

Oil & Natural Gas Technology

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Quarterly Progress Report

Oct 1, 2009- December 31, 2009

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 begin 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 assessing microbial abundance, for DNA extraction and purification, and for PCR assays of key methane-degradation genes. The preliminary work focused on contrasting cores with low and high methane concentrations. Preliminary evidence suggests that aerobic and anaerobic methane-degradation genes are detectable in the high methane core, but are much less prevalent in the low methane core. The next phase of the work will expand on these findings to include more core samples, to obtain sequence information, and to quantitatively estimate the abundance of key 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

Most of this task was completed in August 2009 but there was still some cruise logistic work to be done during the quarter being discussed in this report. In early December 2009, M. Cottrell went to Seattle to oversee the offloading and shipping of equipment left onboard the USCGC *Polar Sea*. The equipment and remaining supplies safely arrived in Delaware.

Task 3: Oceanic Cruise

The cruise was carried out on September 15-25, 2009, thus completing this task. A complete account of the expedition was submitted separately.

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 was started soon after the cruise was finished and was the main focus of this quarter's activity. Since the number of samples greatly exceeds the budget for sequence analysis, it is necessary to collect and to examine other data on various biogeochemical properties to select approach sediment cores for detailed DNA sequence analysis. These data are per se of interest in exploring methane fluxes and degradation in the Alaska coastal region.

One basic property is the number of microbes and variation in these numbers with depth in the sediment core. Microbial abundance is estimated via epifluorescence microscopy after DNA staining. We also refined methods for extracting DNA from these cores and calculated expected yields (% DNA recovered) by assuming 2 fg DNA per microbial cell. The microbial community in these sediments is probably dominated by bacteria and to a less extent, archaea, but other forthcoming molecular analyses will give a more definitive answer.

Total microbial abundance was $1-10 \times 10^9$ cells per gram of sediment, which is about what we expected. An example is given from piston core 10 (PC10) which had low methane concentrations (Fig. 1). In this core, abundance varied greatly with depth, but some of this variation is undoubtedly due to uncertainty in counting microbes in these difficult samples. DNA was extractable from the top layers of the cores and yields were 10-50%, which is the expected level. But we have been unable so far to extract DNA from the deep sections of the cores.

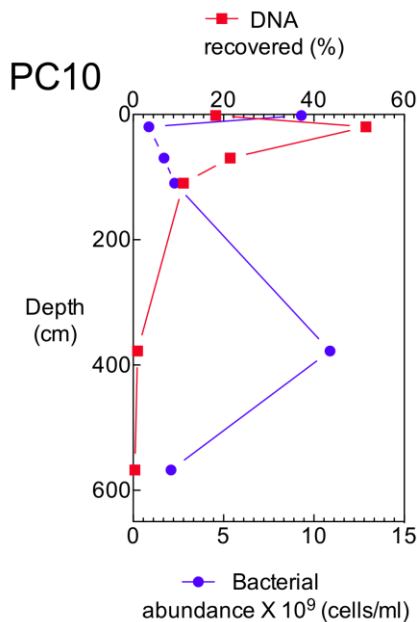


Figure 1 Example of microbial abundance and DNA yields from a sediment core collected during the September 2009 Beaufort Sea cruise.

The next step was to use the DNA in PCR assays of bacteria and archaea and for key methane-degradation genes. Typically, bacteria and archaea are assessed by their 16S rRNA genes. For now the 16S rRNA assay is a positive control for whether the extracted DNA is pure enough for subsequent molecular analyses. Eventually, the sequences of the 16S rRNA genes will be determined to examine the types of microbes present in these samples.

We used 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 anaerobic (no oxygen) 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 (Fig. 2). In contrast, both the aerobic and the anaerobic methane-degradation genes were detected deeper into the core, as deep as 300 cm in the case of *pmoA*, for a core with high methane concentrations (Fig. 3)

