Photochemical Changes in Water Accommodated Fractions of MC252 and Surrogate Oil
Created During Solar Exposure as Determined by FT-ICR MS

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Abstract

To determine effects of photochemical weathering of petroleum, surrogate and Macondo (MC252) crude oils were exposed to solar radiation during the formation of Water Accommodated Fractions (WAF) in sterile seawater. Samples were incubated in either unfiltered sunlight, with ultraviolet radiation blocked (Photosynthetically Active Radiation [PAR] only), or in darkness. WAFs were collected at two time points over the course of a week. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) analyses of water soluble species formed during exposure to sunlight were compared for the different treatments. Photochemical alterations resulted in differences in compound class distributions. In general, surrogate oil was photo-oxidized across a wider carbon number range compared to MC252. While photochemical differences were observed between MC252 and surrogate oils, microbial production in seawater responded similarly to both WAFs from both types of oils with the majority of the inhibition resulting from oil exposure to visible light.

Key words: petroleum; photochemistry; toxicity; FT-ICR MS; weathering; oil spill

Introduction

Since the Deepwater Horizon oil spill, a significant amount of work has focused on the natural weathering processes of petroleum in the environment and the resulting impact on biological systems. To help assess overall ecosystem impact, a key consideration in understanding the mechanism of toxicity is examination of components produced through photochemical processes.

A standard way to conduct these types of experiments is to prepare Water Accommodated Fractions (WAF) (Coelho, 1997), (Rico-Martinez et al., 2013). The WAF is essentially the water soluble fraction from a mixture of oil and water. A commonly used method to generate WAFs is to mix oil and water on a stir-plate in the dark overnight, then to collect the aqueous fraction (Coelho, 1997). While this may be reproducible for laboratory experiments, it may miss several critical environmental factors that include time and the effects of solar radiation. The potential for sunlight to affect the toxicity of crude oils has been long recognized (e.g., Barron et al., 2003) (Barron et al., 2003) but has received minimal attention in light of the Deepwater Horizon Oil Spill (Ray et al., 2014) (Genuino et al., 2012). This seems unusual considering that much of the oil resided in surface waters of the Gulf of Mexico where it was exposed to summer sunlight.

Recent advances in analytical techniques such as tandem GC X GC (Reddy et al., 2012) and high resolution FT-ICR MS (Stanford et al., 2007), (McKenna et al., 2013) allow us to see more clearly molecular changes within complex samples such as petroleum. Prior studies of water soluble species via total fluorescence and GC-FID analysis showed increased toxicity for samples irradiated with simulated sunlight (King et al., 2014).

Published work in this field has previously neglected the individual contributions of both abiotic (photo-chemical) and biotic (microbial/bacterial) degradation of petroleum. Ray et al, 2014 examined the molecular transformations of irradiated crude oil by FT-ICR MS. However, this work used simulated sunlight and pure water and thus the result may differ from those obtained under environmental conditions (Ray et al., 2014). By using sterile seawater, this study examined abiotic structural transformations of petroleum residues providing valuable insight into the environmental degradation mechanism(s) of petroleum which are currently undefined.

In light of the limited availability of the source MC252 crude oil released during DWH spill and huge demand for research projects on DWH oil spill, Marlin Platform oil was selected as the surrogate oil for MC252 oil. The two oils have similar geochemical properties, GC-based hydrocarbon profiles and toxicities. Nevertheless, GC-based methods only identified compounds account for half of the mass of the MC252 oil (McKenna et al., 2013). The current study offers the first molecular level characterization comparison between those two oils provided by GoMRI for oil spill research and their photo-oxidation patterns.

