

High Resolution Mass Spectrometry Advances in Oil Spill Analysis

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Abstract. Oil is a complex mixture of alkanes, cycloalkanes, aromatics, nitrogen/sulfur/oxygen (N/S/O) heterocycles, alcohols, ketones, carboxylic acids, porphyrins, and myriad possible combinations therein. Once introduced into the environment, some weathering processes reduce this complexity through evaporative losses (loss to atmosphere) as well as water washing of low ring number aromatics, N/S/O heterocycles, alcohols, ketones, and carboxylic acids (loss to seawater). However, Gulf of Mexico Research Initiative (GoMRI) supported research has revealed that the contributions of these mechanisms to the reduction of molecular complexity are dwarfed by that of oxidative weathering processes (photo- and bio-oxidation) that increase the compositional complexity of the transformed oil. Because these oxidative processes increase the complexity of an already analytically challenging organic mixture and the boiling point of transformed species is higher than that of the precursors, conventional analytical techniques yield little insight into the identification of oil spill transformation products. Despite the challenge, recent advances in analytical science now allow molecular-level insight into these complex systems irrespective of initial (unaltered) or transformed-product boiling point; these advances were largely made possible by GoMRI supported research efforts. They expose a continuum of oxidized transformation products that span oil-soluble, oil-soluble interfacially-active, and water-soluble species. The isolation and characterization of oil-soluble, interfacially-active species confirm a long-standing theory that photo-oxidation generates oil-soluble surfactant-like species, which limit the effectiveness of dispersants. Furthermore, photo-oxidation specific microcosms are shown to generate unique species that are also found in field samples. Bio-oxidation only microcosms were found to generate very different, oil-soluble species; thus, photo-oxidation is implicated in the formation of transformation products in the field. Finally, analyses of photo-oxidized distillate cuts as well as asphaltene samples confirm prior reports of photo-induced polymerization and photo-cracking of native petroleum molecules. Here, we summarize the advances in the molecular-level understanding of oil over the past 8 years, in the context of oil spill science, by high resolution mass spectrometry, and we highlight potential opportunities for future research, as well as knowledge gaps that must be addressed for future spills.

Introduction

Mass spectrometry (MS) has contributed oil spill analysis for decades (Albaiges & Albrecht, 1979; Guard, Hunter, & DiSalvo, 1975; Hansen, 1975; Hertz, May, Chesler, & Gump, 1976; Lao, Thomas, & Monkman, 1972; E. B. Overton, Patel, & Laseter, 1979; Patel, Overton, & Laseter, 1979; Teal, Burns, & Farrington, 1978; Wilson, Ferrero, & Coleman, 1975), and the once complex, user intense platforms of the '60's and '70's have evolved into benchtop instruments that can largely operate 24/7 unattended. Incredibly, they have even evolved into platforms that can be deployed subsurface to monitor organic species in seawater (Camilli et al., 2010). Despite the advances, traditional oil spill analyses still rely heavily on Gas Chromatography (GC) based mass spectrometry techniques that most commonly inventory the volatile/semi-volatile components of the oil collected from field samples and compare them to the species identified in the suspected parent oil(s). The “book keeping” technique is extremely powerful when a sample of the suspected oil is available, as biomarkers can be used to match the spilled oil to the suspected oil, and the disappearance of species from the parent oil in the saturates, cycloalkanes, and 1-3 ring aromatics can be used to discern weathering patterns (Arey, Nelson, Plata, & Reddy, 2007; Arey, Nelson, & Reddy, 2007; Payne & Driskell, 2018; Stout & Payne, 2016; Stout, Payne, Emsbo-Mattingly, & Baker, 2016; Stout, Payne, Ricker, Baker, & Lewis, 2016). However, as world oil consumption shifts to heavier and heavier crude oils, a larger mass fraction of the oil is left unanalyzed by these techniques. In the absence of a suspected oil sample, GC-based techniques still provide a wealth of compositional information that combined with 40+ years of research, highlight compositional changes expected for evaporative losses, biodegradation, and water washing. GC-based techniques are incredibly

powerful for such analyses as they provide molecular-level information at the isomeric-level of detail.

