Targeted Petroleomics: Analytical Investigation of Oxidation Products of Macondo Well Oil from Pensacola Beach

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■ SUPPORTING INFORMATION

Supporting Information. Additional information as noted in the text. This material is available free of charge via the internet at http://pubs.acs.org.

■ EXPERIMENTAL METHODS

Samples and Preparation. The environmental control sample (blank) collected from St. George Island yielded less than 100 μg of extractable organic material from a 40 gram sample. In comparison, the contaminated beach sediment yielded in excess of 100 mg of extractable organic material from the same 40 gram sample. The (+) ESI FT-ICR mass spectrum of the environmental blank didn't contain any species with a characteristic mass repeat of CH_2 (inherent to petroleum); therefore, we conclude that no MWO impacted the sampled sediment.

ESI and APPI FT-ICR Mass Spectrometry. Extracts were reconstituted in toluene to yield stock solutions at 1 mg/mL concentration. The stock solutions were further diluted to 0.10 mg/mL with 48.75:48.75 (v/v) toluene:methanol and 2.5% formic acid for protonation during ESI. For negative ESI, samples were dissolved in 49:49 (v/v) toluene:methanol with 1% ammonium hydroxide. All samples were pumped through a fused silica capillary at 0.5 μL/min through a 50 μm i.d. fused silica microESI needle (needle, 2.3 kV; tube lens, 350 V; heated metal capillary current, ~5.0 A).

A modified Thermo-Fisher APPI source was coupled to our FT-ICR mass spectrometer through a custom-built interface.[1] Samples were diluted to 0.25 mg/mL in toluene and infused at 50 uL min^{-1} through a 5 mL Hamilton gas-tight syringe by a syringe pump to the heated vapor region (300 °C) of the APPI source, where N_2 sheath gas (50 psi) facilitated nebulization. Gas-phase neutrals were photoionized by a 10 eV (120 nm) ultraviolet krypton gas discharge lamp (Syagen).

FT-ICR Mass Spectrometry and Data Treatment. All FT-ICR mass spectral experiments were performed with a custom-built mass spectrometer equipped with a passively shielded 9.4 tesla superconducting horizontal solenoid magnet.[2,3] Time-

domain data was acquired and processed by a Predator data station,[4] as described elsewhere.[5] Because of the high compositional complexity of the contaminated sand sample (more than twice as many mass spectral peaks as the MWO, including several thousand 1.7 mDa splits,) broadband phase correction[6] was applied to all mass spectra to increase resolving power by up to a factor of 2.[6,7] Peak lists for the samples were generated, organized, and calibrated by use of custom modular software (Predator Data Analysis),[4] enhanced by a recently described walking calibration.[8] Multiplication of IUPAC mass by 14/14.01565 effectively converts the mass of CH_2 from 14.01565 to exactly 14.00000 to yield homologous Kendrick mass series whose members contain the same number of heteroatoms and rings plus double bonds to carbon (DBE, a measure of unsaturation) but differ only by the number of CH_2 groups, thereby facilitating calibration and assignment of abundant series that span the entire mass range.[9] For the blanks, Agilent tuning mix provided internal calibration by use of a dual ESI source described previously.[10]

Model Compounds and Time of Flight Mass Spectrometry. A mixture (30 mM each) of six model compounds (1-naphthol, dibenzofuran, 4,4'dimethylbenzophenone, 7,9 dimethylbenzacridine, 2-aminoanthracene, and 2,6-dimethylquinoline) was prepared in 48.75:48.5:2.5 (v/v/v) toleuene:methanol:formic acid (J.T. Baker, Phillipsburg, NJ) at 1 nM and directly infused into a time-of-flight mass spectrometer (Waters Micromass LCT Premier XE (Manchester, UK)) at 20 μL/min for positive ion ESI. The model compounds were purchased from Sigma Aldrich (St. Louis, MO).

Tandem Mass Spectrometry Experiments. Ions within an m/z range of 700 ± 25 Da from DWH crude oil and PBDWC were quadrupole-isolated and irradiated with a Synrad (Mukilteo, WA) CW CO_2 laser (λ = 10.6 μm). The laser is mounted off-axis (~3.5°) due to the need to accommodate an axially-mounted electron capture dissociation cathode.[3] The factory-specified laser beam diameter is 3.5 mm. To ensure maximum irradiation of the ion cloud throughout the course of the irradiation period (and thus more efficient fragmentation), the beam was carefully

aligned by use of standard compounds at low laser power. A laser power of ~30 W cm^{-2} was used for the IRMPD experiment with an irradiation period of 500 ms.

