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Microbial communities in seawater from an Arctic and a temperate Norwegian fjord and their potentials for biodegradation of chemically dispersed oil at low seawater temperatures

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ABSTRACT

Biodegradation of chemically dispersed oil at low temperature (0–2 °C) was compared in natural seawater from Arctic (Svalbard) and a temperate (Norway) fjords. The oil was premixed with a dispersant (Corexit 9500) and small-droplet oil dispersions prepared. Faster biotransformation of *n*-alkanes in the Arctic than in the temperate seawater were associated with the initially higher abundance of the alkane-degrading genus *Oleispira* in the Arctic than the temperate seawater. Comparable transformation of aromatic hydrocarbons was further associated with the late emergences *Cycloclasticus* in both seawater sources. The results showed that chemically dispersed oil may be rapidly biodegraded by microbial communities in Arctic seawater. Compared to oil biodegradation studies at higher seawater temperatures, longer lag-periods were experienced here, and may be attributed to both microbial and oil properties at these low seawater temperatures.

1. Introduction

The estimated occurrence of undiscovered oil and gas north of the Arctic Circle may be as much as 90 billion barrels of oil and 44 billion barrels of natural gas liquids, most of it in offshore areas (Bird et al., 2008). In addition, reduced ice coverage in the Arctic will result in higher transport activities in this region than today. Strict regulations of oil exploration and production and transport in the Arctic are imposed by responsible governmental bodies, but accidental releases of oil may occur and cause impacts on local marine environments. Oil spilled to the marine environment undergoes a number of weathering processes like evaporation, water-in-oil (w/o) emulsification, dispersion, dissolution of small and charged compounds, and photo-oxidation (NRC 2003).

Biodegradation is an important weathering process that may result in complete mineralization of hydrocarbons (HCs). Oil spills in marine environments may result in blooms of oil-degrading bacteria, increasing dramatically in their abundance (Braddock et al., 1995; Brakstad and Lødeng, 2005; Bælum et al., 2012; Dubinsky et al., 2013; Hazen et al., 2010; Yakimov et al., 2007). Most of these are affiliated to the classes Alphaproteobacteria or Gammaproteobacteria (Yakimov et al., 2007), and several of them are obligate hydrocarbonoclastic, exclusively transforming HCs (Yakimov et al., 2007). Aliphatic HC-degrading bacteria like *Alcanivorax* are typically succeeded by bacteria like *Cycloclasticus*, which attack more slowly biodegradable aromatic HCs (PAH) (Kasai et al., 2002; Röling and van Bodegom, 2014). Several studies have shown that also Arctic seawater (SW) and marine ice contains hydrocarbonoclastic bacteria with the abilities to biotransform oil HCs (Bagi et al., 2014; Bowman and McCuaig, 2003; Brakstad et al., 2008; Deppe et al., 2005; Garneau et al., 2016; Gerdes et al., 2005; McFarlin et al., 2014; Yakimov et al., 2004). In cold SW, alkane degradation is often associated with high abundances of psychrophilic *Oceanospirillales*, like *Oleispira antarctica*, while *Cycloclasticus* is associated with degradation of aromatic HCs both in temperate and cold SW (Coulon et al., 2007; Dong et al., 2015). Members of the genus *Colwellia* are associated with oil-contaminated marine ice and cold SW, Antarctic sediments, and were also abundant in the deep-sea oil plume after the Deepwater Horizon oil spill (Brakstad et al., 2008; Powell et al., 2006; Redmond and Valentine, 2012). Single-cell genomic studies have revealed that *Oceanospirillales* are associated with *n*-alkane and cycloalkane degradation pathways, while *Colwellia* may be associated with gaseous, and simple aromatic HC degradation (Mason et al., 2014; Mason et al., 2012).

