Biodegradation potential of estuarine microbial communities from the Churchill Marine Observatory in a marine oil snow microcosm experiment

Thamali Vidanage, Shiva Lashkari, Durell Desmond, Lisa Oswald, Dustin Isleifson, Eric Collins
Center for Earth Observation Science, University of Manitoba, Winnipeg, Canada

1.0. Background

The Hudson Basin is thought to hold 67.3 million recoverable barrels of oil equivalent, according to the Geological Survey of Canada. The Arctic is becoming more accessible for oil exploration due to the recent decreases in sea ice coverage brought about by climate change. This has also opened up new shipping routes in the region and increased vessel traffic. In light of the increased likelihood of oil pollution resulting from these changes, it is increasingly important to understand the rates and mechanisms of the natural attenuation of oil by microorganisms.

1.1. Marine Oil Snow (MOS)

MOS are oil-entrained flocs composed of small organic and inorganic materials as well as prokaryotic and phytoplanktonic cells. MOS transports hydrocarbons to the ocean floor as well as the microbial communities associated with it. MOS particles harbor a bacterial community that is distinctly different from the free-living community present in the surrounding seawater.

2.0. Objectives

1) Measure oil biodegradation in MOS particles and the water phase in experimental incubations by analyzing changes in the oil composition over time.

2) Describe how seasonal changes in temperature, salinity, and microbial composition affect oil biodegradation within MOS particles and water phases in mesocosm incubations at the Churchill Marine Observatory (CMO).

3.0. Methods and Materials

Estuarine water samples were collected in August 2024 from the Ocean Sea Ice Mesocosm facility (OSIM) located at CMO, with water derived from the Churchill River Estuary. The salinity of the water was 5 PSU. The water was filled to half the volume of Pyrex glass bottles and amended with Churchill Diesel oil at an oil-to-water ratio of 0.1% (V/V). The bottles were incubated for 28 days rotating at a speed of 10 rpm to mimic natural marine snow formation. The microcosms were sampled over a 4-week period.

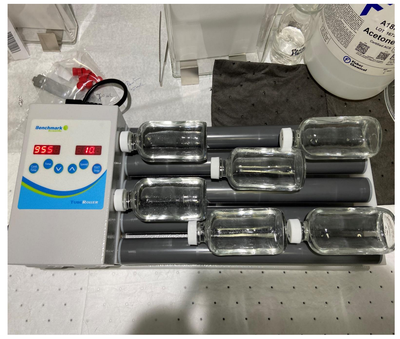

Figure 1 : Roller bottle experiment

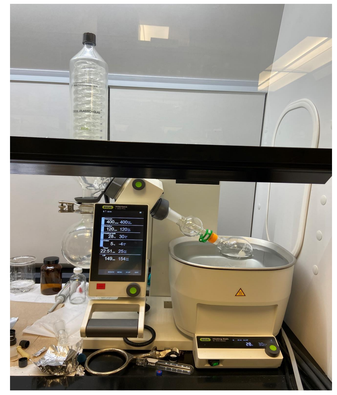

Figure 2: Evaporation of the extract using Rotovap

3.1. Chemical Analysis

Oil was extracted from each microcosm three times with hexane and dichloromethane and then dried with sodium sulfate.
Then the sample was evaporated in the Rotovap until a final volume of 1 mL was retained for GC-MS analysis.
An Agilent 7010B Triple Quadrupole GC-MS (QqQ-MS) was used to analyze alkanes and PAHs using multiple reaction monitoring.

Table 1 : Diagnostic isomer ratios used as indicators of biodegradation.

Ratio	Day 0	4-weeks
C17/Pr (alkane degradation)	0.94	0.77
2MN/1MN (PAH degradation)	0.83	0.75

Table 2 : Percentage loss of select PAHs

Compounds	Day 0	4-weeks
Naphthalene ($C_{10}H_8$)	0	35
Phenanthrene ($C_{14}H_{10}$)	0	5
Fluoranthene ($C_{16}H_{10}$)	0	6

Figure 3: Agilent 7010B Triple Quadrupole GC-MS (QqQ-MS) . Photo credit: Lisa Oswald

