Oil & Natural Gas Technology

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Quarterly Progress Report

January 2010 – March 2010

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

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EXECUTIVE SUMMARY

In October 2008 the University of California at Santa Barbara (UCSB) initiated investigations of water column methane oxidation in methane hydrate environments, through a project funded by the National Energy Technology Laboratory (NETL) entitled: assessing the efficacy of the aerobic methanotrophic biofilter in methane hydrate environments. The sixth quarter of this project was dedicated to the continued processing and analyzing of samples collected during the SEEPS 09 cruise to methane hydrate sites off the coast of California, to the initial work-up of the resulting data, to the development, submission and publication of manuscripts, and to presentations of preliminary results.

During this period project personnel focused on continued processing of samples collected during the SEEPS 09 expedition, and to completion of analyses for samples collected during the summer 2009 field season. Much of this effort was focused on the quantification of methane concentration in water samples, but also isotopic analyses of microbial mat communities collected during the SEEPS 09 cruise. The stable isotope probing experiments conducted with microbial mats during the SEEPS 09 expedition did not fix as high of quantities of methane into biomass as predicted. However, a survey of the natural isotopic composition of mats collected during SEEPS 09 revealed a striking distinction between different mats, and some mats host a clear methanotrophic signal and have become the focus among the mat samples. Project personnel also worked on data analysis aspects of the project, including the continued preparation of three manuscripts, and the submission of one manuscript.

In addition to progress with the direct goals for this project, one value-added experiment was further pursued This experiment involved the addition of isotopically labelled (13C and D) methane to large volume samples of suboxic waters from the Santa Barbara and Santa Monica Basins, and the tracking of isotopic label into the lipids of methanotrophic bacteria. Analysis of the lipids revealed new information about the activities and identities of methanotrophs in suboxic waters.

Project personnel also presented results at two meetings, the 2010 Ocean Sciences meeting in Portland, Oregon, and at the DOE hydrate meeting in Atlanta, Georgia.

PROGRESS, RESULTS AND DISCUSSION

Task 1 - Project Management Plan (PMP)
This task was completed during the first quarter of this award.

Task 2 - Field Sampling of Microbial Mats
Subtask 2.1 - Coal Oil Point Sampling
Subtask 2.2 - Santa Monica Basin Sampling

Task 2 was completed during a previous reporting period.

Task 3 - Turnover Rates for Methane Oxidation in Microbial Mats
Subtask 3.1 - Turnover Rates for Coal Oil Point Samples
Subtask 3.2 - Turnover Rates for Santa Monica Basin Samples
Subtask 3.1 was completed during a previous reporting period. Subtask 3.2 was initiated during a previous reporting period and analysis has continued in this reporting period. Preliminary results were confirmed during this period, demonstrating low methane turnover in selected mats from this site. However, through a survey of mat isotopic composition, we have found that some mats in direct contact with gas hydrate at this site demonstrate a clear methanotrophic signal in their carbon isotope composition.

Task 4 - Molecular Analyses of Methanotrophs
We continue to develop our approach for molecular analysis of methanotrophs and during this reporting period we submitted a manuscript to the peer-reviewed literature that includes our methodology. We have also initiated molecular analyses with methanotrophic mats from Coal Oil Point, from our SIP experiments, and with isotopically depleted mats form the Santa Monica Basin. Specifically we have extracted DNA and generated 16SrRNA gene clones for identification of abundant organisms.

Task 5 - Stable Isotope Probing
Subtask 5.1 - Stable Isotope Probing of Coal Oil Point Samples
Subtask 5.2 - Stable Isotope Probing of Santa Monica Basin Samples
We have completed all the in-situ incubations and isotope labeling experiments with samples from Coal Oil Point, and have completed gradient separations with the resulting DNA. DNA gradients have been purified and have gone into the queue for molecular analyses (Task 4). *Therefore, Subtask 5.1 is now considered complete.* The stable isotope probing experiments of mats from the Santa Monica Basin (Subtask 5.2) were initiated during a previous reporting period and in this period we determined that only small amounts of isotope label were incorporated during these experiments. We will proceed with DNA separation, but there is a high likelihood that yields will be insufficient for downstream sequencing applications. Fortunately, we have identified mats that we think will serve as a suitable replacement for molecular analyses. We analyzed the carbon isotopic composition of the bulk biomass and found these mats to be as depleted as -85 per mille versus VPDB. These values stand in stark contrast to the more isotopically-enriched surface-dwelling mats used shipboard for the SIP incubations. We have

also succeeded in DNA extractions from these mats, with cloning and sequencing of 16SrRNA is planned in a future reporting period.