Effective stimulation of bacterial degradation depends on the bioavailability of the oil compounds in dissolved or dispersed fractions. Chemical dispersants are used as an oil spill response (OSR) method to

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remove oil slicks from the sea surface, by generating dispersions with small droplet size and near-to neutral buoyancies in the seawater column. Despite some controversy about the effect of dispersants on oil biodegradation (Kleindienst et al., 2015b; Lindstrom and Braddock, 2002; Rahsepar et al., 2016), most studies have shown that efficient use of dispersants enhances the biodegradation (Brakstad et al., 2014; Bælum et al., 2012; Lee et al., 2013; McFarlin et al., 2014; Prince et al., 2013; Siron et al., 1995; Techtmann et al., 2017; Venosa and Holder, 2007). Even in Arctic SW at very low temperatures (−1 °C), the use of dispersants facilitated oil biodegradation (McFarlin et al., 2014). However, since the oil dispersibility is related to viscosity and pour point (Brandvik and Faksness, 2009), the dispersibility of many oils becomes reduced in cold seawater.

Since dispersant treatment may be a relevant OSR treatment in the Arctic to prevent the oil from stranding or drifting into ice-covered areas, it is essential to investigate the effect of dispersant treatment on oil biodegradation in Arctic SW. We therefore compared biodegradation of chemically dispersed oil in Arctic and temperate SW at low temperature (0–2 °C) and the relations between community successions and biotransformation of oil compound groups in the SW sources.

2. Materials and methods

2.1. SW sampling

Svalbard (SVB) SW (80 L) was collected beneath the ice in the Van Mijen fjord (77°56′N, 16°43′ E) on April 21, 2016. Holes were drilled in the ice (ice drill) and appr. 10 L SW filled on each of 8 × 20-L Teflon-bags (5-gallon Pail Liners and Lid Protectors made by 2.5 mill modified PTFE film, Welch Fluorocarbon, Dover, NH, USA). The bags were closed with double sets of pull-ties and each bag placed in a 12-L lacquered-lined drum and closed with a locking ring (Air Sea Containers Ltd., Birkenhead, UK). The seawater was stored overnight at 4–5 °C (airport in Longyearbyen), transported by plane the next day, and arrived at our lab in the afternoon of April 22. The SW was then acclimated at 0–2 °C for 5 days until the biodegradation experiment started. Triplicate SW samples (2 L) were also filtered on site (Svalbard) through 0.22 μm Durapore filters (Merck KGaA, Darmstadt, Germany), and the filters transported together with seawater. Volumes of SW (2 L) were filtered through 0.22 μm Durapore filters (triplicate) after arrival to our lab to determine if transport had affected the composition of the microbial communities.

SW (80 L) was also collected from 80 m depth in a temperate fjord, Trondheimsfjord (TRD; 63°26′N, 10°23′E) through a pipeline system supplying our labs (SINTEF Sealab, Trondheim, Norway) with continuous seawater. This SW was collected at the same day as the SVB SW (April 21, 2016), stored at 4 °C until the SVB water arrived at the lab. The TRD SW was then acclimated as described above for the SVB SW. Triplicate SW samples of TRD SW (2 L) were filtered through 0.22 μm Durapore filters when sampled and after storage overnight at 4 °C.

2.2. Biodegradation experiment

Fresh Troll naphthenic oil (batch 2007–0087) and Corexit 9500A dispersant (Nalco, Sugar Land, TX, USA) were used in this experiment. This oil had low viscosity (27 mPas;13 °C), a density of 0.900 g/cm^3, pour point of −18 °C, and low wax (2.0 vol%) and asphaltene (0.2 wt%) contents.

