¹³C (Suess et al. 1999); lipid biomarkers have further linked these formations to AOM (Thiel et al. 1999). Unlike AOM which tends to precipitate carbonate minerals, aerobic CH₄ oxidation (Equation (10)) tends to dissolve carbonates as CO₂ is a weak acid. Carbonate mineral deposits associated with AOM can persist for millions of years (Thiel et al. 2001), and provide some evidence for the persistence of this process through time.



Marine CH₄ seeps and vents give rise to vent communities at the sediment–water interface. Communities generally fall into one of two categories: microbial mats or clam beds. Recent evidence indicates that hydrologic flow patterns dictate the nature of the community (Tryon & Brown 2001). Microbial mats dominate at sites with a consistent net outflow of porewater, while clam beds dominate at sites with a transient flow direction. The surface communities are linked to AOM by sulfide, one of the waste products of AOM (Equation (1)). The microbial mats around CH₄ seeps are composed largely of sulfide oxidizing bacteria, and the mats form over areas of net outflow where sediments exhibit high rates of AOM and high sulfide concentrations (up to 15 mM sulfide). Clam communities tend to die under elevated sulfide and apparently prefer environments with slower rates of AOM and periodic inflow of seawater into the sediments. Additional diversity within the microbial mats may also be related to subsurface AOM, but such a hypothesis is unsubstantiated.

The relation between AOM and CH₄ hydrates is complex and involves physical, chemical, and biological interactions. Prior studies have considered the physical interaction between microbes and hydrates (Lanoil et al. 2001), as well as the chemical influence of high CH₄ on AOM (Boetius et al. 2001). Basic physical and chemical principles are used here to constrain the interactions between hydrates and AOM within marine sediments, and to consider the question: do microbes consume CH₄ hydrates? While the answer to this question is not currently known, the following considerations may apply. In order for a sediment-bound hydrate to be stable it must be

in chemical equilibrium with the surrounding environment, which requires a significant methane concentration ($\sim 10\text{--}20$ mM) in the pore waters. Given equilibrium, there is no energetic impetus for microbes to consume CH_4 directly from the hydrate. Furthermore, CH_4 bound in the hydrate lattice is presumably unavailable to microbes unless the hydrate lattice is broken. Because the rate of hydrate dissociation is likely rapid relative to the rates of AOM there would be little advantage to mining CH_4 from the hydrate directly. Effectively, microbes mediating AOM would facilitate the decomposition of hydrates by creating a disequilibrium between hydrate-bound CH_4 and CH_4 dissolved in the pore water. The closer the AOM community resides to the hydrate, the greater the mass flux of CH_4 and the faster the decomposition (assuming adequate heat flow into the hydrate). However, the AOM community also requires sulfate, which must be transported from the water column to the AOM community. In the case of diffusion limitation, the optimal location for AOM communities to maximize metabolism lies between the hydrate and the sediment–water interface. Future field, laboratory, and modeling studies are needed to determine these complex relationships.

The deep subsurface biosphere

Below Earth's solid surface exists a microbially-dominated biosphere (Whitman et al. 1998). While there is active debate about the nature and magnitude of this biosphere, much of the microbial activity is undoubtedly due to the remineralization of buried organic material. Nearly all of the organic material present in the subsurface is ultimately derived from photosynthetic activity, thus much of the subsurface biosphere is ultimately driven by solar radiation at the surface. In marine sedimentary environments (comprising $\sim 70\%$ of Earth's solid surface) the dominant microbial processes are sulfate reduction and methanogenesis. Sulfate reduction acts to remineralize organic material to CO_2 as the primary product, while methanogenesis acts to remineralize organic material to near-equal amounts of CO_2 and CH_4 ; AOM links these two processes by oxidizing CH_4 to CO_2 at the expense of sulfate. While AOM occurs in subsurface environments and is presumably a chemoautotrophic process, the primary substrates, CH_4 and SO_4^{2-} , co-exist because of the Earth's photosynthetically-driven redox gradient.

Like all biological processes AOM is constrained by environmental extremes including temperature,

redox conditions, and pH. However, AOM seems well adapted to some extreme conditions including high sulfide levels, high pressure, near-freezing temperatures, and low energy conditions. Sulfide levels in CH_4 seeps frequently exceed 15 mM as sulfide is a waste product of AOM. It is not known how hydrostatic pressure influences AOM, though the process occurs at moderate depths in the seafloor (Elvert et al. 2000). AOM occurs at two different energetic extremes, starvation and energy conservation. Geochemical evidence from sediment cores collected by the ocean drilling program indicate that AOM proceeds very slowly, with activity proportional to the CH_4 flux (Borowski et al. 1999). While available data does not allow for the calculation of metabolic rates for individual cells, the population likely lives at the edge of starvation with respect to substrate supply (Harder 1997). Bioenergetic calculations also indicate that AOM occurs with a minimal Gibbs Free Energy yield, and that this process occurs near the biological energy quantum (Hoehler et al. 1994; Hoehler & Alperin 1996; Valentine & Reeburgh 2000). Future studies, including ocean drilling program leg 201, are likely to tighten the link between AOM and the deep subsurface biosphere.

