

# Oil & Natural Gas Technology

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## Quarterly Progress Report

January 2011 – March 2011

### 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 ninth quarter of this project was dedicated to the continued work-up of data resulting from a series of cruises to the Santa Barbara and Santa Monica Basins, the Gulf of Mexico, and an Alaskan lake, to the development, submission and publication of manuscripts resulting from expeditions to study the methane plume originating from MC 252, and Alaskan lakes, and to the publication of a Review on Marine Methane Biogeochemistry.

During this period project personnel focused on the continued analysis of data associated with several aspects of this project, including the analysis of DNA sequence information from samples collected from the Santa Monica Basin, and a lake on Alaska's north slope. Analyses have focused on the identities of the bacteria and archaea active at these locations, along with functional genes used to activate and catabolize methane. Analyses have been expanded to include control locations needed for publication of this research to the peer review literature. Project personnel have also been analyzing data collected from the Santa Barbara Basin in preparation for submission of a manuscript.

In addition to progress with the experimental aspects of this project, one related presentation was given by the PI at Caltech, two papers were published, one in the journal *Science*, and a third paper was submitted to the journal *Continental Shelf Research*.

## **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**

*Task 3 was completed during a previous reporting period.*

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

We continue to develop our approach for molecular analysis of methanotrophs and during this reporting period we published another paper that included our methodology (Kessler, Valentine et al, Science, 2011). We also continue with molecular analyses with methanotrophic mats from Coal Oil Point, from our SIP experiments, and with isotopically depleted mats from the Santa Monica Basin. Specifically we conducted additional analyses of 16SrRNA genes from clones for identification of abundant organisms in both samples, analyzed functional genes, and expanded our sample set to include reference samples that will be needed for publication of the results. We have also been working with additional samples from an Alaskan lake and are in the late stages of manuscript preparation, pending final analysis of 16SrRNA clone libraries from this site.

### **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**

Subtasks 5.1 and 5.2 were completed in a previous reporting period. We are presently analyzing several isotope labeling experiments with samples from the Gulf of Mexico in support of Subtask 5.3. Incubations with methane seemed to have only minimal activity, though uptake results are still pending. These results may be attributable to the fact that our sampling was conducted in June and again in September, which appear to bracket the bloom of methanotrophic bacteria, which appears to have occurred in July and August, as highlighted by the Kessler et al., (2011) paper in Science. We are working with the methane SIP data presently to determine if we can publish the experiments as part of a broader study on the metabolism of bacteria following the Gulf of Mexico oil spill.

### **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**

Subtasks 6.1 and 6.2 were completed in a previous review period. We hope to take additional samples from the bottom water of the Santa Barbara Basin, and are exploring various cruises of opportunity to acquire samples, which we consider Subtask 6.3. Subtask 6.3 is taking shape to include sampling of the bottom water should an appropriate opportunity arise. One potential opportunity is the SEEPS 11 cruise scheduled for September, 2011 with the ROV Jason and the AUV Sentry.

## **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**

We have initiated data analysis of methane oxidation rates and turnover times for methane in the Santa Barbara Basin. These data are being prepared for publication in the peer reviewed literature.

## **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. This data is being analyzed concurrently with data from Task 7 on an as-needed basis. We have brought in additional project personnel to work with us to analyze this data.

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

A draft budget was previously 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. This budget was revised and submitted as part of a paper to Continental Shelf Research. A budget incorporating data from the remainder of the Basin was initiated during a previous reporting period, and analysis continued in 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**

This task was completed in a previous reporting period.

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

These analyses were completed with expected publication in a Ph.D. dissertation during the next reporting period.

Project personnel focused their laboratory efforts on the continued processing of data from ADCP measurements in the Santa Barbara Basin and on the molecular biological analysis of methanotrophy in the mat communities from Coal Oil Point, the Santa Monica Basin, and the Gulf of Mexico.

