METHANE CONSUMPTION IN WATERS OVERLYING A HYDRATE-ASSOCIATED MOUND IN THE SANTA MONICA BASIN: A PROJECT SYNOPSIS

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ABSTRACT

An understanding of the role of methane hydrates in the global carbon cycle, and in past and future climate change, is dependent on an understanding of methane consumption in hydrate-associated environments. In the marine environment, a dual-component microbial biofilter consumes up to 80% of methane produced. Throughout most of the world's ocean the primary component of this biofilter, anaerobic methane oxidation within sediment, prevents significant amounts of methane from leaving the seafloor. However, in areas of elevated methane production, this sedimentary component is overwhelmed, and methane is released to the water column [1]. The water column component of the marine biofilter for methane is arguably the largest uncharacterized global sink for methane. The goal of our work is to utilize a combined geochemical and molecular biological approach to develop a quantitative understanding of methane consumption in the marine water column of the Southern California Bight. Here we will present geochemical data showing that degree of basin enclosure, and basin-scale circulation patterns, are first order controls on methane oxidation rates in the Santa Monica Basin (SMB). We will also present genetic data elucidating similarities and differences in methanotrophic communities in distinctive horizons within the SMB water column

Keywords: seawater, methane consumption, water column sink

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NOMENCLATURE

[L] liters
[m] meters
[μm] micrometer
[nM] nanomolar (nanomoles per liter)
[τ] turnover time

BACKGROUND

In the open ocean methane concentrations range between 0.5-5 nM, tending to be slightly oversaturated in surface waters, and at or below equilibrium saturation at depth [1,2,3]. This difference in saturation state is due to microbial methane consumption (methanotrophy) within the water column. Estimates of methane turnover times in the deep ocean are in the range of 15-50 years [2,3]. In areas with locally elevated methane concentrations. including hvdrothermal vents and hydrocarbon seeps, turnover times as low as 2 weeks to 1.5 years have been reported [4,5]. While methane oxidation rate measurements made in a few discrete areas have provided evidence for the importance of the water column methane biofilter [4, 5, 6,7,8,9], primary controls on the rate and consumption extent of remain uncharacterized.

There have been a handful of studies identifying methanotrophic bacteria in the open ocean, coastal areas, and oxygen minimum zones [10,11,12,13,14]. However, no studies have quantitatively profiled methanotrophic communities or investigated links between community structure/composition and rate/extent of methane oxidation. Hydrocarbon seep environments, including those off-shore of Southern California, provide a natural investigating laboratory for these populations, their level of activity under varying conditions, and controls on their efficacy.

STUDY SITE, SAMPLING, METHODS

The SMB is located on the coastal side of the Southern California Borderland, as part of an interconnected system of 13 submarine basins ranging in depth from about 590 to nearly 2600 m (Figure 1). The SMB is approximately 100 km long, 40 km wide and 900 m deep. During the SEEPS'07 (Studies of the Ecology and Evolution of Petroleum Systems) cruise, the water column overlying a hydrate-associated mound in the SMB was sampled in a series of seven vertical casts over a period of three (nonconsecutive) days. The venting feature targeted by this investigation has been described by others [15,16,17], and provides an ideal study site for our investigation of the buffer zone between marine methane hydrate and the atmosphere.



Figure 1 – The basins of the Southern California Borderland. Map obtained from GeoMapApp (www.marinegeo.org/geomapapp)

The SMB is connected with the San Pedro Basin and San Diego Trough to the South, the Santa Catalina and Santa Cruz Basins to the west and the Santa Barbara Basin to the north (Figure 1). The SMB sill depth is 747 m, below this depth water is restricted from lateral mixing with the other basins and with the open ocean. Between 700-747 m connections with the San Pedro Basin/ San Diego Trough and Santa Cruz Basin open, allowing bottom water to flow through the basin.

At 600 m the western connection between the Santa Monica, Santa Catalina and Santa Cruz Basins begins to open, allowing mixing of intermediate waters.

