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Biodegradation of dispersed oil in seawater is not inhibited by a commercial oil spill dispersant

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ABSTRACT

Chemical dispersants are well-established as oil spill response tools. Several studies have emphasized their positive effects on oil biodegradation, but recent studies have claimed that dispersants may actually inhibit the oil biodegradation process. In this study, biodegradation of oil dispersions in natural seawater at low temperature (5 °C) was compared, using oil without dispersant, and oil premixed with different concentrations of Slickgone NS, a widely used oil spill dispersant in Europe. Saturates (*n*C10-*n*C36 alkanes), naphthalenes and 2- to 5-ring polycyclic aromatic hydrocarbons (PAH) were biotransformed at comparable rates in all dispersions, both with and without dispersant. Microbial communities differed primarily between samples with or without oil, and they were not significantly affected by increasing dispersant concentrations. Our data therefore showed that a common oil spill dispersant did not inhibit biodegradation of oil at dispersant concentrations relevant for response operations.

1. Introduction

Chemical dispersants, consisting of mixtures of surfactants and solvents (Place et al., 2016; Prince, 2015), are used in marine oil spill response operations to reduce the surface tension of the oil by creating a more hydrophilic oil surface (Lessard and DeMarco, 2000). When oil on the sea surface is efficiently treated with dispersants, wave actions will generate dispersions of small oil droplets with near-to neutral buoyancies in the seawater, removing most of the oil from the sea surface (Tkalich and Chan, 2002), and generating dispersions with median oil droplet sizes typically between 20 and 50 μm (Brakstad et al., 2014; Lunel, 1993). Common stockpiled dispersants like Corexit 9500 and Slickgone NS, have shown comparable acute toxicities to marine copepods (*Calanus finmarchicus*), with LC_{50}-values of 21–24 mg/L, being considerably less toxic than the dispersed oil itself, which showed LC_{50} of 0.5–0.8 mg/L to the same copepod (Hansen et al., 2012; Hansen et al., 2014).

Dispersants were also injected near the wellhead during the Deepwater Horizon oil spill in the Gulf of Mexico, resulting in reduced oil surfacing, and generating a deep sea plume of small oil droplets at 900–1300 m depth (Camilli et al., 2010). The expected goals of the dispersant treatment were to prevent large slicks in the area with many ships were gathered to stop the leak, and to prevent the oil from impacting the shoreline (Atlas and Hazen, 2011).

By encouraging the formation of small oil droplets, dispersants increase the surface-to-volume ratio of the oil, and enhance oil biodegradation. Faster *n*-alkane biodegradation was measured in chemically than physically dispersed Macondo oil in a flume basin study at breaking wave conditions, in accordance with smaller oil droplets generated in the chemically (median size < 20 μm) than the physically (median size appr. 80 μm) dispersed oil after 5 h of incubation (Brakstad et al., 2014). Colonized oil droplets eventually generate 'flocs' of biodegraded oil, microbial cells and extracellular polymers (Bælum et al., 2012; Hazen et al., 2010; Macnaughton et al., 2003). Several laboratory studies have shown positive effects of dispersants on oil biodegradation rates in seawater or with enrichment cultures at different conditions (Brakstad et al., 2014; Bælum et al., 2012; Hazen et al., 2010; McFarlin et al., 2014; Prince et al., 2013; Siron et al., 1995; Techtmann et al., 2017; Venosa and Holder, 2007). However, other studies have indicated negligible or uncertain effects of dispersants on oil compound biodegradation rates (Lindstrom and Braddock, 2002; Macnaughton et al., 2003; Swannell and Daniel, 1999). Data from recent studies even suggested that dispersants may suppress the activity of oil-degrading microbes (Kleindienst et al., 2015; Rahsepar et al., 2016). Once the oil is chemically dispersed, rapid dilution will occur in the water column. Environmental concentrations of dispersed oil over

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the period of biodegradation is expected to be below 1 mg/L (Lee et al., 2013). It is therefore important to account for dilutions in biodegradation studies with chemically dispersed oil, by running experiments at low oil concentrations, although compromises may be made to enable measurements of oil compounds biodegradation.

The objective of this study was to determine if increasing dispersant-to-oil ratios (DORs) of a common oil spill dispersant inhibited biodegradation and microbial communities rates of hydrocarbons at low oil concentrations (2–3 mg/L) in natural unamended seawater. At this concentration, biodegradation of *n*C10- to *n*C36-alkanes and 2- to 4-ring aromatic compounds was possible to measure. Slickgone NS is an approved dispersant in several European countries, including the use as a secondary tool in oil spill operations on the Norwegian Continental Shelf, and biodegradation studies were performed at a temperature relevant for this area (5 °C).

2. Materials and methods

2.1. Oil, dispersant and seawater

Fresh Statfjord C paraffinic oil (batch 2014–0081), provided from Statoil ASA, Mongstad, Norway, was pre-heated at 50 °C for 30 min to melt the wax in the oil, and cooled to room temperature. The dispersant Slickgone NS was provided from K. Todnem AS, Sandnes, Norway. The Statfjord oil was pre-mixed with Slickgone in DORs of 1:100, 1:25 and 1:10. Seawater was collected via a pipeline system at 80 m depth (below thermocline, salinity 34‰) in a Norwegian fjord (Trondheimsfjord; 63°26′N, 10°23′E), outside the harbour area of Trondheim. The seawater temperatures was 5.9 °C at the time of collection. The seawater was acclimated overnight to 5 °C before start of biodegradation experiment.