Materials

HPLC-grade solvents (hexanes (95% *n*-hexane), toluene, and methanol) were purchased from J.T. Baker/Avantor Performance Materials (Center Valley, PA, USA). Dichloromethane, hydrochloric acid and sodium hydroxide were purchased from Fisher. ^3H -leucine (54 Ci mmol^{-1}) was obtained from Perkin Elmer. ECOLUM liquid scintillation cocktail purchased from MP Biomedicals. Macondo well crude oil (MC252) was provided by BP oil company. Surrogate crude oil was obtained from AECOM, Ft. Collins, CO (A0063S). Surrogate oil elemental analysis performed by Midwest Microlab, LLC, Indianapolis, IN.

Methods

Creation of the water accommodated fraction: The development of WAFs is accomplished using Teflon bottles which have UVB transparency greater than 86% and UVA transparency greater than or equal to 90% (unpublished data). Each sample is incubated in a 1 liter Teflon bottle in a temperature controlled water table on the roof of the Biology building at the University of West Florida. The effects of photo-degradation on MC252 and surrogate oil were examined in separate

experiments. Aged Gulf of Mexico (collected ~ 40 km offshore Pensacola, FL and > 3 yrs old) seawater was passed through a 0.2 μ m pore-size polycarbonate filter (Millipore), added to the Teflon bottles, and heated at 70°C for >3 hrs (pasteurization) prior to the addition of oil. After cooling, the pasteurized seawater was then amended with oil to contain ~2-4 % Macondo surrogate oil (2%) and MC252 (3.8%). Sample treatments included the following exposures; 1) full solar spectrum exposure (“Full Sun”), 2) Photosynthetically active radiation (PAR) exposure achieved by wrapping bottles in Courtguard™ (50% transmittance cut-off at 400 nm) which blocks both UVB and UVA, and 3) “dark” treatments for bottles wrapped in heavy black plastic or aluminum foil. Bottles were shaken vigorously twice per day and placed back in the water table. At each time point (extending over one week: T1- day 1 and T2 - day 8 for MC252 and day 7 for surrogate), three bottles from each solar treatment were collected and allowed to settle for two hours, then 900 mL the WAF was siphoned from the bottom of each bottle, avoiding the oil/water interface.

Additional WAFs were made with 2% oil of each oil in 25 mL sterile seawater in 35 mL Teflon bottles. These were collected in a time series extending over two weeks. Triplicate WAFs of each oil were collected at each time point and stored frozen (-20° C) until all were collected.

Bacterial production: Surface seawater samples were collected from the end of the pier at Pensacola Beach, FL (30° 19.640' N, 87° 08.514' W) and transported back to our lab at *in situ* temperature in the dark. Three-tenths of a mL of each WAF from the time series exposure was placed in a polystyrene 5 mL snap-cap tube. Triplicate controls received 0.3 mL of seawater filtered through a 0.2 μ m pore size polycarbonate filter. Seawater was amended with ³H-leucine (54 Ci mmol⁻¹ PerkinElmer, Bridgeport, CT) to a final concentration of 20 nM and 3 mL of

labeled seawater added to each tube containing WAF. Tubes were capped, mixed, and incubated in the dark at *in situ* seawater temperature (26°C) for 4 hr. Leucine incorporation was terminated after removing triplicate 1.0 mL subsamples from each snap cap tube to 2 mL microfuge tubes containing 50µL 100% trichloroacetic acid. Tubes were capped and processed according to Smith and Azam (1992) and incorporated ³H-leucine determined by liquid scintillation counting using a Packard Tri-Carb 2900 (Smith and Azam, 1992). Percent inhibition of 3H-leucine incorporation caused by specific WAFs was determined by comparing to the unamended controls.

Solar radiation: Incident solar radiation during the production of WAFs was monitored with a Biospherical Instruments (San Diego, CA) model GUV511 solar radiometer containing five channels at 305, 320, 340, and 380 nm, as well as a channel to measure Photosynthetically Active Radiation (PAR; 400–700 nm).