Comprehensive GC × GC. The development of comprehensive 2D GC has further expanded the compositional information available and addressed problems with co-eluting peaks that complicate data interpretation in 1D GC separations (Aeppli et al., 2014; Booth et al., 2007; Boursier et al., 2013; Dutriez et al., 2013; Frysinger, Gaines, & Reddy, 2002; Frysinger, Gaines, Xu, & Reddy, 2003; Gaines & Frysinger, 2004; Gros et al., 2014; Nelson et al., 2019; Robson, Sutton, McCormack, Chilcott, & Rowland, 2017; Santos, da Cruz, Avila, Pereira, & Azevedo, 2018; Ventura et al., 2011). Furthermore, its recent coupling to high resolution time-of-flight mass spectrometry brings new compositional information and applications that were previously impossible to obtain (Kaefer et al., 2019). However, the main drawback of GC-based techniques is their limited boiling point “window”. Native petroleum saturate (alkane) species are readily detected and resolved up to ~C₃₀–C₃₅ (cryo-modulation) (Gaines & Frysinger, 2004) or C₆₀ (pulsed flow modulation) (Boursier et al., 2013; Duhamel et al., 2015), but the attainable carbon number drops as the molecules become aromatic or polar due to an increase in their respective boiling points. Furthermore, the addition of oxygen (common in the surface weathering (photo-oxidation) of petroleum spills) is not readily detectable due to the large change in boiling point with oxygen addition to the native (unaltered), hydrocarbon dominant petroleum. The oxidized transformation products have been known to exist for decades (Overton et al., 1980), and have been suggested to form by multiple mechanisms: photo-cracking of large molecules, photo-oxidation of the native petroleum species, photo-induced polymerization, and are implicated in the *in situ* production of surfactants (Thingstad & Pengerud, 1983); however, the compositional complexity, combined with their increased boiling point, led to the identification of only a

handful of the lowest carbon number species in past studies (Overton et al., 1980). Thus, there is a need to both expand the analytical window for the analysis of native petroleum molecules as well as facilitate the compositional analysis of transformation products (new species formed largely due to oxidation). Such capabilities could confirm suspected photo-induced weathering mechanisms and characterize potential photo-generated surfactants.

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. Recent results have demonstrated the utility of ultra-high resolution mass spectrometry (Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS)) for the molecular-level analysis of petroleum, and petroleum transformation products from oil spills, regardless of boiling point (Chen et al., 2016; Chen et al., 2018; Corilo et al., 2013; Griffiths, Da Campo, O'Connor, & Barrow, 2014; Lemkau, McKenna, Podgorski, Rodgers, & Reddy, 2014; Ruddy et al., 2014; Thomas et al., 2019). Importantly, this extension of the analytical window to higher carbon number overlaps with that of GC-based techniques at low carbon number (McKenna et al., 2013). Thus, results from both techniques can be used to document, and understand compositional changes due to weathering.

FT-ICR mass spectrometry provides the highest resolution ($\sim 2M$ at $m/z = 400$) and mass accuracy (100-200 ppb) of all commercially available mass spectrometers. (Marshall et al., 2013; Kaiser, Quinn, Blakney, Hendrickson, & Marshall, 2011) The high resolution facilitates the identification of peaks even in cases where the mass difference between 2 adjacent peaks is less than the mass of an electron (~ 0.55 mDa) (McKenna et al., 2014), and the high mass accuracy allows the assignment of elemental compositions based on mass measurement alone. (Marshall & Rodgers, 2008; Marshall & Rodgers, 2004; Rodgers, Schaub, & Marshall, 2005) Combined with the coarse chemical functionality specific ionization of many available ion sources, FT-ICR MS

