Deep-Sea Research II 129 (2016) 108–118

Contents lists available at ScienceDirect

Deep-Sea Research II

journal homepage: www.elsevier.com/locate/dsr2

Biodegradation of crude oil and dispersants in deep seawater from the Gulf of Mexico: Insights from ultra-high resolution mass spectrometry

Michael Seidel [a,b], Sara Kleindienst [a,1], Thorsten Dittmar [b], Samantha B. Joye [a], Patricia M. Medeiros [a,*]

[a] Department of Marine Sciences, University of Georgia, Athens, GA 30602, USA
[b] Research Group for Marine Geochemistry (ICBM-MPI Bridging Group), Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl von Ossietzky University of Oldenburg, 26129 Oldenburg, Germany

ARTICLE INFO

Available online 30 May 2015

Keywords:
Oil-biodegradation
Dispersants
Ultra-high resolution mass spectrometry
Corexit
Gulf of Mexico

ABSTRACT

During the 2010 Deepwater Horizon oil spill in the Gulf of Mexico, three million liters of chemical dispersants (Corexit 9500 and 9527) were directly applied at the discharging wellhead at 1500 m water depth. Such a deep-water large-scale application was unprecedented and the effect of dispersants on oil biodegradation is not yet completely understood. The present study explores the biodegradation of oil, dispersant, dispersed oil or dispersed oil and nutrients at the molecular level using ultra-high resolution Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR–MS) following a laboratory experiment with Gulf deep water. Oil-derived molecular formulae exhibited a specific molecular fingerprint and were mainly observed in the mass range <300 Da. The relative abundance of heteroatom-containing (N, S, and P) compounds decreased over time in the oil-only treatments, indicating that they may have served as nutrients when oil-derived hydrocarbons were metabolized. Relative changes over time in the molecular composition were less pronounced in the dispersed oil treatments compared to the oil-only treatments, suggesting that dispersants affected the metabolic pathways of organic matter biodegradation. In particular, dispersant addition led to an increase of S-containing organic molecular formulae, likely derived from the surfactant di-octyl sulfosuccinate (DOSS). DOSS and several dispersant-derived metabolites (with and without S) were still detectable after six weeks of incubation, underscoring that they were not rapidly biodegraded under the experimental conditions. FT-ICR–MS fragmentation studies allowed tentatively assigning structures to several of these molecules, and we propose that they are degradation products of DOSS and other dispersant components. The present study suggests preferential degradation, transformation and enrichment of distinct dispersant molecules, highlighting the need to include these compounds when tracking Corexit-derived compounds in the environment.

1. Introduction

Between April and July 2010, about 790 million liters of oil were released at a depth of 1500 m during the Deepwater Horizon (DWH) blowout in the northern Gulf of Mexico (hereafter Gulf) (McNutt et al., 2012). During the discharge, 7 million liters of chemical dispersants were used, mostly Corexit 9500A, of which ca. 3 million liters were applied directly to the discharging wellhead (National Response Team, 2011), and smaller amounts of Corexit 9527, which was mainly applied at the sea surface (Graham et al., 2011; Kujawinski et al., 2011). The deep ocean application of dispersant was unprecedented. Many of the biological and physical factors that determine the distribution and degradation of chemically dispersed oil at sea remain poorly constrained (National Research Council, 2005). For example, there is insufficient knowledge about the rates at which dispersed oil binds to sediments, how quickly it is degraded in the ocean, whether and how it is taken up by organisms, and what (final) products are created during the degradation processes (National Research Council, 2005). The long-term fate of dispersant-derived products in the environment is a subject of ongoing research (White et al., 2014).

Chemical dispersants are applied after oil-spills to emulsify the hydrophobic oil-derived molecules in water and to stimulate biodegradation (National Research Council, 2005). Their primary

* Corresponding author. Tel.: +1 706 542 6744.
E-mail address: medeiros@uga.edu (P.M. Medeiros).
[1] Present address: Center for Applied Geosciences, Eberhard-Karls-University Tuebingen, 72074 Tuebingen, Germany.

http://dx.doi.org/10.1016/j.dsr2.2015.05.012
0967-0645/

purpose is to prevent the formation of thick surface oil slicks by enhancing the solution of oil in water and microbial oil degradation by increasing the bioavailability of the oil in the water column. While dispersants are generally assumed to be less toxic than oil (National Research Council, 2005), the increased oil-in-water solubility could make chemically dispersed oil more toxic to aquatic organisms (George-Ares and Clark, 2000; National Research Council, 2005), including algae (Lewis and Pryor, 2013), micro-zooplankton (Almeda et al., 2014) and fish (Ramachandran et al., 2004). Dispersants can also affect microbial degradation of oil-derived hydrocarbons (Lindstrom and Braddock, 2002). The commercially available dispersants Corexit 9500A and Corexit 9527 contain ca. 10% and 17% mass fraction (w/w%) of the anionic surfactant di-octyl sulfosuccinate (DOSS; Kujawinski et al., 2011), respectively, which has been shown to persist for more than four years after application in the deep ocean (Kujawinski et al., 2011; White et al., 2014).

