

# Oil & Natural Gas Technology

DOE Award No.: DE-NT0005667

## Quarterly Progress Report

July 2010 – September 2010

### ASSESSING THE EFFICACY OF THE AEROBIC METHANOTROPHIC BIOFIL- TER 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

October 30, 2010



Office of Fossil Energy

## TABLE OF CONTENTS

Executive Summary.....	2
Progress, Results and Discussion.....	3
Conclusion.....	5
Cost Status.....	6
Milestone Status.....	7
Accomplishments.....	8
Problems or Delays.....	8
Products.....	9
Acknowledgment .....	9
Disclaimer.....	9
Appendix: PLUMES Cruise Update.....	10

## 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 eighth quarter of this project was dedicated to completing the field work in the shallow Santa Barbara Basin, to the continued work-up of the resulting data, to the development, submission and publication of manuscripts, and to rapidly working up results collected during the June, 2010 cruise to MC 252.

During this period project personnel focused on sampling in surface and mid-waters of the Santa Barbara Basin, completing six expeditions to different areas of the Basin in order to define methane sources in support of a basin-wide budget. Project personnel also focused on analyzing samples from these expeditions, most notably for methane concentration. Project personnel also demobilized from the PLUMES expedition during this reporting period, and analyzed over five hundred samples that were returned from the cruise. Project personnel further conducted cloning and sequencing with DNA from microbial mat samples from Santa Barbara Basin, Coal Oil Point, as well as the deep waters from the Gulf of Mexico, including the initial phylogenetic interpretation of sequence data from 16SrRNA clone libraries. Project personnel also worked on data analysis aspects of the project, including the preparation, submission and publication of a manuscript in Science Express based on the behavior of hydrocarbon gases in the Deep Gulf of Mexico, and publication of another paper on isotope probing of seep bacteria with methane and ethane.

In addition to progress with the direct goals for this project, one value-added DNA sequencing effort was continued, with the receipt of sequence information on viromes from both aerobic and anaerobic, methane-oxidizing communities. The sequence efforts are now completed with the Gordon and Betty Moore foundation releasing the final viromes to project personnel. Results will be worked up during subsequent reporting periods. A second value added opportunity was seized during this reporting period, which included participation of the PI and project personnel on a cruise to the Gulf of Mexico. This cruise provided an opportunity to observe the fate of the deep water methane plume that arose from the Deepwater Horizon incident as a follow-on to the PLUMES cruise, and occurred at no cost to DOE. Results are expected to provide insight as to the fate of methane released from large scale methane emission events, such as from gas hydrate, into the deep ocean.

## **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 our methodology in the Redmond et al., 2010 paper. 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 have conducted a second round of sequencing of 16SrRNA genes from clones for identification of abundant organisms in the Santa Monica Basin mat sample, and have collected additional information as to the biomarker distributions in these samples through an international collaboration.

### **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 completed several isotope labeling experiments with samples from the Gulf of Mexico in support of Subtask 5.3 after the PLUMES expedition and have conducted DNA extraction and gradient centrifugation on select samples. We also conducted SIP incubations during the PLUMES II expedition and will begin with DNA extraction in the next 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**

This reporting period covered the bulk of the summer field season in support of subtask 6.1. Trips were conducted either weekly or bi-weekly during July and August, number-

ing six in total. Stations included a number of re-occupations as well as new stations located in the Eastern portion of the Santa Barbara Basin. Now that the surface sampling in the eastern Santa Barbara Channel is completed we plan to resume sampling in the deep waters of the Basin in support of subtask 6.2. Subtask 6.3 will be assessed when the surface and deep sampling plans are completed.

## **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**

Data was collected in support of task 7.1 during this reporting period, but little data analysis was performed. Data analysis is expected to resume in the next 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**

No additional analysis of current velocity data was conducted during this reporting period. This analysis is expected to be performed concurrently with preparation of a budget manuscript for the Santa Barbara Basin.

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

No additional analysis was performed in support of Task 9 during this reporting period. This task will be taken up again in the next 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**

Subtask 10.1, 10.2 and 10.4 were completed during a previous reporting period. To address Subtask 10.3 we are targeting areas within the Santa Barbara Basin which have either not been sampled previously, or where novel methane sources were tentatively identified. More specifically, the former refers to the eastern channel area and the latter to the mid-channel trend. The summer 2010 sampling season targeted primarily these two areas.

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

Data resulting from sensitivity studies conducted in previous review periods was further analyzed during the current review period. These analyses are ongoing and are to comprise a portion of a Ph.D. dissertation written by project personnel. Additional sensitivity tests conducted in the Gulf of Mexico were also analyzed during this review period.

Project personnel focused their efforts during this reporting period on completing field research to the greatest extent possible, both in the Gulf of Mexico and in the Santa

Barbara Basin. Laboratory efforts focused on processing the remaining samples from the PLUMES expedition, and other efforts focused on the rapid publication of major results. With the field season completed, efforts will turn again to data analysis and manuscript preparation.

