Oil & Natural Gas Technology

DOE Award No.: DE-NT0005667

Quarterly Progress Report
(April – June 2009)

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

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Office of Fossil Energy

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EXECUTIVE SUMMARY

In October 2008 the University of California at Stan 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 third quarter of this project was dedicated to field sampling and experimentation, and the continuation of collaborations with other DOE and NETL-funded groups.

During this period project personnel received the three necessary items of equipment needed for summer research: an acoustic Doppler current velocity profiler, a CTD-rosette system with sampling bottles, and an ultra-low temperature freezer. Defects with two of the items have caused delays, but are covered by warranty and being rectified. Our sampling program is underway and both microbial mat and water samples have been collected and analyzed for their methanotrophic potential. Project personnel also participated on a spring sampling expedition to the Arctic, began planning a summer field expedition to the same location, continued planning the details of the SEEPS 09 cruise scheduled for September, 2009, and continued quantifying methane oxidation rates in samples retrieved from the waters of the Eastern Tropical North Pacific. Initial results are being analyzed from several of studies including phylochip analysis of microbial mat communities, and oxidation rate measurements from Alaskan lakes, Coal Oil Point, and the Eastern Tropical North Pacific.

The workup of fresh 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. Results suggest sufficient 13C-methane incorporation for the identification of communities incorporating carbon from methane, and these analyses are ongoing.

In addition to progress with the proposed research, a value added proposal submitted to a private foundation to conduct virome sequencing (that is DNA from the total viral population) of a methanotrophic microbial mat community was approved. These samples will be worked up in coming quarters from incubations conducted during this quarter and may provide up to 50 Mb of sequence data for the viral communities associated with methane consuming bacterial mats.

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. A second set of (6) samples was collected during the third quarter of this award and used in support of task 3.

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 13C 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. Samples collected during the third quarter of this award validate the linear uptake of methane and incorporation of 13C into biomass, and provide rates for each mat collected at COP.

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 were received in the third quarter and analysis began. Analyses are also being conducted with additional samples collected during the third quarter of this award.

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 13C-methane was added to samples from the COP field in preparatory experiments for subtask 5.1. Analyses conducted during the second quarter indicate 13C uptake into biomass, and centrifugation appears to have separated

13C-DNA. The different fractions of DNA were sent to LBL for phylochip analysis, and results were received late in the third quarter. These results are presently being analyzed.

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

Sampling of Santa Barbara waters was conducted during the third quarter. Collection of depth distributions was inhibited by an equipment malfunction, but surface water samples were collected. Depth distributions should become available beginning in the fourth 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

Methane oxidation rates and turnover times were analyzed during the third quarter for surface waters of the Santa Barbara Basin. Data are expected during the fourth quarter.

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

Unusual results from studies in Alaskan lakes hint that methanotrophs may be capable of isotopic exchange, which is important for the interpretation of our rate measurements, particularly with low oxygen conditions. We are initiating laboratory tests to determine whether exchange is possible.

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 during the second quarter of this award. Mr. Paul brought a number of samples with him to work up for analysis, and initial results were received late during the third period of the award. 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 Ms. Monica Heintz participated on a field excursion to Alaska during the third quarter. She quantified methane oxidation rates and collected samples for DNA analysis of the microbial community. The results show staggering potential for methanotrophy in the frozen lakes and will be compared with summer conditions during a forthcoming expedition to the site scheduled for the fourth quarter. Both collaborations add value to current DOE projects.

One value-added proposal submitted to the Moore Foundation's Marine Microbiology Initiative during the previous quarter was approved. This proposal is for a viral metagenome associated with methanotrophic microbial mats. This will add value to this DOE-funded research as it promises to reveal the genomic content of the viruses predating methanotrophic mats, potentially including functional genes important to the community. Sample work-up on mats began in the third quarter, with DNA due in the fourth 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

Field sampling and laboratory studies began in earnest during the present review period enabled by favorable summer conditions and the receipt of needed equipment. Initial scientific results showing linear rates of methanotrophy and 13C-methane uptake were validated for benthic mats at Coal Oil Point and water column samples from shallow waters of the Santa Barbara Basin and the Eastern Tropical North Pacific were analyzed, Important collaborations to this and other DOE projects were advanced, and one field expedition 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 received, but have not all hit the ledger during this period.

