

# Oil & Natural Gas Technology

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## Quarterly Progress Report

April 1, 2011-June 30, 2011

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

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## TABLE OF CONTENTS

Executive Summary .....	1
Progress Report .....	1
Conclusions.....	3
Cost Status.....	4
Products .....	4

## 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. The main activity of the last quarter (the second half of the supplemental period) was to continue the molecular analysis of microbes and genes coding for methane degradation enzymes in sediment cores collected during the cruise. We obtained all of the metagenomic sequence data in January 2011 and submitted those data to the MG-RAST metagenomics analysis server ([metagenomics.anl.gov](http://metagenomics.anl.gov)) in February. The preliminary analyses by MG-RAST include quality control filters, dereplication, and annotations. Three of the five samples were analyzed by MG-RAST last quarter, and we just received the results from the remaining two samples. The delay is due to the transition from MG-RAST v2 to v3, which occurred when we submitted our data. Final analyses can now begin.

The second half of the supplemental period was also spent analyzing two specific genes involved in methane degradation, one for oxygen-dependent degradation (*pmoA*) and the other for oxygen-independent degradation (*mcrA*). In the previous quarter of this project, we found that the PCR assay was positive for not only the *mcrA* gene, which was expected, but surprisingly also for the *pmoA* gene deep into the core, long after oxygen had become undetectable. Subsequent sequence analyses, however, indicated that the *pmoA* results were false-positives. The *mcrA* results appear to be correct. 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. During the last quarter, the milestones for the project were revised and resubmitted to reflect the delay in receiving the second year of funds.

### 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.

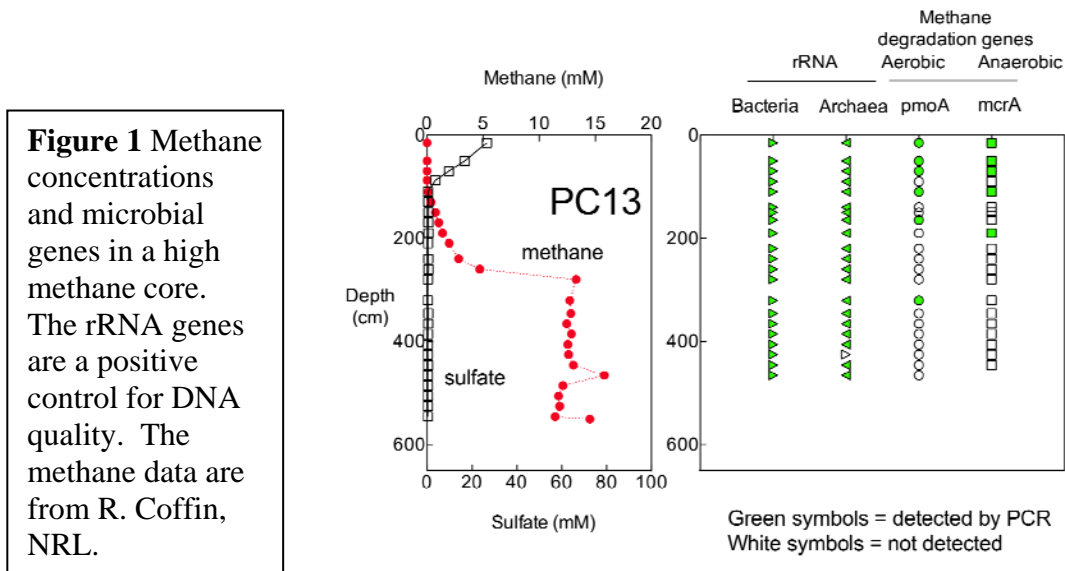
The final metagenomic sequence data were delivered to us by JGI in January 2011 and submitted to the MG-RAST metagenomics analysis server ([metagenomics.anl.gov](http://metagenomics.anl.gov)) in February. The annotations of metagenomes from three of the five sampling depths, including 20 cm, 90 cm and 170 cm, were completed last quarter, and the remaining two samples from 260 cm and 485 cm were just completed last month (late July 2011). Our data were submitted during the transition from MG-RAST server v2 to v3 and that caused some delay in the annotation of our metagenomes. Although we have done some analysis with the data from the first three samples, final analyses can begin now that we have data from all of the samples.

We had to wait for the MG-RAST analyses because we do not have the computation power to carry out these preliminary but essential analyses of the sequence data. MG-RAST does a quality filter, dereplication and annotation, including phylogenetic classification and functional classification. The MG-RAST annotation resources include the SEED, KEGG, GO, INSDC, COGs, eggNOGs and IMG data bases. In addition to classification, the annotation generates abundance profiles for COG categories, SEED subsystems as well as Kegg pathways. Identification of protein coding genes was accomplished using the FragGeneScan gene caller to identify the most likely reading frame and frame shifts for each sequence. The similarity comparisons were then performed on the translated sequences, which is the most evolutionarily sensitive and computationally efficient approach.

While waiting for the MG-RAST analyses, we continued to work on the PCR analyses for specific genes involved in methane degradation. As described in previous quarterly reports, we use PCR assays to examine two key genes related to methane degradation: particulate methane oxidase (*pmoA*), which is used during oxygen-dependent (aerobic) methane degradation, and methyl coenzyme M reductase A

(*mcrA*), which is used by oxygen-independent (anaerobic) degraders of methane. These two genes were detected only in the surface layer in core PC10 which had low methane, even though bacteria and archaea were detectable throughout the core. In contrast, both the aerobic and the anaerobic methane-degradation genes were detected deep into a core with high methane concentrations (Fig. 1).

To confirm these results, PCR products for both *pmoA* and *mcrA* were cloned and sequenced. As expected, the *mcrA* products were similar to known genes, confirming that the PCR assay was correct. Unexpectedly, none of the *pmoA* sequences from these sediment core samples were similar to known *pmoA* genes, implying that the PCR assay had given false-positives.



We are now working out methods for quantitative PCR (QPCR) detection of *mcrA* genes. For QPCR we have obtained positive results with another primer set (ME1 fwd and ME3rev) for *mcrA*. The PCR products from this primer set are now being sequenced to confirm their identity.

## 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, but it also pointed to problems with the standard automated sequence analyses. We also found problems with the standard PCR assay for *pmoA*. Slowly but surely we are making progress in solving the many technical problems with analyzing these data and samples, and we are now poised to complete this project. This information will provide more insights into methane degradation in the Beaufort Sea.

## Cost Status

The “original” indicated in the table below is one half of the funds requested for the supplemental six months of this project. Not shown in the table is the time commitment by Kirchman, supported by the University of Delaware, which was claimed as match to this project.

### Second Half of Supplemental Budget

	<u>Original</u>	<u>Actual</u>
Personnel	\$3,236	\$3,674
Benefits	1,045	1,187
Permanent Equipment	0	0
Expendable Supplies		0
Travel	0	0
Subtotal	\$4,281	\$4,861
Indirect costs (53%)	\$2,269	\$2,576

The actual costs of this second half of the supplemental time are very close to the costs originally estimated for this supplemental period. All of the personnel, especially Dr. Matt Cottrell, worked as much on this project as projected.

## 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.

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