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Biodegradation of crude oil in Arctic subsurface water from the Disko Bay (Greenland) is limited☆

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ABSTRACT

Biological degradation is the main process for oil degradation in a subsurface oil plume. There is, however, little information on the biodegradation potential of Arctic, marine subsurface environments. We therefore investigated oil biodegradation in microcosms at 2 °C containing Arctic subsurface seawater from the Disko Bay (Greenland) and crude oil at three concentrations of 2.5–10 mg/L. Within 71 days, the total petroleum hydrocarbon concentration decreased only by 18 ± 18% for an initial concentration of 5 mg/L. The saturated alkanes *n*C13-*n*C30 and the isoprenoids *i*C18-*i*C21 were biodegraded at all concentrations indicating a substantial potential for biodegradation of these compound classes. Polycyclic aromatic compounds (PACs) disappeared from the oil phase, but dissolution was the main process of removal. Analysis of diagnostic ratios indicated almost no PAC biodegradation except for the C1-naphthalenes. To conclude, the marine subsurface microorganisms from the Disko Bay had the potential for biodegradation of *n*-alkanes and isoprenoids while the metabolically complex and toxic PACs and their alkylated homologs remained almost unchanged.

1. Introduction

In 2012, it was estimated that the West Greenland offshore environment holds a technically recoverable oil resource of 10.7×10^9 barrels of oil and 1.7×10^9 barrels of natural gas liquids (Schenk, 2011). The recent reductions in sea ice coverage due to climate change (Perovich et al., 2013) has made the Arctic increasingly accessible for oil exploration. The Greenlandic government has granted drilling licenses for sites off the coast of Northeast and West Greenland (Government of Greenland a) and exploratory drillings have been at water depths down to 1500 m (Government of Greenland b).

Oil released to the marine environment undergoes physical, chemical and biological removal processes. Biodegradation may be separated from the other processes by using diagnostic ratios that rely on inherent differences in biodegradability between compounds with similar physicochemical properties. This means that biodegradation can be demonstrated by changes over time in ratios of isomers of otherwise similar compounds (Wang et al., 1998, 1999; Christensen et al., 2005). Such diagnostic ratios are available for both alkanes (Wang et al., 1998) and alkylated polycyclic aromatic compounds (PACs). (Christensen et al., 2005; Rowland et al., 1986; Lamberts et al., 2008).

A recent study of biodegradation in Arctic subsurface water found that PACs were biodegraded only to a very limited extent, whereas *n*-alkanes and isoprenoids were extensively biodegraded (Kristensen et al., 2015). The draw-backs of that study were the use of open-top microcosms that allowed entry of oxygen and evaporation of light oil compounds during incubation, and that the oil concentration was 100 mg/L. These conditions are not realistic for a subsurface spill; the replenishment of oxygen will be limited in a subsurface plume, the evaporation of light oil compounds will be absent, and dispersed oil will be at lower concentrations.

The aim of our study was to investigate the potential for biodegradation of crude oil in Arctic subsurface water. Specifically, we wanted to address the effect of limited oxygen supply in subsurface spills. The oil concentrations were therefore chosen so that the lowest concentration (2.5 mg/L) would not induce oxygen limitation, whereas the highest concentration (10 mg/L) would lead to severe oxygen depletion if all oil compounds were fully degraded. We wanted to investigate biodegradation of specific oil compound classes instead of only changes in total oil

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concentrations. Furthermore, we wanted to examine the oil/water partitioning of oil compounds. This study adds to the sparsely investigated field of Arctic marine oil biodegradation.

2. Materials and methods

2.1. Site characteristics

Water samples were collected in the Disko Bay, West Greenland (69°12′ N, 53°30′ W) in June 2012 (Supplementary Information Fig. S1). The Disko Bay is characterized by varying topography and depths ranging from 300 to 500 m (Buch, 1990). During winter, sea ice covers the bay (Hansen et al., 2006) and upon break-up of sea ice in the spring, freshwater is supplied to surface waters. The Disko Bay holds Jakobshavn Glacier, a large outlet glacier from the Greenland Ice Sheet, which is an additional source of freshwater. The water column is stratified due to rising temperatures in the spring and differences in salinity caused by the input of freshwater. During the winter of 2008, a thermocline was reported at approximately 60 m depth and in June 2008 two thermoclines were reported at approximately 20 and 75 m (Dünweber et al., 2010). At our sampling depth of 150 m, temperature, salinity, and nutrient levels show low annual variation and deeper waters are homogeneous across a transect of the Disko Bay (Hansen et al., 2012). The sampling site was located approximately 50 km from the Puilasoq and Orsivik oil exploration sites in the Davis Strait (Nunaoil, 2014) and the sampling site is believed to represent the conditions of the oil exploration sites.

2.2. Sampling

Sampling was performed at a site of approximately 250 m depth and 100 L of water was retrieved from 150 m depth by using 10-L Niskin water samplers (KC Denmark A/S, Silkeborg, Denmark). The sampling depth was chosen to get below the euphotic zone. A conductivity, temperature, depth (CTD) measurement was carried out at the site, see Fig. S2. Water samples were filtered through a 60-μm nylon filter (Sefar Nitex) to remove copepods and meiofauna. Samples were stored in new, untreated 25-L polyethylene containers (Scandrums A/S) and refrigerated when possible during transportation to Denmark. Sample temperature was logged with temperature loggers in two of four containers. The temperature was between 0 and 5 °C for most of the time between sampling and refrigeration in Copenhagen, but reached a maximum temperature of 14 °C, see Fig. S3. Upon arrival in Copenhagen, the samples were aerated with filtered air (0.45-μm filters, Minisart, Sartorius) through new, untreated ceramic aquarium airstones for 12 h and subsequently distributed in microcosms as described below.

