**Hydrothermal Liquefaction Oil and Hydrotreated Product from Pine Feedstock
Characterized by Heteronuclear Two-Dimensional NMR Spectroscopy and FT-ICR
Mass Spectrometry**

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Abstract

Hydrothermal liquefaction (HTL) crude oil and hydrotreated product from pine tree farm waste (forest product residual, FPR) have been analyzed by direct infusion electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) in both positive- and negative-ionization modes and high-resolution two-dimensional heteronuclear ¹H-¹³C NMR spectroscopy. FT-ICR MS resolves thousands of compounds in complex oils and provides unparalleled compositional details for individual molecules for identification of compound class (heteroatom content), type (number of rings plus double bonds to carbon or double bond equivalents (DBE) and carbon number (degree of alkylation). Heteronuclear ¹H-¹³C NMR spectroscopy provides one-bond and multiple-bond correlations between pairs of ¹H and ¹³C chemical shifts that are characteristic of different organic functional groups. Taken together this information provides a picture of the chemical composition of these oils. Pyrolysis crude oil product from pine wood was characterized for comparison. Generally, pyrolysis oil is comprised of a more diverse distribution of heteroatom classes with higher oxygen number relative to HTL oil as shown by both positive- and negative-ion ESI FT-ICR MS. A total of 300 N₁, 594 O₁ and 267 O₂ compounds were observed as products of hydrotreatment. The relative abundance of N₁O₁, N₁O₂, N₁O₃, N₂, N₂O₁, N₂O₂ and O₃ compounds are reduced to

different degrees after hydrotreatment and other higher heteroatom containing species (O_4 - O_{10} , N_1O_4 , N_1O_5 and N_2O_3) are completely removed by hydrotreatment.

Keywords: biofuel, FT-ICR MS, mass spectrometry, pine, hydrothermal liquefaction, hydrotreatment, pyrolysis, NMR, HSQC, bio-crude

Introduction

Production of transportation fuel from biomass has the potential to reduce the dependence on finite petroleum reserves. A second-generation feedstock, forest pine residual (FPR) biofuel is a low-cost supplement to fossil fuel needs that does not compete with food production. As estimated by Billion-Ton Annual Supply study prepared by U.S. Department of Agriculture and Oak Ridge National Laboratory, forestlands in the contiguous United States could sustainably supply about 368 million dry tons of lignocellulosic biomass annually in the form of logging/harvesting residue, thinning material, mill residue and urban wood waste.[1]

Hydrothermal liquefaction (HTL) and fast pyrolysis are two major thermochemical conversion methods for the production of liquid fuel precursors from forest-derived feedstock.[2, 3] Hydrothermal liquefaction is a low temperature (250-350 °C) and high pressure (5-20 MPa) thermochemical process that requires no drying of biomass and converts organic constituents of biomass into an oil, aqueous fraction, gases and solid by-products.[4] Fast pyrolysis is the thermal treatment of biomass in the absence of oxygen at high temperatures (400-600 °C) and atmospheric pressure with short residence period and high heating rate ($\sim 1000\text{ }^{\circ}\text{C min}^{-1}$) where dry biomass is converted to oil, biochar and a gaseous fraction.[5] The major emphasis of these analyses is the characterization of FPR HTL product and a comparison to that of pyrolysis derived oil. Physical and chemical properties of the oils derived from hydrothermal liquefaction and fast pyrolysis are markedly different. The HTL oil is generally a highly viscous oil with limited water solubility whereas fast pyrolysis oil is less viscous and exhibits higher water solubility than HTL products.[6] Wood-derived liquefaction oils have lower oxygen content, lower moisture content and higher heating value than fast pyrolysis oils.[6, 7] High oxygen content in the bio-crude is a major cause for corrosion and thermal instability of the oil. Nonetheless, numerous upgrading strategies are available for converting biomass-derived oils into usable hydrocarbon fuel.[2, 8-10] The removal of oxygen and nitrogen from HTL oil is essential to meet fuel specifications, which have been established to limit corrosivity, minimize NO_x emission upon combustion and reduce molecular weight for improved material transport.[6, 11] Hydrotreatment is the currently preferred technique for treatment of the polar, heteroatom-containing compounds present in biomass-derived oils.[8, 12]

