EVIDENCE FROM ISOTOPICALLY DEPLETED ARCHAEAL AND BACTERIAL BIOMARKERS IN EASTERN MEDITERRANEAN MUD VOLCANOES FOR CARBON CYCLING IN SPATIALLY HETEROGENEOUS ANAEROBIC METHANE OXIDIZING MICROBIAL COMMUNITIES

Josef P. Werne[1]*, Marianne Baas[1], Richard D. Pancost[1], Ellen C. Hopmans[1], Ioanna Bouloubassi[2], Giovanni Aloisi[3], Ralf R. Haese[4], Jaap S. Sinninghe Damsté[1,4]

[1]Dept. of Marine Biogeochemistry and Toxicology, Netherlands Institute for Sea Research, Texel, The Netherlands - werne@nioz.nl
[2]Laboratoire de Physique et Chimi Marines, Université Pierre et Marie Curie, Paris, France
[3]Laboratoire d'Oceanographie Dynamique et de Climatologie, Université Pierre et Marie Curie, Paris, France
[4]Dept. of Geochemistry, Institute of Earth Sciences, University of Utrecht, The Netherlands

Significant amounts of methane are produced in marine sediments, but little escapes from the sediments to the overlying water column or atmosphere. A number of recent studies have implicated anaerobic methane oxidation (AMO) as the primary process responsible for scavenging of methane in the sediments, yet the organisms, mechanisms, and pathways of carbon cycling in anaerobic methane oxidizing microbial communities remain topics of significant debate. The present study is a molecular and isotopic assessment of the spatial variation and pathways of carbon cycling within the anaerobic methane oxidizing microbial community in mud volcanoes in the eastern Mediterranean Sea.

Keywords: Eastern Mediterranean, Mud Volcanoes, Geochemistry, Organic Matter, Bacteria

Mud volcanism in the Eastern Mediterranean Sea has been a subject of considerable investigation for the past several years for several reasons. Among these are the potential contributions of methane from mud volcanoes and related cold seeps to the atmosphere and subsequently to global warming, and also the impact of mud volcanism on the chemical budget of bottom waters1. Recently, multiple expeditions to study mud volcanoes and related cold seeps in the eastern Mediterranean were undertaken by a joint Dutch-French multi-disciplinary investigation (the MEDINAUT and MEDINETH cruises, see 1 for details). One of the major topics of investigation of the MEDINAUT/ MEDINETH expeditions has been the biogeochemical processes involved in scavenging methane that would otherwise be released into the atmosphere, potentially contributing to global warming. A number of recent studies have implicated the process of AMO, performed by a consortium of methane oxidizing archaea and sulfate reducing bacteria, as the primary process consuming methane in anoxic sediments before it is released to the overlying watercolumn (2-4). Authigenic carbonates are often observed to form in conjunction with the anerobic oxidation of methane (5-7).

One of the most diagnostic indicators of AMO has been the identification of organic biomarker compounds linked to methanogenic archaea and sulfate reducing bacteria that are extremely depleted in ^{13}C relative to typical biomass (2,4,6,8,9). For example, $\delta^{13}C$ values in the range of –80‰ to-100‰ have been identified in biomarkers from eastern Mediterranean mud volcanoes (3), cold seeps from the California margin (2), and Miocene carbonate crusts (9), among others. Despite intensive study in recent years, however, much remains unclear about the processes and organisms involved in AMO. Among the questions still under debate are the specific biogeochemical pathways involved in AMO, the specific organisms responsible for AMO, and the possible diversity of the anaerobic methane oxidizing microbial community. The present study attempts to constrain these variables using molecular isotopic studies of sediments and carbonate crusts from eastern Mediterranean mud volcanoes. Specifically, we are investigating spatial variability in the lipid distributions and the relative carbon isotopic signatures of AMO related biomarkers in these different environments.

