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Identifying oil/marine snow associations in mesocosm simulations of the Deepwater Horizon oil spill event using solid-state ^{13}C NMR spectroscopy

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ABSTRACT

The Deepwater Horizon oil spill stimulated the release of marine snow made up of dead/living plankton/bacteria and their exopolymeric polysaccharide substances (EPS), termed marine oil snow (MOS), promoting rapid removal of oil from the water column into sediments near the well site. Mesocosm simulations showed that Macondo surrogate oil readily associates with the marine snow. Quantitative solid-state ^{13}C NMR readily distinguishes this oil from naturally formed marine snow and reveals that adding the dispersant Corexit enhances the amount of oil associated with the MOS, thus contributing to rapid removal from the water column. Solvent extraction of MOS removes the oil-derived compounds for analysis by one and two-dimensional GC/MS and evaluation of potential transformations they undergo when associated with the EPS. The results reveal that the oil associated with EPS is subjected to rapid transformation, in a matter of days, presumably by bacteria and fungi associated with EPS.

1. Introduction

The Deepwater Horizon oil spill event that occurred in the Gulf of Mexico in April–July 2010, released > 4 million barrels of light crude oil into the water column. This event stimulated the production of exopolymeric substances (EPS), transparent exopolymer particles (TEP), and other organic materials from phytoplankton and bacteria (Passow et al., 2012; Quigg et al., 2016). EPS aggregated with themselves and with oil to form a visibly identifiable marine snow called MOS (marine oil snow) (Daly et al., 2016). The association of the aggregated EPS with oil droplets accommodated within the water and dispersed oil droplets (formed from the addition of a dispersant, Corexit) affected the buoyancy of the MOS, leading to precipitation in and around the site of the well blowout (Daly et al., 2016).

Marine snow particles are macroscopic aggregates (> 0.5 mm) composed of living and detrital microbial (phytoplankton and bacteria) organisms, fecal pellets, and inorganic materials whose aggregation may be dependent on microbial activities such as the release of sticky EPS and TEP (e.g., Alldredge and Silver, 1988; Turner, 2015: Quigg et al., 2016). These marine snow aggregates are studied for their role in transporting fixed carbon to the deep ocean as part of the biological pump and have been noted to incorporate oil introduced into the marine environment (e.g., Boehm, 1987; Payne et al., 1987; Passow et al., 2012). The subsequent sedimentation of the oil-incorporated MOS marks a mechanism for the removal of oil from pelagic areas that transfers any negative petroleum impacts from the surface to benthic realms. The overall process has been referred to by Daly et al. (2016) as MOSSFA (marine oil snow sedimentation and flocculant accumulation). In the case of the 2010 Deepwater Horizon oil spill, several independent estimates suggest that MOSSFA accounted for up to 14.4% of oil removal (Valentine et al., 2014; Chanton et al., 2015). With MOSSFA being such an important process in the biological pump, specifically with important carbon cycle and pollution implications, an understanding of the chemical composition and transformations of the complex aggregates and their components will be important for understanding and predicting future sedimentation fluxes.

In attempts to simulate the process of MOS formation, mesocosm experiments were conducted in the summer and fall of 2015 where EPS production from the microbial and plankton community were promoted in the presence and absence of a water accommodated fraction of oil (WAF) as well as chemically enhanced WAF (treated with the dispersant, Corexit; CEWAF) and a dilute CEWAF (DCEWAF). The MOS

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formed and sedimented to the bottom of each mesocosm tank was then collected and subjected to quantitative solid-state ^{13}C NMR to determine the nature and extent of oil accumulation and to characterize the MOS components (e.g., EPS and biological components, oil) produced. It is particularly noteworthy that only small amounts of MOS and EPS could be harvested (< 10 mg) and that quantitative solid-state NMR spectra could be obtained on such small amounts of material. To our knowledge, these are the first such data for MOS. The ability to obtain ^{13}C NMR spectra for such materials allows us to understand the average chemical composition of MOS and could be employed to examine marine snow and marine snow components collected from open ocean samples or sediment traps, where limited amounts of material can be reasonably collected. Moreover, this analytical capability provides the opportunity to gain quantitative information on the amounts and chemistry of marine snow that become associated with oil in the environment.

To achieve such low detection levels in solid-state ^{13}C NMR where one commonly requires 100′s of mg of carbon and long experiment times to obtain quantitative spectra, we employed a new multi-pulse cross polarization technique (Johnson and Schmidt-Rohr, 2014) with a small-diameter sample rotor (2.5 mm). Quantitative solid-state ^{13}C NMR prior to this key publication required high sample volumes, usually in large diameter rotors (4–7 mm), and long experiment times (often days of spectrometer time) using a pulse sequence (direct polarization magic angle spinning, DPMAS) that allowed for sufficient T_1 relaxation of the ^{13}C signals between pulsed acquisitions (30–60 s). The new approach used here is a multi-cross polarization with magic angle spinning (multi-CPMAS) that provides for quantitative solid-state ^{13}C NMR at the time-scale of the relaxation of ^{1}H that is on the order of 1–2 s. This significant time saving allows one to obtain spectra on the order of hours instead of days. More importantly, the use of a small-diameter rotor capable of spinning at > 15,000 Hz provides for an efficient filling factor, because the small sample cavity allows one to fill the rotor fully with only a few mg of material. In larger diameter rotors having larger sample volumes small samples of MOS material would not provide an efficient interaction with the NMR coils that provide the radio frequency signals to and from the sample. Thus, it would be nearly impossible to obtain adequate signal to noise ratios (S/N) for these samples, especially if the organic matter contents are diminished due to incorporation of inorganic materials scavenged from the water column.