Molecular characterization of oil is necessary for feedstock selection, process optimization, and to establish the monetary value of the liquid product. Analytical techniques used to describe oil composition include gas chromatography-mass spectrometry (GC-MS),[13-16] Fourier transform infrared spectroscopy,[15] one-dimensional ^1H and ^{13}C NMR spectroscopy,[13] and ultraviolet fluorescence spectroscopy.[17] However, the limitations inherent with each of these techniques has resulted in only partial characterization of HTL-produced oil.[18] High resolution methods such as Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) [19, 20] or multidimensional heteronuclear NMR spectroscopy can overcome many of these limitations. Electrospray ionization (ESI), in particular when coupled with FT-ICR mass spectrometry, provides preferential ionization and observation of thousands of polar constituents in complex matrices without prior fractionation or derivatization. ESI FT-ICR MS has been successfully used in the characterization of highly complex crude oil [21-24], diesel fuel [25], coal extracts [26, 27], humic and fulvic acids [28-31] and algal lipid extracts [32]. Smith et al. illustrated a comparative compositional analysis of red oak oil using three high-resolution mass spectrometers including ESI FT-ICR, orbitrap, quadrupole-time-of-flight (Q-TOF) and demonstrated that FT-ICR is necessary to resolve thousands of component signals in complex oils of this type.[33] Jarvis et. al have provided compositional analysis of pyrolysis oil derived from pine pellet and peanut hull with negative-ion ESI FT-ICR MS.[34] Recently we have reported a detailed compositional description of hydrothermal liquefaction products from microalga by positive- and negative-ion ESI FT-ICR MS.[18] Multidimensional heteronuclear NMR spectroscopy has been used extensively to characterize lignin [35] and less often to characterize fossil fuels and crude oil [36, 37] but has only recently been applied to characterization of pyrolysis oils derived from biomass.[38]

Here, we utilize both positive- and negative-ion mode high resolution FT-ICR MS and 2-D ^1H - ^{13}C NMR spectroscopy to provide an improved understanding of the composition of FPR HTL oil for further improvement in renewable fuel production via the HTL process. Additionally, pyrolysis oil was characterized to allow a direct compositional comparison to the HTL oil sample. We report the distribution of heteroatom-containing compounds for each oil production mode and identify molecules that resist hydrotreatment, and define the range of functional group diversity present within these oils as a means to facilitate further improvement in the upgrading of FPR HTL oils.

Materials and Methods

Oil Production

The HTL oil was produced in a hybrid bench-scale continuous flow reactor system (**Figure 1, top**) that combines plug flow reactor components with a small continuous stirred tank reactor. Before testing, a feed slurry was prepared from loblolly pine forest product residual chips (including bark). The chips were mixed with HTL byproduct water from a previous test and then ground in an immersion mill to create a 13 wt% solids slurry with 99% of the particles < 0.4 mm. The fine particle size slurry is required to facilitate pumping at the bench scale. After the feed slurry is prepared, sodium carbonate at 1 wt% (total slurry basis) is added and mixed into the slurry to act as a pH buffering agent. The carbonate controls pH excursion to reduce acid catalyzed decomposition reactions leading to char formation in favor of more useful aldol condensation reactions.

In the HTL system, the slurry is brought to operating pressure (3000 psi) using a continuous flow dual-barrel Teledyne Isco syringe pump. After pressurization, the slurry was heated in a heat exchanger then brought to HTL reactor temperature (350°C) in continuous stirred tank reactor (CSTR). Rapid mixing in the CSTR facilitates good heat transfer and accommodates product slurry swelling. The liquefied slurry then proceeds to a tubular reactor that provides the requisite residence time (liquid hourly space velocity used was 2.2 L/L/h). Downstream of the reactors, solids are removed from the product stream by a stainless steel filter. After solids removal, the product is collected in one of two alternating oil jacketed separators/collection vessels. The product is retrieved, cooled and the gravity separable HTL oil is separated from the aqueous phase.

Pyrolysis oil was produced in the pilot scale fast pyrolysis system at PNNL. The system consists of a metered Auger screw feeder assembly, fluidized bed reactor, cyclones for solid char separation, cooling towers for vapor quenching and liquid/liquid separations, filters for aerosol recovery and recirculation loop with pumps for the quench liquid. It has a capacity of 1 kg /hr of biomass feed, pyrolyzed between 450-500°C under near ambient nitrogen atmosphere, within a nominal residence time of 1.6 seconds. Feedstock used was debarked/clean pine (AWF2020) obtained from American Wood Fibers (Schofield, Wisconsin).

Hydrotreated oil was produced by use of a commercial Co/Mo catalyst in a lab-scale hydrotreater specifically designed for collecting detailed mass balances with a previously described approach.[39] A schematic diagram of the lab-scale hydrotreater is shown in Figure 1, bottom and upgrading parameters are provided in **Table 1**. The hydrotreater is configured as a single pass, co-current, continuous, and downflow, trickle bed reactor. Gas samples are analyzed via an on-line GC with multiple columns for measuring fixed gases, light hydrocarbon gases, and H₂S and NH₃.

2-D ¹H-¹³C NMR Spectroscopy

Samples for NMR spectroscopy containing 35-45 mg of oil in 600 μ L DMSO- d_6 (99.9%-D) with 0.05% v/v tetramethylsilane (TMS), (Cambridge Isotope Laboratories, Andover MA) were prepared in 5 mm 535-PP NMR tubes (Wilmad-LabGlass, Vineland NJ).