Table 1 Cumulative Light Exposure

	305 nm (J/(cm² nm))	320 nm (J/(cm² nm))	340 nm (J/(cm² nm))	380 nm (J/(cm² nm))	PAR mol/cm²
T1 (MC252)	0.084	0.557	1.21	1.49	3844
T2 (MC252)	0.639	4.34	9.51	11.75	31258
T1 (surrogate)	0.123	0.760	1.66	2.06	5488
T2 (surrogate)	0.669	4.78	10.7	13.33	35337

Sample preparation for FT-ICR MS analysis: Water-soluble organic species were extracted from each WAF using the liquid-liquid extraction method of Stanford et al. (Stanford et al., 2007) and the triplicate extracts combined. For negative-ion ESI analysis, water-soluble organic acidic extracts were dissolved in toluene to yield stock solutions (1 mg/mL), and further diluted in toluene with equal parts (vol:vol) methanol spiked with 0.25% (by volume) tetramethylammonium hydroxide (TMAH) to final concentration (10-50 µg/mL) prior to

analysis. For positive-ion ESI analysis, 1% formic acid was used. For APPI analysis, samples were directly diluted into toluene.

9.4 T FT-ICR mass spectrometer: Whole MC252 and surrogate crude oil and derived acidic and basic water-soluble organics (WSO) were analyzed with a custom-built FT-ICR mass spectrometer (Kaiser et al., 2011) equipped with a 9.4 tesla horizontal 220 mm bore diameter superconducting solenoid magnet operated at room temperature, and a modular ICR data station (Predator) (Blakney et al., 2011) facilitated instrument control, data acquisition, and data analysis.

Results and Discussion

Photochemical alterations to WAFs cause changes in the chemical content resulting in varied effects on microbial growth. This study examined compositional changes in WAFs made under sterile conditions, allowing for separation of biological degradation from photochemical processes. Although bacterial growth response was generally consistent between MC252 and surrogate WAFs, significant deviations in molecular weight distributions were observed. Based on bacterial growth response, inhibition of 3H-leucine incorporation appears to be driven by PAR exposure during WAF formation (Figure 1).

FT-ICR MS was used to analyze extracted WAFs. Key points we wish to highlight in this study are 1. Fundamental compositional comparisons between parent MC252 and surrogate oils and 2. varied photo-chemical alterations resulting in differences in compound class distributions. ICR is limited to semi-quantitative comparisons and similar ionization efficiencies of compound classes across samples with limited matrix effects are assumed. However, the observed high

concentration of oxygenated species present in these samples can suppress ionization of less acidic species such as N₁, NO and NS heteroatom class compounds.

Initial comparisons of bulk properties, primarily elemental analysis, show similarities between parent oils (MC252 and surrogate) with the exception of oxygen content (Supplemental Table 1). MC252 contains 0.4 % oxygen, whereas surrogate contains 5.36% oxygen (Aeppli et al., 2012).

Parent crude oil comparison: MC252 vs. surrogate

Acidic species determination via negative-ion ESI: Upon FT-ICR MS analysis of MC252 and surrogate crude oil, heteroatom class distributions and compositional differences were observed as shown in figures (S1 and S2). Figure S1 shows the heteroatom class distribution of acidic species derived from negative-ion ESI FT-ICR MS where the major species observed for both oils were hydrocarbon (HC), N₁, NO and O₁. This provides a baseline for comparison of WAFs made from each oil.

Figure S2 shows isoabundance contour plots of double bond equivalents (DBE) vs. carbon number for MC252 and surrogate crude oil for the hydrocarbon, N₁, O₁ and NO heteroatom classes obtained from negative-ion ESI FT-ICR MS. Comparison of relative abundance-weighted average DBE and carbon numbers determined for each elemental class show remarkable similarities between the two crude oils.

Positive-ion APPI: Figure S3 shows the heteroatom class distribution of species derived from positive-ion APPI FT-ICR MS where the major species observed for both oils were hydrocarbon (HC), N₁, O₁ and S₁ for both oils. Surrogate showed increased relative abundance for most heteroatoms classes compared to MC252 with the exception of HC and S₁. APPI analysis showed surrogate oil is enriched in oxygenated species extending to O₆ compared to MC252.

