

The Anaerobic Oxidation of Methane: New Insights in Microbial Ecology and Biogeochemistry

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Abstract: As the major biological sink of methane in marine sediments, the microbially mediated anaerobic oxidation of methane (AOM) is crucial in its role of maintaining a sensitive balance of our atmosphere's greenhouse gas content. Although there is now sufficient geochemical evidence to exactly locate the "hot spots" of AOM, and to crudely estimate its contribution to the methane cycle, a fundamental understanding of the associated biology is still lacking, consequently preventing a thorough biogeochemical understanding of an integral process in the global carbon cycle. Earlier microbiological work trying to resolve the enigma of AOM mostly failed because it was largely focussed on the simulation of AOM under laboratory conditions using cultivable candidate organisms. Now again, understanding the biological and biochemical details of AOM is the declared goal of several international research groups, but this time in a combined effort of biogeochemists and microbiologists using novel analytical tools tailored for the study of unknown microbes and habitats. This review gives an overview on very recent progress in the study of AOM that dramatically advanced this ~ 30-yr-old field. New insights on the quantitative significance of AOM are combined to refine older estimates.

Introduction

Since pioneering reports by Reeburgh (1976) and Barnes and Goldberg (1976), subsequent studies employing stable isotopes, radiotracers, modeling, and inhibition techniques have established that methane in marine sediments is oxidized biologically under anoxic conditions (see review of Valentine and Reeburgh 2000, and references therein). Despite the compelling evidence for the anaerobic oxidation of methane (AOM), details of the related biochemical mechanisms and organisms are still unknown. Zehnder and Brock (1979) showed that incubation of active methanogenic cultures with $^{14}\text{CH}_4$ would result in formation of some $^{14}\text{CO}_2$, thus demonstrating the oxidation of a small fraction of methane under anaerobic conditions. Although no anaerobic methanotroph has ever been isolated, biogeochemical studies have shown that the overall process involves a transfer of electrons from methane to sulfate (Iversen and Jørgensen 1985; Hoehler et al. 1994). The isotopic

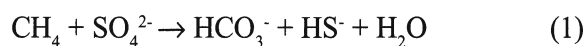
and genetic signature of the microbial biomass in environments enriched with methane shows that this transfer is probably mediated by a microbial consortium that includes archaea and sulfate-reducing bacteria (Hinrichs et al. 1999; Boetius et al. 2000). The distributions of abundant lipid products apparently derived from members of AOM consortia indicate a substantial diversity among the microbial players in different methane-rich environments. However, the intermediate substrate (e.g. $\text{H}_2 + \text{CO}_2$, acetate, formate), which is exchanged between archaeal and bacterial members of the consortium and thereby coupling methane oxidation and sulfate reduction remains unidentified. In several sedimentary environments, AOM can be the dominant sulfate-consuming process, e.g. in sediments from Carolina Rise and Blake Ridge (Borowski et al. 1996) or at Hydrate Ridge (Boetius et al. 2000). Also, AOM may prove to be the oxidative process that penetrates most deeply

into anoxic environments wherever sulfate is available. In the methane budget proposed by Reeburgh (1996), more than 80% of the methane produced annually in marine sediments is consumed mostly in anoxic environments before it can reach the atmosphere. The previously estimated 75 Tg/yr methane consumption is nearly twice the annual increase in the atmospheric inventory of CH₄ (40 Tg/yr). However, our updated compilation of published AOM rates shows that the consumption of methane in anoxic sediments is probably several times higher than previously estimated, implying that methane production estimates are probably too low as well. In contrast to earlier assessments, AOM may have been an important biogeochemical process in earlier stages of Earth history.

The Process

The metabolic process of AOM is still unknown and all reactions discussed in the literature remain speculative as long as the elusive microorganisms involved in AOM are not available for physiological investigations. However, different lines of evidence, including pore water concentration profiles, radioisotope measurements, biomarker studies, phylogenetic analyses, and thermodynamic models serve as a basis to examine the likelihood of the various proposed pathways.

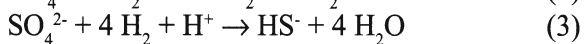
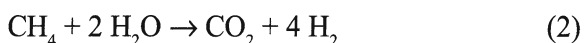
In the mid 70's, it was established that in certain horizons in anoxic sediments and waters at continental margins net consumption of methane occurs (Barnes and Goldberg 1976, Reeburgh 1976). It was observed that AOM peaks coincide with increased sulfate reduction. Thus, Barnes and Goldberg (1976) suggested sulfate as the terminal electron acceptor for this process according to reaction (1).



Thermodynamic models show that this reaction can become favorable at in situ conditions in marine sediments, however, only with a low free energy yield of -25 kJ per mol methane consumed (Hoehler et al. 1994). This is only approximately half the energy required for the formation of ATP. Until

today it has been discussed controversially whether AOM can support microbial growth. However, recently, extremely high amounts of biomass of aggregated archaea and sulfate reducing bacteria involved in AOM were found in surface sediments above marine gas hydrate (Boetius et al. 2000). The biomass of the consortia in the zone of AOM (1-10 cm sediment depth) exceeded the total microbial biomass of surrounding sediments by an order of magnitude and even served as food to some members of the macrofauna community, as indicated by stable isotope analysis (Levin, unpubl. data). Furthermore, the cell-specific rates of methane based sulfate reduction were similar to those of cultivated SRB under optimal culture conditions. These field data indicate that the process of AOM can support significant cell growth and activity.

Counterintuitive to thermodynamic considerations predicting an extremely low energy gain of AOM, the involvement of two or more microorganisms as syntrophic partners was discussed already at the beginning of microbiological research on this process (see review of Valentine and Reeburgh 2000, and literature therein). It was proposed that a consortium of microorganisms involving both methanogens and bacteria may mediate AOM, with the latter oxidizing intermediate products derived from methane. In field experiments conducted by Hoehler et al. (1994), AOM was possible as long as hydrogen concentrations were kept at extremely low levels. The authors concluded that AOM is mediated by two syntrophic partners, which rely on interspecies hydrogen transfer: methanogenic archaea mediating the oxidation of methane with water (reaction 2), and sulfate reducing bacteria scavenging the intermediate hydrogen (reaction 3).



The free energy gain of the reversal of methanogenesis as shown in reaction (2) depends to a large degree on the concentration of dissolved hydrogen in pore waters. For example, under reactant/product concentrations and temperatures typical for Cape Lookout Bight sediments, AOM becomes