

Oil & Natural Gas Technology

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Quarterly Progress Report

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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 and the Gulf of Mexico, and to the development, submission and publication of manuscripts resulting from expeditions to study the methane plume originating from MC 252, and Alaskan lakes.

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 Barbara Basin, Coal Oil Point seeps, the deep plumes from the Gulf of Mexico, 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.

In addition to progress with the experimental aspects of this project, four presentations were given at the Fall Meeting of the American Geophysical Union, two papers were published, one in the journal *Science*, and a third paper was submitted to the journal *Science*. Project personnel also participated on two value added cruises to the Gulf of Mexico to further track the fate of methane in the deep water.

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 two papers that include our methodology (Valentine et al., 2010; Redmond et al 2010), and submitted a second (Kessler, Valentine et al, submitted to Science). 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 are analyzing 16S rRNA genes from clones for identification of abundant organisms in both samples, and are also in the process of analyzing functional genes.

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.

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

Several dozen stations were occupied during the previous review period, and a preliminary analysis of the data indicates that sufficient samples have been collected in support of Subtask 6.1. This subtask is now considered complete. We conducted deep water sampling in the Santa Barbara Basin during this reporting, and the deep water sampling is considered complete. 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. Thus, Subtask 6.2 is now considered complete. Subtask 6.3 is taking shape to include sampling of the bottom water should an appropriate opportunity arise.

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 have now been completed for all samples collected on the SEEPS 09 cruise, and during the summer 2010 field season. Patterns of methane oxidation rates and turnover have been analyzed for the shallow and interior waters of the deep basin, and for the shallow water along the margins. We anticipate beginning data analysis with these oxidation rates during the next reporting period, in preparation for eventual publication.

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. As noted below we have run into difficulties with data quality at some depths and are developing alternative approaches and workarounds. Analysis of this data will continue into future reporting periods.

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 is being revised for submission to the peer reviewed literature. A budget incorporating data from the remainder of the Basin was initiated during a previous reporting period, and analysis will be continued 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

Subtask 10.4 – Gulf of Mexico Water Sampling

Subtasks 10.1, 10.2 and 10.4 were completed during a previous reporting period. To address Subtask 10.3 we targeted 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. This reporting period saw the completion of the summer 2010 field sampling, which included stations in both the eastern basin/shelf area, as well as the mid-channel trend. *Subtask 10.3 is now considered complete.*

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 will be included in a Ph.D. dissertation.

Project personnel focused their laboratory efforts on processing 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. Field efforts included completion of the 2010 summer field season, and two cruises of opportunity to track methane in the deep Gulf of Mexico.

Project personnel gave presentations at the Fall meeting of the American Geophysical Union during this reporting period. Four presentations were given, covering topics of hydrocarbon oxidation rates, methanotrophy in the deep Gulf of Mexico, and methanotrophy in Alaskan lake systems. One paper was published during this review period, in journal *Science* (Valentine et al., 2010). This work quantified the oxidation rates of methane, ethane and propane in the deep Gulf of Mexico following a rapid hydrocarbon irruption, and found methanotrophy to be sluggish relative to ethane and propane consumption. A second paper was submitted during this reporting period and tracked the fate of methane from the same irruption. Publication in *Science* is anticipated in the next reporting period. A third paper was published during this reporting period, in *Applied and Environmental Microbiology*, which outlines the molecular approaches to analysis of methanotrophic communities. Project personnel have also been analyzing data and preparing manuscripts for publication. The first of these manuscripts considers the fate of methane under ice in Alaskan lakes with submission expected in the next reporting period.

The value-added virome sequencing funded by the Moore Foundation's Marine Microbiology Initiative was continued, with value added efforts presently focused on quality control and interpretation of the metagenomes. Subsequent steps will include the search for functional genes associated with methanotrophy.

Conclusion

The current reporting period saw the continued generation of data and its analysis and publication. This focus transitioned from field sampling campaigns in the Santa Barbara Basin and Gulf of Mexico to the analysis of the retrieved samples and the interpretation and presentation of the results.