analysis facilitates the assignment of tens of thousands elemental compositions in a single mass spectrum, and provides insight into their chemical functionality (i.e. acidic, basic, aromatic). The elemental compositions are subsequently sorted and grouped (Marshall & Rodgers, 2008; Rodgers & McKenna, 2011) according to: 1) **heteroatom class**, the number and type of atoms other than hydrogen and carbon (i.e. the hydrocarbon class is HC, and those with a single sulfur atom are S₁) 2) **type**, their hydrogen deficiency relative to an alkane, commonly termed double bond equivalents (DBE). DBE is a measure of the rings + double bonds to carbon in a molecule. For example, hexadecane (a C₁₆ alkane) would have a DBE = 0. Benzene would have a DBE = 4 (1 ring and 3 double bonds). 3) **carbon number**, simply the number of carbon atoms in the elemental composition assigned. An individual heteroatom class is typically visualized as an isoabundance color coded plot of DBE vs. carbon number as shown in **Figure 1** for the Macondo crude oil. It summarizes the type and carbon number data for a given heteroatom class. Inspection of Figure 1 reveals two notable compositional boundaries. A horizontal boundary at DBE = 4, and a diagonal boundary labeled “Planar Polycyclic Aromatic Hydrocarbon (PAH) Limit”. The horizontal boundary at DBE = 4 is imposed by the ionization method employed in the analysis, electrospray ionization (ESI) that preferentially ionizes basic species (6-membered ring nitrogen species/pyridine homologs). Thus, the alkylated pyridines (1-ring aromatic nitrogen species, DBE = 4) are the lowest DBE species that are readily observed. The diagonal boundary is the result of the thermal processes involved in petroleum maturation. Aromatic structures in petroleum are limited to planar polycyclic aromatic hydrocarbons and their heteroatom-containing homologs (PAHs/PANHs/PASHs). Thus, the maximum aromaticity (planar) possible at a given carbon number is denoted by the PAH limit line (Hsu, Lobodin, Rodgers, McKenna, & Marshall, 2011). As one progresses up in DBE = 4 (alkylated pyridines),

the alkyl quinolines and alkyl acridines are highlighted at DBE = 7 and DBE = 10. The relative abundance of each species is assigned by the color code shown in the bottom right. The molecular “footprint” occupied in the plot of DBE vs. carbon number is commonly referred to as its “compositional space” for the given class. The space varies with different crude oils and/or refined products (i.e. light crudes are shifted to lower carbon numbers and DBEs, whereas heavier crudes are shifted to higher carbon numbers and DBEs). Although ultra-high resolution mass spectrometry extends the analytical window into the non-distillables of petroleum (out to C₉₀ in Figure 1) and enables the molecular-level analysis of previously unobserved transformation products, it is important to note that it does so by accurate mass measurement, which in turn enables elemental composition assignment. Thus, all isomers with a given elemental composition are detected as a single peak.

The work presented herein will focus on the identification of previously unidentified transformation products. Thus, laboratory microcosms are employed to capture the water soluble species that are lost to the environment in field samples. Volatile transformation products are not considered, as they can be routinely analyzed by other methods. Since *water* soluble species cannot be routinely captured from field samples, *oil* soluble transformation products from microcosm experiments are compared to field samples to discern the weathering mechanism that most contributes to the transformation products observed.

Methods

Samples and Reagents. South American heavy crude oil was provided by the University of Quindio (Colombia), Arab heavy distillate (263-363°C) was provided by GE Global Research (New York), a residual fuel oil (SRM1621e) and the Macondo crude oil (SRM 2779) were