Two-dimensional ^1H - ^{13}C heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC) spectra were collected at 298 or 313 K on a 600 MHz Agilent (Varian) Inova instrument equipped with a variable temperature Z-axis pulsed field gradient triple resonance [$^1\text{H}/^{13}\text{C}/^{15}\text{N}/^{31}\text{P}$] inverse probe (Varian 5 mm Pentaprobe) using the gChsqc and gHMBC pulse sequences. The HMBC experiment was optimized for detection of $J_{\text{HC}}=8$ Hz couplings. The HSQC spectra were collected in phase sensitive States-Haberkorn-Ruben hypercomplex mode while HMBC spectra were collected in magnitude mode in both dimensions. Separate HSQC spectra optimized for aliphatic, aromatic, or aldehyde regions were collected using values of 0.00145 s and 0.0013 s for tCH and lambda, respectively, for aromatic and aldehyde spectra, and 0.00175 s and 0.0016 s, for aliphatic spectra. Spectra were collected with 1024 (HSQC) or 4096 (HMBC) complex points (Varian parameter np) and 12000 Hz (20 ppm) spectral width with 43 ms (HSQC) or 107 ms (HMBC) acquisition time in the ^1H dimension, and 128-256 increments for spectral widths in the indirect (^{13}C) dimension of 7500-15000 Hz (50-100 ppm) for HSQC spectra and 36000 Hz (240 ppm) for HMBC spectra. Typically 128-256 transients were averaged with a recycle delay of 1.3 seconds between scans. In HSQC spectra, adiabatic (WURST) ^{13}C decoupling was used during the acquisition period.

Spectra were processed in Felix (FelixNMR Inc). For HSQC spectra, a cosine bell window matched to the entire time domain was used with zero-filling to twice the number of points in the real dimension. For HMBC spectra, an unshifted sine bell window was applied to the entire time-domain signal. In the indirect dimension, a matched cosine bell was used for both experiments. Linear prediction of 30% more points was applied in the indirect dimension prior to apodization and zero-filing.

Spectra were referenced to TMS ($\delta = 0.0$ ppm in ^1H and ^{13}C) and residual protonated DMSO ($\delta^1\text{H} = 2.50$ ppm and $\delta^{13}\text{C} = 39.5$ ppm) or CDCl_3 ($\delta^1\text{H} = 7.24$ ppm and $\delta^{13}\text{C} = 77.2$ ppm).

ESI FT-ICR Mass Spectrometry

Oil samples for ESI FT-ICR MS analysis were prepared at a concentration of 3 mg/mL in chloroform:methanol (1:1 v/v). Each sample was further diluted to 0.6 mg/mL in methanol containing either 0.1% formic acid or 0.1% ammonium hydroxide in positive- and negative-ion modes, respectively. Addition of acid or base to the electrospray solution facilitates protonation or deprotonation in each ionization mode. The final dilute samples were centrifuged to remove any suspended particulate matter prior to FT ICR-MS analysis. All solvents were high-performance liquid chromatography (HPLC)-grade and purchased from Sigma-Aldrich (St. Louis, MO).

Oil samples were analyzed with a hybrid linear ion trap 7 T FT-ICR mass spectrometer (LTQ FT, Thermo Fisher, San Jose, CA) equipped with an Advion Triversa Nanomate (Advion Biosystems, Inc.). Ion transmission parameters were optimized by comparison of the molecular weight distribution observed for low-resolution mass spectra acquired with the integral linear ion trap mass spectrometer (LTQ) and that observed for FT-ICR mass spectra. FT-ICR mass spectra were acquired in both positive- and negative-ion modes at a mass resolving power of $m/\Delta m_{50\%} = 400,000$ at m/z 400 (i.e. a time-domain acquisition period of ~ 3 s). A total of 500 and 450 time domain transients were co-added for each sample in positive- and negative-ion modes, respectively to enhance signal-to-noise ratio prior to fast Fourier transformation and frequency to mass-to-charge ratio conversion. FT-ICR mass spectra were internally calibrated with respect to homologous alkylation series of known compounds, whose elemental composition differ by integer multiples of CH_2 . Peak lists were generated from each mass spectrum at $S/N > 10$. Measured masses were converted to the Kendrick mass scale^[40] and sorted by the Kendrick mass defect to facilitate identification of homologous series with the same heteroatom composition and the same double-bond equivalents (DBE, number of rings plus double bonds) but differing in the degree of alkylation. Mass measurement accuracy for elemental composition assignments for pyrolysis oil, HTL oil and hydrotreated oil in both ionization modes are 0.237, 0.134 and 0.165 ppm_{rms}, respectively (see **Figure S1** of the Supporting Information for mass measurement error distribution).

Results and Discussion

2-D NMR

While HTL oil was soluble to at least 40 mg/mL in DMSO, the upgraded hydrotreated oil was not completely soluble in DMSO, and some of the oil formed an insoluble layer on top of the solvent. Analysis of the insoluble layer after dissolution in chloroform showed that it was not enriched in specific compounds but resembled the makeup of the soluble oil, suggesting the limited solubility in DMSO is a bulk property of the upgraded oil.