The ability to obtain solid-state ^{13}C NMR spectra makes available to us the possibility of modeling the spectral data in a manner similar to that reported by Baldock et al. (2004) where the spectral signals can be deconvoluted to determine relative proportions of biological and anthropogenic components which are present as complex mixtures in the isolated MOS. This allows for detailed characterizations of contributing compound classes, quantitative estimations of each compound class, and evaluations of our efficacy for isolation of oil-derived components by solvent extraction. The extracts can then be subjected to detailed molecular characterization by standard and advanced analytical approaches that include GC/MS and GC × GC/MS.

2. Materials and methods

2.1. Mesocosm Experiments and MOS Collection

Experiments were conducted in 130 L borosilicate glass tanks in August (Mesocosm 1 – M1) and October (Mesocosm 2 – M2) of 2015 as has been described elsewhere (Wade et al., 2017). Briefly, seawater was collected offshore Galveston, TX, pre-filtered (charcoal filter) to remove large particulates, and transported to Texas A&M University at Galveston. For M1 and M2, the treatments were prepared in an identical manner except there was one tank per treatment for M1 and triplicates per treatment for M2. WAF was prepared by mixing 25 mL of Macondo surrogate oil with the seawater (130 L) for 18–24 h (Knap et al., 1983; Wade et al., 2017) and then 79 L added to mesocosm tanks in M1 and 87 L for M2. For the CEWAF treatment, Corexit 9500A was mixed with the oil at a 1:20 ratio (v:v) then mixed with 130 L of seawater for 18–24 h, and 79 L and 87 L was added to the CEWAF tanks for M1 and M2. For the DCEWAF treatment, the CEWAF was diluted 1 in 10 with the original seawater and made up to 79 L and 87 L for M1 and M2. A control treatment was also prepared using an equal volume of the original seawater only. A plankton (< 63 μm, 2 L) concentrate collected in nearby coastal waters was added to each tank for all treatments, to mimic the concentration of plankton in coastal areas, just prior to initiation of the experiment. At the start of each experiment, the estimated oil equivalents were determined by fluorescence analysis (Horiba Scientific Aqualog Fluorometer) after calibration with a Macondo surrogate oil standard (Wade et al., 2011, 2017). The mean estimated oil equivalents were 0 mg/L, 3.4 mg/L, 3.6 mg/L and 36 mg/L for the control, WAF, DCEWAF, and CEWAF treatments at the start of M1, respectively. For M2, the mean estimated oil equivalents were 0 mg/L, 0.26 mg/L, 2.74 mg/L, and 41.5 mg/L for the control, WAF, DCEWAF, and CEWAF treatments, respectively (Wade et al., 2017).

Each mesocosm was allowed to run for 96 h. The tanks were maintained at room temperature (20 °C) employing a 12-h light cycle with lamps providing 50–100 μmole-quanta/m^2/s. Aggregates were observed to form rapidly in each of the tanks (< 24 h), and MOS particles sank to the bottom in both mesocosm experiments. MOS particles that sank over the course of M1 (0–96 h) were collected. For M2, particles that sank during hours 0–48 were collected and used for analyses to be presented elsewhere (Xu et al., 2017, in review); only the MOS particles that sank between hours 48 and 96 were collected for solid state ^{13}C NMR analyses. MOS were collected by inserting a syringe (100 mL) attached to a stainless steel needle into the bottom of the tanks and transferring them to pre-cleaned (HCl) and pre-combusted (450 °C, 4 h) glass bottles. MOS was placed on a polycarbonate membrane (0.4 μm pore size) and rinsed three times with 15 mL of nanopure water (18.2 MΩ). The MOS was resuspended in water to separate them from the filter which was then carefully removed. The samples were subsequently freeze-dried prior to analysis. MOS particles from triplicate samples were combined for each treatment in the case of M2 in order to obtain enough material (8–21 mg) for solid state ^{13}C NMR analysis. For evaluation of the contributions of oil to MOS, the freeze-dried samples were extracted using dichloromethane (DCM), a solvent frequently used for the analysis of oil composition (e.g., Frysinger et al., 2003), after initial ^{13}C NMR analysis. DCM-insoluble particles (4.6–18.3 mg) were then isolated, freeze-dried, and saved for ^{13}C NMR analysis.

3. Solid-state ^{13}C NMR Analyses

The freeze-dried solid MOS samples were transferred to a 2.5 mm rotor covered with a Vespel cap for solid-state ^{13}C NMR analysis using a multiple cross polarization magic angle spinning (multiCPMAS) pulse sequence (Johnson and Schmidt-Rohr, 2014). Experiments were conducted on a Bruker Avance II instrument with ^{1}H resonating at 400 MHz and ^{13}C resonating at 100 MHz. Samples were spun at the magic angle (54.7°) at a frequency of 18 MHz. The continuous pulses were optimized at 0.5 s, and a 2 s recycle delay was used. All sample spectra were baseline corrected and calibrated to a glycine external standard (176.03 ppm).

Sample spectra were analyzed using a molecular mixing model (MMM) to estimate contributions from major organic biopolymer components (carbohydrate, protein, lipid, carbonyl) following Baldock et al. (2004). The relative contributions (% of total spectral signal) from major carbon moieties present in each sample were obtained by integrating the spectral signal over chemical shift regions corresponding to those major carbon moieties (methylenic C (CHx): 0–45 ppm, αC in peptides: 45–60 ppm, alkyl-O carbon (HCOH): 60–95 ppm, anomeric C (O—C—O): 95-110 ppm, aromatic C (C═C): 110–145 ppm, aromatic-O