

# Oil & Natural Gas Technology

DOE Award No.: DE-NT0005667

## Quarterly Progress Report

July 2009 – September 2009

### ASSESSING THE EFFICACY OF THE AEROBIC METHANOTROPHIC BIOFIL- TER 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 fourth quarter of this project was dedicated to field sampling and experimentation, culminating the SEEPS 09 cruise to methane hydrate sites off the coast of California.

During this period project personnel completed troubleshooting of project equipment including a CTD/Rosette sampling system, an ADCP current velocity profiler, and an ultra-low temperature freezer, each of which experienced technical problems upon arrival and two of which required hardware repair. Project personnel conducted several field exercises including shallow and mid-water sampling in the Santa Barbara Basin, an expedition to the Arctic in collaboration with another DOE-funded project, and a major field campaign to methane hydrate sites off the margin of Southern California.

The current quarter was dedicated to sampling expeditions with results expected in subsequent quarters. We were able to sample all proposed environments including mid-waters of the Santa Barbara Basin, deep waters of the Santa Barbara and Santa Monica Basins, as well as microbial mats from a methane hydrate site in the Santa Monica Basin. Proposed experiments were also initiated, namely quantification of oxidation rates for samples from the Santa Barbara and Santa Monica Basins, and stable isotope probing of microbial mats from an 800m deep methane hydrate site in the Santa Monica Basin.

In addition to the progress with the direct goals for this project, two value-added experiments were conducted, and several discoveries were made on the SEEPS 09 cruise. These activities occurred at no cost to DOE and included quantifying methane oxidation rates at one site in the Gulf of California, and at one site in Southeast Alaska. Results from these experiments will further add to our growing catalog of methanotrophic potentials for different environments. We also discovered and sampled a new methane hydrate site at 900m water depth in the Santa Monica Basin, as well as a significant seep field at 200-250m water depth in the Santa Barbara Basin.

## **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**

*Subtask 2.1 was completed during a previous reporting period.* During the current period subtask 2.2 was completed by through the collection of microbial mats from an 800m deep methane hydrate site in the Santa Monica Basin. Six plates were collected during a dive of the DSV Alvin, each harboring a microbial mat community. The plates had been exposed to methane at the sea floor for a period of 26 months. These plates were returned to the overlying vessel and the mat material used for experimentation. Additional mats were collected in the vicinity of the benthic plates, primarily by collecting rocks encrusted in mat communities. Task 2 is now complete.

### **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 current reporting period. Samples collected from the 800m deep methane hydrate site were incubated with isotopic label shipboard to assess the methane turnover rates, with results expected during a subsequent reporting period.

### **Task 4 - Molecular Analyses of Methanotrophs**

Additional samples were collected for molecular analysis of methanotrophs, but no additional experimentation was conducted during the current reporting period.

### **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 have confirmed consumption of the stable isotope label, with additional analyses anticipated in subsequent budget periods. We have begun experiments involving stable isotope probing of samples from the Santa Monica Basin (subtask 5.2). Samples collected during the SEEPS 09 cruise were incubated with  $^{13}\text{C}$ -methane for defined time intervals, with results anticipated in a subsequent 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**

Sampling of Santa Barbara Basin waters was conducted during the fourth quarter, addressing both Subtasks 6.1 and 6.2. Several trips were taken using a small boat during this budget period, focusing primarily on waters shallower than 250m. The depth interval between 190-250 meters has long proved enigmatic as it displays a persistent methane maxima, but with no known source. During this reporting period we occupied 13 new stations in order to better constrain the source of this maximum and to guide sampling for the SEEPS 09 expedition. During the SEEPS 09 cruise we sampled shallow and deep water extensively, occupying ten additional stations in the Santa Barbara Basin.

### **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**

Samples were collected during this reporting period and analyses in support of each subtask are underway. Several hundred samples were collected both from small vessels and during the SEEPS 09 cruise, and incubations performed immediately to quantify methane turnover. Measurements for methane concentration are ongoing with results expected during a subsequent budget 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**

Current velocity data was collected during this 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 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**

Extensive water sampling was performed during this reporting period, including those samples described above for the Santa Barbara Basin, as well as from a dozen additional stations located elsewhere along the Southern California Margin. Additional water sampling was targeted to the mid-water methane plume in the Santa Barbara Basin as

well as to the suboxic bottom waters of the Santa Monica and Santa Barbara Basins. Two additional Basins were also sampled, the San Pedro and Santa Catalina Basins, and open water reference stations were also sampled offshore from Monterey and at the base of the Patten Escarpment.