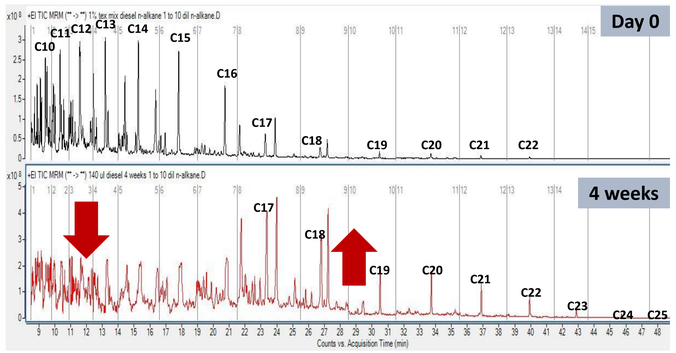

Figure 4: Gas chromatograms of n-alkanes on Day 0 and 4-weeks

4.0. Discussion

No MOS formation was observed during the 28-day incubations. However, the comparison of ratios of C17/ Pristane from Day 0 and Week 4 indicated that biodegradation of alkanes did occur in the water from CMO.

Additionally, the comparison of ratios of 2-methylnaphthalene/ 1-methylnaphthalene indicated potential PAH degradation.
Percentage losses of PAHs were calculated using $[((A_0/C_0)-(A_S/C_S))/(A_0/C_0)] \times 100$, where A_S and C_S are the concentrations of the target compound and conserved compound (2,4-dimethylphenanthrene) in the 4-weeks incubation.

Relative to the high molecular weights, the low molecular weight alkanes degrade over time presumably due to microbial degradation (as shown by the gas chromatograms).

5.0. Future Directions

We will extract DNA from the microbial community present in the water and conduct shotgun metagenomic analysis using nanopore sequencing technology.

Screening for biosurfactant-producing bacterial species will be carried out using three methods:
1. Emulsifying index
2. Biosurfactant hemolytic activity
3. Blue agar plate method

Additional incubations from autumn and spring estuarine waters will be conducted to determine the seasonal cycle of MOS-forming microbes and their biodegradation potentials.

Contact Information

Thamali Vidanage
University of Manitoba
vidanagt@myumanitoba.ca
+204 8819506

References

1. Arnosti, C., Ziervogel, K., Yang, T., & Teske, A. (2016). Oil-derived marine aggregates – hot spots of polysaccharide degradation by specialized bacterial communities. *Deep Sea Research Part II: Topical Studies in Oceanography*, *129*, 179–186. https://doi.org/10.1016/j.dsr2.2014.12.008
2. Dewing, K. E., Lister, C. J., Kung, L. E., Atkinson, E. A., King, H. M., Kalejaiye, A. M., & Krakowka, A. (2023). *Hydrocarbon resource assessment of Hudson Bay, northern Canada* (8989; p. 8989). https://doi.org/10.4095/332028
3. Murphy, S. M. C., Bautista, M. A., Cramm, M. A., & Hubert, C. R. J. (n.d.). Diesel and Crude Oil Biodegradation by Cold-Adapted Microbial Communities in the Labrador Sea. *Applied and Environmental Microbiology*, *87*(20), e00800-21. https://doi.org/10.1128/AEM.00800-21
4. Quigg, A., Santschi, P. H., Xu, C., Ziervogel, K., Kamalanathan, M., Chin, W.-C., Burd, A. B., Wozniak, A., & Hatcher, P. G. (2021). Aggregation and Degradation of Dispersants and Oil by Microbial Exopolymers (ADDOMEx): Toward a Synthesis of Processes and Pathways of Marine Oil Snow Formation in Determining the Fate of Hydrocarbons. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.642160
5. Scheibye, Katrine & Christensen, Jan & Johnsen, Anders. (2017). Biodegradation of crude oil in Arctic subsurface water from the Disko Bay (Greenland) is limited. Environmental Pollution. 223. 10.1016/j.envpol.2016.12.032.
6. Simon, M., Grossart, H.-P., Schweitzer, B., & Ploug, H. (2002). Microbial ecology of organic aggregates in aquatic ecosystems. *Aquatic Microbial Ecology*, *28*(2), 175–211. https://doi.org/10.3354/ame028175

Acknowledgements

We'd like to thank the GENICE II for funding this project. We also acknowledge the Centre for Earth Observation Science for providing resources and facilities. Special thanks to Dr. Gary Stern, Jocelyn Plouffe and Denise Whynot for their guidance and assistance during this study.