This is consistent with the higher oxygen content of surrogate oil. Figures S1, S3 and supplemental Table 1 show how similar MC252 and surrogate oil are upon initial analysis of the parent oils. However, differences are observed for light incubated water accommodated fractions between the two oils.

Acidic water soluble organic (WSO) fraction analysis: Acidic WSO fractions were analyzed via negative-ion ESI FT-ICR MS (Figures 2 and 3). Figure 2 shows alterations in surrogate oil upon incubation over time. When comparing relative heteroatom class distributions within each sample after a week, dark treatments showed formation of N_1 and S_1O_3 species. After a week (T2), PAR incubated samples showed the most substantial increase for the S_1O_4 class. Full sun exposed samples showed a different trend compared to PAR and dark treatments with an increase in N_1 and N_1O_1 and N_1O_2 species over time.

Trends for MC252 varied from those observed for surrogate oil (Figure 3). Dark incubated samples showed no changes in the distribution of species with the exception of S_1O_3 which showed an increase at T2. PAR exposed samples showed a loss of O_2 with increases in O_4 - O_6 and $N_1O_{3.5}$ over 6 days. Full sun incubated samples showed increases in higher number O species (O_4) and formation of S_1O_4 , S_1O_4 , N_1O_4 , and N_1O_5 over 6 days. In comparison, differences between MC252 and surrogate oil are related to S_1O_x and N_1 species behavior.

By comparison, Ray et al. examined photochemical changes to MC252 WAFs created under dark, non-sterile incubation after 7 days. The WAFs were then exposed to artificial sunlight (3 day full sun equivalent exposure). Post exposure acid fractions showed predominate O_5 species formation which have a higher water solubility (Ray et al., 2014). This study examined WAFs created under varied light exposures (PAR and full sun). Although WAF preparation varied between these two studies, trends for O_2 loss, increases in O_4 - O_6 and

formation of N_1O_x species are consistent with those reported by Ray et al. for MC252 water soluble acidic species when exposed to simulated sunlight (Ray et al., 2014). However, higher percent relative abundances were observed for this study ($O_3= 9\%$, $O_4= 13\%$, and $O_5= 8\%$) compared to those reported by Ray ($O_3= 3\%$, $O_4=5\%$, $O_5= 5\%$) for negative mode ESI of the light exposed acid fraction for MC252.

Figure 4 shows the heteroatom class distributions for acidic species derived from negative-ion ESI FT-ICR MS for MC252 and surrogate oil full sun exposures T1 and T2. Surrogate oil did not show variation in S_1 and S_1O_x distributions over 6 days of exposure. However, N_1 and lower N_1O_x species (N_1O_1 and N_1O_2) formation was observed. This is in contrast to the behavior of MC252 where increases in N_1O_x with greatest increase observed for higher oxygen species (N_1O_4 and N_1O_5) at T2. MC252 also showed formation of S_1O_3 and S_1O_4 at T2.

Relative isoabundance-contoured plots of double bond equivalents vs carbon number for acidic water soluble organic species O_1 - O_5 for MC252 and surrogate oil for T1 and T2 exposures (dark, PAR and full sun) are shown in Figures 5 and 6. Average carbon number and DBE values for Figure 5 are shown in Table 2.

After one day exposure (T1), surrogate oil showed enrichment in O_{3-5} classes in light exposed (PAR and Full sun) samples compared to those incubated in the dark. This is opposite to the trend observed for MC252 which did not show enrichment of oxygenated species with light exposure by T1. At approximately one week incubation (T2), MC252 begins to show formation of oxygenated species for PAR and full sun exposed samples compared to dark controls. However, surrogate oil continues to have very different reactivity with formation of O_1 - O_5 with a shift towards higher DBE for PAR and Full sun exposures.

