Oil & Natural Gas Technology

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Quarterly Progress Report

April 2010 – June 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 seventh quarter of this project was dedicated to completing the processing and analyzing of samples collected during the SEEPS 09 cruise to methane hydrate sites off the coast of California, to the continued work-up of the resulting data, to the development, submission and publication of manuscripts, to the initiation of the 2010 field season in the Santa Barbara Basin, and to a cruise of opportunity to the MC 252 site in the Gulf of Mexico.

During this period project personnel focused on completion of analyses for water column samples collected during the SEEPS 09 expedition, and to preparation for upcoming field studies in the Santa Barbara Basin and the Gulf of Mexico. Project personnel also continued cloning and sequencing with DNA from microbial mat samples from Santa Barbara Basin and Coal Oil Point including the initial phylogenetic interpretation of sequence data from 16SrRNA clone libraries. Multiple field expeditions were mounted including a 10 day cruise to the MC 252 site in which samples were collected for analyses of methane concentration, oxidation rate, microbial community structure, and stable isotope probing studies. Project personnel also worked on data analysis aspects of the project, including the continued preparation of two manuscripts, the submission of one manuscript, and revision of another manuscript.

In addition to progress with the direct goals for this project, one value-added DNA sequencing effort was pursued. This effort involved an agreement with the Gordon and Betty Moore foundation to sequence three additional viromes from methanotrophic environments. These viromes arise from both aerobic and anaerobic methanotrophic environments, and specifically include viral DNA from SIP experiments – including 13C labeled DNA fractions originating from methane. Resulting sequence information is expected to reveal DNA sequences from viruses that infect methanotrophs, and also to reveal key genes specific to microbes important to the methanotrophic ecosystem. These sequencing efforts are at no cost to DOE.

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 in the last period results confirmed slow methane turnover and only minor uptake of 13C. In place of this sample, downstream analyses are utilizing microbial mats found in direct contact with gas hydrate at this site that demonstrate a clear methanotrophic signal in their carbon isotope composition. *Subtask 3.2 is now considered complete.*

Task 4 - Molecular Analyses of Methanotrophs
We continue to develop our approach for molecular analysis of methanotrophs and during this reporting period we revised a manuscript for the peer-reviewed literature that includes our methodology. We also continue with 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 conducted a first round of sequencing of 16SrRNA genes from clones for identification of abundant organisms in the Santa Monica Basin mat sample.

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
Subtask 5.3 - Stable Isotope Probing of Gulf of Mexico Water Samples

Subtask 5.1 was completed in a previous reporting period. As reported previously the isotopic incorporation into mats from the Santa Monica Basin was insufficient, and gradient centrifugation was not possible with these samples. We have replaced this sample with a natural mat for further downstream analyses and with this substitution we *now consider Subtask 5.2 to be complete*. We have completed several isotope labeling experiments with samples from the Gulf of Mexico in support of Subtask 5.3, to the point of terminating incubations and harvesting biomass. Several other incubations continue. We expect to begin DNA extraction during the next 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

Planning and sampling for Subtask 6.1 were resumed during this budget period and the first trips of the 2010 field season were taken. Initial sampling targeted the eastern Santa Barbara Basin where no samples had previously been collected. Upon completion of the surface sampling in the eastern Santa Barbara Channel in the next reporting period we plan to resume sampling in the deep waters of the Basin in support of subtask 6.2. Subtask 6.3 will be assessed when the surface and deep sampling plans are once completed.

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

Methane oxidation rates have now been completed for all samples collected on the SEEPS 09 cruise, and turnover times have been calculated. Patterns of methane oxidation rates and turnover have been analyzed for the shallow and interior waters of the deep basin, though sampling continues in shallow water along the margins, and in interior waters around the mid-channel region where a large seep area has been tentatively identified. Sampling will continue through the next reporting period and analysis through additional reporting periods.

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 and initiated during previous reporting periods was continued in this reporting period. Continued analysis supports initial observations of 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 was 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 a previous reporting period, and analysis continued into this reporting period.