A sediment core was taken for this study from Kazan mud volcano in the Anaximander Mountains area (MNLBC19, 35°25.950'N, 30°33.679'E, water depth 1673 m). The core was analyzed for archaeal and bacterial biomarkers that could be associated with AMO. Archaeal biomarkers identified include glycerol diethers such as archaeol and hydroxyarchaeol, saturated and unsaturated isomers of the irregular isoprenoid pentamethylicosane (PMI), and a series of glycerol dialkyl glycerol tetraethers (GDGT's) which had previously been attributed to anaerobic methane oxidizing archaea (10). All of these compounds had concentration maxima at a depth corresponding to the current depth at which AMO was expected to be occurring based on pore water profiles of methane and sulfate concentration (Fig. 1). The carbon isotopic compositions of these compounds ranged from –85‰ to –105‰, suggesting the possibility of either contributions from different archaea utilizing methane or its derivatives, spatial or temporal variations in the δ13C of methane, or variations in carbon isotopic fractionation associated with the pathways of methane-derived carbon assimilation and biosynthesis of different lipids.

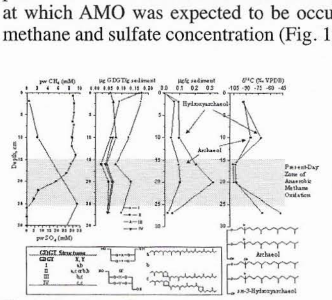

Fig. 1. Depth profiles for pore water methane and sulfate, concentrations of archaeal derived biomarkers (GDGT's and dlycerol diethers) and carbon isotopic compositions of archaeal diethers.

A number of bacterial biomarkers were also identified, including C30 hopanoids such as tetrahymanol, which has not previously been associated with AMO, and a series of dialkyl glycerol diethers previously associated with AMO as well as previously unidentified glycerol diethers (Fig. 2). The carbon isotopic compositions of these bacterial markers lie in the range of –70‰ to –93‰. The fact that these biomarkers are slightly less depleted than those attributed to archaea supports the hypothesis that the bacteria are utilizing methane-derived carbon provided by the methane oxidizing archaea, and in fact, this series of non-isoprenoid diethers is extremely likely to be derived from sulfate reducing bacteria. Previously, these diethers have only been identified in carbonate crusts related to AMO, where they were a significant fraction of the extractable organic matter. Parallel gene surveys of the crust revealed unknown archaeal and bacterial strains most closely related to methanogens and sulfate reducers. The distributions of these archaeal and bacterial biomarkers vary significantly over very small spatial scales of only 10 cm. AMO related biomarkers such as both archaeal and bacterial diethers, for example, dominate the polar fraction of the extracted organic matter at 20 cm, and decrease significantly in shallower sediments. Also, the relative concentrations of archaeol and hydroxyarchaeol vary significantly over the 30 cm length of the core, and the carbon isotopic compositions of these compounds vary over a range of more than 30‰. The existence of AMO related biomarkers in near surface sediments, however, suggests that the zone of AMO has migrated within the sediments over time, and that what we currently observe is merely a snapshot of current conditions rather than an accurate representation of long term conditions. Thus, these observations suggest differences not only in the availability of methane and the size of the methane oxidizing archaeal community over just a few centimeters sediment depth, but also over potentially short temporal scales. In addition, at least one biomarker was identified, 5a-cholestane, which is probably derived from eukaryotes and has a carbon isotopic composition of –52‰, suggesting that even eukaryotic organisms may be ultimately partially utilizing methane-derived carbon sourced from cold seeps associated with eastern Mediterranean mud volcanism. Finally, the presence of this series of bacterial diethers, which we have previously identified only in cold seep related carbonate crusts, suggests that similar organisms may be responsible for AMO in multiple environments. Conversely, the relative distributions of lipids in crusts and sediments are very different, suggesting that the overall microbial community probably varies considerably between different environments in which anaerobic methane oxidation is occurring.

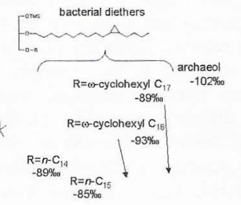

Fig. 2. Partial gas chromatogram of polar fraction of extractable organic matter form Kazan mud volcano, showing isotopicallydepleted archaeal (archaeol, hydroxyarchaeol) and bacterial (tetrahymanol, non isoprenoid dialkyl glycerol diethers) biomarhers, indicating that AMO is a sigificant influence on carbon cycling within the cold seep community.

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