Table 2 Average C number/Double Bond Equivalent Values by Treatment (from figures 5 and 6)

Species	MC252 Dark (T1;T2)	Surrogate Dark (T1;T2)	MC252 PAR (T1;T2)	Surrogate PAR (T1;T2)	MC252 Full Sun (T1;T2)	Surrogate Full Sun (T1;T2)
O ₁	*, C ₂₁ /8	C ₂₆ /11; C ₃₂ /11	*, C ₁₈ /10	C ₂₀ /9; C ₂₀ /10	C ₂₁ /11; C ₁₉ /10	C ₂₀ /9; C ₃₂ /11
O ₂	C ₂₁ /7; C ₂₃ /8	C ₁₈ /5; C ₁₉ /11	C ₁₈ /8; C ₁₉ /10	C ₁₈ /6; C ₂₂ /9	C ₁₉ /8; C ₂₀ /10	C ₁₉ /7; C ₂₇ /10
O ₃	C ₂₃ /10; C ₂₃ /11	C ₂₃ /8; C ₂₅ /9	C ₂₄ /12; C ₂₀ /10	C ₂₀ /9; C ₂₁ /10	C ₂₃ /12; C ₂₁ /10	C ₂₁ /9; C ₂₅ /11
O ₄	C ₂₅ /10; C ₂₄ /11	C ₂₉ /10; C ₂₅ /9	C ₂₉ /15; C ₂₁ /10	C ₂₃ /9; C ₂₂ /10	C ₂₈ /14; C ₂₂ /10	C ₂₃ /10; C ₂₆ /12
O ₅	C ₂₅ /8; C ₂₄ /8	C ₂₇ /12; C ₂₅ /10	C ₃₃ /17; C ₂₅ /11	C ₂₅ /11; C ₂₄ /11	C ₃₂ /16; C ₂₆ /12	C ₂₅ /11; C ₂₈ /13

*indicates no species > 0.5% relative abundance were detected

Figures S4 and S5 are a comparison of carbon number and DBE values for acidic water soluble organic species N₁ and NO₁₋₄ for MC252 and surrogate oil for day 1 exposures (dark, PAR and full sun). Initially, MC252 parent oil an increased N₁ relative abundance (~38%) compared to surrogate oil (30%) (Figure S1). After one day of full sun exposure, MC252 WSO had no detectable N₁ species with greater than 0.5% relative abundance. In contrast, surrogate oil of the same exposure treatment showed persistence of the N₁ class which is most likely pyrrolic species (Rodgers et al., 2005).

Surrogate oil shows a very different response for dark and full sun incubations compared to MC252. Dark surrogate oil had increasing distributions of nitrogen containing species with a shift towards higher average carbon number over time. Full sun N₁, NO and NO₂ also had a shift towards higher average carbon numbers (C₃₆, C₂₉, C₂₈ respectively) by T2. Furthermore, surrogate oil is being oxidized across a wider carbon number range compared to MC252 as evidenced by the distribution of NO_x species.

Table 3 Relative-abundance weighted Average C number/Double Bond Equivalent Values by Treatment (Figures S4 and S5)

