

Oil & Natural Gas Technology

DOE Award No.: DE-NT0005667

Quarterly Progress Report

January 2009 – March 2009

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

Submitted by:
University of California
Santa Barbara CA 93106

Principal Investigator: David L. Valentine

Prepared for:
United States Department of Energy
National Energy Technology Laboratory

April 20, 2009



Office of Fossil Energy

TABLE OF CONTENTS

Executive Summary.....	2
Progress, Results and Discussion.....	3
Conclusion.....	4
Cost Status.....	6
Milestone Status.....	7
Accomplishments.....	8
Problems or Delays.....	8
Products.....	8

LIST OF FIGURES

Figure 1: Project costing profile.....	6
Figure 2: Project cumulative costs.....	6

LIST OF TABLES

None

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 second quarter of this project was dedicated to preparation for Spring and Summer field seasons, and the continuation of collaborations with other DOE and NETL-funded groups.

During this period project personnel ordered the three necessary items of equipment needed for summer research: an acoustic Doppler current velocity profiler, a CTD-rossette system with sampling bottles, and an ultra-low temperature freezer. One item arrived during this period and the other two are expected early in the next quarter, at which point field testing will begin. Project personnel also worked with the group at UAF to coordinate a spring sampling expedition to the Arctic, traveled to Lawrence Berkeley National Lab to learn DOE phylochip technology, began planning the details of the SEEPS 09 cruise now scheduled for September, 2009, and began quantifying methane oxidation rates in samples retrieved from the waters of the Eastern Tropical North Pacific. The one week trip to LBL involved laboratory work for a graduate student to learn the process behind phylochip technology and is enabling processing of samples at the UCSB-funded lab. Initial results are expected in the next review period including the application of the new generation 3 phylochip to preliminary samples.

The workup of microbial mat samples continued through the quarter and included the centrifugation of samples for isotope separation, and the use of DNA from these samples for phylochip application. Preliminary results suggest sufficient ^{13}C -methane incorporation for the identification of communities incorporating carbon from methane, and continued work with these samples is warranted.

In addition to progress with the proposed research, a value added proposal was submitted to a private foundation to conduct virome sequencing (that is DNA from the total viral population) of a methanotrophic microbial mat community. If funded this level of sequencing (up to 50 Mb) will provide useful insight into the viral communities associated with methane consuming bacteria overlying methane hydrate sites.

PROGRESS, RESULTS AND DISCUSSION

Task 1 - Project Management Plan (PMP)

This task was completed during the first quarter of this award. During September and October the Principal Investigator revised and submitted a PMP, which was approved by DOE. This document outlined the course of the entire project. In addition to this document the PI worked with the Program Manager (PM) to develop a project summary suitable for general viewing. The PI further wrote and submitted a Technology Status Assessment highlighting the state of current technology in relation to methanotrophy and methane hydrates.

Task 2 - Field Sampling of Microbial Mats

Subtask 2.1 - Coal Oil Point Sampling

Subtask 2.2 - Santa Monica Basin Sampling

During the pre-award period, nine samples of microbial mats were collected from the sea floor at Coal Oil Point in support of subtask 2.1. These samples were incubated in-situ at ~63 feet water depth for one month prior to collection – in the gas plume of a massive methane seep.

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

Experiments were conducted with the samples collected at COP to quantify the turnover rate of ^{13}C methane by the microbial mats collected – in support of subtask 3.1. Analyses conducted during the second quarter of this award indicate linear turnover rates for methane associated with these mats, suggesting that the observed metabolism during laboratory incubation is similar to in-situ activity. Sample analysis is continuing to confirm these results.

Task 4 - Molecular Analyses of Methanotrophs

During the first quarter of this award extractions were performed from samples collected at Coal Oil Point to enable further molecular analyses. During the second quarter these samples were taken to LBL by project personnel, and prepared for DOE's phylochip. Initial results are expected in the third quarter.

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

During the first quarter of this award ^{13}C -methane was added to samples from the COP field in preparatory experiments for subtask 5.1. Analyses conducted during the second quarter indicate ^{13}C uptake into biomass, and centrifugation appears to have separated ^{13}C -DNA. Further analyses are ongoing.

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

These tasks are scheduled to begin in the third quarter.

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

These tasks are planned for a future reporting period.

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

These tasks are scheduled to begin in the third quarter.

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

This task is planned for 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

This task is planned to start in the third quarter.

Task 11 - Sensitivity Testing of Methane Oxidation Rates

This task is planned for a future reporting period.

