Nutrient Amended Biodegradation of Hydrocarbon Contamination along Canada's Labrador Coast

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Introduction

- Future oil production (Fig. 1) combined with growing vessel traffic[1] (Fig. 2) increase the risk of an oil spill event off the coast of Labrador.
- Sea ice and iceberg floes may impede conventional oil spill response measures[2].

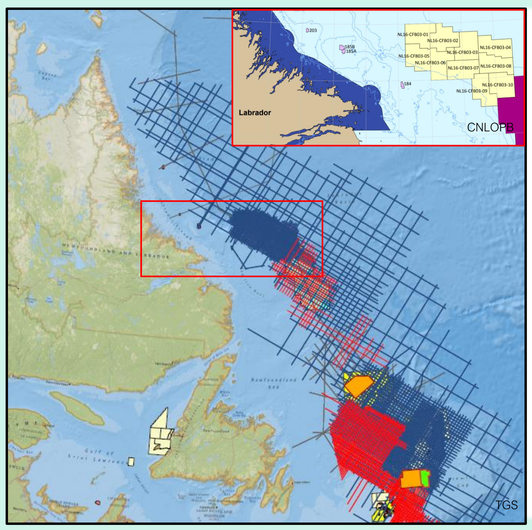


Figure 1.

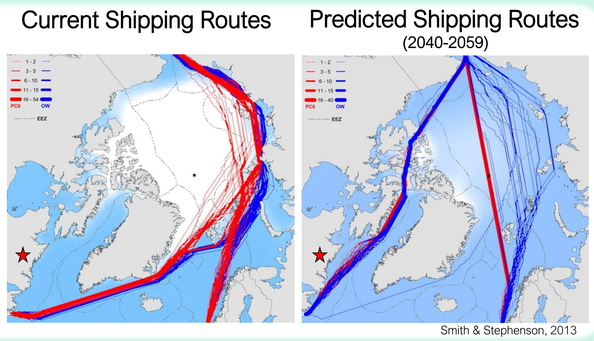
The Labrador Shelf is the focus of ongoing oil and gas exploration via one of the world's largest seismic survey programs.

Seismic lines shown in blue and red along the Labrador Shelf. Red box indicating parcels up for bid in 2018.

Maps modified from TGS (www.tgs.com) and CNLOPB (www.cnlopb.ca)

Figure 2.

As Canada's Northwest Passage becomes increasingly ice free, shipping routes are predicted to increase along the Labrador coast[1] (★)



Smith & Stephenson, 2013

- Bioremediation of spilled oil by indigenous hydrocarbonoclastic bacteria (HCB)[3] is essential for eliminating oil that enters marine systems[4].
- Biostimulation, supplementation of nutrients (e.g., nitrogen and phosphorous) to stimulate HCBs, has proven to be effective for nearshore marine oil spills[5].
- Whether indigenous microbial communities can effectively biodegrade spilled hydrocarbons off the coast of Labrador remains poorly understood.
- The purpose of this research is to probe the coast of Labrador for a microbial response to hydrocarbon contamination, focusing on biostimulation as an approach to spilled oil in cold, ice impacted marine environments.

Objectives

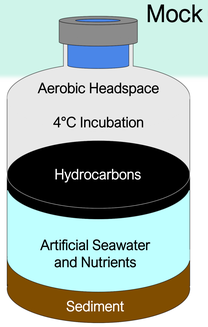
1. Uncover baseline microbial community composition of Labrador Shelf sediment
2. Demonstrate response of indigenous microbes to biostimulation in mock oil spill microcosms incubated at aerobically at 4°C
3. Assess temporal shifts in microbial communities during nutrient and hydrocarbon amended experiments

Materials & Methods

Sediment Collection

Figure 3. Sediment obtained from Labrador Shelf[6] (Station 640[A]) aboard the *CCGS Amundsen*[B] in 2015 by box corer[C]

Microcosm Experiment



Hydrocarbons: Diesel Fuel 0.1 or 1% v/v

Nutrients: Nitrogen + Phosphorus; Low or High concentrations

Controls: Sediment Free; Unamended; Killed

Figure 4. Microcosm components. Four treatment conditions vary by diesel and nutrient concentrations.

Amplicon Sequencing

Illumina MiSeq

Figure 5. In-house Illumina MiSeq used for 16S rRNA gene sequencing of amplicons prepared with 731F and 806R primers.

