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Detailed investigation into the asphaltene fraction of hydrothermal liquefaction derived bio-crude and hydrotreated bio-crudes

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Abstract

Hydrothermal liquefaction (HTL) is a wet thermo-chemical biomass conversion technology for the production of e.g. liquid transportation fuels. The process yields a bio-crude with properties similar to petroleum crude, but also with significant differences. Particularly the higher heteroatom content and high viscosity render bio-crudes unsuitable for a direct use in the current infrastructure as a drop-in fuel without an upgrading step. The presented work investigates the composition of an HTL bio-crude produced from lignocellulosic biomass at pilot scale as well as the upgraded

fuels obtained via catalytic hydrotreatment. Pentane solvent extraction is employed to fractionate the bio-crude and its hydrotreated counterpart into a light fuel extract and an asphaltene residue. The asphaltene fraction, which at room temperature is a solid material constituting 60 wt.% of the bio-crude, is reduced upon hydrotreating to 34 wt.%. The pentane extracts reveal a superior fuel quality in terms of higher heating value and composition. Detailed molecular and structural analysis via nuclear magnetic resonance (NMR), gas chromatographymass spectrometry (GC-MS) and ultra high pressure liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) revealed an aromatic and heteroatomic structure of the asphaltene fraction. UHPLC-HRMS analysis indicated an average molecular weight ranging from 150 till 300 Da, which was affected by hydrotreating. A novel analytical strategy for UHPLC-HRMS, including weighted kernel density estimation, was developed. The superior quality of solvent extracted fuels suggests this could be a simple step in improving the capability of HTL derived bio-crudes in a refinery context. Additionally, the difficulty in hydrotreating the asphaltenes suggests that removal of this fraction via solvent extraction prior to the upgrading could be a viable option for improving the efficiency of this post-HTL treatment step.

Introduction

Hydrothermal liquefaction (HTL) is a direct thermochemical route for conversion of wet biomass into bio-crude: a liquid transportation fuel and a renewable replacement for petroleum¹ and chemicals.² The advantages of the process include high conversion rates, catalytic effect of the reaction medium, improved fuel properties, and flexibility with regard to feedstocks.¹ Wet biomass streams such as micro- and macro-algae are of particular interest for HTL due to the high organic content combined with the lack of requirements for the energy intensive biomass drying step common for pyrolysis and gasification. The HTL technology is still in the relatively early technology readiness stages and two important areas under development

include up-scaling of HTL plants (as discussed by Elliott et al.³) and optimization of the final fuel upgrading steps.

Although petroleum oil is also derived from biomass and was produced at conditions similar to HTL in terms of increased pressure and temperature, it differs significantly from the bio-crudes in terms of composition and properties.^{4,5} From the fuel perspective, particularly the heteroatom content of the bio-crudes is troublesome, with oxygen generally in the range of 10-20% and nitrogen as high as 7% for feedstocks rich in protein such as microalgae or waste.^{6,7} Bio-crudes additionally exhibit a large fraction of distillation residue ($> 375\text{ }^{\circ}\text{C}$) as shown by Uhrenholz et al. of 43 wt.%⁸ or 51.6 % over $350\text{ }^{\circ}\text{C}$,⁹ compared to approximately 33% for a Brent petroleum crude.¹⁰ Therefore, upgrading of HTL bio-crudes is a necessary step prior to using them as a direct replacement for gasoline, diesel and aviation fuels. The heavy asphaltenic fraction is the main source of both the increased viscosity and the high heteroatom content compared to bio-crude, and as such is both undesirable and more difficult to upgrade. Previous work has shown that upgraded bio-crudes exhibit a smaller fraction of the heavy boiling point material. Biller et al. (2015) investigated the upgrading of microalgae bio-crudes and showed a fraction of approximately 60 wt.% boiling over $340\text{ }^{\circ}\text{C}$ compared to 70 % in the original bio-crude.¹¹ Furthermore, increasing the hydrotreatment temperature from 350 to $405\text{ }^{\circ}\text{C}$ resulted in a reduction of the heavy material to $\approx 25\text{ wt.}\%$. Yu et al. (2016) upgraded lignocellulosic bio-crudes and showed a 40 wt.% fraction of material boiling $>340\text{ }^{\circ}\text{C}$.¹²