Project personnel have also been analyzing data and preparing manuscripts for publication. The Redmond et al 2010 paper was submitted in revised form to Applied and Environmental Microbiology and published then published during this reporting period. This manuscript details our SIP protocols and uses SIP to identify genes similar to methane monooxygenase that are associated with the oxidation of ethane and potentially propane. A second manuscript (Valentine et al., 2010) was prepared, submitted to *Science*, revised, and ultimately published in Science Express all during this reporting period. An additional manuscript is in the final stages of preparation which considers patterns and controls on methanotrophy in the Santa Monica Basin.

The value-added virome sequencing funded by the Moore Foundation's Marine Microbiology Initiative was completed during this reporting period, with the final batch of sequences provided to project personnel through the initiative. This has occurred at no cost to DOE, and provides a significant value added aspect to the project with metagenomic data from methane-consuming mats as well as isotopically-informed sequence information.

Project personnel also continued collaboration with other researchers funded through the methane hydrate program, making significant progress in drafting a publication on methanotrophy in arctic lake systems.

Project personnel also participated on a second cruise to the Gulf of Mexico. The cruise was a follow-on to the PLUMES cruise and while at no cost to DOE, allowed project personnel to better assess the fate of methane in the deep ocean following a massive release event. Results from this expedition are expected to dovetail nicely with the results from the PLUMES expedition and together provide the needed temporal behavior to understand the fate and impact of methane released suddenly into the deep ocean.

## **Conclusion**

The current reporting period saw the continued sampling in the Santa Barbara Basin, as well as analysis of samples from the Gulf of Mexico, as well as the publication of that data in the journal *Science Express*. With the sampling season ending project personnel will turn back to laboratory analysis of collected samples as well as 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.

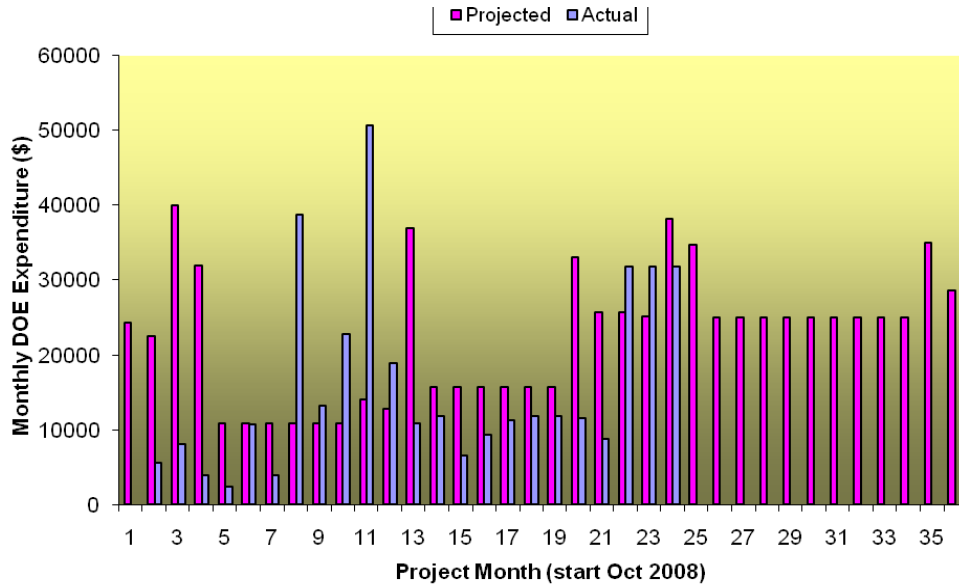


Figure 1. Project costing profile

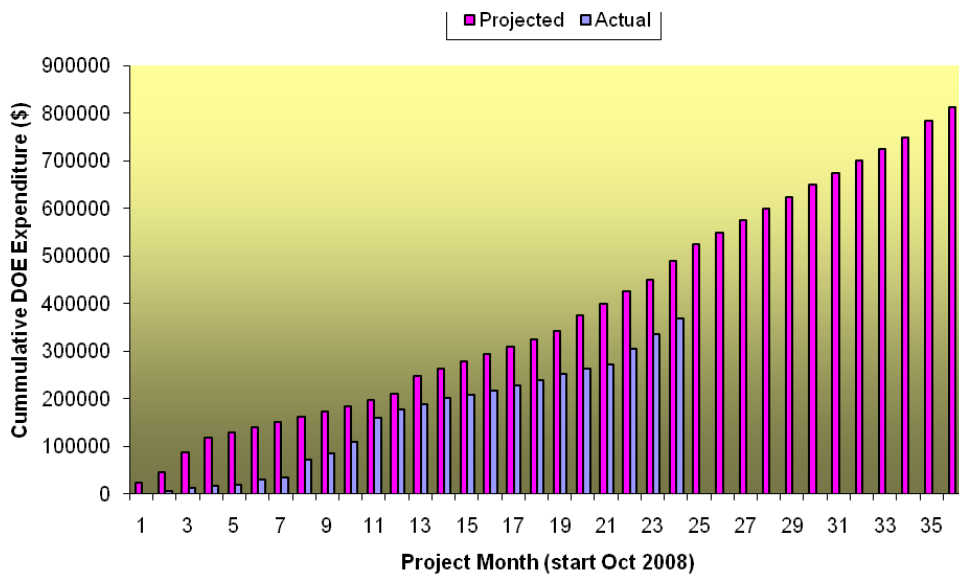


Figure 2. Project cumulative costs

## 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: Samples for oxidation rates have been analyzed now that all concentration measurements are completed. Progress with analysis of the ADCP has slowed on account of the extensive laboratory effort that followed the PLUMES cruise and the need for project personnel to conduct extensive trouble shooting with