Task 6 - Field Measurements in the Santa Barbara Basin

Subtask 6.1 - Shallow Water Sampling and Measurements, Santa Barbara Basin

Subtask 6.2 - Deep and Bottom Water Sampling and Measurements, Santa Barbara Basin

Subtask 6.3 - Repeat Sampling, Santa Barbara Basin

No sampling expeditions were conducted during this reporting period. Additional sampling will be conducted during the summer 2010 field season, based on results from the summer 2009 field season and the SEEPS 09 expedition. Planning and sampling will begin during the next reporting period.

Task 7 – Analysis of Methane Oxidation Rates and Methane Turnover Times Throughout the Santa Barbara Basin

Subtask 7.1 - Shallow Water

Subtask 7.2 - Interior Water

Subtask 7.3 –Targeted Measurements

During the current reporting period numerous analyses were conducted with samples from SEEPS 09. All turnover rate measurements are complete, and oxidation rate calculations are awaiting the completion of concentration measurements expected in a subsequent quarter. Unfortunately a late eluting peak in the SEEPS 09 samples has slowed analytical throughput by a factor of 4.

Task 8 - Analysis of Current Velocity Data

Subtask 8.1 – Current Velocity Analysis for the Shallow Santa Barbara Basin

Subtask 8.2 - Current Velocity Analysis for the Deep Santa Barbara Basin

Analysis of the current velocity data collected during previous reporting periods was initiated in this reporting period. Preliminary analysis indicates opposing flow directions within some plume horizons, likely separating plumes vertically, and potentially explaining the variability observed for the depth of the mid water methane maximum in this basin. Analysis of this data will continue into future reporting periods.

Task 9 - Development of a methane budget for the Santa Barbara Basin

A draft budget is still being developed for the northern margin of the Santa Barbara Basin based on samples previously collected. The focus of this budget is the Coal Oil Point and the underlying plume at 200-250m. A budget incorporating data from the remainder of the Basin was initiated during this reporting period, in a preliminary form to guide future sampling. Natural distinctions between the deep basin, mid water and surface water suggest a natural division into three discrete depth horizons for budgeting purposes.

Task 10 - Field Sampling of Waters

Subtask 10.1 - Santa Barbara Basin Water Sampling

Subtask 10.2 - Southern California Margin Water Sampling

Subtask 10.3 - Targeted Water Sampling

Subtask 10.1 and 10.2 were completed during a previous reporting period. Results worked up from the summer 2009 field season and SEEPS 09 expedition suggest that additional sampling will be required for the Summer 2010 field season. Specifically, the mid-channel plume will be again targeted with high-resolution transects in order to better quantify the flux sustaining this feature.

Task 11 - Sensitivity Testing of Methane Oxidation Rates

Data resulting from sensitivity studies conducted in previous review periods was further analyzed during the current review period. These analyses are ongoing and expected to lead to a manuscript in a future reporting period.

Project personnel have focused their primary laboratory efforts on processing the remaining samples from the SEEPS 09 expedition and the summer 09 field season. Analyses have been ongoing for the entire reporting period and include quantifying methane concentration in all water samples collected during the SEEPS 09 expedition as well as earlier in the summer 2009 sampling season. Because of a perpetual delay caused by a late eluting peak, we are continuing with our four new undergraduate assistants who work in shifts to quantify methane concentration in these samples. We anticipate clearing the sample backlog during the next reporting period.

Project personnel gave presentations at two meetings during this reporting period. The PI travelled to Atlanta, GA in January to present at the DOE Methane Hydrate meeting. The PI also travelled to Portland, OR in February to present progress on the Santa Barbara Basin methane budget at the AGU Ocean Sciences meeting, and to chair three sessions on marine methane biogeochemistry.