Separation of HTL bio-crudes into heavy and light fractions is a promising strategy for additionally enhancing the quality of the original and the upgraded fuels. In HTL of microalgae, it has been shown via the use of hexane extraction, that more severe reaction conditions lead to higher amounts of the light bio-crude with fewer heteroatoms (O and N).^{13,14} This approach of solvent extraction employed is similar to the process of asphaltene

extraction used in petroleum refining to avoid contamination and poisoning of catalyst with sulphur.¹⁵ Biller et al. (2015) performed pentane extraction on microalgae bio-crudes and their catalytically hydrotreated counterparts and showed that the remaining oxygen and nitrogen contents of the upgraded fuels could be removed further via solvent extraction with a limited penalty on the total extracted fuel mass.¹¹ Despite the intensity of research on the subject, the mechanisms behind the formation and the detailed composition of the heavy bio-crude fraction itself (i.e. asphaltene) are still largely unknown. Savage et al. performed ^1H and ^{13}C NMR on heavy and light bio-crude fractions and was able to show a larger abundance of aliphatics in the light fraction.¹⁴ Recently, thermochemolysis and stepwise pyrolysis of solid residue was published by Madsen et al.¹⁶ Other than this, there has been no characterization of the heavy fraction beyond elemental composition and GC-MS, published.

It is necessary to understand composition of heavy asphaltene compounds representing a significant fraction of the HTL bio-crudes. Since it is difficult to upgrade asphaltenes, the yields of the produced drop-in fuels are reduced. In this work, we investigated the characteristics of both the light as well as the heavy fractions of lignocellulose derived HTL bio-crudes and their counterparts from catalytic hydrotreatment. Despite the fact that lignocellulose is, due to its high availability and the relative ease of harvesting, the most commonly applied feed for HTL on a pilot scale,^{17,18} only a limited number of studies have investigated upgrading options for lignocellulosic derived bio-crudes.^{12,19,20} Most of the upgrading research to date has been carried out on microalgae derived bio-crudes.¹⁸ By application of pentane extraction combined with multiple state-of-the-art analytic instruments, and advanced data processing tools, we investigate the detailed composition of the HTL bio-crudes from lignocellulosic biomass, including the fate of the asphaltene fraction during upgrading. The results are important for the development of an optimized bio-crude upgrading strategy in a future HTL bio-fuel scheme.

Material and Methods

Bio-crude production

Bio-crude was produced from aspen wood via HTL at supercritical conditions at the continuous bench scale system (CBS1) at Aalborg University, Denmark.²¹ In short the following conditions were used: 300350 bar, 390420 continuous production of 150 kg bio-crude with K_2CO_3 at pH 8; details can be found in Jansen et al.²¹. The mass and energy recoveries from wood to oil were 45.3 wt% and 85.6%, respectively. Catalytic hydrotreating of the bio-crude was carried using batch reactors and is described in detail in a previous publication.¹² Pentane extraction of the upgraded bio-oil was performed using a mass ratio of 20:1 pentane to bio-oil. Bio-crude was weighed into a 14 mL centrifuge and the appropriate amount of pentane was added. The tubes were closed and placed in an ultrasonic bath for 1 h. After centrifugation, the pentane extract was pipetted to a new pre-weighed sample container. The procedure was repeated once more with fresh pentane and the extract was combined with the first extract. Pentane was evaporated at room temperature under a stream of nitrogen for approximately 8 h. The asphaltene fraction is defined as the residue after pentane extraction of the upgraded bio-crude.