### **Task 11 - Sensitivity Testing of Methane Oxidation Rates**

Sensitivity studies were initiated during the SEEPS 09 expedition. Notably, the impact of temperature on oxidation was probed using samples from the Deep Santa Barbara Basin, which were incubated at five different temperatures to develop a temperature response curve. Also, sensitivity and versatility of the experimental method was tested by performing a sequential delay experiment to assess the impact of short term storage and transport on the consistency of oxidation rate measurements.

In addition to the formal tasks associated with the project, collaboration was continued with a DOE-funded group investigating methane release from permafrost environments, and Ms. Monica Heintz participated on a field excursion to Alaska again during the fourth quarter. She again quantified methane oxidation rates and collected samples for DNA analysis of the microbial community. Preliminary results show significant differences associated with ice coverage, and the results are being worked up for publication. This collaboration adds significant value to current DOE projects.

Two additional sampling expeditions were also conducted to quantify methane oxidation rates. The first expedition was to the Gulf of California, and quantified methane oxidation rates at a single station near active gas seeps in the Northern Gulf, offshore from San Felipe. The second expedition was to bubbling lakes and bays in southeastern Alaska, and methane oxidation rates were quantified in one bubbling lake. The one to two day delay between sampling and radiotracer injection, likely to be an issue at most locations, spurred us to assess the impact of transport and storage of samples on the quantification of rates, as mentioned under Task 11.

The value-added virome sequencing funded by the Moore Foundation's Marine Microbiology Initiative was continued with the submission of a viromic DNA associated with aerobic microbial mats. The sample was successfully submitted and is awaiting sequencing. This occurred at no cost to DOE, and provides a significant value added aspect to the proposal with metagenomic data from methane-consuming mats.

The SEEPS 09 cruise provided a platform to achieve sampling goals, and also led to important or useful discoveries and data products of direct benefit to the methane hydrate program. A new major seep field was discovered and sampled in the Santa Barbara Basin, and will greatly improve the accuracy of the methane budget being developed for this basin. A mound structure explored during the SEEPS 09 cruise also proved to be a hydrate-related feature. This mound is located at 900m water depth, contains free methane gas, and is covered in microbial mats. The feature is suspected to have formed from the crystallization of gas hydrate in the subsurface, similar to an arctic pingo. This site lies in suboxic water and holds potential as a readily accessible location for repeat sampling, being only 20 miles from the port of Los Angeles. In colla-

boration with other investigators, we also applied in-situ mass spectrometry to our explorations during the SEEPS 09 cruise. Three instruments were utilized, one on the ship's CTD system, one mounted to the DSV Alvin, and a third mounted to the AUV Sentry. These spectrometers quantify the relative concentration of methane and provided methane heat maps for several deep sites along the California margin. Experimentation with the AUV and mass spectrometer showed that the AUV could use the mass spectrometer to find methane seeps and to conduct fine resolution sampling of them – in an automated fashion. While not a direct goal of this project, these aspects of our work provide a value-added capacity to the hydrate program.

## **Conclusion**

Extensive field investigations were conducted during the present reporting period, including sampling from small vessels as well as the SEEPS 09 expedition. The water and microbial mat samples collected are being used to investigate the methanotrophic potential of waters from the Santa Barbara Basin and surrounding areas of the California borderland, as well as mats that cover methane hydrate sites in the deep Santa Monica Basin. Results from these measurements and experiments are expected during coming reporting periods. The discovery of significant seeps areas in the Santa Barbara Basin will greatly improve the methane budget being generated for this area.

