Oil & Natural Gas Technology

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Quarterly Progress Report

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

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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. The main activity of the last quarter was to continue the molecular analysis of microbes and genes coding for methane degradation enzymes in sediment cores collected during the cruise. We obtained more metagenomic sequence data in October and November 2010 and are containing the analyses of these data. In addition to providing insights into methane degradation not otherwise possible, the new data could lead to modifications of the assays originally planned as part of the NETL project. Our preliminary analysis of the metagenomic data revealed a complete pathway for sulfate reduction at depths but less than half of the expected methane degradation genes in the metagenomic data analyzed so far. It is still unclear if these genes are being missed by standard metagenomic sequence analyses or are simply not present. We have also analyzed total prokaryote abundance by direct microscopy and abundance of bacteria and archaea by quantitative PCR. 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 the previous quarterly report, 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 number of samples (five) was set by JGI. The data used to select these samples included methane and sulfate concentrations and PCR-based assays for methane degradation genes (Fig. 1). Surprisingly, genes for aerobic methane degradation (*pmoA*) were found through the sediment core, even in the top layer were sulfate reduction is high (data not shown) and in the bottom layers were oxygen is absent. In contrast, genes for anaerobic methane degradation (*mcrA*) were detectable only from about 75-225 cm, even though this process was measurable by 14C-degradation experiments. It is not clear if these genes are truly absent or if there is a problem with the PCR assay; one potential problem is that the PCR primers, which were taken from previous studies, do not match the actual genes in these samples. The metagenomic data would help answer this question.

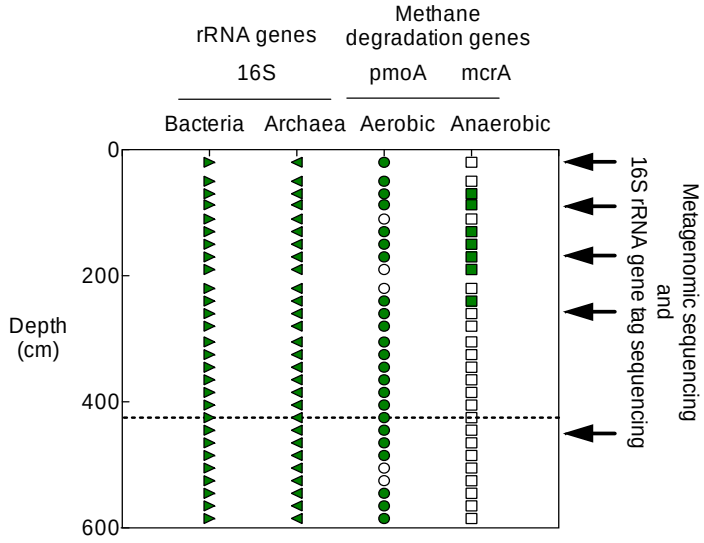

Figure 1. PCR-based assays for rRNA genes (for identifying microbes) and methane degradation genes. The filled-in symbols indicate a positive reaction.

We are still in the early stages of analyzing the metagenome data for genes involved in methane degradation, but a few observations can be made at this time using the standard JGI automated data analysis tools. The last quarterly report mentioned results for the aerobic methane degradation pathway. This report will highlight two other pathways. The metagenomic data revealed all of the genes involved in dissimilatory sulfate reduction (Figure 2). This pathway is significant, because it is the major pathway for the mineralization of organic carbon in anoxic environments and because some sulfate reducing bacteria are involved in anaerobic methane degradation, the second

