

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

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

Principal Investigator: David L. Kirchman

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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 on 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. During that quarter, we received data on the metagenomes of microbial communities from five depths in a methane-rich sediment core, and some very preliminary analyses were initiated. These analyses confirmed the previous 16S rRNA gene sequence data indicating that microbes in this core were very diverse at all depths. Preliminary analysis also turned up genes similar to those in sulfate-reducing bacteria capable of hydrocarbon degradation. The next step of the analysis is to find in the metagenomic data key genes involved in aerobic and anaerobic methane degradation. These data will be important in designing better methods for quantifying methane degradation genes. Ultimately, the metagenomic and 16S rRNA gene data will be combined with other microbial and biogeochemical data to obtain the most complete picture possible 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

Parts of this task are completed, 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 have been in contact with her to compare data sets.

Task 5 - DNA Sequence Analysis

This task continues to be the main focus of project and was the main focus of the previous quarter. 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. We are still analyzing the 16S rRNA data to obtain a complete picture of which microbes were in these cores.

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. All available data were used to help in the selection of these samples, including the pyrosequence results just discussed and the abundance of methane degradation genes (*pmoA* and *mcrA*). Most important were data on sulfate and methane concentrations (Fig. 1). These data were used to calculate net sulfate reduction (loss of sulfate), methane degradation, and methanogenesis. After much discussion, the five samples were selected to cover the range of biogeochemical conditions encountered with depth in the sediment core (Fig. 1). The top sample at 20 cm is most likely to have some aerobic methane degradation. The sample at 90 cm is at an apparent peak of net sulfate reduction, while the next sample at 170 cm is at a depth of apparent methane degradation (concentrations decrease here). Methane degradation probably also occurred at the next sample taken at 260 cm and perhaps also at the bottom sample at 485 cm.

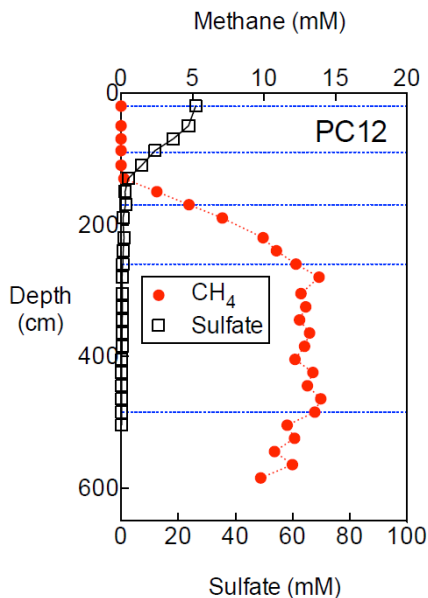


Figure 1. Methane and sulfate concentrations in sediment core PC12. The dotted horizontal lines indicate the five depths examined by a metagenomic approach. The methane and sulfate data are from R. Coffin, NRL.

The final DNA extractions from the five samples were completed during the last quarter and the DNA sent to JGI. We had to deal with several questions from JGI about the quantity and quality of the DNA; extracting DNA from these core samples was difficult. Finally, however, JGI approved the samples and started sequencing.

Overview of metagenomic data We just received the first installment of the metagenomic data from JGI. A second installment of the data, which will be about the same size as the first, is promised to arrive in early October 2010. JGI needs also to place the data in their genome website (<http://img.jgi.doe.gov/cgi-bin/m/main.cgi>) before we begin analysis of the data. However, we can make a few observations about the data and the microbes found in this methane-rich core (Table 1).

Although JGI personnel felt that the number of sequences per sample was rather low, we still have about 1.0×10^8 base pairs (bp) of genomic data per sample and 6×10^8 bp in total. Assuming 1000 bp per gene and a genome size of 2×10^6 bp, this sequencing effort has yielded information on about 6×10^5 genes and over 300 genomes. The 16S rRNA tag data indicated that these sediment microbial communities were highly diverse, and we can see the impact of that diversity in the metagenomic data. The vast majority of reads (a sequenced segment of the genome) were singletons (>98%) and occurred only once in the data set, consistent with a very diverse community. If the community were dominated by just a few organisms, we would see fewer singletons.

Table 1 Sequencing statistics for microbial communities at five depths in a methane-rich core taken from the Beaufort Sea. A “read” is sequenced segment of DNA. For these data, each read was of about 270 DNA bases. A singleton is a read that occurred only once in the dataset. The “top hit” is the organism or taxonomic group whose genes are represented most frequently in the metagenomic data.

Depth (cm)	Number of		%	
	Bases	Reads	Singletons	Top Hit
20	1.26E+08	436,068	98.9	<i>Desulfatibacillum alkenivorans</i> AK-01
90	2.14E+08	603,364	91.7	Planctomycetales
170	1.54E+08	546,129	98.7	Deltaproteobacteria
260	6.48E+07	322,286	99.2	<i>Desulfatibacillum alkenivorans</i> AK-01
485	6.55E+07	333,013	99.2	<i>Desulfatibacillum alkenivorans</i> AK-01

The data provide a very preliminary look at the organisms and their physiologies in these five microbial communities. The top “hit” given in Table 1 is the organism or taxonomic group whose genes are represented most frequently in the metagenomic data. In fact, the top hit was actually the category of “not known”, i.e. sequences not matching any known gene. The number of genes in this category will greatly decrease as the data are examined in more depth, although often a high proportion (as much as

40%) of genes remain unknown even after extensive analysis of genomes from well-characterized organisms. Another caveat is again about the diversity of these communities and of the data. The “top” hit is only very slightly more abundant than the next 10 hits in the analysis.

Even with these caveats in mind, the top hits are interesting. Genes from *Desulfatibacillum alkenivorans* AK-01 were most abundant in three of the five depth samples. This organism is a member of the Deltaproteobacteria and is a sulfate-reducing bacterium, as are many Deltaproteobacteria. It was isolated from the Arthur Kill waterway, which has a history of contamination from nearby petrochemical industries. It is capable of using C18 alkanes for growth. An unknown deltaproteobacterium was the top hit for the sample from 170 cm. Genes from bacteria in the Planctomycete phylum are abundant in all of these communities, especially the one from 90 cm. Some of these bacteria are capable of anaerobic ammonium oxidation. There may be relationships between ammonium and methane oxidation.

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

Analysis of the metagenomic data will take much time because of the size and complexity of the data sets, but we anticipate learning more soon about key genes involved in methane degradation. This information will help us design better methods (better PCR primers) to analyze these genes and to gain more insights into methane degradation in the Beaufort Sea.

Cost Status

The table below gives the project expenses for the second quarter of Year 2 as originally budgeted (“Original”) and actual expenditures (“Actual”), as of July 17, 2010.

Second Quarter of Second Year Budget

	<u>Original</u>	<u>Actual</u>
Personnel	\$14,950	\$1,084
Benefits	4102	350
Permanent Equipment	0	0
Expendable Supplies	2537	1987
Travel	0	0
Subtotal	21588	3421
Indirect costs (53%)	11442	1813
Total	\$33,030	\$5,234

The large difference between actual expenditures and the original budget is due to personnel costs. As explained in last quarter's report, in the original proposal, all expenditures were projected to be high initially and then to decrease during the year. We now expect a much more even rate of expenditures over the second year of this project. This more even rate reflects how the University distributes personnel money (especially for Kirchman), but it also is closer to how the work actually proceeds.

Products

- 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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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