Quantifying the chemical composition of petroleum remains an analytical challenge (Marshall and Rodgers, 2008). Gas-chromatography (GC) is capable of resolving only a small fraction of oil-derived molecules and most of the molecules that remain at oil-contaminated sites after weathering fall outside the GC-amenable analytical window (Aeppli et al., 2012). Almost 60% of the total mass of organics in Macondo crude oil is not detectable by conventional GC analyses (McKenna et al., 2013). Ultra high-resolution mass spectrometry is an analytical method that is capable of determining the primary molecular composition of oil (Marshall and Rodgers, 2003; Ruddy et al., 2014). The high mass accuracy of Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR–MS) allows the determination of thousands of molecular formulae in petroleum and dissolved organic matter (DOM; Marshall and Rodgers, 2008). While petroleum characterization via FT-ICR–MS has been the subject of several studies, the molecular characterization of the water-accommodated fraction (WAF) of oil or dispersant-derived compounds has received less attention, and previous studies on oil weathering and biodegradation focused mainly on oil-derived molecules from particulate organic matter (McKenna et al., 2013; Ruddy et al., 2014).

The present study extends the work of Kleindienst et al. (2015b) who explored the effects of dispersants on the activity and composition of oil-degrading microorganisms in a laboratory experiment. In their study, Kleindienst et al. (2015b) found that the application of dispersant significantly changed the microbial community composition within a week and did not enhance rates of hydrocarbon degradation (determined by 14C-hydrocarbon tracer experiments) or overall microbial activity (estimated by 3H-leucine incorporation rates). Here, we address the molecular compositional changes of the WAF of oil during biodegradation in more detail aiming to answer the following questions: how does the application of dispersant influence the biodegradation of individual compounds in the WAF of oil? Which oil- and dispersant-derived compounds are degraded and which accumulate in the DOM pool? To address these questions, we simulated an oil-infusion into deep seawater from the Gulf using laboratory microcosms and applied FT-ICR–MS including collision-induced fragmentation of single molecular formulae to reveal the transformations of DOM on the molecular formula and structural level, including a multitude of dispersant- and oil-derived compounds.

2. Experimental methods

2.1. Experimental design

Experimental microcosms were established using seawater collected from ca. 1200 m water depth at an active natural hydrocarbon seep located at the Green Canyon, block 600 (GC600) in the Gulf of Mexico in March 2013. All treatments and controls were prepared and sampled in triplicate as described in detail by Kleindienst et al. (2015b). In short, Gulf deep water samples were amended with oil-only (Macondo surrogate from the Marlin platform), dispersant-only (Corexit 9500), dispersed oil, and dispersed-oil + nutrients (ammonium, nitrate, phosphate, and trace metals). Oil-only WAFs were prepared with 0.85 L of sterile seawater amended with 0.15 L Macondo surrogate oil and dispersed oil WAFs were additionally amended with 0.015 L of Corexit 9500. These treatments were mixed on a magnetic stirrer for 48 h at room temperature in the dark. The mixtures were allowed to settle for 1 h and afterwards, the aqueous phase was sub-sampled avoiding the oil or dispersant phases. Seawater controls were either incubated abiotically (0.2 μm filtered and pasteurized, abiotic control) or as biotic control without any nutrient, oil or dispersant addition. All treatments were employed in triplicate in 2 L pre-combusted glass bottles, placed on a roller table, and incubated under oxic conditions (oxygen concentrations were measured at each sampling point) at 8 °C in the dark, which matched the *in situ* temperature for these samples (Kleindienst et al., 2015b). Incubations were performed at atmospheric pressure since the majority of deep sea and subseafloor prokaryotes appear to be able to grow at atmospheric pressure with little difference in community composition and incubation pressure (Jannasch et al., 1982; Parkes et al., 2009). Sampling was conducted at 8 °C in the cold room where the incubations were performed. Aliquots (200 mL) for DOM characterization via FT-ICR–MS analysis were collected at the start of the experiment (time zero = T_0), after one week (sampling point 1 = T_1) and after six weeks (sampling point 4 = T_4).

2.2. FT-ICR–MS analysis

Experimental bottles were sampled by inverting the bottles several times to mix them and then withdrawing the necessary volume of water for a given analysis (Kleindienst et al., 2015b). For DOM analysis, a filtered (0.7 μm pre-combusted glass fiber filters, Whatman) seawater sample (ca. 200 ml) was acidified to pH 2 (HCl, p.a.) and DOM was extracted using cartridges filled with a modified styrene divinyl benzene polymer (Agilent Bond Elut PPL, 0.2 g) (Dittmar et al., 2008). The extraction efficiencies were determined as the dissolved organic carbon (DOC) content of an extracted volume of original sample vs. the DOC content in the SPE-DOM (by evaporating an aliquot of methanol extract at 40 °C and re-dissolving the dried extract in ultrapure water). The methanol extracts were stored frozen to avoid esterification reactions between the SPE-DOM and methanol (Flerus et al., 2011). Methanol extracts were diluted 1:1 (v/v) with ultra-pure water to yield a DOC concentration of 12 mg L^{-1} for the analysis with FT-ICR–MS. Samples were analyzed with a 15 Tesla solariX FT-ICR–MS (Bruker Daltonik GmbH, Bremen, Germany) equipped with an electrospray ionization source (ESI) in negative mode. This mode was chosen because anionic surfactants such as DOSS are readily analyzable by negative ESI–MS (e.g., Kujawinski et al., 2011; Place et al., 2010, 2016). Instrument settings and molecular formulae assignments are described in detail in Seidel et al. (2014). In short, the capillary voltage was 4 kV in negative mode. Ions were accumulated in the hexapole for 0.3 s and data were acquired in broadband mode using 4 megaword data sets and a scanning range of 150–2000 Da with 500 scans accumulated per mass spectrum. Mass spectra were calibrated internally with a list of known CHO compounds in the targeted mass range (achieved mass accuracy < 0.1 ppm). Molecular formulae were assigned to peaks with a signal-to-noise ratio > 4 applying the criteria described by Koch et al. (2007) with a mass tolerance of < 0.5 ppm. The areas of peaks with assigned molecular formulae were