Species	MC252 Dark (T1;T2)	Surrogate Dark (T1;T2)	MC252 PAR (T1;T2)	Surrogate PAR (T1;T2)	MC252 Full Sun (T1;T2)	Surrogate Full Sun (T1;T2)
N ₁	*	C ₃₆ /15;C ₃₈ /14	*	C ₂₀ /10;C ₂₂ /11	*	C ₂₀ /10;C ₃₆ /14
NO	C ₁₈ /9;C ₁₇ /8	C ₃₂ /12;C ₃₄ /15	C ₁₈ /8;C ₂₁ /10	C ₂₁ /11;C ₂₃ /11	C ₁₈ /10;C ₂₁ /11	C ₂₂ /11;C ₂₉ /13
NO ₂	C ₂₁ /9;C ₂₁ /9	C ₂₀ /10;C ₂₇ /13	C ₁₉ /10;C ₂₀ /10	C ₂₁ /11;C ₂₂ /12	C ₂₀ /10;C ₂₁ /11	C ₂₂ /12;C ₂₈ /13
NO ₃	C ₂₁ /9;C ₂₁ /9	C ₂₁ /9;C ₂₆ /15	C ₂₂ /10;C ₂₃ /11	C ₂₂ /11;C ₂₃ /12	C ₂₂ /10;C ₂₃ /11	C ₂₃ /12;C ₂₆ /12
NO ₄	C ₂₄ /10;C ₂₅ /10	C ₃₁ /10;C ₂₈ /10	C ₂₅ /11;C ₂₅ /12	C ₂₅ /12;C ₂₅ /13	C ₂₅ /12;C ₂₅ /13	C ₂₆ /12;C ₂₈ /13

*indicates no species > 0.5% relative abundance were detected

Basic species characterization of surrogate oil by positive-ion ESI: Figure S6 represents the isoabundance contour plots of double bond equivalents (DBE) vs. carbon number for the hydrocarbon, N₁, N₁O₁, and N₂ heteroatom classes obtained from positive-ion ESI FT-ICR MS. Relative abundance-weighted average DBE and carbon numbers from elemental compositions are indicated for each heteroatom class. The most abundance species was N₁ (44%) with most abundant molecular formula DBE 14, average C₄₂ corresponding to pyridinic species (Stanford et al., 2007).

Basic water soluble organic (WSO) fraction analysis: The water soluble organic fractions described previously were also analyzed using positive ion ESI FT-ICR MS. Isoabundance contour plots of double bond equivalents (DBE) vs. carbon number for the major heteroatom classes obtained from positive-ion ESI FT-ICR MS at various exposure times are represented in Figure S7 (T1) and Figure S8 (T2). Relative abundance-weighted average DBE and carbon numbers from elemental compositions are indicated in Table 4. After a week of exposure, all samples showed N₁ as the most abundant species: dark (33%), PAR (19%), and Full sun (26%). Figures S7 and S8 show no obvious difference between the three treatments. This is not

surprising due to the recalcitrant nature of pyridinic N which are very stable (McKenna et al., 2013).

Table 4 Average C number/Double Bond Equivalent Values by Treatment (Figures S7-S8)

Species	Surrogate Dark (T1;T2)	Surrogate PAR (T1;T2)	Surrogate Full Sun (T1;T2)
N ₁	C ₄₈ /15;C ₄₈ /15	C ₅₀ /16;C ₄₈ /15	C ₄₉ /14;C ₄₁ /14
NO	C ₄₈ /17;C ₄₈ /18	C ₄₉ /18;C ₄₆ /16	C ₄₆ /16;C ₄₅ /16
NO ₂	C ₄₄ /17;C ₄₁ /16	C ₄₃ /17;C ₃₉ /17	C ₃₈ /15;C ₃₇ /16
NO ₃	*,*	C ₃₄ /15;C ₃₃ /16	C ₃₂ /15;C ₃₂ /15
NO ₄	*,*	C ₃₆ /16;C ₃₆ /17	C ₃₅ /16;C ₃₄ /16
NO ₅	*,*	*,C ₃₇ /18	*, C ₃₆ /17
NO ₆	*,*	*,*	*, C ₃₅ /16

*indicates no species > 0.5% relative abundance were detected

Conclusion

Even though MC252 and surrogate oils share similar geochemical properties such as elemental compositions and bulk properties, the complexity of the petroleum influences its response to photochemical degradation due to compositional differences. In this study, sterile seawater was used to prepare WAFs which were then incubated under various conditions (full sun, PAR and dark). Acidic fractions analyzed for surrogate and MC252 had higher DBE, corresponding to aromatic acids. Differences in heteroatom class distribution for full sun incubated WAFs were observed between surrogate and MC252. After prolonged exposure, surrogate oil had increasing percent relative abundance of nitrogen containing species (primarily N₁, NO₁, NO₂, NO₃, and NO₄) compared to MC252. Surrogate oil is being oxidized across a wider carbon number range compared to MC252 as evidenced by the distribution of NO_x species.