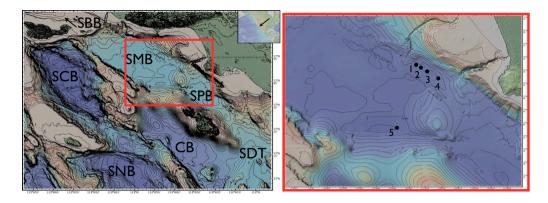


Figure 2 – Location of vertical casts conducted during the SEEPS'07 cruise in the Santa Monica Basin. The hydrate associated mound lies directly under station #3. Map obtained from GeoMapApp (www.marine-geo.org/geomapapp).

At water depths less than 200-250 m surface waters of all of the basins, except the Santa Barbara Basin which is restricted on its southern edge by the Channel Islands, are fully interconnected. Generally, surface waters in this region have a northern origin and flow, in the Southern California eddy, equatorward as the California Current and poleward as the California Countercurrent, while bottom waters have a southern origin and flow poleward as an undercurrent [18,19].

In July, 2007, samples for determination of ambient methane concentrations and methane oxidation rates and for methanotrophic community analyses were collected throughout the water column at five stations (Figure 2), a total of three casts were conducted at station #3. Methane concentrations of an equilibrated headspace were measured on a gas chromatograph equipped with a flame ionization detector [5]. Methane oxidation rate measurements were made using a tritium-tracer method, based on measuring fraction of radiolabeled methane converted water aerobic to by methanotrophy (CH₄ + 2O₂ \rightarrow CO₂ + 2H₂O + {biomass}) [5]. Following a defined incubation period (approximately 24 hours), the activity of the radiolabeled product (³H-H₂O) was measured, and a fractional turnover rate calculated. Methane oxidation

rates were calculated as the product of the fractional turnover rate and ambient methane concentration. Turnover times were calculated as the inverse of the fractional turnover rate. Ten 20 L water samples for methanotrophic community analyses were collected. A peristaltic pump was used to filter these samples through a 3 µm pre-filter and 0.2 µm sterivex filter in series. Filters were preserved in a sucrose storage buffer, DNA was extracted using phenol/chloroform purification method. Two key genes (encoding for pmoA and Type I 16S rRNA) have been amplified, and clone libraries have been constructed. The pmoA gene encodes for the alpha-subunit of the first key enzyme in aerobic methanotrophy, particulate methane monooxygenase. This gene was targeted with a commonly used primer pair A189/mb661 [20,21]. A portion of the 16S rRNA gene that is conserved among known Type I methanotrophs was amplified with the primer set TypeI R/TypeI F [22]. Sequence analysis, amplification of other methanotroph-specific genes and semiquantitative community profiling of these genes across all samples is underway; up-todate data will be presented at the conference.

PRELIMINARY FINDINGS

Preliminary analysis of methane concentration and oxidation rate data shows a strong link between physical oceanography and methanotrophic activity in this semi-enclosed, coastal basin. Although our sampling program targeted a specific venting feature, our results elucidate trends that appear to be controlled by broad-scale circulation patterns.

Concentration and oxidation rate profiles at the five sampling stations, and on all three days at station #3, are strikingly similar. Methane concentrations show maxima below sill depth (747 m: 10-40 nM) and at 50 m; (10 nM). Methane concentrations are stable, and range from 2.5-5 nM, between 700-50 m and above 50 m. Turnover time (τ) is the time required to fully deplete the sample methane pool at the observed oxidation rate. While this measure has important caveats in extrapolation to environmental settings, it is a useful way to compare methanotrophic activity between samples with known methane concentrations. Four distinct horizons are apparent from turnover time profiles. Below sill depth, rapid turnover times are observed. on the order of 3 weeks to 1.5 months. These are some of the shortest turnover times yet reported for the marine water column. Just above sill depth, turnover times begin to increase (representing a decrease in methanotrophic activity) with decreasing depth. This approximately linear increase continues to 500 m ($\tau = 2$ years). Between 500 m and 200 m turnover times show a nearly linear decrease (increase in activity) to a minimum of 4 months. Above 200 m. turnover time is on the order of 1-2 months. except at the 50 m mixed layer methane maxima where anomalously long turnover times are observed (6 months to 3 years). Interestingly, the four distinct horizons observed in turnover time data also correspond to distinctive regions observed in temperature, salinity and oxygen concentration profiles. It is evident from these profiles that waters below sill depth have uniform characteristics in the area sampled, and that three distinct water masses exist between sill depth and the seasurface.