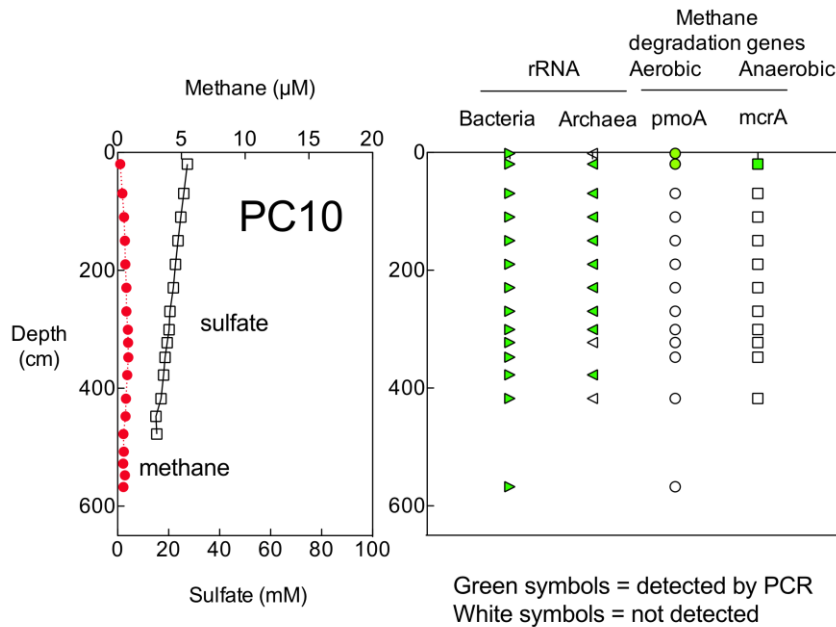
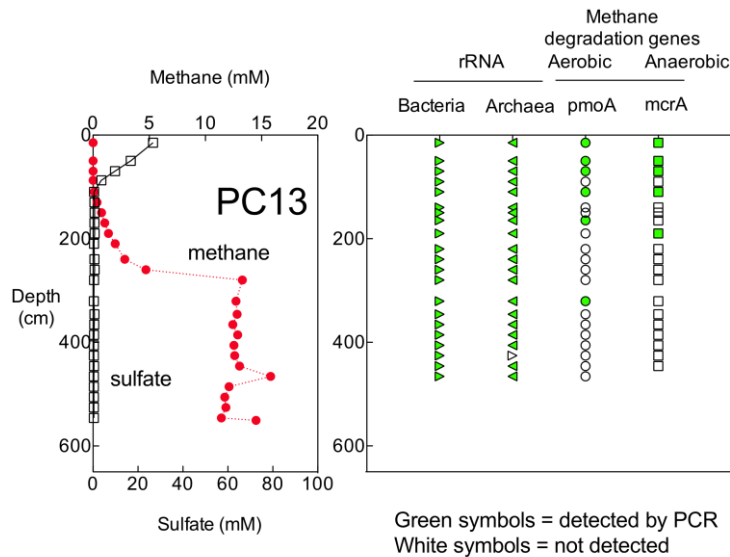


Figure 2 Methane concentrations and microbial genes in a low methane core. The rRNA genes are a positive control for DNA quality. The methane data are from R. Coffin, NRL.

Figure 3 Methane concentrations and microbial genes in a high methane core. The rRNA genes are a positive control for DNA quality. The methane data are from R. Coffin, NRL.



Many more analysis must be completed before any conclusions can be reached, but some speculation about these preliminary results may be useful. The apparent correlation between methane degradation genes and methane concentrations has implications for understanding the contribution of microbes to methane degradation over various time scales. Given their long generation time (days to months), these microbes would not contribute substantially to degradation of methane released episodically on short time scales. It is likely that methane would have to be released continuously over months to years before sufficient methane-degrading communities build up. If borne out by more extensive analyses, these findings have implications for understanding methane fluxes in coastal systems like that of Alaska.

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

The methods are now in place for determining microbial abundance, DNA extractions, and PCR screening for microbes and methane-degradation genes, although more refinement may be necessary. These methods will be applied to more samples during the next phase of the project. The next step will be to obtain actual sequence data and to estimate quantitatively the abundances of the 16S rRNA and methane-degradation genes.

Cost Status

The table below gives the project expenses for the second quarter as originally budgeted ("Original") and actual expenditures ("Actual"), as of January 7, 2010.

Fourth Quarter Budget

	<u>Original</u>	<u>Actual</u>
Personnel	\$ 12,434	\$ 17,347
Benefits	3486	4,468
Permanent Equipment	0	0
Expendable Supplies	3000	1,820
Travel	0	2,808
Subtotal	18,919	26,443
Indirect costs (53%)	10,027	14,015

The actual expenditures were higher than originally budgeted, but overall money is being spent as anticipated. In three of the previous reports, this cost status showed that actual expenditures were less than the original budget; the surpluses from previous quarters now even out the deficits shown here. The biggest difference in this quarter's

budget is for personnel. This reflects the labor needed to analyze the samples collected on the Beaufort cruise. Travel costs were more than originally expected because a trip to Seattle was necessary to oversee shipping of equipment from the ship back to the lab in Delaware. In addition, the airfare for the January NETL meeting was bought in December.

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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