Oil & Natural Gas Technology

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Quarterly Progress Report

October 2009 – December 2009

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 fifth quarter of this project was dedicated to processing and analyzing samples collected during the SEEPS 09 cruise to methane hydrate sites off the coast of California.

During this period project personnel returned from the SEEPS 09 expedition and began the process of organizing, prioritizing, archiving and analyzing samples from the cruise and from the summer 2009 field season, as well as returning the home laboratory into a functional state after equipment was returned from the SEEPS 09 cruise.

The current quarter was otherwise dedicated to initiating analyses from the summer field season and SEEPS 09 expedition, namely the quantification of methane concentration and methane turnover. Samples were queued and analyzed for turnover time, and were queued also for methane concentration, with analyses initiated. Progress was also made with the preparation of isotopically labeled samples for subsequent molecular analyses. Project personnel also worked on data analysis aspects of the project, culminating in submission of one manuscript, and partial preparation of three others.

In addition to the progress with the direct goals for this project, one value-added experiment was 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.

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 the previous reporting period and analysis has continued in this reporting period. Preliminary results suggest the low uptake of methane from the mats collected at 800m depth. More conclusive results and potential explanations are expected in a subsequent reporting period.

Task 4 - Molecular Analyses of Methanotrophs
We continue to develop our approach for molecular analysis of methanotrophs and during this reporting period were actively working on publishing our methodology in the peer-review literature.

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
Results from stable isotope probing of microbial mat samples from Coal Oil Point (Subtask 5.1) are ongoing. We previously confirmed consumption of the stable isotope label, and during this reporting period performed DNA gradient separation of samples. Molecular analyses of the DNA are planned for subsequent reporting periods. We continued during this reporting period with experiments involving stable isotope probing of samples from the Santa Monica Basin (subtask 5.2). Incubations from SEEPS 09 were terminated and DNA extracted from the samples. Gradient separation and molecular analyses are planned for upcoming reporting periods.

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
One sampling trip was conducted after the SEEPS 09 cruise to the shallow Santa Barbara Basin. Additional sampling will be conducted during the Summer 2010 field sea-

son, based on analyses from the summer 2009 field season and the SEEPS 09 expedition.

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. The majority of 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

Current velocity data was collected during a previous reporting period for both the shallow and deep water of the Santa Barbara Basin. Detailed analysis is anticipated in subsequent 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 a combination of data collected in this reporting period and older data. 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 is anticipated in a future 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

Sampling of the deep Santa Barbara Basin (subtask 10.1) and other locations in the California Margin (Subtask 10.2) were completed during the SEEPS 09 expedition, and those subtasks are now considered complete. Targeted water sampling may be required in subsequent reporting periods based on results worked up from the summer 2009 field season and SEEPS 09 expedition.

Task 11 - Sensitivity Testing of Methane Oxidation Rates

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

Following the completion of the SEEPS 09 expedition on September 29th 2009, project personnel focused on sample preservation, as well as the mundane aspects of returning from a major research cruise – such as returning the home lab to a functional state to

begin analyses. Analyses have been ongoing since October 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 have brought on four new undergraduates to work in shifts to quantify methane concentration in these samples. This is increasing throughput, albeit progress is still slow and many months will be required to clear the sample backlog.

Project personnel also analyzed results from experiments in which isotopically labeled methane, ethane or propane were fed to aerobic surface sediments from a hydrocarbon seep. The results reveal novel groups of bacteria not previously implicated in the oxidation of these compounds. Of significant interest is an apparent group of ethane oxidizing bacteria that otherwise have all the biomarker signatures of methanotrophs.

Project personnel have also been analyzing data and preparing manuscripts for publication. The first of these manuscripts was submitted in revised form to Geo-Marine Letters with a decision expected in the next review period. This work explores the relationships of methane, ethane and propane in the plume originating from Coal Oil Point. Three additional manuscripts are in the final stages of preparation, including one analyzing methanotrophy in the Santa Monica Basin, one using stable isotope probing to identify the organisms and genes responsible for methane, ethane and propane oxidation in oxic sediment layers, and one generating a methane loss budget for the northern margin of the Santa Barbara Basin.

The value-added virome sequencing funded by the Moore Foundation's Marine Microbiology Initiative was continued with the submission of viromic DNA associated with deep samples from the Santa Barbara Basin and methanotrophic communities from seep sediments in the same basin. Two of our submitted samples have been pyrosequenced, though results are not yet available. This occurred at no cost to DOE, and provides a significant value added aspect to the proposal with metagenomic data from methane-consuming mats.

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. A positive result would confirm that bacteria oxidize methane in deep suboxic water, and would place constraints on carbon flow into the bacterial community. After incubation the samples were filtered shipboard. During the present reporting period the samples were extracted and analyzed by GC-FID and GC/MS by project personnel during a visit to Caltech. A similar experiment was also performed using microbial mats from Coal Oil Point and is being analyzed along with the deep samples from the Basins. 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.

Conclusion

The current reporting period follows an intense summer field season that included numerous sampling trips and culminated with a 17 day research cruise. The focus of the current period was primarily on analyzing samples and data from these expeditions. New project personnel were brought in to address analytical needs dealing with the volume of samples collected, and existing project personnel 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.