Previously, it was determined that the most prevalent water soluble acidic species in South American crude oil belonged to heteroatom classes O, O₂, O₃, O₄, OS, O₂S, O₃S, O₄S,

NO₂, NO₃, and NO₄ (Stanford et al., 2007). Mapolelo et al. reported the most acidic species belong to the group of naphthenic acids corresponding lower DBE value (DBE 1-3) (Mapolelo et al., 2008). The results of this study have much higher average DBE for nitrogen and oxygen containing species at the same carbon number, indicating a higher level of aromaticity such as condensed aromatic structures containing carboxylic acid moieties (Stanford et al., 2007), (Ruddy et al., 2014).

Although WAF preparation for this study varied from Ray et al., some similarities were observed such as O₂ loss, increases in O₄-O₆ and formation of N₁O_x species for light exposed MC252. However, this study found higher percent relative abundances for oxygenated species compared to Ray et al. (Ray et al., 2014). This further emphasizes the importance WAF formation may have on species formed from photochemical exposure. Future efforts will focus on identifying specific sub classes formed during PAR exposure that contribute towards the observed toxicity trend.

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Figure 1. (A) Inhibition of bacterial production by MC252 WAFs made under different solar exposures. Lack of inhibition by the WAF made in the dark demonstrates the photochemical reactivity of the WAF. Similar results between Full Sun and PAR treatments indicate that the photochemical reactivity is caused by visible light and not ultraviolet radiation. (B) Percent inhibition of bacterial production from MC252 WAFs (●) and surrogate oil WAFs (□) as a function of exposure to sunlight during WAF formation over two week periods. Both oils yield similar response to sunlight exposure.

Figure 2: Heteroatom Class Distribution of Acid Fraction Species Derived from Negative-Ion ESI FT-ICR Mass Spectra Light Treatments (T1, T2) Surrogate Oil

Figure 3: Heteroatom Class Distribution Acid Fraction Species Derived from Negative-Ion ESI FT-ICR Mass Spectra Light Treatments (T1, T2) MC252 Oil

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Figure 4: Direct comparison for MC252 and Surrogate for Heteroatom Class Distribution Acid Fraction Species Derived from Negative-Ion ESI FT-ICR Mass Spectra Light Treatments (T1, T2)

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Figure 5: Isoabundance contour plots of double bond equivalents (DBE) vs. carbon number for the O₁- O₅ heteroatom classes obtained from negative-ion ESI FT-ICR MS acidic fraction water-soluble organics (Comparison Surrogate and MC252) T1 exposure

Figure 6: Isoabundance contour plots of double bond equivalents (DBE) vs. carbon number for the O₁- O₅ heteroatom classes obtained from negative-ion ESI FT-ICR MS acidic fraction water-soluble organics (Comparison Surrogate and MC252 O₁-O₅ species) T2 exposure