Project personnel have also been analyzing data and preparing manuscripts for publication. The first of these manuscripts was submitted to Geo-Marine Letters during a previous reporting period and is now published on-line with print publication expected in a future reporting period. This work explores the relationships of methane, ethane and propane in the plume originating from Coal Oil Point. A second manuscript was submitted to Applied and Environmental Microbiology during this reporting period. This manuscript details our SIP protocols and uses SIP to identify genes similar to methane monooxygenase that are associated with the oxidation of ethane and potentially propane. Three additional manuscripts are in the final stages of preparation, including one analyzing methanotrophy in the Santa Monica Basin, and one generating a methane loss budget for the northern margin of the Santa Barbara Basin, and a review considering emerging topics in marine methane biogeochemistry.

The value-added virome sequencing funded by the Moore Foundation's Marine Microbiology Initiative was continued, with additional viromes sequenced. The data has not yet been released, but is expected in a future reporting period. This has occurred at no cost to DOE, and provides a significant value added aspect to the proposal with metagenomic data from methane-consuming mats.

Project personnel also participated on a cruise to methane seep and gas hydrate sites off the coast of Chile, which spanned the current and subsequent reporting periods. This occurred at no cost to the award, but provides significant potential benefit by enabling quantification of methane oxidation rates in the water column and with benthic materials acquired through trawling.

Project personnel also met with and continued collaboration with other researchers funded through the methane hydrate program, laying the groundwork for a publication on methanotrophy in arctic lake systems.

Additionally, project personnel analyzed samples from additional experiments conducted during SEEPS 09 that may directly benefit this award. One such experiment involved addition of nM levels of 13C-methane and or CH3D to 30L volumes of suboxic water from the Santa Barbara and Santa Monica Basins, in order to track the consumption of methane into bacterial lipids. The lipids were extracted and analyzed by GC-FID and GC/MS by project personnel during a visit to Caltech during a previous reporting period. During this reporting period these samples were analyzed using GC-IRMS, both for carbon and hydrogen isotope ratios. Strong incorporation was seen for both isotopes into monounsaturated fatty acids, consistent with the importance of type 1 methanotrophic bacteria in these suboxic ocean waters. This work is expected to contribute significantly to Tasks 3, 5 and 11, and will further benefit Task 4 by providing a quantifiable context for methane carbon incorporation into bacterial lipids. A similar experiment was also performed using microbial mats from Coal Oil Point and samples are still being analyzed.

Conclusion

The current reporting period saw the continued generation of data and its analysis. The focus of the current period was primarily on analyzing samples and data from expeditions conducted in summer, 2009. Additional project personnel are addressing analytical needs dealing with the volume of samples collected, and senior project personnel are focused on data analysis and preparation of publications.

COST STATUS

There are no subcontracts to this award. All funds are being expended by UCSB. Financial report under separate cover.