Community complexity: arguments for a secondary community

The combination of 16S rDNA gene surveys and isotope analysis of lipid biomarkers applied to methanotrophic communities in CH_4 seeps has provided evidence for a complex community structure. Based primarily on lipid isotope evidence, some authors have proposed that a variety of organisms and mechanisms may be active at any given site. While available evidence is consistent with a variety of organisms performing AOM, evidence is also consistent with the presence of a secondary microbial community living from the waste products and remains of the primary methanotrophic community.

High rates of AOM will supply significant amounts of organic carbon to the sediment in the form of microbial biomass and waste products. With high rates of metabolism in CH_4 seeps (Boetius et al. 2000), and correspondingly high cell densities, a significant buildup of CH_4 -derived carbon is expected. Lipid biomarkers are but one example of such a buildup. Assuming a constant CH_4 supply, the population should eventually reach a steady state in which cell growth

and cell death occur at the same rate. The event of cell death will provide labile organic material to the porewater, and such CH₄-derived carbon would be available to the remainder of the sediment microbial community. If certain heterotrophic microbes specialize in consuming a particular class of compounds produced by the primary methanotrophic community, they could be expected to maintain the isotopic abundance of the primary community as heterotrophs tend to retain the isotopic signature of their food source. Other microbes might acquire organic material from mixed sources, and could be expected to maintain an isotopic content intermediate between their food sources. Still other microbes may grow autotrophically and acquire their cellular carbon from CO₂. However, most of the CO₂ in CH₄ seeps is generated from CH₄, and many of these organisms may show moderate isotopic depletions themselves.

The relative importance of endogenous sediment organic material and CH₄-derived organic material to the secondary microbial community need not be proportional to the pool size. Organic material deposited with the sediment is likely to be more refractory than many of the products from the methanotrophic community, which receives a continual supply of carbon. The relatively rapid turnover of carbon from the methanotrophic community could continually supply the secondary community at a higher rate than the larger pool of endogenous sedimentary organic material. In such a scenario, the secondary microbial community could take on an isotopic content similar to the primary community. The relative isotopic influences of other sedimentary organic material and CO₂ would depend on the organic content of the sediment, the availability of CH₄ and sulfate, and the flow history of the seep. Additional evidence indicates that remineralization of sedimentary organic material is retarded in seep environments (Hinrichs et al. 2000), providing additional evidence that much of the metabolic activity is likely based on CH₄.

Advective CH₄ flow through marine sediments generally occurs along faults and fractures and exhibits transience. Both the rates and direction of seepage can change on short time scales (Tryon et al. 1999; Tryon & Brown 2001). The methanotrophic community living within such sediments undoubtedly undergoes significant changes concurrently with changes in substrate supply. In addition to the general trends in flow, there is extensive heterogeneity around the seeps themselves. Seep environments are neither consistent nor homogenous, and the associated microbial

communities likely share these traits. Lipid biomarker evidence seems to differentiate highly active communities from less active, dying, and dead communities (Hinrichs et al. 1999; Peckmann et al. 1999; Boettius et al. 2000; Elvert et al. 2000; Hinrichs et al. 2000; Pancost et al. 2000, 2001; Bian et al. 2001; Orphan et al. 2001a; Thiel et al. 2001). However, this tool indicates little about the past history of the seep and associated microbial community, and the past history likely confuses the interpretation of biomarker evidence. In the event of complete substrate limitation, it is likely the primary microbial community will die off over time, and release much of their organic carbon to the sediment porewaters. Accompanying this situation is likely an enhancement of the secondary microbial community, a dispersment of ¹³C-depleted carbon to the secondary community, a decrease in the absolute quantity of ¹³C-depleted biomarkers, and an increase in the abundance of ¹³C-depleted lipid breakdown products (i.e., crocetane; Elvert et al. 2000).

Future studies

Given recent observations of the ANME-1 and ANME-2 archaea involved in AOM, it seems likely that these organisms will eventually be isolated either in pure culture or in coculture. The isolation of these organisms will allow for a variety of novel physiological, biochemical, and genomic studies of AOM. Such studies will also provide a unique opportunity to compare the well-characterized ecology of AOM to the behavior of axenic cultures. Environmental genomic studies are also likely to yield tremendous insight into AOM. Large quantities of genetic sequence data may be generated from bacterial artificial chromosome libraries (Beja et al. 2000) or using other methods. It may also be possible to sequence the entire genome of one or more key organism. Additional environmental studies focussing on mRNA transcripts, coenzymes or cofactors also have the potential to yield insight into AOM. Future biogeochemical studies also hold the potential to further our understanding of this process.

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