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 10.4 – Gulf of Mexico Water Sampling

Subtask 10.1 and 10.2 were completed during a previous reporting period. To address Subtask 10.3 we are targeting areas within the Santa Barbara Basin which have either not been sampled previously, or where novel methane sources were tentatively identified. More specifically, the former refers to the eastern channel area and the latter to the mid-channel trend. Planning began for the summer 2010 field season in these two areas, and sampling was initiated for the eastern channel. Subtask 10.4 was addressed during cruise CH-06-10 to the Gulf of Mexico. Water was sampled at 31 stations in the vicinity of MC 252, including samples for methane concentration, oxidation rate, DNA, and as inoculums for stable isotope probing experiments*. Subtask 10.4 is now considered complete.*

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 a manuscript is in preparation. Additional sensitivity tests were also conducted in the Gulf of Mexico.

Project personnel have focused their laboratory efforts on processing the remaining samples from the SEEPS 09 expedition and the summer 09 field season. With these analyses completed, efforts shifted toward data analysis and planning for the 2010 field season.

Project personnel gave presentations at one meeting during this reporting period. The PI and three other project personnel travelled to the annual Southern California Geobiology Symposium at the California Institute of Technology where project personnel presented one oral talk and one poster presentation. The oral presentation by Ms. Monica Heintz was awarded the honor of best presentation at the meeting.

Project personnel have also been analyzing data and preparing manuscripts for publication. The first of these manuscripts was published in print in Geo-Marine Letters during this review period. A second manuscript submitted to Applied and Environmental Microbiology in the previous reporting period was returned for minor revisions and will be submitted in revised form early in the next 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. Two additional manuscripts were submitted during this review period, 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. An additional manuscript is in the final stages of preparation which considers patterns and controls on methanotrophy in the Santa Monica Basin.

The value-added virome sequencing funded by the Moore Foundation's Marine Microbiology Initiative was continued, with approval and submission of additional virome sequencing from stable isotope probing experiments with methanotrophs. Sequence data

was also released for a methanotrophic virome we submitted in a previous reporting period, with additional sequence data expected in a future reporting period. This has occurred at no cost to DOE, and provides a significant value added aspect to the project with metagenomic data from methane-consuming mats, and now isotopically-informed sequence information.

Project personnel also continued collaboration with other researchers funded through the methane hydrate program, beginning the drafting process for a publication on methanotrophy in arctic lake systems.

Project personnel also participated on a cruise to the Gulf of Mexico, as described in greater detail in the appendix.

Conclusion

The current reporting period saw the continued generation of data and its analysis. The initial focus of the current period was primarily on completing instrumental analyses and analyzing data from expeditions conducted in summer, 2009. This focus transitioned into field sampling campaigns in the Santa Barbara Basin and Gulf of Mexico. Additional project personnel focused on molecular and genetic analyses of methanotrophic communities.