purchased from NIST. Seawater was collected from the Gulf of Mexico by the Louisiana Universities Marine Consortium (35 ppt, pH 8.3) and filtered through combusted (450°C) 0.27 μm glass microfiber filters (Advantec). It was photo-irradiated prior to use in experiments for 12 h to decrease DOC concentration (Final [DOC] \sim 1 ppm) and remove any terrestrial signatures. All glassware was acid cleaned and combusted at 500°C in a muffle furnace prior to use. Toluene, (HPLC)-grade dichloromethane (DCM), heptane, and pentane were all purchased from VWR. For isolation and analysis of interfacial material (IM), high-performance liquid chromatography (HPLC)-grade DCM, methanol (MeOH), *n*-heptane, toluene, and water were obtained from J.T. Baker (Phillipsburg, NJ). The pH \sim 11 water was prepared by dropwise addition of Sigma-Aldrich (St. Louis, MO) ammonium hydroxide solution (28% in water). Chromatographic-grade silica gel (Fisher Scientific, 100–200 mesh, type 60 Å) was dried overnight in an oven at \sim 110°C.

Sample preparation. Thin oil films (120 μm) were created with 325 mg light oil, and spread over 50 mL of pre-irradiated seawater (Zito et al., 2019). The oil films were exposed to simulated sunlight using an Atlas Suntest CPS solar simulator for 24 hours (equivalent to 6 days of natural sunlight) in 250 mL jacketed beakers thermostatically controlled at 27°C. Simulated solar exposure experiments were performed in triplicate, whereas there was a single dark control. The oil was removed from the surface of the water with a glass rod. The interfacial material (IM) was isolated from each sample following methods previously reported (Clingenpeel, Robbins, Corilo, & Rodgers, 2015; Jarvis, Robbins, Corilo, & Rodgers, 2015). Briefly, 40g of HPLC water (pH = 11) was added dropwise to 60 g of oven-dried silica gel. After hydration, the silica gel remained a free flowing powder. Approximately 250 mg of oil was dissolved in 5 mL of heptol (1:1 (v/v) *n*-heptane:toluene) and 1 g of the hydrated silica was added to generate a slurry.

The slurry was loaded into a 5 mL borosilicate glass pipet (the column) with glass wool packed in the end. The column was washed with excess heptol (~10 mL) to remove unretained species (fraction 1). The species that interacted with the hydrated silica (IM) were eluted with 10 mL of 10:25 (v/v) MeOH:toluene and collected as fraction 2. The oil and IM were blanketed with argon gas, and stored at -3°C in the dark until analysis. Dissolved organic matter was collected by separating the water from each oil after exposure. Water samples were subsequently filtered through 0.27 µm glass microfiber filters and stored under the same conditions as the oil and IM. Ketones were isolated and derivatized from field and microcosm samples by a strong cation exchange method previously reported (Krajewski et al., 2017; Krajewski et al., 2019).

Mass spectrometry. Dissolved organic matter (DOM_{HC}) was collected and preconcentrated by a previously reported solid-phase extraction technique (Dittmar, Koch, Hertkorn, & Kattner, 2008). The extracts, along with the oil and IM samples, were stored in the dark at 4°C in pre-combusted glass vials until analysis by negative-ion electrospray ionization coupled with a custom-built Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) equipped with a 9.4 tesla (Kaiser et al., 2011) superconducting magnet (National High Magnetic Field Laboratory (NHMFL), Florida State University, Tallahassee). Each mass spectrum was internally calibrated with a “walking” calibration equation (Savory et al., 2011) with internally developed software provided by the NHMFL (Predator) (Blakney, Hendrickson, & Marshall, 2011) followed by molecular formula assignment by PetroOrg (Corilo, 2014).

Results and Discussion.

