Oil & Natural Gas Technology

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Quarterly Progress Report

July 2011 – September 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 eleventh 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 revision of manuscripts resulting from expeditions to study the methane plume originating from MC 252, methane in the basins offshore southern California, Alaskan lakes, and to a final oceanographic cruise to the Santa Barbara and Santa Monica Basins.

During this period project personnel focused on the revision of manuscripts for publications in a special issue of the Proceedings of the National Academy of Sciences on the Deepwater Horizon incident, on the revision of an article previously submitted to Limnology and Oceanography, and to the revision of an article previously submitted to Continental Shelf Research. We continue to polish a manuscript on methane oxidation in ice covered Arctic lakes and plan submission in the next period. We also continued to analyze data from microbial mats of the Santa Barbara and Santa Monica Basins. Project personnel also participated in a cruise aboard the R/V Atlantis to the borderland basins off Southern California, which included studies of methane in the Santa Barbara and Santa Monica Basins.

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.

<u>Task 3 - Turnover Rates for Methane Oxidation in Microbial Mats</u>

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 expand our approach for molecular analysis of methanotrophs and during this reporting period one of our manuscripts was accepted to the Proceedings of the National Academy of Sciences that highlights two of our approaches: stable isotope probing, and comparative community analysis by combined T-RFLP and clone libraries. We also continued our collaboration with the laboratory of V. Orphan (Caltech) to analyze mmo using alternative approaches including an intergenic spacer analysis and quantitative PCR. We have also conducted additional sequencing on samples from an Alaskan lake and continue manuscript preparation.

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

Task 5 was completed in a previous reporting period.

Task 6 - Field Measurements in the Santa Barbara Basin

<u>Subtask 6.1 - Shallow Water Sampling and Measurements, Santa Barbara Basin</u>

<u>Subtask 6.2 - Deep and Bottom Water Sampling and Measurements, Santa Barbara Basin</u>

Subtask 6.3 - Repeat Sampling, Santa Barbara Basin

Subtasks 6.1 and 6.2 were completed in a previous review period. We conducted additional sampling during the SEEPS 11 cruise during September, 2011 aboard the R/V Atlantis with the ROV Jason and the AUV Sentry. We repeated sampling as central locations in the Santa Monica and Santa Barbara Basins that included methane concentrations and methane oxidation rates. In collaboration with John Kessler and Rich Camilli we conducted surface surveys for methane concentration and sea-air flux throughout the Santa Barbara and Santa Monica Basins. Subtask 6.3 is now considered complete.

<u>Task 7 - Analysis of Methane Oxidation Rates and Methane Turnover Times</u> Throughout the Santa Barbara Basin

Subtask 7.1 - Shallow Water

Subtask 7.2 - Interior Water

Subtask 7.3 – Targeted Measurements

We continue with 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

<u>Subtask 8.1 – Current Velocity Analysis for the Shallow Santa Barbara Basin</u> <u>Subtask 8.2 - Current Velocity Analysis for the Deep Santa Barbara Basin</u>

Task 8 was completed in a previous reporting period

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

We continue to develop a draft methane budget for the Santa Barbara Basin. We also received favorable reviewer comments on the budget manuscript revised and submitted to Continental Shelf Research.

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

This task was completed during a previous reporting period.

Project personnel focused their primary efforts on data analysis and revising four manuscripts returned from peer review. Significant efforts were needed to prepare for the SEEPS 11 cruise, which occurred from Sep 13-29. Further data analysis was completed with continued analysis of sequence data from Alaskan lakes, and from microbial mats. We further analyzed samples using alternative approaches for analysis of mmo, and are assessing the utility of restriction fragmentation, quantitative PCR and single cell genomics to address questions of methanotrophic ecology.

Conclusion

The current period saw the continued analysis of data, the revision of multiple submitted papers, acceptance of one paper in the Proceedings of the National Academy of Sciences and a final research expedition to the Santa Barbara and Monica Basins. We continue to 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.