field equipment during the 2010 summer field season. This research is behind schedule and will be conducted concurrently with the planned drafting of a manuscript covering the Santa Barbara Basin.

*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: All samples are now in-hand to address this milestone. Clone libraries have been generated for mats from both Santa Monica Basin and the Coal Oil Point seeps and sequencing efforts are underway for 16SrRNA genes. This research is on schedule.

*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: This research has not yet begun and is on schedule.

## **ACCOMPLISHMENTS**

- Published a paper (Redmond et al., 2010) identifying putative methane and ethane-oxidizing bacteria in seep environments.
- Published a cover image for the journal Applied and Environmental Microbiology related to Redmond et al., 2010.
- Published a paper in Science (Valentine et al., 2010) based on the Gulf of Mexico spill research, demonstrating that ethane and propane induced an immediate microbial response, followed by methane.
- Project personnel completed a Masters thesis (Farwell, 2010) including isotope studies of methanotrophy in the Santa Barbara Basin.
- Participated on a return cruise (PLUMES II) to the Gulf of Mexico to track the far-field fate of methane and constrain the timing of the methanotrophic response.
- Completed the 2010 field sampling season.
- Submitted abstracts to the Fall 2010 AGU meeting

## **PROBLEMS OR DELAYS**

We have experienced some minor delay with the analysis of ADCP, but do not see this interfering with progress on this work, as the data can be analyzed while the relevant results are being prepared for publication.

## PRODUCTS

→ Seventh Quarterly Report Submitted

→ Publication: Valentine DL, JD Kessler, MC Redmond, SD Mendes, MB Heintz, C Farwell, L Hu, FS Kinnaman, S Yvon-Lewis, Mengran Du, EW Chan, F Garcia-Tigreros, CJ Villanueva (2010) Propane respiration jump starts microbial response to a deep oil spill, **Science Express** (2010); DOI: 10.1126/science.1196830.

→ Publication: Redmond, MC, DL Valentine and AL Sessions (2010) Novel Methane, Ethane, and Propane Oxidizing Bacteria at Marine Hydrocarbon Seeps Identified by Stable Isotope Probing. **Applied and Environmental Microbiology** 76(19) 6412-6422.

**Acknowledgment:** "This material is based upon work supported by the Department of Energy under Award Number DE- NT0005667."

**Disclaimer:** "This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof."

Appendix : Summary of additional accomplishments for cruise CH-06-10.

Cruise Title: CH-06-10, PLUMES (Persistent and Localized Underwater Methane Emission Study).

Cruise Dates: June 11-21, 2010

Cruise Location: Departure from Gulfport Mississippi, scientific operations at MC 252, Arrival in Gulfport Mississippi.

Project Participants Involved: David Valentine (cruise participant); Monica Heintz (cruise participant), Chris Farwell (cruise participant), Stephanie Mendes (transportation), Sarah Bagby (data support). Other cruise participants included 8 scientists from Texas A&M University.

Other funding sources: The vessel time and other shipboard scientific projects were funded by the National Science Foundation.

Update: Complete work-up of the data from the PLUMES cruise was accomplished during this reporting period. This included isotopic analyses of all samples, including both natural levels and samples treated with stable isotope tracers, concentration analyses of hydrocarbon gases, as well as DNA extraction from bacteria followed by cloning and sequencing of 16S rRNA genes and mmo genes. These results were compiled into a manuscript that was submitted to Science during this project period. Expedited review and high interest also led to this paper being published in Science Express during this review period. Findings presented in this work indicate that methanotrophy occurred only slowly in the plumes of dissolved methane that surrounded the Deepwater Horizon spill, but that rates of methanotrophy began to increase as other hydrocarbon gases, notably ethane, became depleted.

Follow-on expedition: The PI and other project personnel were given the opportunity to participate on a second expedition to the Gulf of Mexico, focused on tracking the fate of methane after flow from the Macondo well had ceased. This cruise was funded by NOAA and occurred at no cost to DOE. This afforded project personnel the opportunity to investigate the fate of methane, the timescales of methanotrophy, and response of the methanotrophic community to massive inputs of methane to the marine subsurface. This cruise was completed at the end of September, and data will be worked up into the next reporting period.

## **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](http://www.netl.doe.gov)

Customer Service:  
1-800-553-7681

