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Technology Status Assessment

ASSESSING THE EFFICACY OF THE AEROBIC METHANOTROPHIC BIOFILTER IN METHANE HYDRATE ENVIRONMENTS

Submitted by:
University of California
Santa Barbara CA 93106
Principal Investigator: David L. Valentine

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Current State of Information and Technology

Current Stat of Information

No comprehensive studies dedicated to understanding the aerobic methanotrophic biofilter have been conducted and no authoritative works are available on the topic. Several studies of limited applicability have been conducted, and these studies guide our present understanding of this process. A recent review of oceanic methane by Reeburgh (2007) highlights the current understanding of the marine methane cycle and represents the state-of-the-art for marine methanotrophy. An important distinction for the proposed research is separating anaerobic processes of methane production and anaerobic oxidation of methane from the aerobic process of methanotrophy (microbial methane oxidation with oxygen as terminal electron acceptor). The anaerobic processes occur typically within seafloor sediments or in waters of highly restricted basins such as the Black Sea and Cariaco Basin (KESSLER et al., 2006a; KESSLER et al., 2006b; REEBURGH et al., 1991). AOM in particular has been the subject of several hundred investigations over the past decade, including several major efforts funded by the European Union. The anaerobic processes often dominate their local environment and as a result are relatively easy to investigate in-situ. In contrast, aerobic methanotrophy occurs broadly in oceanic water columns, and the knowledge base is not as well established as methanotrophy occurs as a trace process superimposed over numerous other microbial processes such as heterotrophic respiration and photosynthesis. An example of our limited knowledge is that we do not currently know the identity of organisms comprising the aerobic methanotrophic biofilter.

Only a handful of studies have directly considered aerobic methanotrophy in the ocean, with a number of these studies targeting hydrothermal plumes (DEANGELIS et al., 1993), permanently anoxic basins (REEBURGH, 1976; REEBURGH et al., 1991; WARD et al., 1987), or the surface layers of the open ocean (KARL and TILBROOK, 1994; NIHOUS and MASUTANI, 2006; TILBROOK and KARL, 1995). Only a few investigations referenced their work toward seeps and methane hydrate as a potential methane source (GRANT and WHITICAR, 2002; KESSLER et al., 2006a; KESSLER et al., 2005; VALENTINE et al., 2001), and a similarly small number have quantified methane oxidation rates (REEBURGH et al., 1991; VALENTINE et al., 2001; WARD and KILPATRICK, 1993; WARD et al., 1987). The knowledge gained from previous investigations of marine methanotrophy has been used to constrain the modern methane source strength of the marine subsurface only to within a factor of ≈3 (KVENVOLDEN et al., 2001). The best guess estimate for present methane flux into the ocean is around 30 Tg (KVENVOLDEN et al., 2001) but remains poorly constrained. Despite shortcomings in our quantification of methane cycling in oxic waters, it is well established from global budget considerations that only a small fraction of marine methane enters the atmosphere; that is, the marine methanotrophic biofilter is known to be highly effective at preventing dissolved methane from breaching the boundary of the sea surface *at the present time*. How the methanotrophic biofilter might react to changes in ocean methane input, temperature, oxygenation or circulation is simply not known. Our knowledge base for the operation of this process in the present and past is insufficient to trust predictions of future behavior.

In addition to the importance of aerobic methanotrophy in the water column, this process may also be important at the sea floor. A large but poorly constrained fraction of subsurface methane enters the ocean through areas of active seepage that occur commonly along continental margins. While numerous constraints have been placed on these fluxes through investigation of anaerobic methane oxidation in the subsurface, the scientific community has

generally considered that methane surviving past this process is free to enter the ocean. Recent research indicates this may not be so, because microbial mat communities living at the interface of the sediment and overlying water may harbor dense methanotrophic populations (DING and VALENTINE, 2008; SOMMER et al., 2006) capable of intercepting methane before it reaches the overlying water. Recent observations from a deep gas-hydrate containing environment support these works and indicate a potentially-important role for benthic mats as part of the methanotrophic biofilter (ELVERT and NIEMANN, 2008). As in the overlying water, neither the quantities of methane removed through this process, nor the abundance of these communities are well established.

