

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 tenth 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, methane in the basins offshore southern California, Alaskan lakes, and to the publication of a new method to quantify methane oxidation rates.

During this period project personnel focused on the preparation of publications for a special issue of the Proceedings of the National Academy of Sciences on the Deepwater Horizon incident, on the submission of an article to Limnology and Oceanography, and the publication of two other articles including a technical response to our 2011 paper in Science, where we provide further evidence supporting our hypothesis that nearly all the methane from Deepwater Horizon event was consumed within a matter of months. 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.

In addition to progress with the experimental aspects of this project, one related presentation was given by the PI at as a plenary lecture at the American Society for Microbiology annual meeting in New Orleans, including multiple press appearances.

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 a paper defending our approach (Kessler et al., 2011) and submitted a paper that supports our previous published work (Valentine et al submitted to PNAS). We also collaborated with the laboratory of V. Orphan (Caltech) to analyze mmo using alternative approaches including an intergenic spacer analysis. We have also conducted additional sequencing on samples from an Alaskan lake and are in the late stages of 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

Subtasks 5.1 and 5.2 were completed in a previous reporting period. During this period we analyzed samples from the Gulf of Mexico and submitted a paper to the Proceedings of the National Academy of Sciences, presenting these results (Redmond and Valentine, submitted). We found little uptake of methane in Gulf samples, but we did find uptake of ethane, propane and benzene, consistent with the timing proposed in our previously published works. Subtask 5.3 is now considered complete.

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 plan additional sampling during the SEEPS 11 cruise scheduled for September, 2011 aboard the R/V Atlantis 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 completed in this reporting period. We have been using this data to define the movement of methane plumes in the Santa Barbara Basin and plan to include these analyses in a forthcoming publication.

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 and are awaiting reviewer comments on the budget manuscript currently in review at 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, and is now published in the Ph.D. dissertations of Dr. Monica Heintz (UCSB) and Dr. Mary Pack (UC Irvine), and in the recent publication by Pack et al (2011).

Project personnel focused their primary efforts on completing data analysis tasks and preparing manuscripts for preparation. Significant efforts were needed to meet the deadline for submission to the Proceedings of the National Academy of Sciences special issue on the Deepwater Horizon, and two manuscripts were submitted there. Efforts were also needed to complete a manuscript highlighting methanotrophy in the Santa Monica Basin and other basins offshore California, which was submitted to Limnology and Oceanography. Further data analysis was completed with 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 the various approaches.

Conclusion

The current period saw the continued analysis of data, the publication of two papers, and the submission of three papers likely to garner broad interest. 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.