In addition to the formal tasks associated with the project, two collaborations were continued. Collaboration with scientists at LBL involved a member of the project team, Mr. Blair Paul, traveling to LBL where he worked for one week in the laboratory of Dr. Gary Andersen. Mr. Paul brought a number of samples with him to work up for analysis. The primary goal of the trip was to learn the sample processing for DNA prior to application of the phylochip, such that much of the processing could be conducted at UCSB. This goal was achieved and we are awaiting the preliminary results of phylochip analyses to guide the next set of analyses. A future trip to LBL is warranted to learn the data analysis tools associated with phylochip results. A second collaboration with a DOE-funded group investigating methane release from permafrost environments was also continued, and plans formalized for Ms. Monica Heintz to participate on two field excursions to Alaska, in spring and summer, 2009. We plan to quantify methane oxidation rates and collect samples for DNA analysis of the microbial community. Both collaborations add value to current DOE projects.

One value-added proposal was submitted to the Moore Foundation's Marine Microbiology Initiative. This proposal was for a viral metagenome associated with methanotrophic microbial mats. If funded this will add value to this DOE-funded research as it promises to reveal the genomic content of the viruses predated methanotrophic mats, potentially including functional genes important to the community. Announcements are expected in the third quarter. This was one of five methane-related viromes proposed by the PI, the others being associated with anaerobic methane oxidation, methanogenesis, and suboxic basin waters – all of which are important for methane hydrate research, but only tangentially related to this award.

One member of our project team, Ms Monica Heintz, also participated on a cruise to the Eastern Tropical North Pacific to investigate methane cycling in relation to the suboxic zone and methane plume originating from Central America. Participation was independently funded and this award did not pay any associated costs, but significant benefit is expected as this expedition provides important comparison results to the planned studies of water column methanotrophy. Several hundred samples are currently being analyzed for methane oxidation rates with promising trends emerging.

Conclusion

A majority of the work conducted during this second quarter was preparatory in nature and included purchasing of field equipment for spring and summer field season, and preparation for summer sampling. Initial scientific investigations were continued with benthic mats at Coal Oil Point and in the water column of the Eastern Tropical North Pacific, and all preliminary observations suggest our proposed approaches are viable for these samples and environments. Important collaborations to this and other DOE projects were advanced, and one trip was made in support of these collaborations.

COST STATUS

There are no subcontracts to this award. All funds are being expended by UCSB. Financial report under separate cover. Note that all three equipment items have been ordered, and encumbrances to this award total \$77,748 (equipment did not hit the ledger during this period).