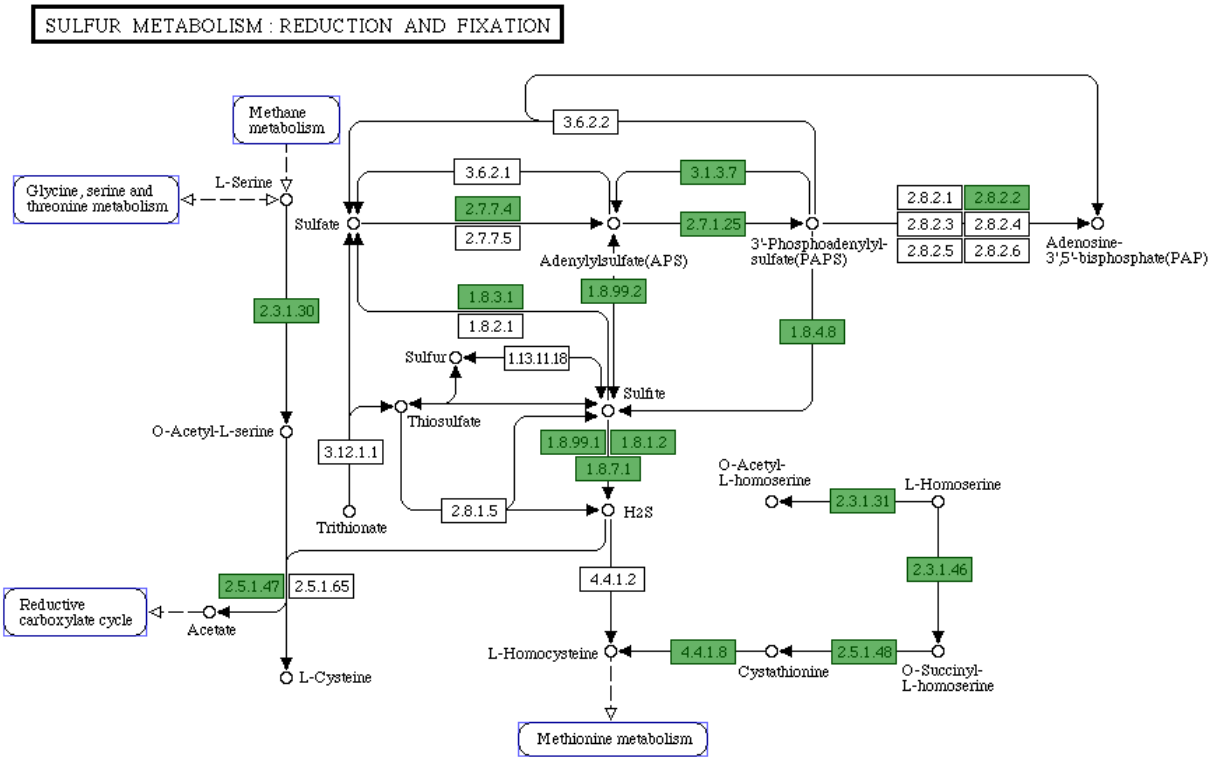

Figure 2. The pathway for sulfate reduction. The green-highlight boxes indicate enzymes found in the metagenome at 20 cm.

pathway to be highlighted here. We were able to find some genes for this methane degradation pathway (Figure 3), but not key ones, such as methyl-coenzyme M reductase (MCR) and methyl H_4MPT:CoM methyltransferase (Mtr).

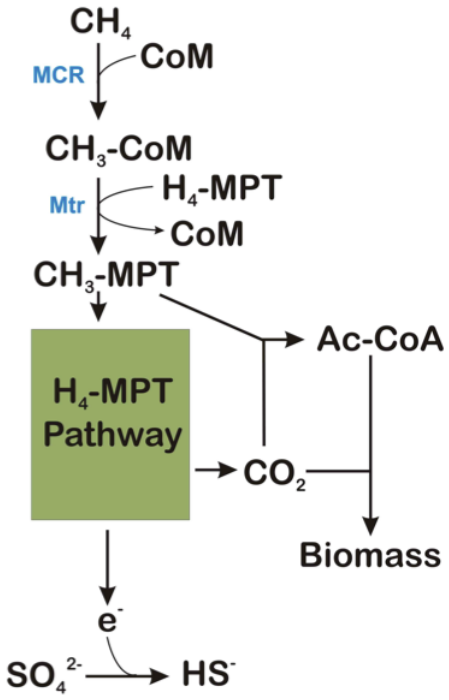

Figure 3 Pathway for anaerobic degradation of methane. Some of the genes for this pathway have been found, but not the key ones, such as such as methyl-coenzyme M reductase (MCR) and methyl H_4MPT:CoM methyltransferase (Mtr).

It is quite possible that the standard MG-RAST annotator used by JGI is missing the unusual genes for anaerobic methane degradation.