The oil was pre-mixed with Corexit 9500 in a dispersant-to-oil ratio (DOR) of 1:100, and oil dispersions prepared in an oil droplet generator (Brakstad et al., 2015a; Nordtug et al., 2011). Two oil droplet stock dispersions (concentration of 200 mg/L and median droplet size of 10 μm) were prepared in the droplet generator system, with acclimated SW from SVB or TRD. Based on oil droplet concentration measurements (Coulter Counter; see below), each stock dispersion was diluted in natural acclimated SW (0–2 °C) from their respective source (SVB or TRD) to reach final nominal concentrations of 2 mg/L oil droplets. This oil concentration did not require additional mineral nutrient amendment, as previously shown (Brakstad et al., 2015a; Prince et al., 2013). The dispersions were distributed in baked (450 °C) and autoclaved flasks (2 L; Schott), completely filled and capped without headspace or air bubbles, and flasks were mounted on a carousel system with slow continuous rotation (0.75 r.p.m), as previously described (Brakstad et al., 2015a). The carousel system was maintained at 0–2 °C for 64 days in the dark. Triplicate flasks of dispersions in natural SW from both SVB and TRD were sacrificed for analyses after 30 min incubation (0 days). Flasks were then sacrificed for analyses after 7, 14, 21, 28, 42 and 64 days of incubation as described for 0-day samples. Flasks with SW blanks without oil or dispersant, were incubated at the same conditions as the oil dispersions, and one flask of each SW blank sacrificed at the same times as the dispersions.

2.3. Microbiology analyses

2.3.1. Total cell concentrations and most probable number determinations

Total prokaryote concentrations were determined by epi-fluorescence microscopy analyses of samples stained by the nucleic acid stain 4′,6-diamidino-2-phenylindol (Porter and Feig, 1980). Most probable number (MPN) concentrations of heterotrophic prokaryotes (HP) and oil-degrading prokaryotes (ODP) were determined as previously described (Brakstad et al., 2008), except for the incubation conditions. Incubations were performed at 0–2 °C for 7 days, followed by 20 °C for 3 days (HP) or 7 days (ODP).

2.3.2. 16S rRNA gene amplicon sequencing

SW blanks and oil dispersions (approximately 500 mL) were filtered through 0.22 μm filters (Millipore), and DNA was extracted from filters by employing FastDNA Spin kit for soil (MP Biomedicals), according to the manufacturer's instructions. DNA quantification was performed by Qubit 3.0 fluorometer (Thermo Fisher Scientific Waltham, MA, U.S.A.), with dsDNA High Sensitivity kit (ThermoFisher Scientific, MA, U.S.A.).

16S rDNA amplicons were generated according to Illuminas “16S Metagenomic Sequencing Library Preparation” protocol using S-D-bact-0341-b-S-17 and S-bact-0785-a-A-21 primer set (Klindworth et al., 2013). Amplicons generated by PCR were isolated using magnetic beads (Agencourt Ampure XP Beads, Beckman Coulter Inc.; Brea, CA, USA). Libraries were quantified using Quant iT Picogreen Dye and the Fragment Analyzer (Advanced Analytical), as well on Agilent's Bioanalyzer. All amplicons were pooled equimolar and then sequenced paired-end on the Illumina MiSeq platform, 2 × 300 nt, following the manufacturer instructions.

Raw pair-end reads were assembled with fastq-join in QIIME 1.9.1 (Caporaso et al., 2010b). Assembled sequences were demultiplexed and quality filtered to remove low quality reads (Phred score < 20; −q 19). UCHIME was employed for chimera detection on assembled quality filtered reads (Edgar et al., 2011). Operational Taxonomic Units (OTUs) were determined by clustering assembled sequences on 97% nucleotide identity using UCLUST (Edgar, 2010) with open reference clustering option. Representative sequences were aligned with PyNAST (Caporaso et al., 2010a), and taxonomy assignment was performed with RDP classifier (Wang et al., 2007), based on SILVA-123 database (Klindworth et al., 2013). To evaluate for potential differences in the dynamics of microbial communities between different samples and sample groups at separate time points, multivariate statistics in the form of principal coordinate analysis (PCoA), based on un-weighted UniFrac distance metrics was carried out. Prior to that, relative abundances of OTUs were calculated, and OTUs with < 0.01% of relative sequence abundance were removed. Statistical analysis was performed within the Phyloseq package v.1.12.2 (McMurdie and Holmes, 2013) in R-studio v.3.2.2. For visualization of taxonomical composition, for each taxon (on genus or family level) cut-off of 3% was applied for incubation samples, while for source water samples cut-off was set at 2% of