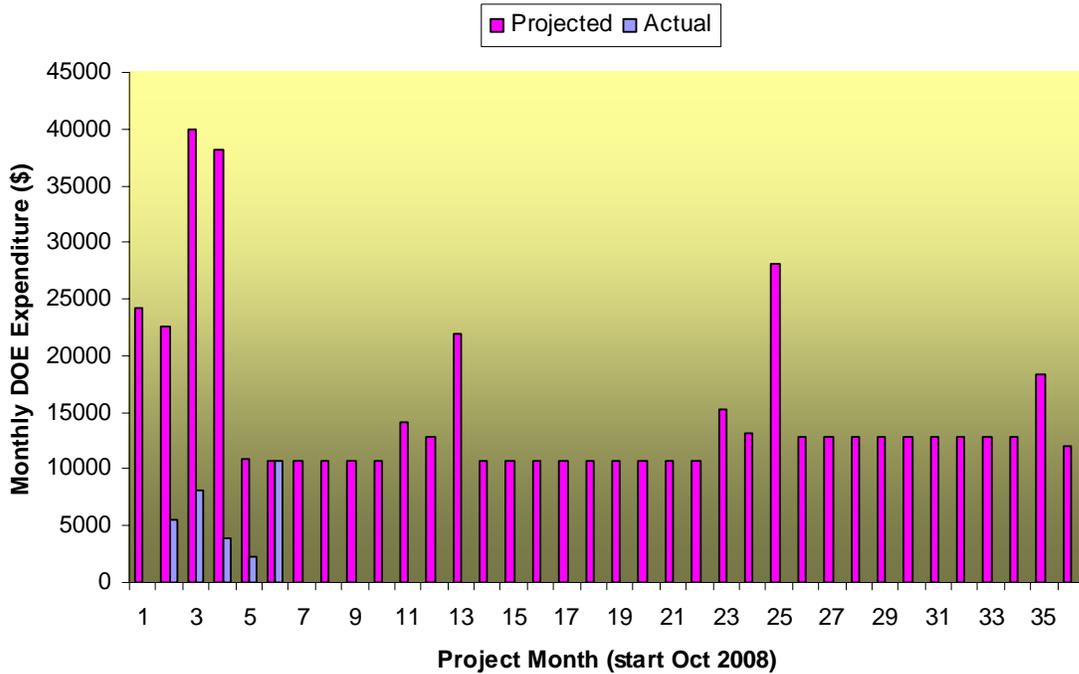


Figure 1. Project costing profile

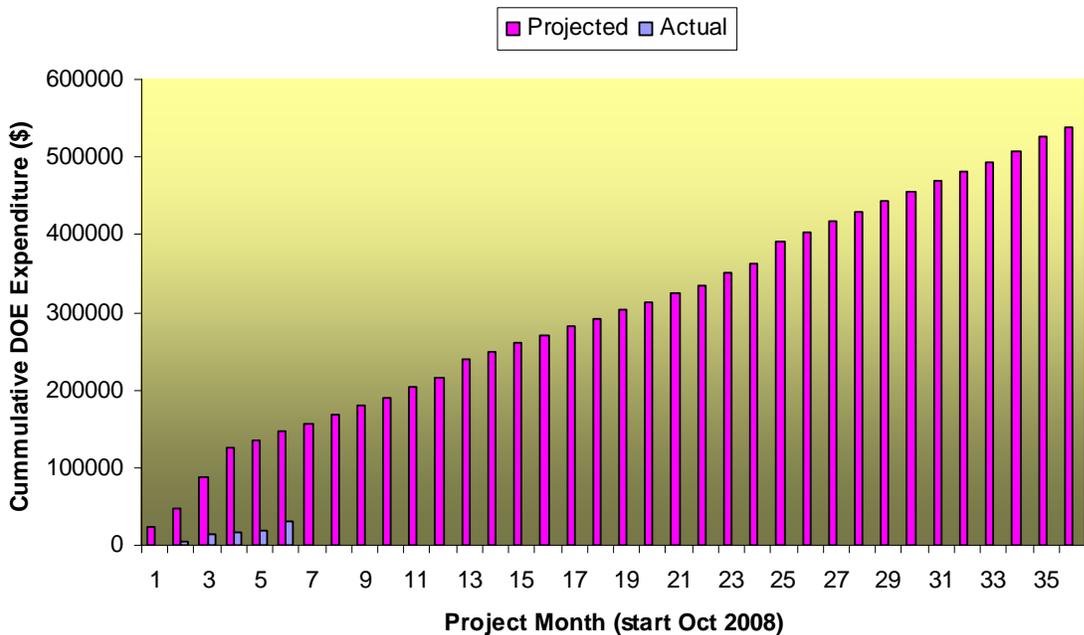


Figure 2. Project cumulative costs

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 ordered with arrival of the last item expected early in the third quarter. Missing the fall field season relieved the rush on these items, and the current time frame will allow them to be tested prior to the Spring field season.

Milestone 2: Confirmation of $^3\text{H-CH}_4$ oxidation and $^{13}\text{C-CH}_4$ 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: Samples collected during the pre-award time are being used in pursuit of this milestone. Missing the fall field season may delay this milestone slightly, depending on weather and storm conditions in the early spring that may set back in-situ colonization of sea floor deployments. The work-up of results from the preliminary experiments suggests rapid and linear rates of methane uptake with further sample analysis planned.

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: Planning began for the SEEPS 09 cruise, after the Atlantis schedule was finalized in November, 2008. Unfortunately, the schedule was later scrapped by UNOLS on account of delays in the production of components needed for a different cruise. Revised dates were finalized for SEEPS 09 and announced in March of 2009, and the cruise is now scheduled for September 13-29, 2009. We have initiated planning around these dates.

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: This research has not yet begun and is on 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: This research has not yet begun and 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

- Ordering of all major equipment items needed for field research program.
- Demonstration that methanotrophic mat communities from Coal Oil Point oxidize and incorporate ¹³C-methane at a linear rate in laboratory incubations
- Preparation and submission of preliminary samples for Phylochip analysis at LBL
- Completion of a one-month expedition to the Eastern Tropical North Pacific, and preliminary analysis of methane turnover for more than 100 samples.
-

PROBLEMS OR DELAYS

During the first quarter of the award there was a slight delay in the timing of award announcements and a delay in arrival/processing of funds. As a result the Fall 2008 field season was missed. This has pushed initial field work to the Spring of 2009, but will only cause minor delays in conducting tasks and meeting milestone deadlines. This delay was partially compensated by pre-award spending that enabled incubations during Summer/Fall 2008 in-situ at the Coal Oil Point seep field. This delay also caused a delay in employment of project personnel as key academic deadlines were missed. These issues have since been sorted out and will cause only a slight lag in the cumulative expenditure. There were no significant problems or delays that occurred during the second quarter of this award.

PRODUCTS

- First Quarterly Report Submitted

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
Fairbanks, AK 99709

Visit the NETL website at:
www.netl.doe.gov

Customer Service:
1-800-553-7681