Two-dimensional ^1H - ^{13}C HSQC and HMBC spectra were collected to characterize the molecular structural composition of the oils in light of the empirical formulae derived from FT-ICR mass spectral data. In ^1H - ^{13}C spectra, cross-peaks indicate ^1H - ^{13}C correlations derived from the strong (125-160 Hz) one-bond J_{HC} -coupling in HSQC spectra, or from weak (< 12 Hz) multiple bond J_{HC} -couplings in HMBC spectra. Peaks in HSQC spectra group into two general regions that correspond to the aliphatic region ($\delta^1\text{H}$ ca. 0.8-5.5 ppm and $\delta^{13}\text{C}$ ca. 8-80 ppm) and the aromatic region ($\delta^1\text{H}$ ca. 4.5-10.0 ppm and $\delta^{13}\text{C}$ ca. 95-140 ppm) and can be used to assign functional classes to mixtures such as oils.

Aliphatic Region

The aliphatic region of the 2-D ^1H - ^{13}C HSQC spectrum for HTL oil is shown in **Figure S2, panel A** of the supporting information. The spectra are notably devoid of peaks in the alcohol/ether region from $\delta^1\text{H}$ 3.5–5.0 ppm and $\delta^{13}\text{C}$ 50–80 ppm, indicating that neither biomass carbohydrates nor their hydroxylated degradation products survive the HTL process, with the exception of a few discrete compounds. The major feature in this region corresponds to aromatic methoxy ether moieties remaining on fragments of deconstructed lignin. Unassigned peaks include a distinct pair of strong peaks at the same carbon shift ($\delta^{13}\text{C}$ 72.5 ppm) and distinct proton shifts ($\delta^1\text{H}$ 3.37, 3.75 ppm) indicative of a diastereotopic methylene group bound to an alcohol, ether, or ester oxygen, and a cluster of primary alcohol peaks around $\delta^1\text{H}$ 3.42 and $\delta^{13}\text{C}$ 60.3 ppm. Other notable features in the aliphatic region include a range of benzylic or allylic methyl and alkyl groups, and possibly CH_x groups adjacent to carbonyls. A notable cluster of weak peaks at a distinct region of ^1H and ^{13}C chemical shifts is attributed to the alkyl α -protons of N-alkylated pyrroles or indoles. We find no evidence for other N-heterocycles (pyridine, pyrimidine, purine, imidazole) unless the heterocyclic rings are poly-substituted and do not have aromatic hydrogens. The HMBC spectrum (data not shown) of HTL oil shows evidence of carbonyl carbons at ^{13}C chemical shifts characteristic of both ketones ($\delta^{13}\text{C}$ 206–212 ppm) and amides or carboxylic acids ($\delta^{13}\text{C}$ 166–178 ppm), with cross peaks from aliphatic protons only ($\delta^1\text{H}$ 0.8–2.3 ppm). No aldehyde proton resonances are evident in 1-D ^1H spectra (data not shown).

The aliphatic region of the ^1H - ^{13}C HSQC spectrum for hydrotreated HTL oil is shown in **Figure S2, panel B**. A comparison of the aliphatic region of the 2-D NMR spectra for the HTL oil and the upgraded product are shown in **Figure 2, panel A**. A broad hump of overlapping weak peaks that underlies a number of much stronger peaks in the crowded region of the HSQC spectrum of HTL oil is no longer present in the spectrum of the upgraded oil. We observe elimination of signals that correspond to Ar-OCH_3 , ether, and alcohol functionality upon hydrotreatment. An increase in upfield ($\delta^1\text{H}$ 0.5–2.0 ppm) proton resonances correlated with downfield ($\delta^{13}\text{C}$ 30–45 ppm) carbon shifts probably indicates more branched alkanes. Overall, the aliphatic region of spectrum for the upgraded product is similar to that of the feedstock oil, but with a general reduction in compound diversity. In HMBC spectra, none of the ketone carbonyl groups observed in HTL oil remain, unless they are no longer correlated with protons, and the only remaining carbonyl cross peaks are from upfield aliphatic protons ($\delta^1\text{H}$ 0.8 – 1.3 ppm) to carboxylic acids or amides ($\delta^{13}\text{C}$ 165–180 ppm).

Aromatic Region

The aromatic region of the ^1H - ^{13}C HSQC spectrum of HTL oil is shown in **Figure S2, panel C**. The chemical shifts of the cross peaks suggest a composition predominantly consisting of substituted phenolic rings, but also some non-phenolic aromatic and polyaromatic compounds. The spectra lack any contribution from furan derivatives (region not shown). We observe a series of distinct strong peaks attributed to 3-alkylated cyclopentenones ($\delta^1\text{H}$ 5.9–6.0 ppm, $\delta^{13}\text{C}$ 125–130 ppm) and *cis* carbon-

carbon double bonds in extended alkyl chains ($\delta^1\text{H}$ 5.3 ppm, $\delta^{13}\text{C}$ 130 ppm). Two isolated cross peaks with distinctly downfield proton ($\delta^1\text{H}$ ca. 8.0 ppm) but not carbon ($\delta^{13}\text{C}$ ca. 122 ppm) chemical shifts are characteristic of phenanthrenes, whose protons at the 4 and 5 positions on the concave side of the tricyclic structure are deshielded by the ring current of the aromatic ring on the opposite side. These two peaks and others in the spectra correspond well with expected shifts of 1-methylphenanthrene and we tentatively assign these peaks as such.