2.2. Biodegradation experiment

The three pre-mixed dispersions, and the oil without dispersant, were prepared in natural unfiltered seawater at room temperature with an oil droplet generator, as previously described (Brakstad et al. 2015a; Nordtug et al. 2011). Oil droplet concentrations of 200 mg/L and droplet sizes of 10 µm were pre-set for the droplet generator system. Based on oil droplet concentration measurements (Coulter Counter; see below), each stock dispersion was diluted in natural unfiltered seawater (5 °C) to reach a final nominal concentration 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). Dispersant in seawater without oil (0.2 mg/L; corresponding to concentration in DOR 1:10) was run through the oil droplet generator and distributed on carousels similarly to the oil dispersions. The carousel system was placed in a temperature-controlled room at 5 °C for 64 days in the dark, and flasks were sacrificed for analyses after 30 min incubation (0 days) in triplicate (oil without dispersant and premixed oil in DOR 1:25), duplicate (premixed oil in 1:100), or as single samples (premixed oil in DOR 1:10). Flasks were then sacrificed for analyses after 7, 14, 21, 28 and 64 days of incubation, as described for 0-day samples. Flasks, completely filled with unfiltered seawater without oil or dispersant, were incubated at the same conditions and used as seawater blanks.

2.3. Analyses

2.3.1. Oil droplet size and concentrations

Concentrations and size distributions of oil droplets were determined by Coulter Counter measurements (Beckman Multisizer 4; Beckman Coulter Inc., Brea, CA, U.S.A.), fitted with either 100 µm or 280 µm apertures. These apertures were used for measurements of droplets within a diameter range of 2–60 µm or 5.6–100 µm, respectively. Oil only and DOR 1:100 were measured with 280 µm aperture, while DORs 1:25 and 1:10 were measured with 100 µm aperture. Sterile-filtered (0.22 µm) seawater was used as electrolyte in the Coulter Counter. Droplet concentrations were determined from volume concentrations (μm^3/ml) and recalculated (mg/L), based on the density of the fresh oil (0.834 g/cm^3).

2.3.2. Chemical analyses

Samples of dispersions and seawater blanks were solvent-solvent extracted (dichloromethane) for measurements of semivolatile organic compounds by gas chromatographic methods. The flask glass walls were also rinsed with DCM after removal of dispersions to extract material attached to the glass walls. Extracts of dispersions and glass walls were pooled. Total extractable organic carbon (TEOC) was analyzed by GF-FID, while quantification of 96 individual targeted compounds (*n*C10-*n*C36 alkanes, decalins, phenols, 2- to 5-ring polycyclic aromatic hydrocarbons (PAH) and 17α(H),21β(H)-Hopane) was performed by GC–MS analyses, as previously described (Brakstad et al., 2014). In the GC–MS analyses, response values for individual target analytes were determined, and based on a signal-to-noise ratio of > 10, the lower limit of detections (LOD) varied from 0.001 µg/L to 0.01 µg/L for individual oil compounds. Target analytes were normalized against 17α(H),21β(H)-Hopane (Prince et al., 1994; Wang et al., 1998).

2.3.3. Analyses of total cell concentrations and most probable number determinations

Total prokaryote concentrations (epifluorescence microscopy) and most probable number (MPN) determinations of culturable heterotrophic prokaryotes were performed as previously described (Brakstad et al., 2008).

2.3.4. 16 S rRNA gene amplicon sequencing

Seawater samples without oil 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 a Qubit 3.0 fluorometer (Thermo Fisher Scientific Waltham, MA, U.S.A.), with dsDNA High Sensitivity kit (ThermoFisher Scientific, MA, U.S.A.).

Microbial community compositions in samples were analyzed by 16S rRNA gene amplicon sequencing, using a primer combination targeting the V3 and V4 hypervariable regions of bacterial 16S rRNA genes and 2 × KAPA HiFi HotStart ReadyMix (Klindworth et al., 2013). Both forward and reverse primers were tagged with Illumina adapter overhang, and amplicons generated by PCR (Eppendorf Mastercycler), were isolated using PCR clean-up spin columns (Qiagen; QIAquick Gel Extraction Kit), and thereafter used in index PCR with Illumina NEXTERA XT indexes (Nextera XT Index Primer 1 (N7xx) and 2 (S5xx)) and 2 × KAPA HiFi HotStart ReadyMix. Indexed 16S rRNA amplicons were isolated using PCR clean-up spin columns (Qiagen; QIAquick Gel Extraction Kit), and thereafter verified and quantified using Qubit 3.0. All DNA indexed amplicons were diluted to 4 nM in Tris-HCl (10 mM, pH 8.5) and pooled for generation of a library for sequencing. DNA library and Illumina positive control PhiX samples were thereafter denatured and incubated at 96 °C for 2 min, before running in an Illumina MiSeq sequencer, according to Illumina's 16S rRNA sample preparation guideline. Raw pair-end reads were assembled with fastq-join in QIIME 1.9.1. 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. Operational Taxonomic Units (OTUs) were determined by clustering assembled sequences on 97% nucleotide identity, using UCLUST with open reference clustering option. Representative sequences were