Microcosm Studies. To determine which weathering mechanism (biodegradation or photo-oxidation) contributes to the oxidized species detected in field samples (Chen et al., 2016; Ruddy,

Blakney, Rodgers, Hendrickson, & Marshall, 2013; Ruddy et al., 2014), photo- and bio-only microcosm studies were performed in the laboratory. Oil samples were collected and analyzed after a collection of weathering periods (0 - 30 days for biodegradation and 0 – 120 h for photo-oxidation). Direct analysis of the oil after 24 hours of photo-oxidation reveals the molecular changes induced by irradiation. **Figure 2** highlights these changes at a single nominal mass for a South American crude oil ($m/z = 400$), but are also present at every nominal mass (Ruddy et al., 2014). Photo-oxidation increases the compositional complexity (from ~30 peaks per nominal mass to greater than 50 peaks per nominal mass) through the formation of oxidized products, a few of which are highlighted in red: hydrocarbons (HC) are converted to oxygen-containing (O_x) species and sulfur-containing species (S_1 and S_2) are converted to SO_x species. The effect of photo-oxidation is not limited to crude oils, as distillates and blends display similar behavior. In a separate experiment performed on a NIST residual fuel oil, abundant O_x species are readily identified after only 24 hours of irradiation. **Figure 3** summarizes the newly formed O_x species in a van Krevelen plot of H/C vs. O/C ratio of the elemental compositions assigned from the FT-ICR MS data (Wu, Rodgers, & Marshall, 2004) before (dark) and after 24 h of irradiation. Thus, ultra-high resolution mass spectrometry readily allows molecular-level characterization of oil soluble photo-transformation products not possible by other methods. However, prior research implicates photo-oxidation in the generation of other products such as water soluble species and oil soluble surfactants (Harriman, Zito, Podgorski, Tarr, & Suflita, 2017; Ray, Chen, Podgorski, McKenna, & Tarr, 2014; Thingstad & Pengerud, 1983). In fact, the photo-irradiation of the residual fuel oil raised the dissolved organic carbon from just over 10 ppm prior to irradiation to over 110 ppm after 120 h of irradiation. Suspected oil soluble surfactants were isolated from the photo-irradiated oil via the wet silica method that isolates non-interfacially active oil components

from interfacially active species (Jarvis et al., 2015). The analysis of all three fractions (oil soluble non-interfacially active, oil soluble interfacially active, and water soluble species) are presented in **Figure 4**, and reveal an oxygen content dependence on solubility and behavior. Photo-oxidation products with a low number of oxygen atoms remain oil soluble and are not interfacially active (black bars). Products with higher oxygen content (red bars) remain oil soluble but behave as surfactants (they interact with both oil and water). Those with the highest number of oxygen atoms per molecule partition out of the oil and become water soluble species. The large degree of overlap between all three heteroatom distributions is due to the wide carbon number range of the original residual fuel oil. Simply, high carbon number species require a higher number of oxygens to become interfacially active, and even greater numbers to yield water solubles.

Although the photo-microcosm results confirm the *in situ* generation of surfactants and reveal the oxygen dependence of chemical behavior, they do little to reveal what weathering process (bio- or photo-) is responsible for oxidized transformation products observed in field samples. To address this goal, a chemical functionality dependent method was employed to target ketone/aldehyde transformation products, as both biodegradation and photo-oxidation have been shown to produce them (Krajewski et al., 2017; Lao et al., 1979; Overton et al., 1980; Ruddy et al., 2014). Oil from a bio-only and photo-only microcosm as well as multiple field samples collected after the Deepwater Horizon oil spill, were fractionated by strong cation exchange chromatography to isolate potential ketones/aldehydes (Krajewski et al., 2017). They were subsequently subjected to a ketone/aldehyde specific chemical derivatization and analyzed by FT-ICR mass spectrometry (Alhassan & Andersson, 2013). The results are presented in **Figure 5**. Oil isolated from the bio-only microcosm contains ketones that span a narrow carbon

number range (15 – 35) and an average DBE ~10. However, the oil isolated from the photo-only microcosm contains ketones that span a wide carbon number (15 – 65) and DBE (0 – 20) range, nearly identical to those identified in field samples (Niles et al., 2019). The similarity, combined with supporting similarity of naphthenic acids identified in field and photo-only microcosms (data not shown), strongly suggests that photo-oxidation is the dominant mechanism for the formation of oil soluble oxidized transformation products. Thus, the molecular composition of oil soluble oxidized species could potentially be used to reveal the photo-oxidation history of field samples.