Hydrocarbon Degradation

Gas Chromatography Mass Spectrometry

Figure 6. Microbial respiration (CO_2 and O_2) in microcosm headspace monitored by GC as indicator of hydrocarbon degradation. GC-MS used to compare hydrocarbon profiles before and after microbial degradation incubations.

Community Composition

Bioinformatics

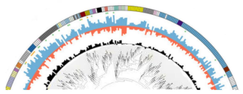


Figure 7. Bioinformatic analysis using in-house MetaAmp[7] pipeline.

Results

Hydrocarbon Degradation

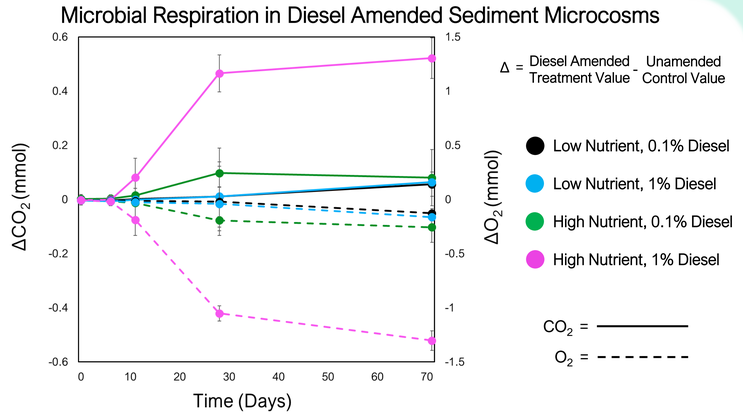


Figure 8. Microbial respiration, indicated by O_2 consumption and CO_2 production, in sediment microcosms incubated at 4°C and amended with either 0.1 or 1% v/v diesel fuel, with either low or high concentrations of N and P (either 15 or 4670µM for P, and either 2 or 1470µM for N). Mean values from triplicate incubations are plotted, with error bars showing standard deviations. High nutrient with 1% diesel treatments had five times the CO_2 production, with full oxygen consumption after 71 days of incubation compared to the other three treatment conditions.

Microbial Community Composition

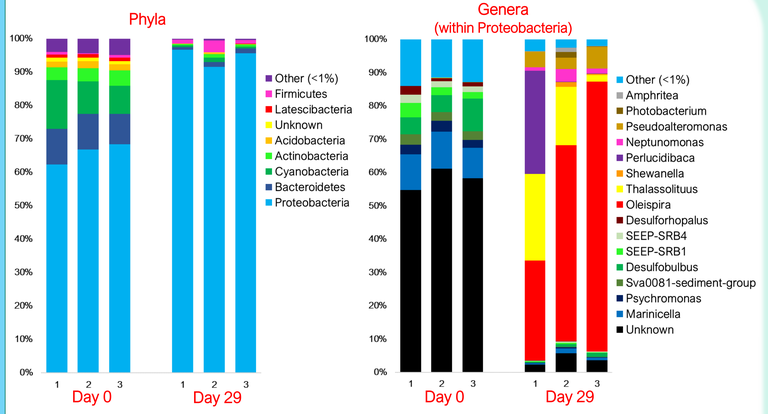


Figure 9. Bacterial community composition revealed by 16S rRNA gene amplicon libraries prepared from Labrador Shelf sediment microcosms amended with high levels of nutrient (4670µM: P 1470µM: N) and 1% (v/v) diesel. Triplicate samples were assessed at day 0 and day 29 of a 71-day incubation at 4°C. Incubations showed increases in the relative abundance of Proteobacteria, specifically genera known to include HCBs: *Oleispira, Thalassolituus, Perlucidibaca*, and *Neptunomonas*.

Summary & Future Work

- Genera containing HCBs are present in Labrador Shelf sediment and shown to increase in relative abundance when incubated with diesel and high nutrients.
- Biodegradation in the environment with ambient nutrient concentrations may be slow without supplementation of nitrogen and phosphorus.
- Future work involves hydrocarbon profiling with GC-MS to observe degradation before and after incubation, as well as repeating this experiment using crude oil.

Hypotheses

1. Hydrocarbon degrading microbes are present in Labrador Shelf sediments and poised to respond in the event of an oil spill
2. Indigenous microbes are capable of degrading hydrocarbons aerobically at 4°C
3. Biostimulation with nitrogen and phosphorous will increase the rate of biodegradation

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References

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