State of Technology

The primary tools available to investigate aerobic methanotrophy in the ocean are indirect. Classically, concentration distributions of methane are used to infer methanotrophic activity. Such distributions were originally used to estimate the turnover time for methane in the open ocean (SCRANTON and BREWER, 1978), and have more recently been integrated with independent measures of water mass age (HEESCHEN et al., 2004; REHDER et al., 1999), and with detailed process models (NIHOUS and MASUTANI, 2006) to understand methane dynamics in the open ocean. Concentration distributions have also been used to track methane sources in seep and hydrate environments (CYNAR and YAYANOS, 1992; DAMM et al., 2005; GRANT and WHITICAR, 2002). A second indirect measure of aerobic methanotrophy is sequencing of functional genes associated with the process, particularly the gene encoding particulate methane monooxygenase (pmoA). While infrequently applied, this approach promises to reveal the genetic diversity of uncultivated marine methanotrophs, but suffers from a lack of specificity and quantification. A third indirect approach involves quantifying the natural isotopic composition (typically ^{13}C, but also ^{2}H and ^{14}C) of methane dissolved in marine waters, and results can be used to infer the source and subsequent processes acting on methane (GRANT and WHITICAR, 2002; KESSLER et al., 2006b; VALENTINE et al., 2001). A fourth indirect approach involves the enrichment and isolation of methanotrophic bacteria from seawater. To our knowledge this approach has been successfully applied only a handful of times (KIMURA et al., 1999; LIDSTROM, 1988; SIEBURTH et al., 1987), but without linking isolates to the natural environment, the relevance of the isolates to the marine methane cycle is uncertain. A fifth indirect approach involves the analysis of biomarker molecules and their isotopic composition in order to infer methanotrophic activity. This approach has enabled investigation of methane cycling in the ancient past (HINRICHS et al., 2003), in modern waters (ELVERT and NIEMANN, 2008), and has recently been applied by the PI to identify methanotrophic microbial mats thriving at the sea floor in areas of active methane seepage (DING and VALENTINE, 2008).

The quantification of methane turnover in marine waters by tracer incubation is the most direct method available to quantify methanotrophic activity. Two methods have been developed to quantify rates of methanotrophy, one involving the use radiocarbon and the other involving tritium-labeled methane. Both methods involve collecting water samples from a location of interest, and incubating the sample with a small amount of radiotracer in the dark near in-situ temperature. The radiocarbon approach has been applied to investigate hydrothermal plumes, as well as oxic and anoxic waters (DEANGELIS et al., 1993; REEBURGH et al., 1991; WARD et al., 1987; WARD et al., 1989). The tritium based approach has been applied in the aerobic surface waters of the Black Sea (REEBURGH et al., 1991), by the PI in a known methane hydrate area, the Eel River Basin (VALENTINE et al., 2001), and more recently in the Santa Barbara Channel (unpublished). The tritium-based technique is considered advantageous by the PI. While the radiocarbon technique does allow carbon to be tracked from methane to both CO_2 and to biomass, the experimental protocol is more cumbersome, and, importantly, the specific activity

of ^{14}C is sufficiently low that tracer level additions of methane (for marine waters ≈2-1000 nM) cannot be quantified using scintillation counting, thus incubations require ≈3 orders of magnitude more methane than is present at background levels. The addition of such high levels departs significantly from natural conditions and calls into question the validity of the measured rate. Because tritium has a much higher specific activity, tracer level addition of <10 nM can be quantified, resulting in a realistic estimate of methane oxidation rate.