COST STATUS

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

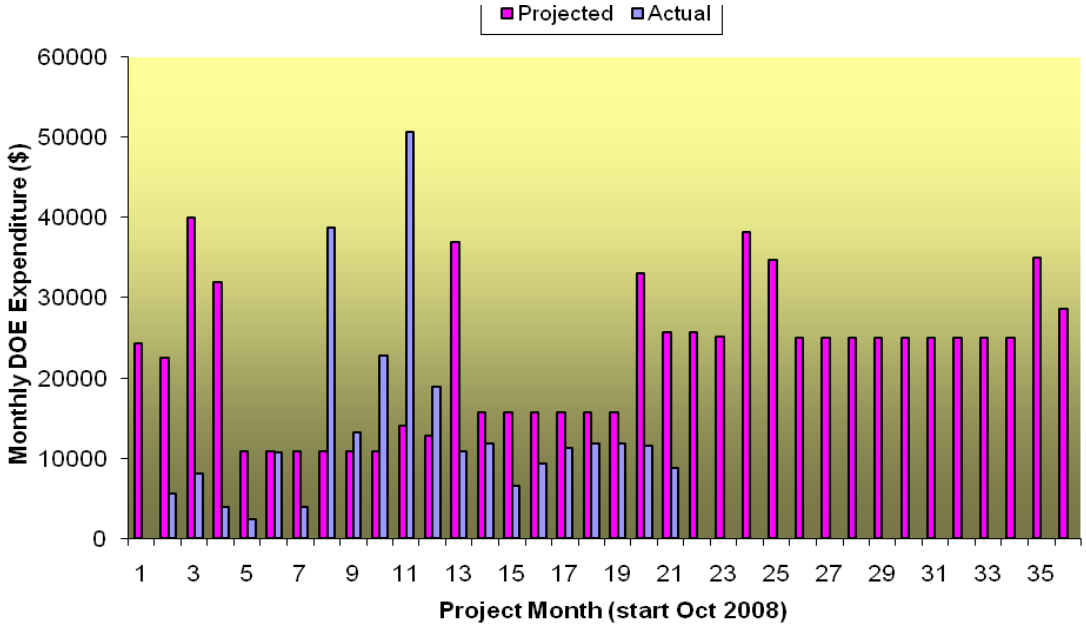

Figure 1. Project costing profile

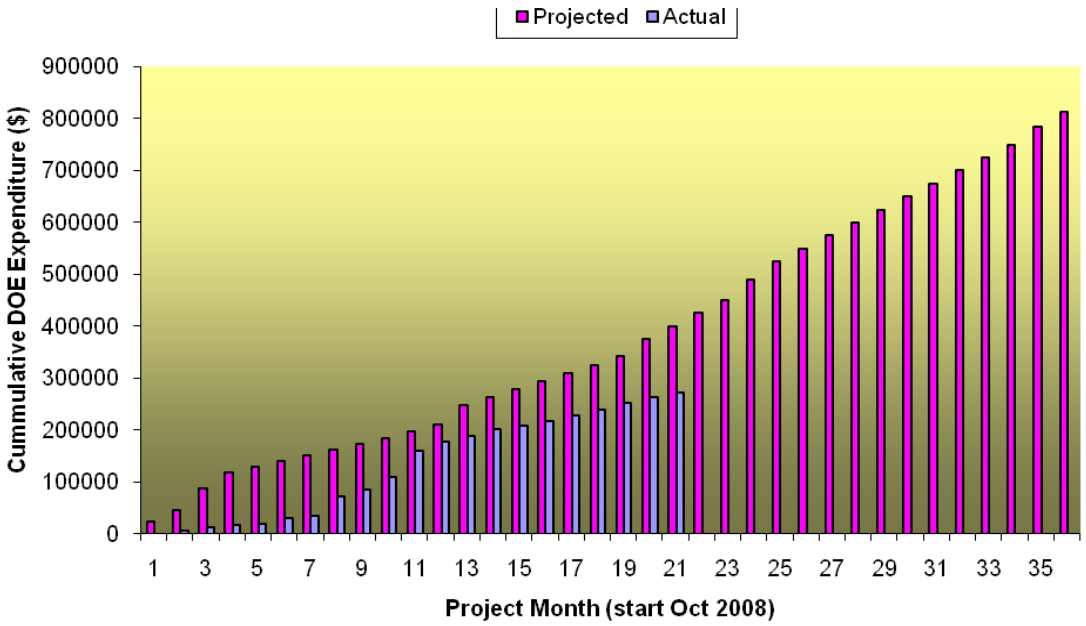

Figure 2. Project cummulative costs

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: This milestone was completed during a previous reporting period.

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: This milestone was completed during a previous reporting period.

Milestone 4: Completion of the Gulf of Mexico (GoM) cruise. The GoM cruise presents an unprecedented opportunity to track the fate of methane from a massive methane plume. During this cruise aboard the *R/V Cape Hatteras* samples will be collected for methane concentration, methane oxidation rates, methane stable isotopes, microbial cells, and large volume filtrates for DNA. The estimated completion of this milestone is 6/30/10 and is associated with tasks 5.3 and 10.4.

Status: The PLUMES (Persistent and Localized Underwater Methane Emission Study) expedition was conducted aboard the R/V Cape Hatteras in the Gulf of Mexico from June 11-21, 2010. Samples were collected throughout the water column near the site of MC 252 for methane concentration, methane oxidation rates, methane stable isotopes, microbial cells, and large volume filtrates for DNA. Samples and equipment were being shipped back to UCSB at the termination of reporting period and this milestone is now considered complete.