Project personnel gave a presentation at the California Institute of Technology during this reporting period. Two papers were published during this review period, one in the journal *Science* (Kessler et al., 2011), and one in the journal *Annual Review of Marine Science* (Valentine 2011). The first paper, on which the PI is a co-lead author, developed the hypothesis that methanotrophs consumed all methane emitted from the Deepwater Horizon incident within a period of months after the flow of methane ceased. The rapid biological response is surprising given the slow inherent methane oxidation rates in the Gulf of Mexico, and raises fundamental questions about how the community can respond so rapidly to the input of methane. The second paper was a comprehensive review that considered marine methane biogeochemistry, with a focus on emerging questions, including those related to gas hydrate. Project personnel have also been analyzing data and preparing manuscripts for publication. The manuscript considering the fate of methane under ice in Alaskan lakes was furthered with additional analyses of clone libraries that should bolster the claims made in the manuscript, with submission expected in the next reporting period. A second manuscript considering methane oxidation in the Santa Monica Basin was also furthered with submission expected within 3-5 months.

## **Conclusion**

The current reporting period saw the continued analysis of archived samples and data, and the publication of two papers likely to garner broad interest. We anticipate a continued transition toward data analysis and publication as this award draws to a close.

# COST STATUS

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

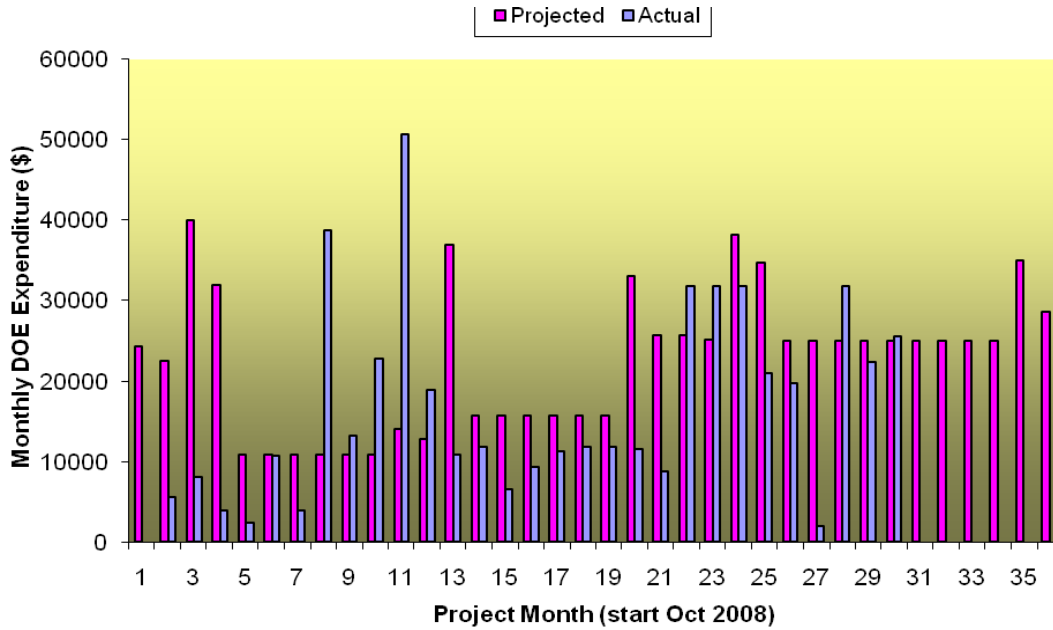


Figure 1. Project costing profile

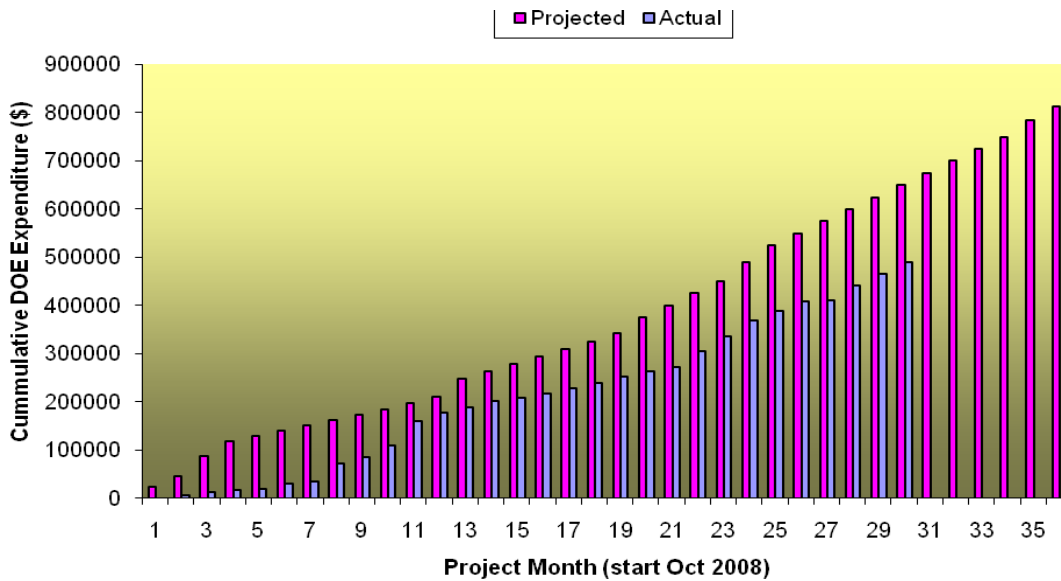


Figure 2. Project cumulative costs

## 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: This milestone was completed during a previous reporting period.

*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: 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: This milestone was completed during a previous reporting period.

*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: We have made substantial progress with the ADCP data and are integrating the data analysis with the preparation of a manuscript on the Santa Barbara Basin. The analysis of oxidation rate data is completed. We anticipate completion of this milestone by 9/30/11.



