

# 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, "Characterization of Methane Degradation and Methane-Degrading Microbes in Alaska Coastal Water", began on October 1, 2008. The main activity of the last quarter was to continue the molecular analysis of microbes and genes coding for methane degradation in sediment cores collected during the September 2009 Beaufort Sea cruise. The work consisted of refining methods for large-scale DNA extraction and purification, preparation of DNA for community analysis, and preliminary analyses of sequence data. These analyses revealed a very diverse community of bacteria through the sediment core, including microbes similar to those found in other methane-rich sediments. The next phase of the work will continue the analysis in order to examine microbes and genes encoding key steps in methane degradation.

### **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**

Parts of this task are completed, but much remains to do. 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 are being analyzed in Germany by our collaborator, Dr. Tina Treude. We have been in contact with her to extend our collaboration into other experiments with live sediment samples from the Beaufort Sea cruise.

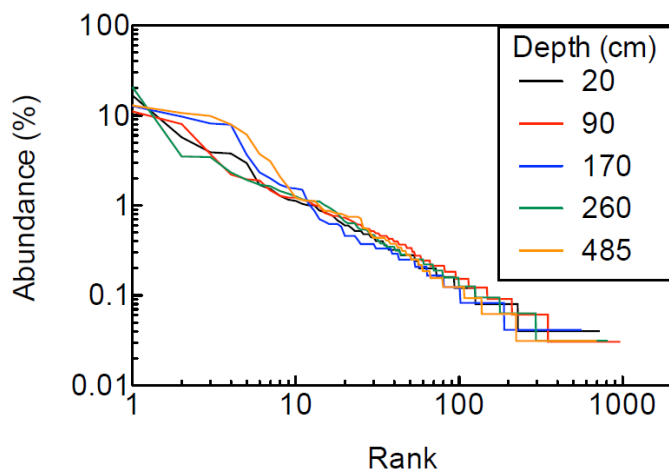
#### **Task 5 - DNA Sequence Analysis**

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. The number of samples (about 40) was set by DOE's Joint Genome Institute (JGI) who did the sequencing.

**Preliminary pyrosequence results** The sequencing resulted in about 210,000 sequences of which there were about 10,000 types of microbes along the length of the two cores. This huge diversity is evident in the very flat rank-abundance curves (Fig. 1). That is, a few types were very abundant and most were rare. This huge diversity will need to be examined in more detail during the rest of the project, but a couple observations can be made now. First, it was surprising to us that few archaea were found by the pyrosequencing analysis. This prokaryotic group of microbes is involved in the anaerobic degradation of methane and in methanogenesis and was expected to be abundant, roughly half of the total. Further analysis of the PCR primers used to extract the microbial sequences indicated biases against archaea, indicating that they are probably underrepresented in these data. Other analyses will examine this problem in more detail over the upcoming months.

The sequence analysis did reveal many bacteria that had been seen in other methane-rich environments and in deep-sea sediments. These include Chloroflexi and Deltaproteobacteria, the latter known for being sulfate-reducers. Also of interest were sequences from phyla that are known only by their 16S rRNA gene sequence. Examples of these include OP11 and WS3. We are in the process of classifying all sequences using the GreenGenes and RDP in order to compare our findings with the classification results provided by JGI.

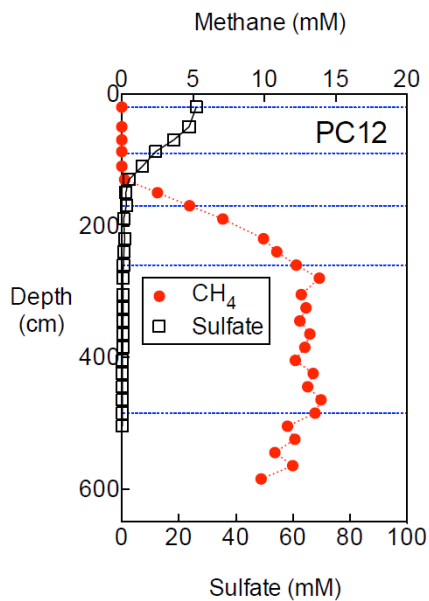


**Figure 1.** The relative abundance of each bacterial type in sediment core PC-12 from the Beaufort Sea, arranged by rank from the most abundant to the least.

**Metagenomic samples and DNA extraction** The other major activity of the last quarter was to identify five samples to be analyzed by metagenomic approaches, 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. 2). 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. 2). 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 2.** Methane and sulfate concentrations in sediment core PC12. The dotted horizontal lines indicate the five depths to be examined by a metagenomic approach. The methane and sulfate data are from R. Coffin, NRL.

The next step was to begin extracting DNA from these samples for metagenome sampling. The sequencing requires high quality and large amounts of environmental DNA. A literature search revealed a couple of approaches that we used as starting points for our work with the Arctic Ocean sediments. We compared the two most commonly used approaches that are based on commercial DNA extraction kits originally designed for soils, which are available from MO Bio, Inc. and MP biotech, Inc. Yields were typically 4-fold higher with the MP biotech kit, which uses unique chemistry and aggressive bead beating so we purchased the necessary device and reagents from this vendor. One hurdle that we had to overcome was shearing of the sediment DNA that occurred with the MP kit, a problem that does not occur with soil. Tweaking the buffer conditions and substituting the phosphate buffer supplied by the vendor with a Tris-EDTA buffer that we devised solved the DNA shearing problem. The MP kit yielded a sufficient amount of DNA from a single extraction for the tag sequencing. However, inhibitory compounds present in these DNA extracts poisoned PCR reactions, including those required for the tag sequencing. An agarose gel electrophoresis clean up step proved to be effective at removing the inhibitors and straight forward enough to apply to the 40 samples that we processed for tag sequencing. The metagenomic sequencing

needs microgram quantities of DNA and therefore required pooling of 20 to 40 individual sediment extractions. These pooled extracts also contained inhibitors that had to be removed, but the pooled sample volumes were too large for the gel electrophoresis approach. The solution was cesium chloride gradient centrifugation. This approach can handle milliliter volumes necessary to process the crude DNA extract pools and it effectively removes inhibitors.

### 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

Pyrosequencing of nearly 40 samples from two sediment cores has added to the growing data set about microbes potentially involved in methane degradation in the Beaufort Sea benthos. In addition to continuing to analyze these data, DNA for metagenomic sampling is now being prepared and will soon be sent to JGI for sequencing.

### Cost Status

The table below gives the project expenses for the first quarter of Year 2 as originally budgeted (“Original”) and actual expenditures (“Actual”), as of April 9, 2010.

#### First Quarter of Second Year Budget

	<u>Original</u>	<u>Actual</u>
Personnel	\$ 30,539	\$ 6,400
Benefits	\$ 8,379	\$ 1,924
Permanent Equipment	\$ -	\$ -
Expendable Supplies	\$ 2,576	\$ 1,960
Travel	\$ 2,606	\$ 569
Subtotal	\$ 44,099	\$ 10,853
Indirect costs (53%)	\$ 23,373	\$ 5,752
Total	\$ 67,472	\$ 16,605

There is a large difference between actual expenditures and the original budget, mainly due to personnel costs. 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. In addition to personnel expenses, travel costs were substantially lower than originally anticipated. In part the difference is

due to the purchase of airfare during the previous quarter to attend the NETL contractors' meeting in January.

## **Products**

- Presentation by Matt Cottrell at the NETL contractors' meeting in Atlanta, GA, January 2010.

- 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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