References

- Aeppli, C., Carmichael, C.A., Nelson, R.K., Lemkau, K.L., Graham, W.M., Redmond, M.C., Valentine, D.L., Reddy, C.M., 2012. Oil weathering after the Deepwater Horizon disaster led to the formation of oxygenated residues. *Environ. Sci. Technol.* 46, 8799-8807.
- Barron, M.G., Carls, M.G., Short, J.W., Rice, S.D., 2003. Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope crude oil to Pacific herring eggs and larvae. *Environ. Sci. Technol.* 22, 650-660.
- Blakney, G.T., Hendrickson, C.L., Marshall, A.G., 2011. Predator data station: A fast data acquisition system for advanced FT-ICR MS experiments. *Int. J. Mass Spectrom.* 306, 246-252.
- Coelho, G.M.a.D.V.A., 1997. Proceedings of the Seventh Meeting of the Chemical Response to Oil Spills: Ecological Effects Research Forum, in: Coelho, G.M., Aurand, D.V. (Eds.). Ecosystem Management & Associates, Inc., Purcellville, VA.
- Genuino, G., Horvath, D.T., King'onde, C.K., Hoag, G.E., Collins, J.B., Suib, S.L., 2012. Effects of visible and UV light on the characteristics and properties of crude oil-in-water (O/W) emulsions. *Photochem. and Photobio. Sci.* 11, 692-702.
- Kaiser, N.K., Quinn, J.P., Blakney, G.T., Hendrickson, C.L., Marshall, A.G., 2011. A Novel 9.4 Tesla FTICR Mass Spectrometer with Improved Sensitivity, Mass Resolution, and Mass Range. *J. Am. Soc. Mass Spectrom.* 22, 1343-1351.
- King, S.M., Leaf, P.A., Olson, A.C., Ray, P.Z., Tarr, M.A., 2014. Photolytic and photocatalytic degradation of surface oil from the Deepwater Horizon spill. *Chemosphere* 95, 415-422.
- Mapolelo, M.M., Stanford, L., Rodgers, R., Yen, A.T., Debord, J.D., Asomaning, S., Marshall, A., 2008. Chemical Speciation of Calcium and Sodium Naphthenate Deposits by Electrospray Ionization FT-ICR Mass Spectrometry. *Energy Fuels* 23, 349-355.
- McKenna, A.M., Nelson, R.K., Reddy, C.M., Savory, J.J., Kaiser, N.K., Fitzsimmons, J.E., Marshall, A.G., Rodgers, R.P., 2013. Expansion of the analytical window for oil spill characterization by ultrahigh resolution mass spectrometry: beyond gas chromatography. *Environ. Sci. Technol.* 47, 7530-7539.
- Ray, P.Z., Chen, H., Podgorski, D.C., McKenna, A.M., Tarr, M.A., 2014. Sunlight Creates Oxygenated Species in Water-Soluble Fractions of Deepwater Horizon Oil. *J. Hazard. Mater.* 280, 636-643.
- Reddy, C.M., Arey, J.S., Seewald, J.S., Sylva, S.P., Lemkau, K.L., Nelson, R.K., Carmichael, C.A., McIntyre, C.P., Fenwick, J., Ventura, G.T., Van Mooy, B.A.S., Camilli, R., 2012. Composition and fate of gas and oil released to the water column during the *Deepwater Horizon* oil spill. *PNAS* 109, 20229-20234.

Rico-Martinez, R., Snell, T.W., Shearer, T.L., 2013. Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A to the *Brachionis plicatilis* species complex (Rotifera). *Environ. Pollut.* 173, 5-10.

Rodgers, R.P., Schaub, T.M., Marshall, A.G., 2005. Petroleomics: MS Returns to its Roots. *Anal. Chem.* 77, 20A-27A.

Ruddy, B.M., Huettel, M., Kostka, J.E., Lobodin, V.V., Bythell, B.J., McKenna, A.M., Aeppli, C., Reddy, C.M., Nelson, R.K., Marshall, A.G., Rodgers, R.P., 2014. Targeted Petroleomics: Analytical Investigation of Macondo Well Oil Oxidation Products from Pensacola Beach. *Energy Fuels* 28, 4043-4050.

Smith, D.C., Azam, F., 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using (3)H-leucine. *Marine Microbial Food Webs* 6, 107-114.

Stanford, L., Kim, S., Klein, G., Smith, D., Rodgers, R., Marshall, A., 2007. Identification of Water-Soluble Heavy Crude Oil Organic-Acids, Bases, and Neutrals by Electrospray Ionization and Field Desorption Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Environ. Sci. Technol.* 41, 2696-2702.