A second type of work carried out during the last quarter provides some basic data on about the abundance of the main prokaryotic groups in the sediment cores being sequenced. These data are needed for interpreting the metagenomic data and the methane degradation rates. The data per se also provide some clues about the microbes carrying out methane degradation and other biological processes. One set of analysis is based on quantitative PCR (QPCR) of the 16S rRNA genes for both bacteria and archaea. The QPCR has been done, but the data have not been analyzed. A more basic analysis is total number of prokaryotes counted by epifluorescence microscopy. This analysis is routine many samples, but very difficult for these sediments, and there is much error (Fig. 3). There is some indication of higher abundance in the top layer of the sediment core, reflecting higher biological activity. It is harder to explain the abundance maximum at the bottom of the core, although this maximum may not be real due to the variation in the data. Other cores will be examined to determine if the pattern observed in this core (PC10) is seen elsewhere.

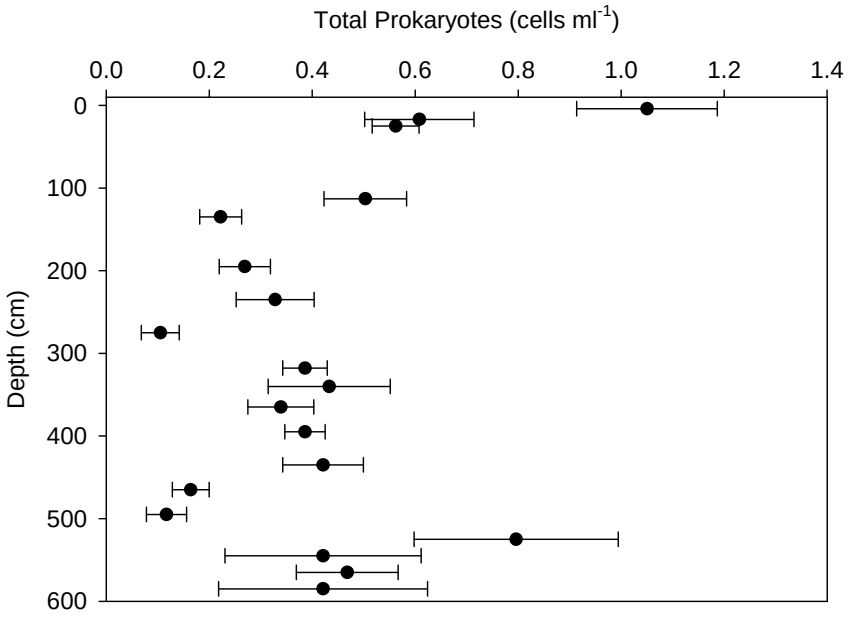

Figure 3 Prokaryote abundance in PC10 as determined by direct count microscopy.

A final activity of the last quarter was to collaborate with investigators at the National Institute of Oceanography in Goa (India) who are examining methane production in the Arabian Sea. Because of Kirchman's input, he was included as a co-author of a paper from this work.

Other Tasks

The remaining parts of Task 4 and Tasks 5-7 are scheduled to be completed during the next year 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 are working at correcting these problems as we continue to analyze the data. We are also starting QPCR analyses of total prokaryote abundance and of specific genes involved in methane degradation. This information will provide more insights into methane degradation in the Beaufort Sea.

Cost Status

The table below gives the project expenses for the fourth quarter of Year 2 as originally budgeted ("Original") and actual expenditures ("Actual"), as of January 7, 2011.

Fourth Quarter of Second Year Budget

	Original	Actual
Personnel	$2,441	$27,285
Benefits	$670	$7,385
Permanent Equipment	$0	$0
Expendable Supplies	$414	$233
Travel	$0	$1,133
Subtotal	$3,525	$36,036
Indirect costs (53%)	$1,868	$19,099

The large difference between actual expenditures and the original budget is due to personnel costs. As explained in the last two quarterly reports, in the original proposal, all expenditures were projected to be high initially and then to decrease during the year. In fact, expenditures have been more even, which is a more accurate reflection of the work load.

Products

Gonsalves, M.-J., C. Fernandes, S. Fernandes, D. L. Kirchman, and P. a. L. Bharathi. in press. Effects of composition of labile organic matter on biogenic production of methane in the coastal sediments of the Arabian Sea. Environ Monit Assess.

• 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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