The aromatic region of the ^1H - ^{13}C HSQC spectrum of upgraded HTL oil is shown in **Figure S2, panel D**. The aromatic region for HTL oil and the upgraded product are shown superimposed in **Figure 2, panel B**. Clearly, hydrotreatment largely removes phenolic compounds and dramatically reduces alkene content. Additionally, the cyclopentenone and *cis*-alkene signals (bottom right) are absent in spectra of the hydrotreated material. Polycyclic aromatic compounds remain and appear as the dominant chemical functionality following hydrotreatment. An increase in the diversity of tricyclic phenanthrene peaks downfield of $\delta^1\text{H}$ 8 ppm is observed for the hydrotreated sample, suggesting that alkyl substitution of 1-methyl phenanthrene from HTL oil takes place or that hydrotreatment induces formation of additional substituted phenanthrenes. Also, similarly with what was observed in the aliphatic HSQC spectra, a broad hump of overlapping low-intensity peaks in the crowded region of the HTL oil spectrum is not as evident in spectra of the upgraded product. The HMBC spectra for the upgraded sample (data not shown) show an intense signal for aromatic protons and carbons and indicate an absence of ketones for upgraded HTL oil. Other strong signals in the HMBC spectra of hydrotreated HTL oil indicate possible acids, amides, imides, and alkylaromatics.

HTL and Pyrolysis Oil Comparison, NMR

The NMR spectra of HTL oil reported here were compared to recently published spectra of pyrolysis oil.[38] We note that both oils contain significant fractions of *ortho*-methoxyphenolic components, indicated by the $-\text{OCH}_3$ and aromatic ring peaks characteristic of guaiacyl-like moieties. However, the pyrolysis oil spectra show a substantial component of carbons bearing hydroxyl groups, including anomeric carbons, indicating that pyrolysis-treated lignocellulosic biomass retains some carbohydrate-like character. This is in contrast to HTL oil that appears to be entirely free of hydroxylated carbons aside from a small group of overlapping peaks suggestive of a primary alcohol, and a single abundant component apparently containing a diastereotopic methylene group that is presumably part of an ether, ester, or alcohol functional group (though its ^{13}C chemical shift is more downfield than expected for a simple primary alcohol). Moreover, HSQC spectra of HTL oil contain abundant peaks indicative of non-phenolic aromatics, while those of pyrolysis oil contains comparatively fewer peaks for non-phenolic aromatics. Whether this is due to cyclization and aromatization of dehydrated aliphatic carbon chains in HTL oil or to loss of phenolic hydroxyl groups is not clear. Another significant difference observed is

that the reported spectra of pyrolysis oil appear to contain furans while HTL oil does not. Finally, the pyrolysis oil contains aldehyde signals, of several varieties, including 2-furaldehyde derivatives, while HTL oil does not. Similar features were found in our own HSQC spectra of pyrolysis oil (data not shown), in general agreement with those reported.[38]

FT-ICR MS

Negative ions

Broadband negative-ion ESI FT-ICR mass spectra of pyrolysis oil, HTL oil, and hydrotreated oil are shown in **Figure S3**. More than 4,700 polar compounds between 100 and 750 Da were resolved and identified for each sample in the negative ion mode. This observed compositional complexity requires ultrahigh mass resolving power and high mass accuracy provided by FT-ICR mass spectrometry to resolve and unambiguously assign elemental composition to all peaks observed in each mass spectrum. The insets in **Figure S3** show low-resolution linear ion trap mass spectra that confirm accurate ion abundance distribution across the observed m/z range for FT-ICR spectra of each oil. Heteroatom class analyses for pyrolysis oil, HTL oil and hydrotreated HTL oil derived from negative- and positive-ion ESI FT-ICR MS are shown in **Figure 3**. Relative abundance for each class is calculated by summing the peak magnitudes of all observed ions for each heteroatom class and dividing by the summed peak magnitudes of all assigned monoisotopic masses.

Oxygen-Containing Compounds

The negative-ion mode class distribution (Figure 3, top) demonstrates that both pyrolysis oil and HTL oil are dominated by O_x species. Distribution of these various oxygenates in the HTL oil depends on the feedstock, process type and operating conditions.[7] Pyrolysis oil contains a broader range of oxygenates (O_1 - O_{15}), whereas HTL oil contains oxygenates with up to 10 oxygen atoms per molecule. These results show that for the same feedstock, pyrolysis produces oil with higher oxygen-containing compounds than HTL and therefore hydrotreatment processing of pyrolysis oil will require more hydrogen than the HTL oil.

Double bond equivalent (DBE) distributions for the most abundant heteroatom classes observed for HTL oil and pyrolysis oil from negative-ion ESI FT-ICR MS are shown in **Figure 4**. For both O_4 and O_6 classes observed in pyrolysis oil, DBE distributions generally shift to lower value in comparison to the HTL oil and that trend is observed for all heteroatom classes.