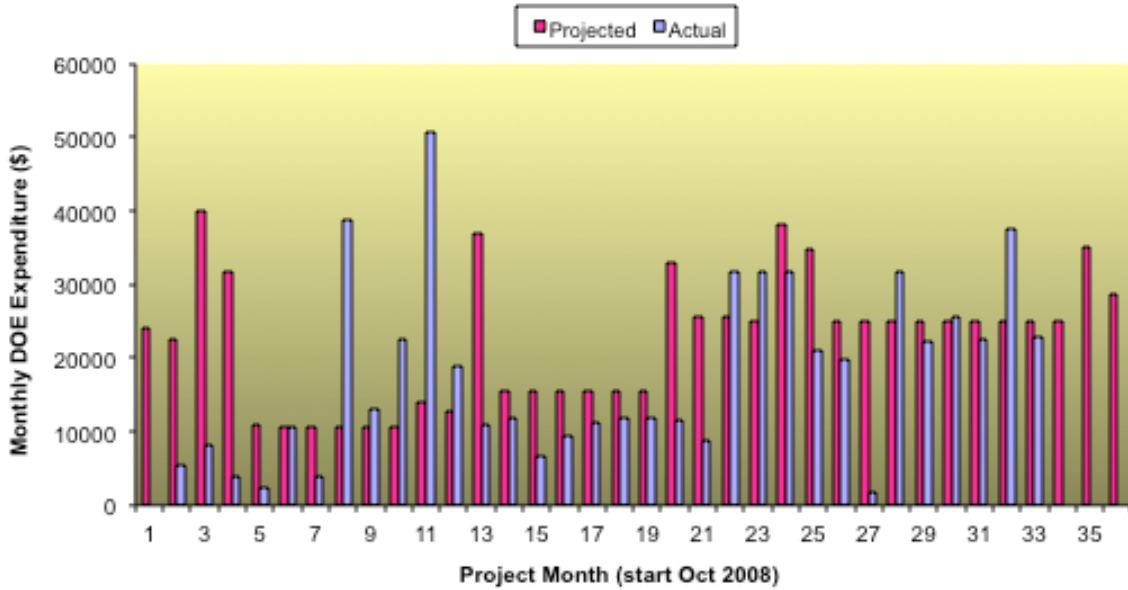


Figure 1. Project costing profile

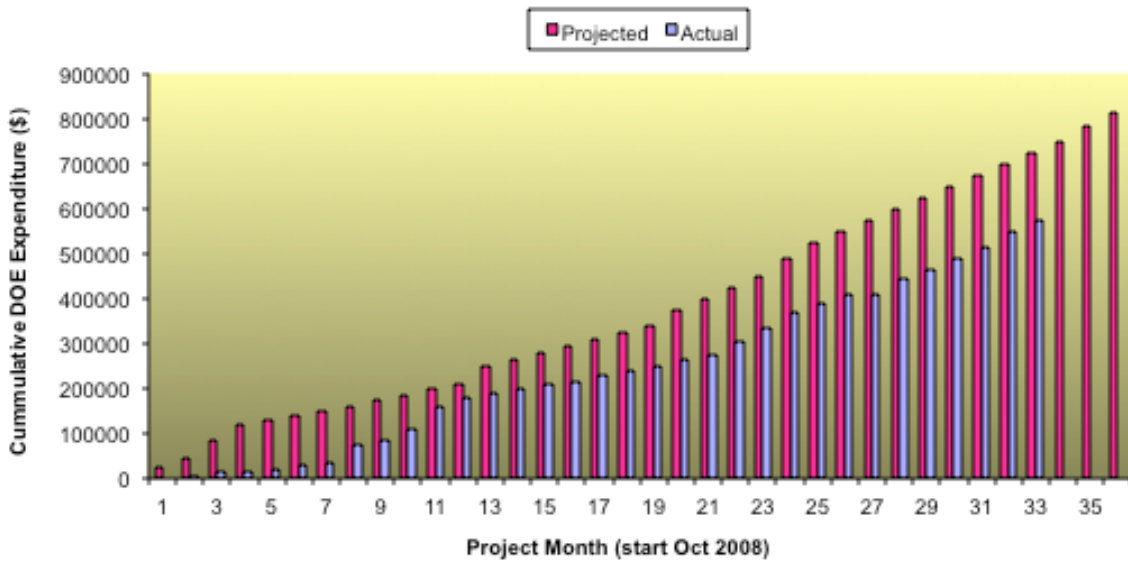


Figure 2. Project cumulative costs

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 were integrating the data analysis with the preparation of a manuscript on the Santa Barbara Basin. The analysis of oxidation rate data is completed. However, we were forced

to set this aside to allow project personnel to complete and submit manuscripts during this review period. 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: We have completed the preliminary analysis for mmo and 16SrRNA for Santa Monica Basin and Coal Oil Point seeps. This milestone is now considered complete. We are, however, exploring new approaches developed since our proposal was written, that provide significantly more information on mmo, and may apply these tools for ultimate publication.

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 have decided that we have sufficient data for further analysis. This Milestone is now considered complete.

ACCOMPLISHMENTS

- Published a peer reviewed response to a technical comment in Science (Kessler et al., 2011) based on the Gulf of Mexico spill research, further demonstrating the rapid timescale of methane consumption by bacteria in the deep Gulf of Mexico.
- Published a paper in the journal Limnology and Oceanography: Methods (Pack et al 2011) presenting a new approach to quantify the rates of biological methane oxidation in marine waters.
- Submitted a paper 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.
- Submitted a paper 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 paper to Limnology and Oceanography (Heintz et al) on methanotrophy in the Basins offshore southern California.
- Gave a keynote presentation at the annual meeting of the American Society for Microbiology on the fate of methane and other hydrocarbons following the Deepwater Horizon event.

→ Conducted several interviews for print media and a 30 minute webcast discussing the fate of methane and other hydrocarbons from the Deepwater Horizon incident.

PROBLEMS OR DELAYS

None

PRODUCTS

→ Tenth Quarterly Report Submitted

→ Submitted: **Redmond MC and DL Valentine (submitted) 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.**

→ 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.**

→ 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.**

→ Publication: **Kessler JD, DL Valentine, MC Redmond and M Du. (2011) Response to Comment on "A Persistent Oxygen Anomaly Reveals the Fate of Spilled Methane in the Deep Gulf of Mexico" Science 332, 1033 (2011); DOI: 10.1126/science.1203428**

→ Publication: **Pack MA, MB Heintz, WS Reeburgh, SE Trumbore, DL Valentine, X Xu and ERM Druffel (2011) A method for measuring methane oxidation rates using low-levels of ¹⁴C-labeled methane and accelerator mass spectrometry. Limnology and Oceanography: Methods 9. 245-260. DOI 10:4319/lom.2011.9.245**

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