COST STATUS

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

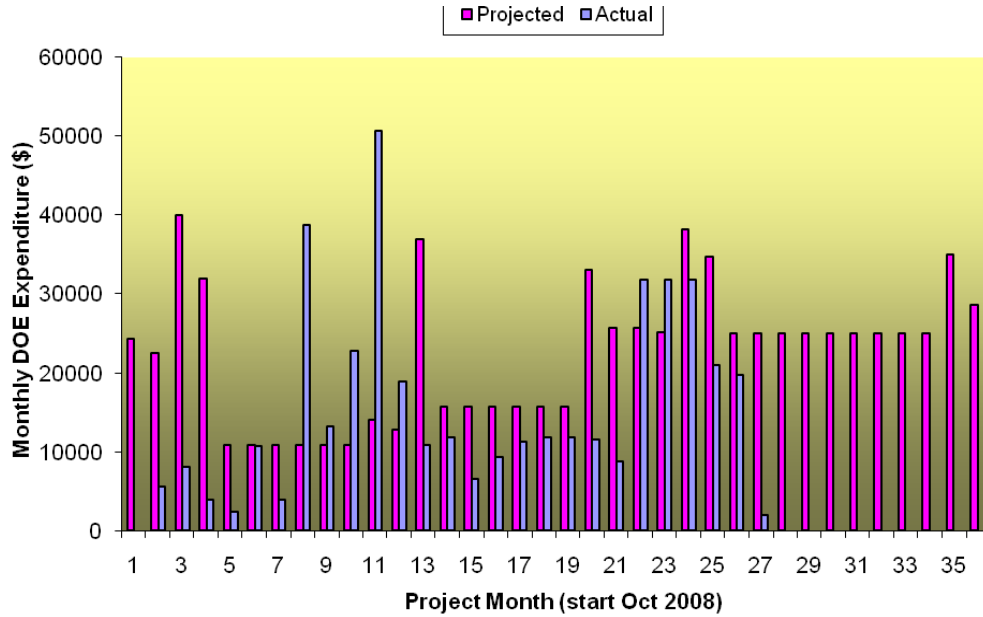


Figure 1. Project costing profile

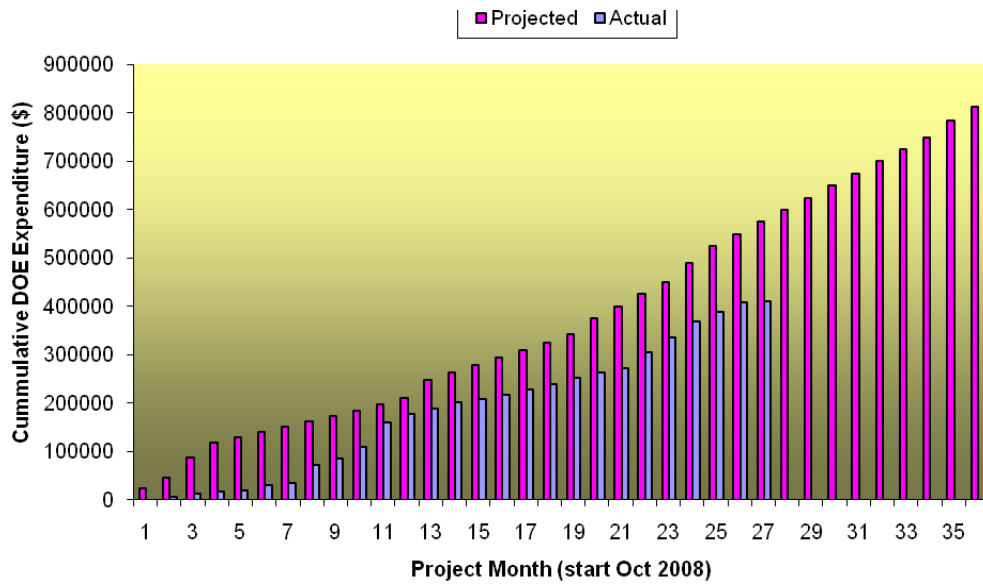


Figure 2. Project cumulative costs

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: Progress with analysis of the ADCP has slowed on account of the extensive analyses that followed the PLUMES cruise, the need for project personnel to conduct extensive trouble shooting with field equipment during the 2010 summer field season, and because of difficulties with quality assurance of data from 150-

250m depth. 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: 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 nearly completed for SMB, and we are targeting alternate genes for methanotrophy in the mat community. This milestone is slightly behind schedule with a revised anticipated completion date of May 1, 2010.

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 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.
- Participated on two value added return cruises to the Gulf of Mexico to track the far-field fate of methane and constrain the timing of the methanotrophic response.
- Submitted a paper to the journal Science describing the fate and impacts of sudden and massive methane release in the deep Gulf of Mexico
- Presented 4 papers at the Fall 2010 AGU meeting
- Conducted dozens of radio, TV and print media interviews discussing the fate of gases from the Deepwater Horizon incident.

PROBLEMS OR DELAYS

We continue to experience delays with the analysis of ADCP data, but are making progress with the analysis. The primary problem seems occurs with samples in the depth range of 150-250m, where the quality of data is poor. However, this range is im-

portant for methane-budgeting purposes as a significant seep feature was identified in this depth range. We are attempting various work arounds and fixes.

PRODUCTS

→ Eighth Quarterly Report Submitted

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

→ 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**; 330 208-211.

→ In press: 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**.

Abstract: **OS22B-07**. Microbial Community Response to the Deepwater Horizon Oil Spill. M. C. Redmond; D. L. Valentine; S. B. Joye, presented at 2010 Fall Meeting, AGU, San Francisco, Calif., 13-17 Dec.

Abstract: **OS33B-1476**. Microbial Consumption of Natural Gases Released from the BP Deepwater Horizon Oil Spill. S. D. Mendes; D. L. Valentine; C. Farwell, presented at 2010 Fall Meeting, AGU, San Francisco, Calif., 13-17 Dec.

Abstract: **GC51J-03**. Ice Cover Enhances Methane Consumption in Alaskan Thermokarst Lakes. M. B. Heintz; J. Pohlman; M. J. Wooller; M. Elvert; C. D. Ruppel; D. L. Valentine, presented at 2010 Fall Meeting, AGU, San Francisco, Calif., 13-17 Dec.

Abstract: **OS22B-02**. A Horizon of Natural Gas in the Deep Gulf of Mexico Dominates the Microbial Landscape (*Invited*). D. L. Valentine; J. D. Kessler; M. C. Redmond; S. D. Mendes; M. B. Heintz; C. Farwell; L. Hu; F. Kinnaman; S. A. Yvon-Lewis; M. Du; E. W. Chan; F. Garcia Tigreros; C. Villanueva. presented at 2010 Fall Meeting, AGU, San Francisco, Calif., 13-17 Dec.

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