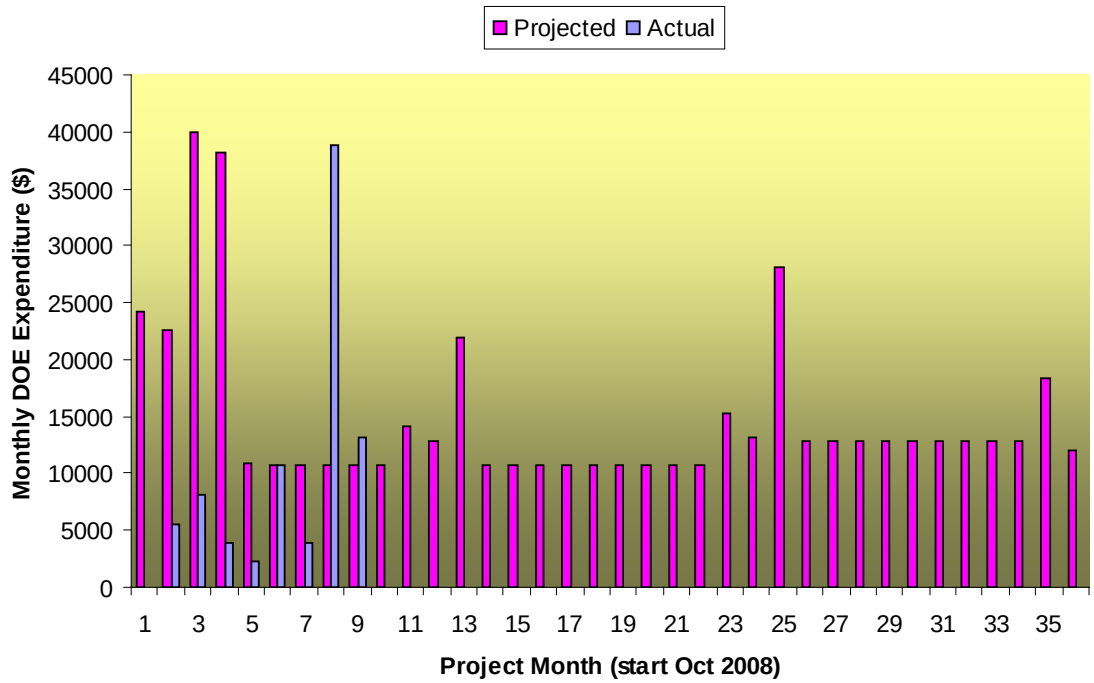

Figure 1. Project costing profile

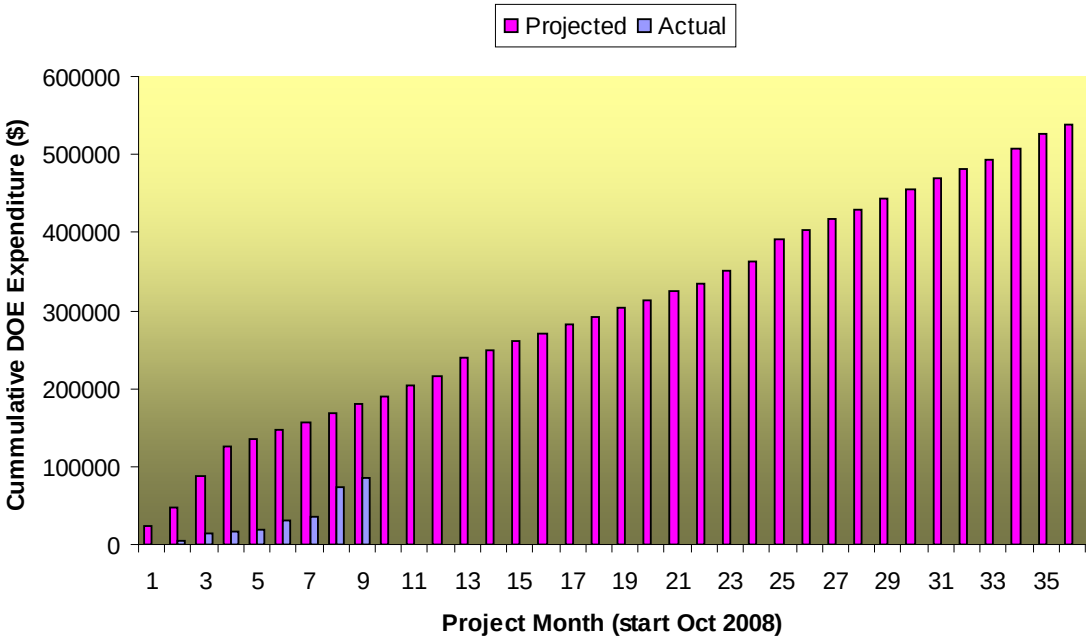

Figure 2. Project cummulative costs

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 ordered with arrival of the last item early in the third quarter. Missing the fall 2008 field season because of the delay in award announcements relieved the rush on these items. All items were put into use immediately, but the CTD Rosette had significant problems. The freezer unit was warped and gradually became unable to hold temperature. This unit did not function properly, with sample bottles not triggering at depths below ~3m. Project personnel interacted extensively with the manufacturer to fix the issue, and after 4 failed sampling trips, the manufacturer sent a partial replacement unit. That unit is currently under testing, with good preliminary results. Final testing will be completed early in the next quarter and we expect this milestone to be completed in July 2009 as planned.

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 is now complete. Samples collected during the pre-award time and during the third quarter were used in pursuit of this milestone. Our results consistently demonstrate linear uptake rates of methane and incorporation into biomass. This has now been confirmed by direct quantification of methane and by quantification of $^{13}C\text{-}CH_4$ uptake. The consistent and rapid rates measured by these two means obviate the need for use of tritium. Completion of this milestone has set the stage for manipulative experiments with this system.

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, and recently submitted a draft dive plan including the retrieval of mats form the Santa Monica Basin and allowing for the planned water column studies.

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

→ Receipt and testing of all major equipment.
→ Initiated field sampling program at Coal Oil Point, including mats and waters
→ Confirmation that methanotrophic mat communities from Coal Oil Point oxidize and incorporate 13C-methane at a linear rate in laboratory incubations
→ Completed a second round of 13C-SIP incubations of mat samples from COP
→ Preliminary analysis of DNA Phylochip results from LBL.
→ Completion of a one-month expedition to the Eastern Tropical North Pacific, and preliminary analysis of methane oxidation rates for more than 100 samples.
→ Completion of a field expedition to study methane oxidation in Alaskan lakes, and preliminary data analysis

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 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. Additional delays during the third quarter of this award included two major equipment malfunctions, both of which are the fault of the manufacturer and are being rectified as such. These include an electrical glitch with the CTD system that prevented closure of bottles at depth, and warping of the -80 freezer that prevented it from holding temperature. Both items are expected to be fixed or replaced by the beginning of the 4th quarter of this award, but both caused delays in terms of futile effort and lost sampling expeditions.

PRODUCTS

→ Second Quarterly Report Submitted

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