Several additional techniques are available to investigate marine methanotrophy, but have not yet been applied in marine or hydrate systems. These techniques include stable isotope probing in which methanotrophs are fed isotopically-heavy methane (^{13}C) as a pulse label, and then cellular components such as DNA, RNA or lipids are separated and analyzed to assess which organisms take up the isotopic label and are therefore responsible for the oxidation. This technique holds promise as being highly specific, but suffers from the problem that high concentrations of methane are typically required to label enough cells for analysis, thus limiting applicability in environments with high ambient methane. Another direct technique is the quantification of functional gene abundance - such as pmoA using quantitative real-time PCR. This approach holds the potential to reveal the number of methanotrophic organisms living in an environment with high sensitivity, but suffers from being specific to a small number of sequences (thus potentially missing organisms with divergent sequences for the gene of interest (i.e., pmoA).

Development Strategies

The global methane reservoir in the form of gas hydrate is estimated at 500 – 10,000 Gt (KVENVOLDEN, 1995; MILKOV, 2004). This pool of carbon resides in permafrost and sub-seafloor settings. Neither the rates of methane generation or loss from the reservoir are known, nor is it known if the reservoir is currently growing, shrinking or at steady state. Evidence suggests that the hydrate reservoir has been unstable in the geologic past (JAHREN et al., 2001; THOMAS et al., 2002), and the reservoir is now considered as a capacitor on geologic timescales (DICKENS, 2003). Given the magnitude and potential instability of this reservoir, combined with the potency of methane as a greenhouse gas, it is critical to understand the natural processes that act to control the release of subsurface methane to the ocean and atmosphere. Our present understanding of biological controls on the marine methane cycle is simply insufficient. We do not know the efficiency of the ocean as a filter for methane today or in the past, and we lack a predictive capacity for how the ocean might respond to changes in methane input from hydrate or other reservoirs.

In order to "improve the scientific understanding of the role methane and gas hydrates play in global carbon cycling and/or in climate change, either in the geologic past, at the present, or in the future" we will conduct detailed investigations of the primary sink for marine methane – aerobic methanotrophy. Specifically: 1) we will determine the importance of benthic microbial mats in preventing the flux of methane from the subsurface through the sea floor, 2) we will close a methane budget for the test case of a methane-replete marine basin, and 3) and we will determine primary physical and chemical controls on methane consumption in marine waters. In conducting this research we will also develop and improve technologies to investigate methane hydrates, more specifically the processes active in the marine methane cycle. This research will significantly improve our knowledge base as to the fate of methane in the ocean, particularly methane derived from gas hydrates.

Future

Completion of this research will provide much-needed knowledge relating submarine methane hydrates and gas seepage to the fate of methane in marine waters and is therefore linked to climate and the global carbon cycle. Specifically, each of the three proposed objectives is likely to provide impacts and benefits. The likely benefit from Objective 1 will be an understanding of how benthic microbial communities impact the flux of methane from subsurface reservoirs to the ocean. Knowledge will also be gained as to what organisms are involved, how active they are in different settings, and how they go about metabolizing methane. Novel approaches to investigating these benthic communities will also be developed. The likely benefit from Objective 2 is a closed methane budget for the Santa Barbara Basin – a classic methane hydrate locality that has served as the study site for numerous prior studies. Such budgets have not previously been developed at the regional scale for oxic marine waters, and results can be fed into a new generation of carbon cycle models (focusing on past, present or future) seeking to incorporate a marine methane input (i.e., hydrate destabilization). The third objective is likely to provide the broadest benefit by revealing major controls on methane oxidation in the ocean. An understanding of how chemical, biological and physical factors impact the aerobic biofilter is critical to predicting the fate of methane released from gas hydrate, and to modeling the global carbon cycle during periods of large scale methane release to the oceans (i.e., gas hydrate dissociation). The primary deliverable products from this research will include novel experimental methods, data, and publications.

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National Energy Technology Laboratory

626 Cochrans Mill Road
P.O. Box 10940
Pittsburgh, PA 15236-0940

3610 Collins Ferry Road
P.O. Box 880
Morgantown, WV 26507-0880

One West Third Street, Suite 1400
Tulsa, OK 74103-3519

1450 Queen Avenue SW
Albany, OR 97321-2198

2175 University Ave. South
Suite 201
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