Primary oxygenated species observed in hydrotreated oil in negative ion mode include O_1 - O_3 compounds, with O_1 being the most abundant heteroatom class observed (Figure 3, top). The relative abundance of mono-oxygen compounds increased from ~1% to 62% after hydrotreatment. This is presumably due to the conversion of higher

oxygen-containing species from the original mixture that generate new O₁ compounds during hydrotreatment. For example, we observed 663 individual O₁ species in the hydrotreated oil, however only 69 O₁ compounds were observed for HTL oil. The same trend was observed for di-oxygen compounds where the number of O₂ compounds increased from 169 to 436 after the hydrotreatment with a two-fold increase in the relative abundance of that class.

Mass scale expanded segments (0.25 Da mass window) for pyrolysis oil, HTL oil and hydrotreated HTL oil from negative-ion ESI FT-ICR MS (**Figure 5**) illustrate the compositional complexity of the oil samples. Both pyrolysis oil and HTL oil contain essentially the same lower oxygen-containing species, whereas higher oxygenates (i.e. C₁₇H₁₅O₆⁻, C₁₆H₁₁O₇⁻ and C₁₃H₁₅O₉⁻) are only observed for the pyrolysis oil. Hydrotreatment eliminates higher oxygen-containing compounds from the HTL oil. In the selected mass segment, only the C₂₂H₂₀O₂ compound (i.e. C₂₂H₁₉O₂⁻ ion) was found to be resistant to hydrotreatment, whereas all other higher oxygenates present in the original HTL oil sample are either completely removed or reduced significantly. New species observed in the oil after the hydrotreatment include C₂₃H₂₃O₁⁻, C₂₂H₃₅O₁⁻ and C₂₄H₁₁O₁⁻. These compounds are possibly formed by conversion of multiple oxygen containing compounds into mono-oxygen species during hydrotreatment as mentioned above.

Abundance-contoured plots of DBE versus carbon number that illustrate compositional distributions for mono- and di-oxygen species observed in hydrotreated oil and HTL oil are shown in **Figure S4**. We observe 594 and 267 O₁ and O₂ compounds, respectively, that are produced during hydrotreatment (i.e. not present in the HTL oil). Both oils exhibit highly aromatic oxygenates (i.e. high-DBE value/low carbon number). Ovals highlight the dominant O₁ and O₂ species present in the HTL oil and are overlaid on the hydrotreated oil mass spectral images to compare the compositional features of the two heteroatom classes observed in each sample. The O₁ and O₂ compounds observed for hydrotreated oil span a broader compositional space with a shift to higher carbon number and higher DBE compared to HTL oil.

Figure 6 shows negative-ion ESI abundance-contoured plots of DBE versus carbon number for various oxygenates observed in HTL oil and pyrolysis oil. Again, the pyrolysis oil is enriched with low DBE and low carbon number species relative to HTL oil. HTL oil exhibits a shift to higher DBE and carbon number with increasing oxygen number. For pyrolysis oil, DBE=10-11 compounds appear to be the dominant species in O₂-O₆ classes, whereas DBE=15 species dominate for >O₆ classes. Proportionate increase in DBE with carbon number observed at “hotspots” and at local maxima for both samples suggests that predominant compounds in a given class are structurally correlated and the dominant motif propagates with successive addition of benzene rings to the parent structure. Major structural constituents in wood biomass feedstock include oxygen-containing polymeric compounds such as cellulose, hemicellulose and

lignins.[41] Simultaneous depolymerization and fragmentation of cellulose, hemicellulose and lignins at high temperature and condensation with other molecules result in numerous chemically distinct compounds forming a complex oil as the end product. Five major groups of compounds identified in wood pyrolysis oil include hydroxyaldehydes, hydroxyketones, sugars, carboxylic acids and phenolics.[7, 41] Pyrolysis of cellulose and hemicellulose produce polyhydroxyl cyclic hydrocarbons and their derivatives, whereas phenolic compounds are mainly derived from lignins.[33] Therefore, the distribution of higher oxygen-containing hydrocarbons shown in Figure 6 reveal the extent of CH₂ incorporation for holocellulose and lignin derivatives.

Nitrogen Compounds

Positive-ion ESI FT-ICR MS class distribution highlights basic nitrogen-containing compounds in each oil sample (Figure 3, bottom). Primary nitrogen-containing species observed for HTL oil contain up to 5 oxygen atoms per molecule. Pyrolysis oil comprises a more diverse collection of compound classes including N₁₋₃ and N₁₋₃O_x. Mono-nitrogen (N₁) species are dominant in the hydrotreated oil. Relative abundance of N₁ class increased from ~12% to 41% after hydrotreatment. Previous reports show that mono-nitrogen compounds are highly resistant to hydrotreatment.[12, 42] The increase in the relative abundance of N₁ species after the hydrotreatment is due to the removal of other heteroatom compounds. **Figure S5** shows mass-scale expanded segments (0.14 Da) for HTL oil (top) and hydrotreated HTL oil (bottom). C₁₇H₂₄N₁O₁⁺ and C₁₆H₂₀N₁O₂⁺ abundances are reduced by hydrotreatment, whereas C₁₅H₁₆N₁O₃⁺ and C₁₆H₂₁O₂¹³C₁⁺ are removed. The species forming the C₁₈H₂₈N₁⁺ ion is highly resistant to hydrotreatment. Additionally, C₁₉H₁₆N₁⁺ is only observed in the hydrotreated oil (absent in the original HTL oil) confirming that this compound has been produced during hydrotreatment.

