Oil & Natural Gas Technology

DOE Award No.: DE-NT0005666

Quarterly Progress Report

July 1, 2011-September 30, 2011

Characterization of Methane Degradation and Methane-Degrading Microbes in Alaska Coastal Water

Submitted by:
School of Marine Science and Policy
University of Delaware
Lewes, Delaware 19958

Principal Investigator: David L. Kirchman

Prepared for:
United States Department of Energy
National Energy Technology Laboratory

October 31, 2011

Office of Fossil Energy

TABLE OF CONTENTS

EXECUTIVE SUMMARY

This National Energy Technology Laboratory (NETL) project consists of an expedition to the Beaufort Sea in September 2009 and analyses of samples collected from that expedition. An additional six months, from January 1, 2011 to June 30, 2011, was added to the project. Since June 30, the project has been in a no-cost phase. The main activity of the last quarter (the first half of the no-cost phase) was to continue the molecular analysis of microbes and genes coding for methane degradation enzymes in sediment cores collected during the cruise. The work done during the last quarter has two parts. The first part consists of continuing the analysis of the metagenomic data from low-methane and high-methane sediment cores collected during the Beaufort Sea cruise. This work is starting to reveal differences in the genetic makeup between the cores, although surprisingly, very few genes specific for methane degradation were detected. We suspect that the paucity of these genes in the metagenomic data is explained by the low abundance of methanotrophic microbes in these samples, even in cores with high methane concentrations.

The second part of the work has continued to focus on the gene for oxygen-independent degradation of methane (*mcrA*). We have been working on a quantitative PCR (QPCR) for this gene which will enable us to enumerate the numbers of anaerobic methane-degrading microbes in sediment core samples with various levels of methane. According to preliminary QPCR analyses, *mcrA* gene abundance is about an order of magnitude higher in methane-rich samples than in cores without any detectable methane. Ultimately, the metagenomic and 16S rRNA gene data will be combined with other microbial and biogeochemical data to obtain a complete picture of methane degradation and other biogeochemical processes in these methane-rich sediments.

Progress Report

Task 1: Project Management Plan

This task was completed.

Task 2: Cruise Logistics and Planning

This task was completed.

Task 3: Oceanic Cruise

This task was completed.

Task 4 - Methane Degradation Analysis

Methane degradation rates have been calculated, but detailed analyses and synthesis of the data remain to be done. The incubations for methane degradation were done on the ship as soon as possible after the sediment cores were available. The

incubations were then killed and subsampled on the ship. As planned, these subsamples have been analyzed in Germany by our collaborator, Dr. Tina Treude. We are in the process of comparing data sets.

Task 5 - DNA Sequence Analysis

This task continues to be the main focus of this phase of the project. During the end of the first year of this project, we had analyzed microbial and biogeochemical data from several sediment cores in order to select a couple for more detailed analyses by gene sequencing. The microbial data include genes for aerobic (*pmoA*) and anaerobic (*mcrA*) methane degradation. From these data, two cores were selected for detailed analysis of the 16S rRNA gene, which is used to identify microbes. About 40 samples were analyzed, a number set by DOE's Joint Genome Institute (JGI) who did the sequencing. As discussed in previous quarterly reports, data on the methane-rich cores were examined to select five samples for metagenomic sequencing, i.e. a complete list of genes found in the microbial communities.

The work done during the last quarter and continuing to the end of the project has two parts. The first part consists of continuing the analysis of the metagenomic data from low-methane and high-methane sediment samples collected during the Beaufort Sea cruise. These large data sets can be analyzed at many levels. One of the first things we did was look for the two diagnostic genes known to be involved in methane degradation (*pmoA* and *mcrA*). We were surprised to see very few sequences similar to these two genes (Table 1). The metagenomic analyses of all samples revealed only 9 and 15 sequences similar to known *mcrA* and *pmoA* sequences, respectively. These numbers are low compared to the total number of genomes (about a thousand) represented in the data set. We are continuing to analyze the metagenomic data sets, including searches for other genes possibly involved in methane degradation.

Table 1. Metagenomic sequences similar to genes involved in aerobic (*pmoA*) and anaerobic (*mcrA*) degradation of methane. "% Identity" refers to how similar the metagenomic sequence is to known genes.

gene	Methane Concentration	Number of Sequences	Range in % Identity
mcrA	High	4	63-90
	Medium	3	62-88
	Low	2	43-54
pmoA	High	4	55-76
	Medium	4	44-59
	Low	7	39-81

The abundance of *mcrA* and *pmoA* sequences is more accurately estimated by QPCR approaches. In brief, this involves using two segments of the targeted genes in a PCR assay in which formation of the products (the "amplicons") is monitored quantitatively. Before QPCR analyses, we sequenced the amplicons from regular PCR assays for both *pmoA* and *mcrA*. The sequence data indicated that the *pmoA* PCR assay for these samples at least yielded false positives. The amplicons were not similar to known *pmoA* genes. The assay for *pmoA* and will not be pursued further because of the false-positive and because we do not expect aerobic oxidation of methane to be important in these anoxic sediments.

However, the *mcrA* assay was confirmed by the sequencing data, and our initial QPCR assays are encouraging (Table 2). We found that the abundance of *mcrA* genes in the methane-rich core (PC12) was about 10-fold higher than in the methane-poor core (PC10), with the exception of one sample 323 cm. These data have implications for relationship between abundance and activity of these methane-degrading microbes and for the time scale of methane release in these sediments.

Table 2 Abundance of the anaerobic methane degradation gene (*mcrA*) in low (PC10) and high (PC12) methane cores.

Core	Depth (cm)	Mean (pg/ul)	SD
PC10	2	0.101	0.003
	110	0.017	0.002
	190	0.280	0.024
	323	1.408	0.152
PC12	20	2.165	0.232
	70	1.679	0.164
	130	5.393	0.587
	505	3.307	0.227

Other Tasks

The remaining parts of Task 4 and Tasks 5-7 are scheduled to be completed during the final months of the project.

Conclusions

Preliminary analyses of the metagenomic and tag data indicate the potential of this approach for revealing new insights into methane degradation and related processes, like sulfate reduction. We are making process in analyzing the metagenomic data and refining the QPCR assays, and we anticipate writing up these results for publication over the upcoming months. This information will provide more insights into methane degradation in the Beaufort Sea.

Cost Status

This project is now in a no-cost period. The work discussed here used supplies purchased during previous periods of the grant. The labor was supported by matches from the University of Delaware.

Products

Gonsalves, M.-J., C. Fernandes, S. Fernandes, D. L. Kirchman, and P. a. L. Bharathi. 2011. Effects of composition of labile organic matter on biogenic production of methane in the coastal sediments of the Arabian Sea. Environ Monit Assess. DOI 10.1007/s10661-011-1883-3

• Revised Web site

A Web site outlining work in the Arctic by Kirchman lab, including the NETL project (http://www.ocean.udel.edu/cms/dkirchman/Arctic/), has been revised.

• Contacts and lectures with the general public

Kirchman's seminars on climate change in polar environments are available on his Arctic web site.

• Inclusion of information and pictures from the Beaufort cruise in a general course for undergraduates and graduate students on marine biology and biological oceanography taught by Kirchman.

Acknowledgment: This material is based upon work supported by the Department of Energy under Award Number DE-NT0005666.

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

Customer Service:
1-800-553-7681