# COST STATUS

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

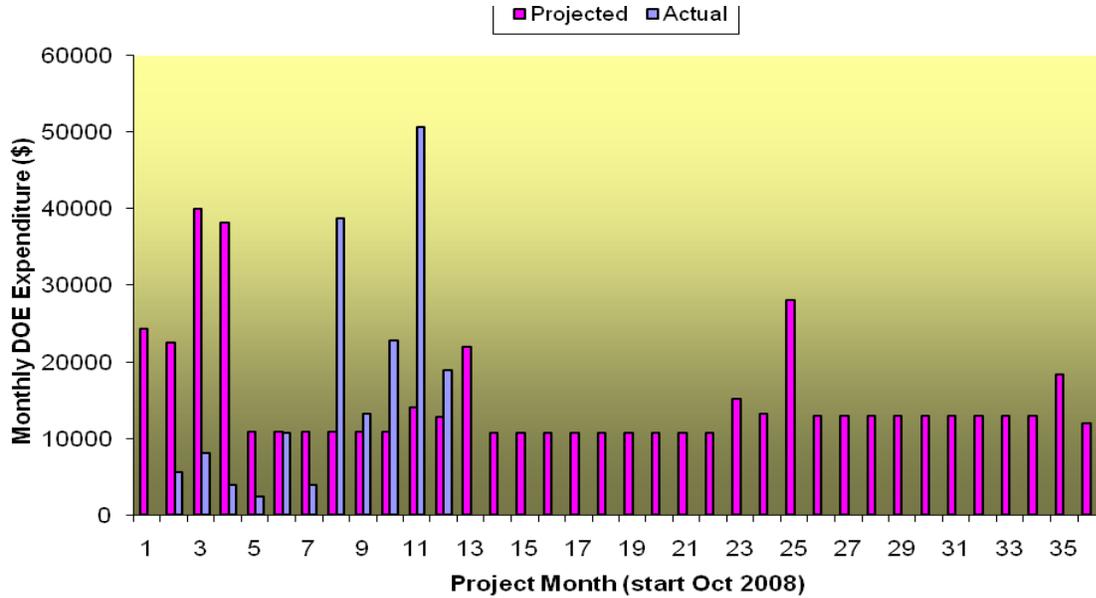


Figure 1. Project costing profile

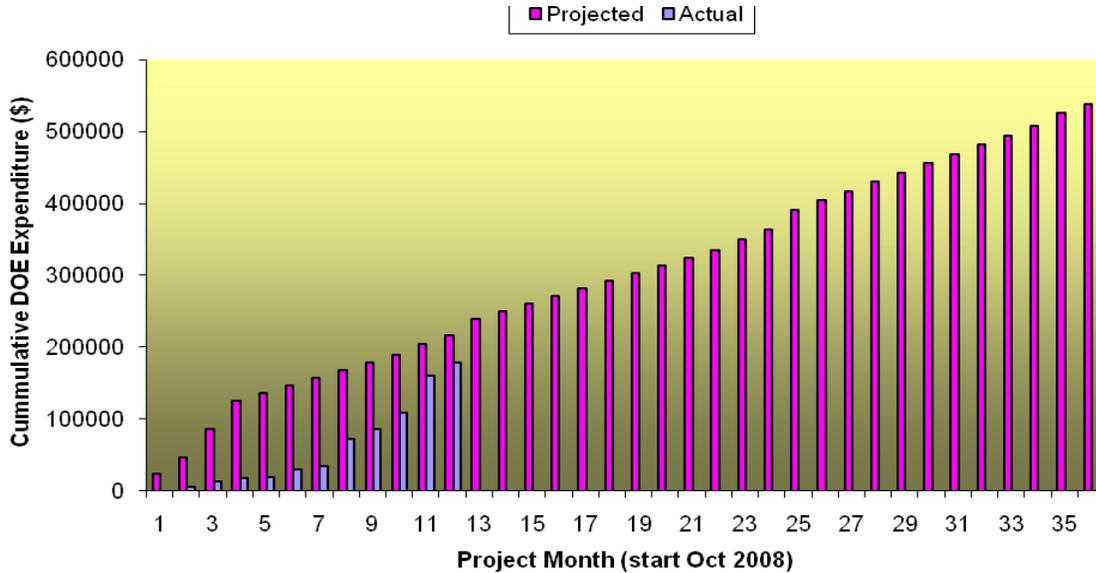


Figure 2. Project cumulative costs

## 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 previously received and are now functioning and were used for sampling during this reporting period. This milestone is now complete.

*Milestone 2:* Confirmation of  $^3\text{H-CH}_4$  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: 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 during this reported 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: 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**

- Extensive field sampling of waters from the Santa Barbara Basin
- Collection of Microbial Mats from the Santa Monica Basin
- Collection of water samples from select locations in the southern California borderland including several offshore basins and reference waters.
- Initiating oxidation rate measurements from collected waters and mats
- Completion of the SEEPS 09 Expedition
- Completion of a field expedition to study methane oxidation in Alaskan lakes, and preliminary data analysis
- Discovery of new gas seeps and hydrate-related features in the Santa Barbara and Santa Monica Basins

## **PROBLEMS OR DELAYS**

We are progressing well and would be on schedule if not for initial project delays.

## **PRODUCTS**

- Third Quarterly Report Submitted

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