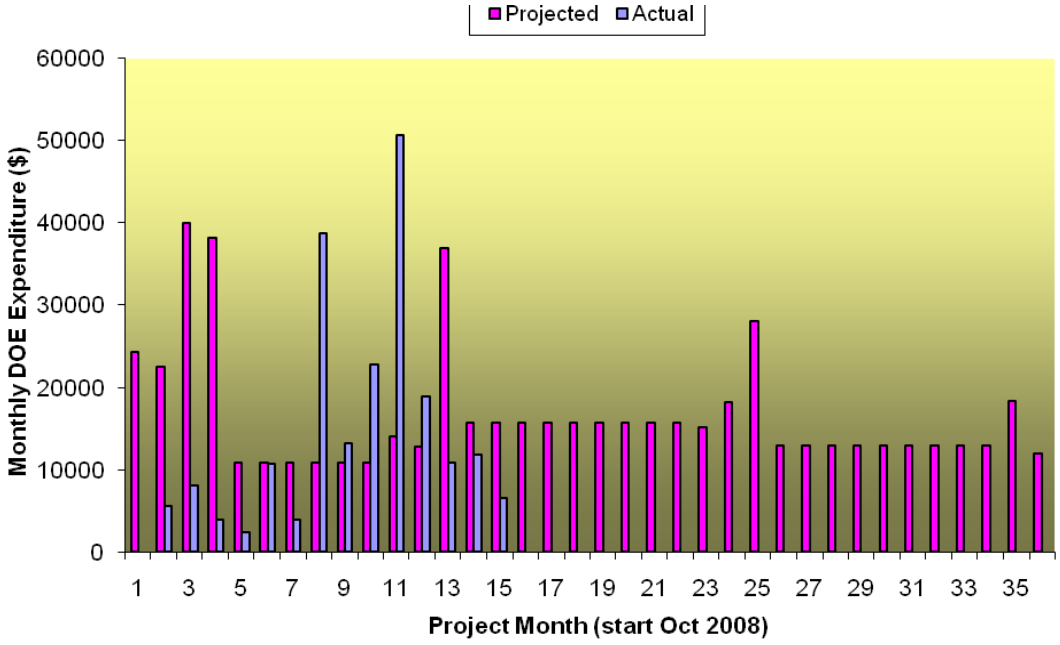

Figure 1. Project costing profile

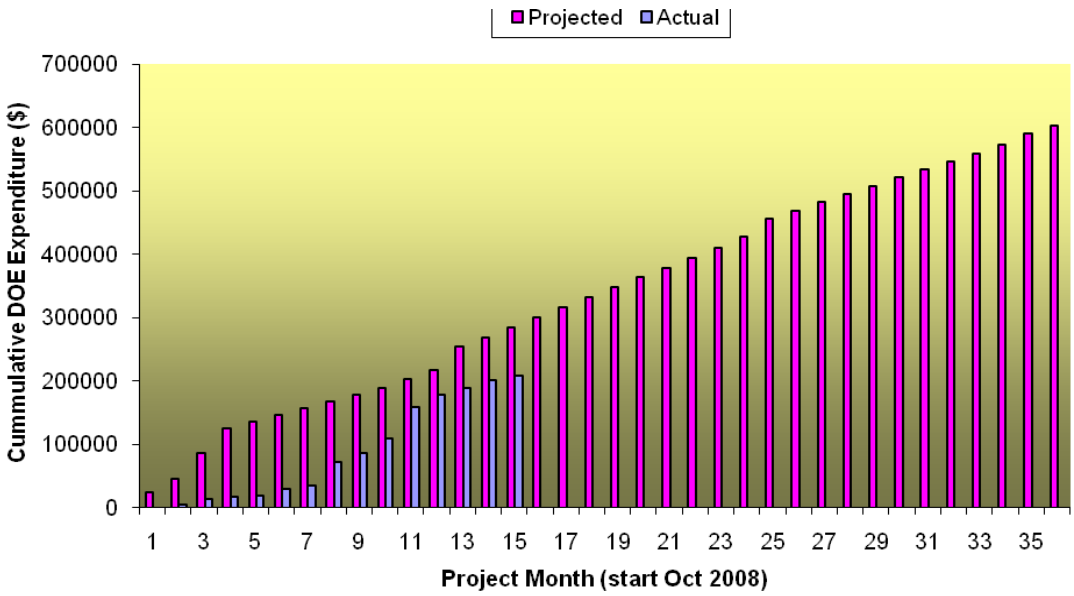

Figure 2. Project cummulative costs

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 the last days of the previous reporting period; many project personnel did not return to UCSB until this 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 will be initiated in a forthcoming review period. This research is slightly behind schedule.

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 now collected and are being analyzed. ADCP data was collected shipboard and is available for use. 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

→ Initiated analyses of samples from SEEPS 09 expedition
→ Conducted SIP experiment to track methanotropic biomass in suboxic waters
→ Identified putative ethane-oxidizing bacteria.
→ Submission of a revised manuscript to Geo-Marine Letters
→ Preparation of three additional manuscripts

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 myriad samples from SEEPS 09 and existing backlog from summer 2009 expeditions, we are several months behind. To address this we have brought on 4 new personnel (undergraduate researchers) to analyze these samples in shifts.

PRODUCTS

→ Fourth Quarterly Report Submitted

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