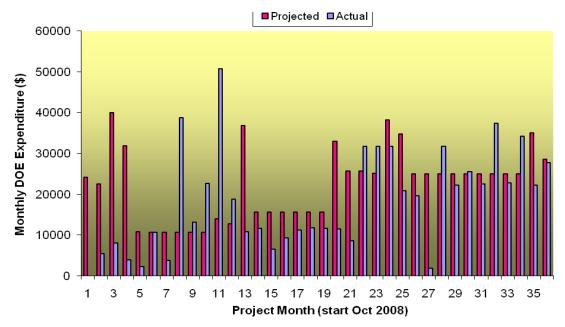


Figure 1. Project costing profile

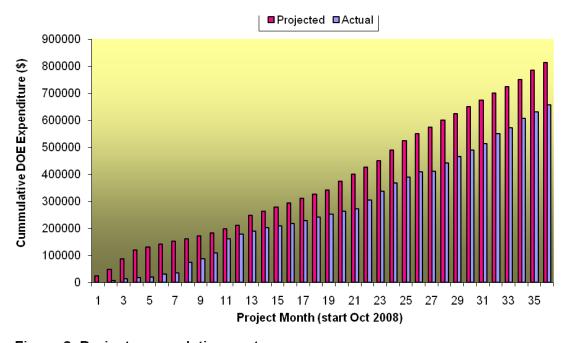


Figure 2. Project cummulative costs

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: This milestone was completed during a previous reporting period.

Milestone 2: Confirmation of ³H-CH₄ oxidation and ¹³C-CH₄ 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: The analysis of oxidation rate data was completed during a previous review period. During this review period we completed the preliminary analysis of the ADCP data from the SEEPS 09 cruise, with the goal of integrating this data into a

publication on the methane budget of the Santa Barbara Basin. This milestone is now considered complete.

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: This milestone was completed during a previous reporting period.

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 milestone was completed during a previous reporting period.

ACCOMPLISHMENTS

- → Completed the SEEPS 11 research cruise aboard the R/V Atlantis, which included conducting repeat methane oxidation rate measurements in both the Santa Barbara and Santa Monica Basins
- → Submitted a revised manuscript to the journal Proceedings of the National Academy of Sciences (Redmond and Valentine) highlighting the changes in microbial community structure that followed the irruption of hydrocarbon gases into the ocean following the Deepwater Horizon event. The manuscript was accepted and is now in press.
- → Submitted a revised manuscript to the journal Proceedings of the National Academy of Sciences (Valentine et al) developing a coupled physical oceanographic and microbial metabolic model to explain the patterns, timing and impacts of hydrocarbon release to the deep ocean during the Deepwater Horizon event.
- → Submitted a revised manuscript to Limnology and Oceanography (Heintz et al) on methanotrophy in the Basins offshore southern California.
- → Submitted a revised manuscript to Continental Shelf Research highlighting a methane budget along the Northern margin of the Santa Barbara Channel.

PROBLEMS OR DELAYS

None

PRODUCTS

→ Eleventh Quarterly Report Submitted

- → Manuscript revised and accepted for publication: Redmond MC and DL Valentine (in press) Natural gas and temperature structured a microbial community response to the Deepwater Horizon oil spill. Submitted to the Proceedings of the National Academy of Sciences, USA.
- → Revised and submitted: Valentine DL, I Mezić, S. Maćešić, N. Črnjarić-Žic, S. Ivić, P Hogan, V Fonoberov and S Loire (submitted) Dynamic auto-inoculation and the microbial ecology of a deep water hydrocarbon irruption Submitted to the Proceedings of the National Academy of Sciences, USA.
- → Revised and submitted: Heintz M, S Mau and DL Valentine (submitted) Oceanographic Control on Methanotrophic Potential in the Santa Monica Basin, Southern CA Borderland. Submitted to Limnology and Oceanography.
- → Revised and submitted: Mau S, M Heintz and DL Valentine (submitted) Quantification of CH₄ loss and transport in dissolved plumes of the Santa Barbara Channel, California. Submitted to Continental Shelf Research

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