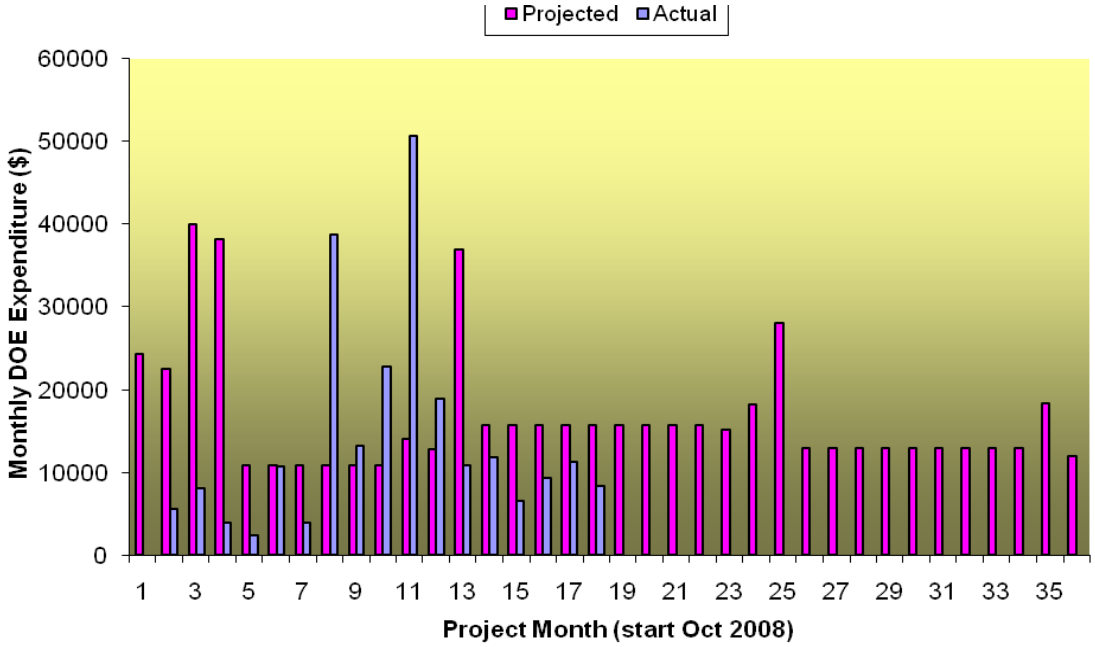

Figure 1. Project costing profile

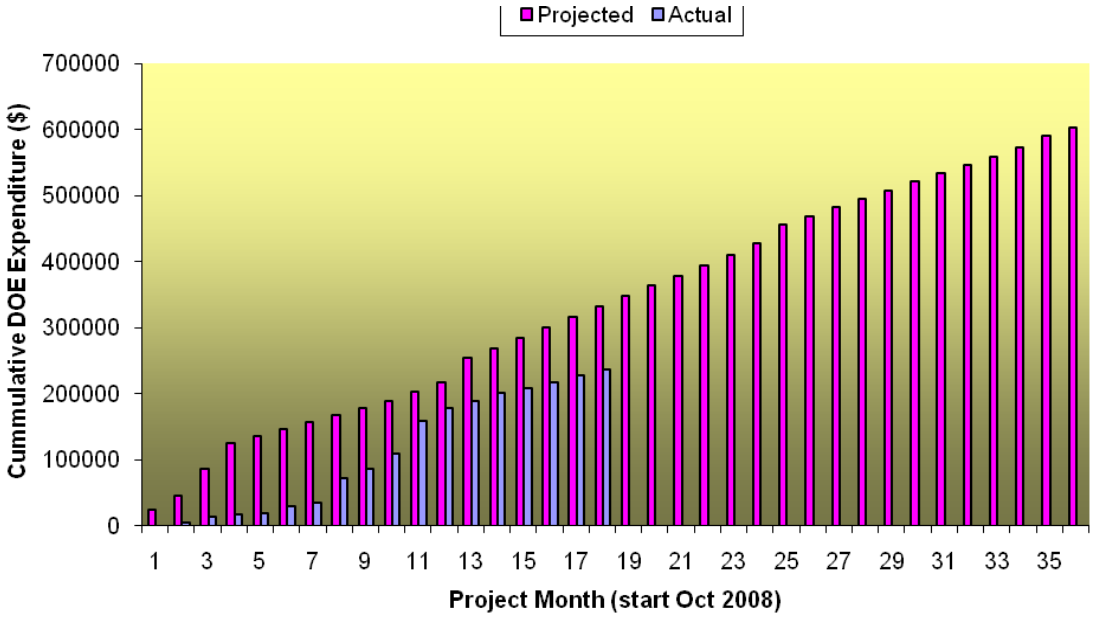

Figure 2. Project cummulative costs

MILESTONE STATUS

Milestone 1: Successful installation and sea trial of the CTD rosette system and ADCP. This milestone relates closely to Tasks 6.1, 6.3, 10.1, and 10.3, and must be reached to enable sampling in support of tasks 7.1, 7.3, 8.1, and 11. The estimated completion date for this milestone is 4/1/09, but may be pushed back until June/July, 2009 on account of missing the fall, 2008 weather window.

Status: These items were previously received and are now functioning and were used for sampling during this reporting period. This milestone is now complete.

Milestone 2: Confirmation of $^{3}H\text{-}CH_4$ oxidation and $^{13}C\text{-}CH_4$ uptake by benthic microbial mats from Coal Oil Point seeps. This milestone relates directly to Tasks 2.1, 3.1, and 5.1 and will further facilitate the completion of tasks 4, and 5.2. The estimated completion date for this milestone is 7/1/09.

Status: This milestone was completed during a previous reporting period.