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 10/1/10.

Status: Samples for oxidation rates have been analyzed now that all concentration measurements are completed. ADCP data is presently being analyzed by project personnel. This research is on schedule.

Milestone 6: 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 12/1/10.

Status: All samples are now in-hand to address this milestone. Clone libraries have been generated for mats from both Santa Monica Basin and the Coal Oil Point seeps and sequencing efforts are underway for 16SrRNA genes. This research is on schedule.

Milestone 7: 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 4/1/11.

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

ACCOMPLISHMENTS

→ Completed analysis of methane oxidation rates for samples from the SEEPS 09 cruise.
→ Identified bacteria present in microbial mats from the Santa Monica Basin hydrate study site.
→ Identified bacteria in novel methanotrophic mats in contact with gas hydrate.
→ Began the 2010 field season in the Santa Barbara Basin, completing three coastal trips.
→ Revised a manuscript identifying putative ethane-oxidizing bacteria.
→ Published a manuscript in Geo-Marine Letters.
→ Submitted a manuscript describing a partial methane budget for the Santa Barbara Basin.
→ Participated on cruise CH-06-10 aboard the R/V Cape Hatteras, to the MC 252 site.
→ Submitted a short cruise description to FiTI.
→ Drafted and submitted year 3 renewal letter.

PROBLEMS OR DELAYS

We have no current delays to report.

PRODUCTS

→ Sixth 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-Marine Letters 30(3-4) 367-378. DOI 10.1007/s00367-010-0185-z.

→ Presentation: Ice Cover Modulates Methanotrophic Efficacy in Arctic Thermokarst Lakes. Heintz, M.B., Pohlman, J.W., Valentine, D.L., Wooller, M.J. (2010) 7th Annual Southern California Geobiology Symposium. CALTECH. Pasadena, CA (oral presentation).

→ Presentation: Kinnaman F., Heintz M., and Valentine D. (2010 April). Identifying the source and strength of a Santa Barbara Channel mid-water methane plume. 7th Annual Southern California Geobiology Symposium. CALTECH. Pasadena, CA (poster presentation).

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Appendix : Summary of efforts and accomplishments for cruise CH-06-10.

Cruise Title: CH-06-10, PLUMES (Persistent and Localized Underwater Methane Emission Study).

Cruise Dates: June 11-21, 2010

Cruise Location: Departure from Gulfport Mississippi, scientific operations at MC 252, Arrival in Gulfport Mississippi.

Project Participants Involved: David Valentine (cruise participant); Monica Heintz (cruise participant), Chris Farwell (cruise participant), Stephanie Mendes (transportation), Sarah Bagby (data support). Other cruise participants included 8 scientists from Texas A&M University.

Other funding sources: The vessel time and other shipboard scientific projects were funded by the National Science Foundation.

Sampling: Thirty stations were occupied during the cruise, all within seven nautical miles of the Macondo well in MC 252. CTD casts were conducted and water collected at each of these stations. A subset of these samples were collected by project personnel for quantification of methane concentration, stable isotopes of methane, methane oxidation rates, and filtration for DNA collection. Samples were also collected to serve as starting material for stable isotope probing experiments with 13C methane. Over 100 samples were collected for methane oxidation rates and methane concentrations, with additional samples collected for concentration analyses by collaborating scientists. Over two dozen samples were collected for isotopic analyses, in addition to samples being collected by other cruise participants. Multiple stable isotope probing incubations were conducted shipboard, ranging in volume from 10 liters to 250 mL. Three such incubations were completed shipboard and several others were transferred to the home lab to continue incubation. DNA was also filtered from several large volume samples shipboard.

Preliminary observations: The cruise identified depth horizons with elevated methane concentrations, on the order of 10,000 times greater than ambient background levels. While sampling was performed throughout the water column, these features became the primary targets for sampling and experimentation. The features were relatively easy to identify based on oxygen anomalies, fluorescence, and light scattering, for which real-time sensor data was available.

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