2.3. Chemicals

A light crude oil from Draugen (Statoil) with low sulfur content and a density of 0.83 kg/L was selected for this study. Further information can be found on Statoil's webpage (Statoil). An internal standard solution consisting of nine deuterated PACs was used; naphthalene-d8 (7.41 μg/mL, 99%), dibenzothiophene-d8 (7.75 μg/mL, 98%), acenaphthene-d10 (9.56 μg/mL, 98%), phenanthrene-d10 (8.56 μg/mL, 98%), pyrene-d10 (8.42 μg/mL, 98%), fluorene-d10 (7.95 μg/mL, 98%), chrysene-d12 (7.28 μg/mL, 98%), benzo[k]fluoranthene-d12 (7.28 μg/mL, 98%), and benzo [g,h,i]perylene-d12 (8.23 μg/mL, 98%) from Cambridge Isotope Lab. HPLC grade dichloromethane (DCM), pentane, acetone, and methanol from Rathburn were used for sample extraction. Hydrochloric acid (37%, Merck) and ammonium hydroxide (28–30%, Sigma-Aldrich) were used for acidification and sample extraction. For quality control of the GC-MS, a mixture of decafluorotriphenylphosphine (DFTPP) (99.3%, Sigma, Supelco), 4,4'- dichlorodiphenyltrichloroethane (4,4′-DDT) (99.7%, Sigma), pentachlorophenol (99.9%, Sigma, Supelco), and benzidine (99.9%, Sigma) was used (50 ng/μL each). An *n*-alkane mixture (Florida TRPH standard, 500 μg/mL, RESTEK) was used to prepare calibration standards for GC-FID analysis of total petroleum hydrocarbon (TPH) concentrations and boiling point fractions (BPFs). All glassware was washed and subsequently rinsed in ethanol to remove organic residues prior to use. Attention was paid to microcosm flasks to ensure that ethanol had evaporated before microcosm set up.

2.4. Microcosms and oil extraction

Microcosms were set up 5 days after sampling in 1-L glass flasks that were sealed with Teflon lined caps (Grathwol, Denmark). One L of water was transferred to each flask followed by the addition of 0, 2.5, 5, or 10 mg of crude oil by using a 50-μL glass syringe (SGE Analytical Science). Flasks were sealed immediately after oil addition to limit oxygen replenishment and evaporation, and were thoroughly shaken. Control microcosms were prepared by mixing 1 L of autoclaved water with 5 mg crude oil. The volume of water in the flasks left a headspace of approximately 15 mL. A total of 90 microcosms were set up with triplicates of each oil concentration (0, 2.5, 5, and 10 mg/L) and autoclaved controls for six incubation times: day 0, 8, 15, 29, 50, and 71. Potential oxygen consumptions were estimated from the theoretical amount of oxygen in fully aerated seawater at 2 °C (0.34 mmol O_2/L) (Benson and Krause, 1984) and two degradation scenarios, where the added oil was either fully mineralized to CO_2, or half of the oil was mineralized and half was incorporated directly into the microbial biomass. The oxygen consumption was calculated (see the SI) from the general oil metabolic reaction below, where the mass of mineralized oil was represented by $(CH_2)_n$:

$$2(CH_2)+3\ O_2 \rightarrow 2\ CO_2+2\ H_2O$$

The microcosms were stored in the dark at 2 °C, and oil appeared as a thin surface film. The microcosms were shaken thoroughly once a week. Samples incubated for 0 days were sacrificed between 2 and 5 h after microcosm set up. Microcosms were acidified with 10 mL of 1 M hydrochloric acid before extraction. The bulk water phase (914 ± 10 mL) was extracted using solid phase extraction (SPE) with Strata X-AW polymeric weak anion exchanges (500 mg/6 mL, Phenomenex). This extraction may have included some emulsified oil, and the water phase extract therefore corresponds to the water-accommodated fraction rather than a water-soluble fraction in which only water-soluble molecules are present (Singer et al., 2000; Girling, 1989). A vacuum manifold with PTFE tubes (1/8" × 1/16", Mikrolab) and polyethylene adaptor caps (Phenomenex) were used for the SPE. The PTFE tubes were carefully inserted into the flasks to avoid touching the sides or bottom of the flasks, and the water was extracted from below the surface. SPE cartridges were eluted in one fraction with 10 mL pentane:acetone (1:1) + 5% ammonium hydroxide. Two hundred μL of internal standard solution was added to eluents. The solvent was exchanged to DCM prior to GC-MS analysis by mixing 1 mL extract with 5 mL DCM and adding anhydrous sodium sulfate as a drying agent. The organic extracts of the water phase are referred to as the water phase in the subsequent text. The remaining water (86 ± 10 mL) and oil residues in microcosms were extracted three times with DCM, and 200 μL internal standard solution was added to the extracts. The extracts were then adjusted to 5 mL by evaporation and transfer to volumetric flasks. These extracts are subsequently referred to as the oil phase. The extracts were stored in 30-mL

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