Three-dimensional mass spectral images for the N₁, N₁O₁, N₁O₂ and N₁O₃ heteroatom classes from HTL oil and hydrotreated HTL oil are shown in **Figure 7**. The abundance scale in each plot is scaled relative to the most abundant compound observed in the two samples for each class. The number of individual N₁ species observed for HTL oil and hydrotreated oil (208 vs. 508, respectively) further confirms the generation of new N₁ compounds during the hydrotreatment. Interestingly, the same trend was observed for N₁O₁ species, where HTL oil and hydrotreated oil contain 254 and 308 individual N₁O₁ species, respectively. The compounds correspond to the relative abundance maximum at DBE = 6, carbon number = 15 observed for N₁ class in HTL oil are preserved during hydrotreatment. Both data sets exhibit an increase in DBE with oxygen addition while carbon number is unchanged which suggests that predominant compounds in each N₁O_x class are oxygenated versions of N₁O_{x-1} compounds with oxygen bound to the molecule as carbonyl oxygen. For example, “hotspot” at DBE = 7, carbon number = 20-22 for N₁ compounds in the HTL oil is observed for N₁O₁, N₁O₂

and N₁O₃ compounds at the same carbon number but with a DBE increase of one (i.e. DBE = 8, 9 and 10, respectively).

Positive-ion mode ESI FT-ICR MS comparison of N₁O₃, N₁O₄ and N₁O₅ heteroatom classes from HTL oil and pyrolysis oil are shown in **Figure S6**. For a given number of nitrogen atoms the number of species in each class decreases with increasing oxygen number for HTL oil. The regions that include abundant N₁O₃, N₁O₄ and N₁O₅ species in the HTL oil are highlighted and those ovals are overlaid on the pyrolysis oil to compare DBE and carbon number limits between two oils. The HTL oil is enriched with higher DBE and higher carbon number compounds. Pyrolysis products in each N₁O_x class occupy only a small fraction of the range that observed for HTL oil. Dominant compounds in hydrothermal liquefaction oil exhibit increased aromaticity with oxygen addition, whereas pyrolysis oil preserves the “hotspot” at DBE=9, carbon number=15-17 for N₁O₃₋₅ classes.

In addition to the heteroatom classes shown in the positive-ion mode class distribution, O₁-O₁₅, O₁-O₉ and O₁-O₃ classes were observed for pyrolysis oil, HTL oil and hydrotreated oil, respectively as both [M+H]⁺ and [M+Na]⁺. The total relative abundance of observed O₁-O₁₅, O₁-O₉ and O₁-O₃ compounds in the positive-ion ESI are 78%, 19% and 25%, respectively. However, 60%, 75% and 97% of the O₁-O₁₅, O₁-O₉ and O₁-O₃ compounds (respectively) observed in the positive-ion ESI, are also observed in the negative-ion mode as deprotonated ions. Therefore, those oxygenates were omitted from the positive ion mode class analysis, as they are already described by the negative-ion ESI data (Figure 3, top). A complete chemical characterization of these oils cannot be achieved only with positive-ion ESI FT-ICR MS, because only 26%, 42% and 41% of total oxygenates present in HTL oil, pyrolysis oil and hydrotreated oil, respectively are observed as proton adducts and/or sodium adducts in the positive-ion ESI spectra.

Conclusion

We have demonstrated a comprehensive chemical characterization of hydrothermal liquefaction oil from pine by both 2-D ¹H-¹³C NMR spectroscopy and positive- and negative-ion ESI FT-ICR mass spectrometry. Combination of these two methods provides a unique description of the HTL oil in several ways. First, NMR observations are used to restrict the molecular structure assignments that fit for FT-ICR MS derived elemental compositions. For example, in the case of molecular formulae that contain two nitrogen atoms NMR data eliminate pyrimidine, purine or imidazole structural motifs and rather suggest those compounds to be combinations of pyrrole, indole, amide and amine groups. Secondly, FT-ICR MS data indicate that the majority of HTL bio-oil constituents are poly-functional (as indicated by the presence of high numbers of heteroatoms for the majority of observed compounds). Two-dimensional NMR reveals that oxygen containing compounds in the HTL oil largely do not include primary alcohol, ether (with the exception of O-methyls) or furan functional groups

but rather are polyphenols, aliphatic carboxylic acids, ketones and amides. The FT-ICR MS observation of relatively low DBE value N_1O_1 compounds in the HTL oil support the observation of amide functionality by NMR. Both data sets show substantial contribution from degraded lignin (and presumably some reaction/degradation products of carbohydrates) for the HTL bio-oil where NMR data show distinct phenolic and aromatic methoxy signals and FT-ICR MS clearly demonstrates the resultant distributions of oxygen, carbon and hydrogen that derive from these multimer assemblages of monolignols, for example (e.g. oxygen compounds, Figures 3, 4 and 6).