Our interpretation of these observations is that the three distinct horizons observed above sill depth define southern bottom waters, northern surface waters and a broad mixing zone between these two distinct water masses. This interpretation is supported by previously published work [18,19]. The close correlation, between transition zones in property profiles and depth at which basin enclosure decreases, presents the question of the role of basin-scale circulation as a control on the activity of the water column methane biofilter.

We postulate that the differences in the trend of methanotrophic activity (increasing vs. decreasing) with decreasing depth are indicative of community concentration, dilution and seeding. Below sill depth, methanotrophic bacteria thrive. They have a consistent supply of carbon and energy, and advection of the active community away from their local energy source is limited by complete basin enclosure; these are concentrated communities.

Above sill depth bottom waters flow through the basin, from the San Diego Trough, towards the Santa Cruz Basin. As depth decreases, the San Pedro and Santa Cruz basin connections widen, and the area through which these waters flow increases. Bottom waters flowing through the basin from the south appear to have low methanotrophic activity. This could be because these waters have not been in recent contact with a sustained source, and are thus methane depleted, and unable to sustain a methanotrophic community. We hypothesize that increased flow of these waters through dilutes the methanotrophic basin community between 700 and 500 m depth. We plan to test this hypothesis with quantitative methanotrophic community profiling.

At 500 m there is distinct transition to higher variability in temperature, salinity and oxygen with depth, this zone extends to approximately 200 m water depth. A broad mixing region in this depth range is

characteristic of the SMB [18,19]. In this zone, both the mixing ratio of northern to southern water and methanotrophic activity increase with decreased depth, suggesting that surface waters of northern origin seed a methanotrophic community in intermediate waters of this region. The seed community may be derived from either the Santa Barbara Basin or the Santa Monica Bay coastal environment. This hypothesis will be tested with comparisons of community composition and source methanotrophic activity between these three sites.

Methanotrophic activity in waters above 200m is likely controlled by the reportedly [23] laver dynamic mixed methane maximum. however the relationship between this concentration maxima and the correlative turnover time minimum is not vet understood. More detailed analyses of surface circulation patterns, an investigation of the methanotrophic community, and comparison with other surface water sites (including the Santa Barbara Basin) will likely increase our understanding of controls on methane consumption in this depth interval.

CONCLUSIONS

Our observations in the SMB water column emphasize the importance of physical oceanographic characteristics and processes to the potential for microbial methane consumption in the marine water column. The contrast observed between methane concentration and methane turnover time profiles suggests that while methane concentration is a first-order control on methanotrophic activity, community concentration, dilution and seeding control the broad scale efficacy of methane consumption. These processes are dependent on local geologic methane sources, source area bathymetry and current patterns in and around areas of elevated methane flux from the seafloor.

We are currently expanding our understanding of these processes in the SMB

with a semi-quantitative genetic screening approach and through a comparison (both geochemical and molecular) with the Santa Barbara Basin. The next step that we will pursue in addressing water column biofilter efficacy in this area is quantitative methanotrophic community profiling over space and time.

REFERENCES

- [1] Reeburgh, W.S. *Oceanic methane biogeochemistry*. Chemical Reviews 2007; 107:486-513.
- [2] Rehder, G., Keir, R.S., Suess, E., Rhein, M. Methane in the northern Atlantic controlled by microbial oxidation and atmospheric history. Geophysical Research Letters 1999; 26(5):587-590
- [3] Scranton, M.I., Brewer, P.G. Consumption of dissolved methane in the deep ocean. Limnology and Oceanography 1978; 23(6):1207-1213.
- [4] DeAngelis, M.A., Lilley, M.D., Olson, E.J., Baross, J.A. Methane oxidation in deep-sea hydrothermal plumes of the Endeavour Segment of the Juan de Fuca Ridge. Deep-Sea Research I; 40(6):1169-1186.
- [5] Valentine, D.L., Blanton, D.C., Reeburgh, W.S., Kastner, M. Water column methane oxidation adjacent to an area of active hydrate dissociation, Eel River Basin. Geochimica et Cosmochimica Acta 2001; 65(16):2633-2640.
- [6] Ward, B.B., Kilpatrick, K.A. Methane oxidation associated with mid-depth methane maxima in the Southern California Bight. Continental Shelf Research 1993; 13(10):1111-1122.
- [7] Ward, B.B., Kilpatrick, K.A., Novelli, P.C., Scranton, M.I. Methane oxidation and methane fluxes in the ocean surface layer and deep anoxic waters. Nature 1987;327:226-229.
- [8] Reeburgh, W.S., Ward B.B., Whalen, S.C., Sandbeck, K.A., Kilpatrick, K.A., Kerkhof, L.J. *Black Sea methane geochemistry*. Deep-Sea Research 1991; 38(supp. 2):S1189-S1210.
- [9] Sansone, F.J., Martens, C.S. Methane oxidation in Cape Lookout Bight, North