Photo-oxidation products. Past studies have proposed multiple reactions that can occur in the photo-oxidation of surface oil (Cao & Tarr, 2017; Ray & Tarr, 2014b, 2014a, 2015) and include direct/indirect photo-oxidation, photo-induced polymerization, and photo-cracking of large molecules (Overton et al., 1980). Although previous data were presented above in support of direct/indirect photo-oxidation of a residual fuel oil, we revisit the photo-oxidation results from the South American crude oil to reveal the heteroatom class diversity of the photo-induced oxidative transformation products and highlight results that support both direct and indirect photo-oxidation mechanisms. **Figure 6** summarizes the heteroatom class information for the parent South American crude oil (black), the oil soluble photo-oxidation products after 24 h of photo-irradiation (red), and the oxidized water soluble species (green). Combined, more than 50 different heteroatom classes are identified, and as previously discussed, an obvious oxygen dependence on solubility is revealed. Species with a low number of oxygens remain oil soluble, whereas higher oxygen content leads to water soluble species. Furthermore, the relative abundances of the HC, S₁, S₂, and N₁ classes drop after photo-oxidation, as these species are converted to O_x, SO_x, S₂O_x, and NO_x species. A pair of DBE vs. carbon number plots for the

water soluble O_x and SO_x heteroatom classes strongly suggests that these species are formed by both direct and indirect photo-oxidation processes, as abundant transformation products are detected at low DBE. These species lack a chromophore (300 – 800 nm radiation) and therefore must be formed via indirect photo-reaction mechanisms.

Evidence for photo-polymerization is presented in **Figure 7**. We used a distillate cut (263-363°C) to restrict the carbon number range of the starting material, as evident by the narrow ($C_{10} - C_{20}$) distribution of the O_1 class in the starting material (Figure 7, middle, left). The class graph (top) summarizes the changes in the heteroatom distributions before (black) and after photo-oxidation (yellow). As previously highlighted, the HC and S_1 species decrease in relative abundance as they are converted to O_x and SO_x species. For the DBE vs. carbon number plots below, red dashed lines mark the highest carbon number and DBE of the starting material. After photo-oxidation, abundant O_x species are detected with carbon numbers greater than 30. However, there were no species in the parent crude with carbon numbers greater than ~25 (alkane) or 20 (mono and di-aromatics). Additionally, bimodal distributions are clearly evident in the O_4 and O_5 class photo-oxidation products. Thus, photo-induced polymerization of the starting material is confirmed.

Finally, photo-cracking of large aromatic structures is confirmed through the analysis of the photo-chemical behavior of petroleum asphaltenes. Asphaltenes are the most aromatic species in petroleum, contain the highest heteroatom contents, and are those species that are insoluble in excess heptane, but soluble in toluene. We employ a similar approach, but opposite rationale to the photo-polymerization study; asphaltenes were used in this study to isolate high carbon number species so that any potential production of low carbon number species by photo-cracking could be easily confirmed. **Figure 8** contains the DBE vs. carbon number plots for 7

classes of species observed in the asphaltene photo-oxidation experiments (HC – O₆). Mass spectral analysis of the parent asphaltenes (prior to irradiation) reveals that there are no species with a carbon number below 20 (top row). Thus, any photo-products that occupy the lower left quadrant of the red dashed lines, must originate from photo-cracking of the asphaltene molecules. The water soluble species generated by the photo-irradiation of the asphaltene sample (bottom row) clearly occupy carbon number ranges that are not present in the starting material. Thus, the photo-cracking of large aromatics (asphaltene in this case) is supported by the compositional information obtained via FT-ICR mass spectrometry.

Future Directions.