Milestone 3: Completion of the SEEPS 09 cruise. The SEEPS 09 cruise presents an unrivaled level of access to recently discovered methane hydrate sites in the Santa Monica Basin and to water column sites throughout the Southern California margin including the deep Santa Barbara Basin. The cruise and associated sampling relate closely to Tasks 2.2, 6.2, and 10.2, and will facilitate completion of tasks 3.2, 4, 5.2, 8.2, 9, and 11. The estimated completion date for this milestone is 1/1/10, but the timing will necessarily depend on the UNOLS scheduling of this (already approved) cruise.

Status: The SEEPS 09 cruise took place September 13-29, 2009 and was completed in a previous reporting period. The cruise included 59 personnel, involved 14 dives of the DSV Alvin, 9 deployments of the AUV Sentry, over 30 hydrocasts, and 1 gravity core. Valentine served as the chief scientist. An exceptional amount of samples and data was collected and work-up is ongoing. This milestone is now complete.

Milestone 4: Conduct a preliminary analysis for mmo and 16SrRNA gene sequences for putative methanotrophs from the Santa Monica Basin, and compare to sequences from Coal Oil Point seeps. This milestone relates directly to Tasks 4, 5.1, and 5.2, and will determine the approach taken in completing Tasks 4 and 5. The estimated completion date for this milestone is PY 7/1/10.

Status: All samples are now in-hand to address this milestone, and the analyses were initiated in this reporting period. This research is slightly behind schedule and sequence data may not be fully interpreted by the 7/1/10 deadline.

Milestone 5: Complete a preliminary analysis of current velocity data and oxidation rate data from the SEEPS 09 cruise. This milestone must be achieved to address

Tasks 6.3, 7.3 and 11. The estimated completion date for this milestone is PY 10/1/10.

Status: Samples for oxidation rates are being analyzed, but require the completion of all concentration measurements, which are lagging because of a late-eluting peak. ADCP data is presently being analyzed by project personnel. This research is on schedule.

Milestone 6: Complete the ocean-going sampling program, and perform preliminary analysis of all physical and chemical data to ensure sufficient data for further analysis. This milestone relates directly to Tasks 6.3, 7.3, and 10.3 and will facilitate the completion of Tasks 9 and 11. The estimated completion date for this milestone is PY 4/1/11.

Status: This research has not yet begun and is on schedule.

ACCOMPLISHMENTS

→ Conducted methane concentration measurements on over 200 water column samples from SEEPS 09 and summer 2009 cruises.
→ Successfully tracked isotope label into methanotropic biomarkers in suboxic waters.
→ Identified novel methanotrophic mats in contact with gas hydrate.
→ Submitted a manuscript identifying putative ethane-oxidizing bacteria.
→ Acceptance of a manuscript to Geo-Marine Letters.
→ 2 presentations given at professional meetings.

PROBLEMS OR DELAYS

We face one minor delay in that a late-eluting peak has increased our analysis time for methane concentration by a factor of 4. Given the remaining samples from the SEEPS 09 expedition, we are several months behind, but expect to finish the analyses during the forthcoming reporting period. These intense efforts are likely to push us back slightly with Milestone 4, probably by one reporting period.

PRODUCTS

→ Fifth Quarterly Report Submitted

→ Publication: Mau S, MB Heintz, FS Kinnaman, DL Valentine (2010) Compositional variability and air-sea flux of ethane and propane in the plume of a large, marine seep field near Coal Oil Point, CA. Geo-Marien Letters DOI 10.1007/s00367-010-0185-z

→ Oral presentation at DOE Methane Hydrate Meeting in Atlanta, GA

→Presentations at AGU Ocean Sciences:

Valentine, DL, MB Heintz, S Mau, F Kinnaman, S Bagby, R Camilli, D Yoerger, O Pizarro, M Jakuba (2010), Methane Biogeochemistry of the Santa Barbara Basin, *Eos Trans. AGU, 91*(26), Ocean Sci. Meet. Suppl., Abstract CO44A-04

Heintz MB, S Mau and DL Valentine (2010) Methane Consumption in the Water Column of the Santa Monica Basin: A Comparison From Two Sampling Expeditions *Eos Trans. AGU, 91*(26), Ocean Sci. Meet. Suppl., Abstract CO45B-12.

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