- Carolina. Limnology and Oceanography 1978; 23(2):349-355.
- [10] Sieburth, J.M., Hohnson, P.W., Eberhardt, M.A., Sieraccki, M.E., Lidstrom, M.E., Laux, D. *The first methane-oxidizing bacterium from the upper mixing layer of the deep ocean: Methylomonas pelagica sp. nov.* Current Microbiology 1987; 14:285-293.
- [11] Lidstrom, M.E. Isolation and characterization of marine methanotrophs. Antonie van Leeuwenhoek 1988; 54:189-199.
- [12] Lees, V., Owens, N.J.P., Murrell, J.C. *Nitrogen metabolism in marine methanotrophs*. Archives of Microbiology 1991; 157:60-65.
- [13] Hayashi, T., Obata, H., Gamo, T., Sano, Y., Naganuma, T. Distribution phylogenetic characteristics of the genes encoding enzymes relevant to methane oxidation in oxygen minimum zones of the Eastern Pacific Ocean. Research Journal of Environmental Sciences 2007; 1(6):275-284. [14] Schubert, C.J., Coolen, M.J.L., Neretin, L.N., Schippers, A., Abbas, B., Durisch-Kaiser, E., Wehrli, B., Hopmans, E.C., Damste, J.S.S., Wakeham, S., Kuypers, M.M.M. Aerobic and anaerobic methaneotrophs in the Black Sea water column. Environmental Microbiology 2006; 8(10):1844-1856.
- [15] Normark, W.R., Piper, D.J.W. Preliminary evaluation of recent movement on structures within the Santa Monica Basin, offshore Southern California. U.S. Geological Survey Open-File Report 98-518. http://pubs.usgs.gov/of/1998/of98-518. [16] Hein J.R., Normark, W.R., McIntyre, B.R., Lorenson, T.D., Powell Methanogenic calcite, ¹³C-depleated bivalve shells, and gas hydrate from a mud volcano offshore Southern California. Geology 2006; 34(2):109-112.
- [17] Paull, C.K., Normark, W.R., Ussler, W. III, Caress, D.W., Keaten, R. Association among active seafloor deformation, mound formation, and gas hydrate growth and accumulation within the seafloor of the Santa Monica Basin, offshore California.

- Marine Geology 2008; doi:10.1016/j.margeo.2008.01.011
- [18] Hinkley, B.M. Circulation over the Santa Monica-San Pedro Basin and Shelf. Progress in Oceanography 1992; 30:37-115. [19] Emery, K.O. The sea off Southern California. New York: John Wiley and Sons, Inc., 1960.
- [20] Holmes, A. J., Costello, A., Lidstrom M.E., Murrell, J.C. *Evidence*
- that particulate methane monooxygenase and ammonia monooxygenase may
- be evolutionarily related. FEMS Microbiology Letters 1995; 132:203–208.
- [21] Costello, A.M., Lidstrom, M.E. Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. Applied and Environmental Microbiology 1999;65(11):5066-5074.
- [22] Chen,Y., Dumont, M.G., Cebron, A., Murrell, J.C. *Identification of active methanotrophs in a landfill cover soil through detection of expression of 16S rRNA and functional genes*. Environmental Microbiology 2007; 9(11):2855-2869.
- [23] Ward, B.B. *The subsurface methane maximum in the Southern California Bight*. Continental Shelf Research 1992; 12(5/6):735-752.

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