The results highlighted herein expose the compositional complexity of photogenerated transformation products from oil spills. They exist as both oil- and water-soluble species which have known, and yet to be determined effects on the physical and chemical behavior of oil in the environment. Although the understanding of the compositional dependent toxicity of photogenerated water soluble species is in its infancy and will continue to expand in future research, analytical techniques and methods now exist for the analysis of high boiling, oxygen rich photomodified hydrocarbons. Thus, the pace of future research should be faster than the previous 10 years. A fraction of the oil soluble phototransformation products have been shown to be interfacially active, and thus contribute to the increased viscosity and emulsion-forming tendency of surface oil. These species also reduce the effectiveness of dispersants. This knowledge, combined with the ability to isolate these species offers an opportunity to tune dispersant packages for aged, photoirradiated surface slicks, as they contain “surfactants” generated by the *in situ* oxidation of the parent oil. Therefore, perhaps emulsion breaking (EB) chemistries could be employed to return the effectiveness of dispersant / EB formulations.

Finally, the compositional and chemical diversity of phototransformation products demands continued research, as determination of specific oxygen functionalities in these polyoxygenated species remains a challenge.

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Figure Legends.

Figure 1. Class specific (N_1 class) high resolution FT-ICR mass spectrometry data visualization via a plot of DBE vs. carbon number where the relative abundance of each elemental composition (pixel) is color coded according to the scale in the bottom right. Commonly encountered PANH cores are shown on the right at their corresponding DBE values. The greatest DBE possible for a planar PAH molecule at a given carbon number is plotted as a red dashed line (Planar PAH Limit).

Figure 2. Zoom mass insets (single nominal mass) of the mass spectra obtained from the analysis of a South American heavy crude oil before (top) and after (bottom, negative peaks) reveal the increased molecular complexity of the photoirradiated oil. The increased complexity (from 33 to 52 peaks at the nominal mass, $m/z = 400$) is a result of newly-formed oxidized species, a few of which are highlighted in red.

Figure 3. van Krevelen diagram (H/C vs. O/C) of the elemental compositions identified in the mass spectral analysis of a residual fuel oil (RFO) before (top) and after (bottom) photoirradiation. The plot reveals the marked progression of species at higher O/C due to photooxidation of the native RFO molecules.

Figure 4. Heteroatom class graph for O_x species identified after the photoirradiation (24h) of a residual fuel oil. Black bars are for oil soluble, non-interfacially active species, red bars for oil soluble, interfacially active species, and blue bars for water soluble species.

Figure 5. The three DBE vs. carbon number plots summarize the ketones identified in a biodegradation only microcosm (left), photo-only microcosm (center), and field sample collected 57 days post-spill. The remarkable similarity between the photo-only and field sample ketones strongly suggests that photo-oxidation is the primary source of O_x transformation products in weathered oil samples.

Figure 6. Heteroatom class graph for the parent South American oil, oil soluble photooxidation products, and water soluble photooxidation products reveals the wide range of oxidized products formed and clearly demonstrates the oxygen content dependence on oil- and water-solubility. The two DBE vs. carbon number plots (O_x left, and SO_x right) reveal the low DBE of the photoproducts, which strongly suggests that they were formed by indirect photooxidation.

Figure 7. Heteroatom class graph (top) of an Arab distillate cut before (black bars) and after (yellow bars) photo-irradiation. The DBE vs. carbon number plots below reveal that higher oxygen-containing classes ($O_4 - O_7$) contain dimers of approximately twice the DBE and carbon number of the lower oxygen-containing species ($O_1 - O_3$). Given the carbon number range of

the starting distillate cut ($< C_{20}$), the presence of such species suggest that photo-polymerization has occurred.

Figure 8. DBE vs. carbon number plots of an asphaltene sample before (top) and the water soluble species (bottom) generated after photoirradiation confirms that photocracking of the parent asphaltene molecules has occurred. The dotted red lines define the DBE and carbon number ranges of the most abundant parent asphaltene molecules (upper right quadrant) as well as that of the water soluble species (bottom left quadrant).