*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: Clone libraries have been generated for mats from both Santa Monica Basin and the Coal Oil Point seeps and sequencing has been conducted for 16SrRNA genes at both locations. Samples from the Santa Monica Basin showed virtually no indication of aerobic methanotrophs, and surprisingly indicated an anaerobic community of methanotrophs dwelling in the microbial mat. Clone libraries are now completed for SMB, and we are targeting nearby control samples presently to bolster our interpretation. This milestone is on schedule for the revised anticipated completion date of May 1, 2011 (note that the revised date of May 1, 2010 was in obvious error).

*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: We have completed the preliminary analysis of all physical and chemical data and are assessing whether the data collected to date is sufficient, or whether additional sampling is needed. This schedule is on task.

## **ACCOMPLISHMENTS**

- Published a paper in Science (Kessler et al., 2011) based on the Gulf of Mexico spill research, demonstrating the rapid timescale of methane consumption by bacteria in the deep Gulf of Mexico.
- Published a paper in the Annual Review of Marine Science highlighting the forefront of marine methane biogeochemical studies.
- Submitted a paper to the journal Continental Shelf Research highlighting a methane budget for the Coal Oil Point seep area and revealing a new methane source area in the Santa Barbara Basin.
- Gave a presentation at the California Institute of Technology on the fate of methane and other hydrocarbons released from Deepwater Horizon.
- Conducted dozens of radio, TV and print media interviews discussing the fate of methane from the Deepwater Horizon incident.

## **PROBLEMS OR DELAYS**

None

## PRODUCTS

→ Ninth Quarterly Report Submitted

→ Publication: \*Kessler, J. D.; \*Valentine, D. L.; Redmond, M. C.; Du, M.; Chan, E. W.; Mendes, S. D.; Quiroz, E. W.; Villanueva, C. J.; Shusta, S. S.; Werra, L. M.; Yvon-Lewis, S.; Weber, T. C., A persistent oxygen anomaly reveals the fate of spilled methane in the deep Gulf of Mexico. **Science** **331** 312-315. \*Co-lead authors.

Publication: Valentine, D.L. (2011) Emerging Topics in Marine Methane Biogeochemistry, **Annual Review of Marine Science** **3**: 147-171. doi:10.1146/annurev-marine-120709-142734.

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