Anion Exchange Separation. We devised an anion exchange separation method based on prior separation of ketones and acids.[11] 20 mg of extracted beach sediment from 5.5-7.0 cm depth (June 30, 2010 sampling) was loaded onto a previously conditioned SPE cartridge, packed with a strong anion exchange sorbent (MEGA BE-SAX, 2 g, 12 mL) from Agilent Technologies (Santa Clara, CA). Four fractions were obtained by successive elution of the retained components with solvents (10 mL each) in the following order: 100% *n*-hexane followed by 100% dichloromethane, 90:10% (v/v) dichloromethane:methanol, and finally 90:10% (v/v) methanol:acetic acid. The fractions were desolvated with dry nitrogen gas. The total recovery (the ratio of total dry weight of all fractions to the weight of a sample loaded onto the SPE cartridge) was 99%.

GC×GC/MS Analysis. Two dimensional gas chromatographic mass spectrometry was conducted with an Agilent 7890 GC and time-of-flight mass spectrometer (Pegasus 4D, LECO, St. Joseph, MI). Samples were dissolved in dichloromethane at 5 mg/mL and 1 μL was injected at split ratio 1:10. Helium carrier gas flow rate was 1.5 mL/min. The gas chromatograph inlet temperature was 300°C. The main (first) oven temperature was initially 50 °C, held for 0.5 min, then ramped at 5 °C/min to 300 °C and held at maximum temperature for 10 min. The secondary oven was offset by +10 °C from the first oven, and followed the same time profile with start temperature, 60°C (held for 0.5 min), 5 °C/min ramp rate, and final temperature, 330°C held for 10 min. The modulation period was 5 s. The first column was a BP-1 (60 m long, 250 μm ID, 0.25 μm film thickness), and the second column was BPX50 (1.5 m long, 100 μm ID, 0.1 μm film thickness). Both columns were purchased from SGE (Ringwood, VA, Australia). The ion source was held at 200 °C. Electron impact (EI) ionization (electron energy, 70 V) was used, and detection of positive ions occurred at an acquisition rate of 200 spectra/s. The TOF operates at nominal mass resolution and the mass tolerance for the selected ion chromatogram construction was +/- 500 mDa. Assignments of ketones were verified by a match of the mass

spectrum to the NIST mass spectral library. GC×GC flame ionization detector (FID) instrument conditions and methods can be found elsewhere.[12]

Elemental Analysis. Elemental analysis was performed with a Thermo Finnigan Elemental Analyzer (Flash EA 1112) to determine CHNS (run 1) and O content (run 2) in two separate experiments. For CHNS analysis, 1.5-3.0 mg of sample was placed in a tin cup, crushed to form a sphere, and placed in the autosampler. All samples were analyzed in quadruplicate. Calibration of the instrument is provided by the analysis of standards and all quadruplicate runs included a separate, independent standard not used in the initial calibration. A similar procedure was followed for oxygen measurements but with a silver cup.

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Figures:

Figure S1. Photographs of the sampling site at 30°19'32.08"N, 87°10'30.55"W on Pensacola Beach, showing the initial excavation (top, right), a zoom of the contamination zone (background), and the collected sediment samples as a function of depth (center).

Figure S2. GC×GC-FID zoomed biomarker regions from Figure 1 (left). Top: Two-dimensional isoabundance contours for the June 30, 2010 Pensacola Beach contaminant (5.5-7.0 cm depth); Bottom: three-dimensional representations for the contaminant and MWO.

Figure S3. Positive ion TOF mass spectrum for an equimolar mixture of six representative model compounds. A mass scale-expanded segment from the (+) ESI FT-ICR MS analysis of the Pensacola Beach contaminant is shown in the inset.

Figure S4. Positive ESI FT-ICR MS heteroatom O_x class distributions for each of four anion exchange chromatographic fractions from the June 30, 2010 Pensacola Beach contaminant (5.5-7.0 cm).

Figure S5. Zoom inset of the (+) ESI O_1 class isoabundance-contoured double bond equivalents (DBE = number of rings plus double bonds to carbon) vs. carbon number plot, reveals alkyl ketones.