These data indicate that polycyclic aromatic compounds, including alkylated phenanthrenes and condensed polycyclic mono-nitrogen compounds are resistant to hydrotreatment (similar to the previous reports on petroleum crude oil,[12, 42]) and the double bond and carbon number distributions of hydrotreatment-resistant molecules are presented. That conclusion is also supported by 2-D NMR observation of increased polycyclic aromatic functionality (e.g. endo protons of phenanthrenes) for hydrotreated sample. Oxygenates containing more than 3 oxygen atoms per molecule are readily removed by hydrotreatment as indicated by FT-ICR MS and supported by the observation that phenols and aromatic O-methoxy group signals disappear completely in NMR spectra of the hydrotreated material. With FT-ICR MS, we observed more than 850 new mono- and di-oxygen species that are formed during the hydrotreatment of the HTL oil. The relative abundance of N_1O_1 , N_1O_2 , N_1O_3 , N_2 , N_2O_1 and N_2O_2 compounds are diminished significantly after hydrotreatment. The specificity of electrospray ionization highlights the residual polar nitrogen and oxygen-containing compounds that are present in upgraded HTL material and not present in sufficient quantity to be observed by NMR. Ongoing work aims to describe the non-polar composition for upgraded samples (which constitutes the bulk of that material) by atmospheric pressure UV photoionization combined with FT-ICR MS detection.

Additionally, pyrolysis oil was characterized to allow a direct compositional comparison to the HTL oil sample. Both positive- and negative-ion ESI results show that hydrothermal liquefaction produces oil with significantly lower oxygen content than pyrolysis and is largely free of hydroxylated carbons and furans, contains significant non-phenolic aromatics, unlike the pyrolysis oil.

Ultrahigh mass resolving power and high mass accuracy afforded by FT-ICR MS enable identification of thousands of species in a single mass spectrum permitting unequivocal elemental composition assignments for comparative analyses (at molecular level) of complex mixtures. Two-dimensional NMR spectroscopy compliments elemental composition information from mass spectrometry by suggesting (and eliminating) chemical functionality possibilities. The chemical characterization shown here provides improved understanding of the composition of oil for further improvement in process optimization and upgrading strategies. Potential

use of hydrothermal liquefaction oil as transportation fuel with the required standard specifications necessitates intensive upgrading processes for further removal of the remaining polar compounds from the oil. Optimization of different operating conditions such as reactor pressure, temperature, catalyst and liquid hourly space velocity will further improve the quality of resultant bio oil and direct improvement in the efficiency of the hydrotreatment process.[43, 44]

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Table Caption

Table 1. Upgrading parameters for hydrotreated HTL oil.

Figure Captions

Figure 1. Schematic of the hydrothermal liquefaction apparatus (top) and hydrotreater (bottom) at PNNL.

Figure 2. Superposition of the 2-D ^1H - ^{13}C HSQC NMR spectra of HTL oil (blue peaks) and upgraded oil following hydrotreatment (red peaks). The aliphatic region is shown in panel A, left, and the aromatic region is shown in panel B, right. Peaks and regions of the spectra corresponding to specific functional groups of interest are labeled. Notable changes in the spectra following hydrotreatment are circled or indicated with arrows, respectively, for peaks that go away or appear following hydrotreatment.

Figure 3. Heteroatom class distribution for pyrolysis oil, hydrothermal liquefaction oil and hydrotreated HTL oil derived from negative ion mode (top) and positive ion mode (bottom) ESI FT-ICR mass spectra.

Figure 4. Double bond equivalents (DBE) distribution for (a) O_4 and (b) O_6 compounds observed in pyrolysis oil and hydrothermal liquefaction oil.

Figure 5. Mass scale expanded segments at $m/z = 315$ for pyrolysis oil (top), hydrothermal liquefaction oil (middle) and hydrotreated HTL oil (bottom) from negative-ion ESI FT-ICR mass spectra.

Figure 6. Negative-ion ESI FT-ICR MS abundance-contoured plots of DBE versus the carbon number for O_3 - O_{13} classes in hydrothermal liquefaction oil (top) and pyrolysis oil (bottom).

Figure 7. Color-coded abundance-contoured plots of DBE versus the carbon number for N₁, N₁O₁, N₁O₂ and N₁O₃ classes for hydrothermal liquefaction oil (top) and hydrotreated HTL oil (bottom) derived from positive-ion ESI FT-ICR MS.

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Catalyst	Ketjen CoMoS _x /F-Al ₂ O ₃ (30-60 mesh), sulfided
Catalyst bed size, ml	20
Catalyst mass, g	16.0
Pressure, psig	1500
Temperature, °C	400
H ₂ / bio-oil ratio	1280 cm ³ H ₂ /cm ³ bio-oil
Basis of reported LHSV	0.25 cm ³ / cm ³ -cat/hr

Fig. 1

Fig. 2

(a) O₄ Class (b) O₆ Class

Fig. 5

Fig. 7

HTL Bio-Oil

Hydrotreated Bio-Oil

Carbon Number