GC-MS analysis

GC-MS analysis was carried out on an Agilent 7890B gas chromatograph coupled with an Agilent 5977A quadrupole mass spectrometer. Approximately 10 mg of bio-crude or treated bio-oils were diluted with 900 μ L dichlormethane (DCM) and 100 μ L of internal standard. The standard was 4-bromotoluene (B82200 Sigma-Aldrich) with a concentration of approximately 200 ppm in DCM to make a final sample concentration of 20 ppm. A 1 μ L volume of solution was injected in split mode with a split ratio of 20:1. Analytes were separated using a VF-5 MS column (60 m x 0.25 mm x 0.25 mm, with 5 m EZ-Guard). The GC inlet temperature was held at 280 °C. The column was initially held at 40 °C for 5 min,

ramped to 100 °C at 10 °C /min, ramped to 280 °C at 4 °C /min, then ramped to 300 °C at 10 °C /min and held for 10 min. Electron impact ionization was employed at 70 eV and data were acquired in scan mode (35 - 500 m/z). Compound identification was performed by using the NIST 11 mass spectral database.²² Each chromatogram was normalized to the sample mass analyzed. The concentration of aliphatic hydrocarbons from C7 to C24, selected aromatic hydrocarbons and oxygen-containing compounds in the bio-crude and treated bio-oils was quantified by analysis of calibration standards of known concentrations. Using suitable dilutions, calibration curves were plotted for each standard compound using the Agilent MassHunter software package to determine the concentration of the compounds in the oil samples.

NMR Analysis

NMR spectra were acquired on a Varian AS 400 spectrometer running at 400 MHz for ^1H , using a standard method. The spectrometer was equipped with a Bruker AVANCE III autosampling system. Chemical shifts (σ) are reported in ppm relative to residual solvent signal (CHCl_3 , $\sigma=7.26$ ppm for ^1H NMR). 2.5 wt% solutions of the bio-crude, as well as pentane extract and asphaltene fraction of upgraded bio-crude were prepared with deuterated chloroform (CDCl_3) for analysis. Compound classes were assigned according to standard chemical shifts.²³

Thermogravimetric analysis (TGA)

TGA experiments were carried out by using a TGA/DSC (METTLER TOLEDO, Switzerland) instrument controlled by STARe software (version 13a). The sample weight loss was recorded continuously as functions of time and temperature. Typically, 15 mg of sample was uniformly spread into 400 μl aluminium oxide crucible and automatically weighed. The argon gas was used as protective gas with a flow rate of 80 ml/min. During the heating from 25 to 900 °C, at the heating rate of 20 K/min, argon was used as inert gas at 20 ml/min in

order to prevent any oxidation. After reaching the setting value of 900 °C, the instrument was switched to isothermal mode and oxygen at 20 ml/min was used as reactive gas. A blank run with empty crucible was automatically subtracted from the sample runs.

Ultra high pressure liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS)

All solvents were from Sigma Aldrich (Switzerland) at the highest purities available (>99.5%). The samples (bio-crude, upgraded bio-crude, pentane extracts, and the asphaltene fraction) were diluted 1:100 in a solvent mix consisting of methanol (MeOH), acetone, toluene, and chloroform (1:1:1:1 v/v), filtrated (0.22 μ m, PTFE Hydrophobic, BGB Switzerland), and the ^{13}C -labeled vanillin was added as internal standard. In order to prevent carry-over the blank samples were performed after each sample run. The internal standard (IS, ^{13}C -vanillin labeled at benzene ring - mass difference 6 Da) was added prior to the analysis, which took place in a Thermo Scientific Dionex Ultimate 3000 Series RS system (Thermo, Switzerland) including a pump, a column compartment, and an auto-sampler. The following program using mobile phase A (1 % acetonitrile (ACN) and 0.2 % formic acid in high purity water) and mobile phase B (1 vol. % high purity water in ACN) at a flow rate of 0.250 ml/min was applied: 1 % B (2 min), from 1 to 99 % B (10 min), and 99 % B (4 min). 1 μ l of the prepared solutions was injected. Separation of the analytes was achieved with an ACQUITY UPLC CHS TM C18 VanGuard TM pre-column and column (150 mm x 2.1 mm x 5 mm, particle size 1.7 μ m) from Waters (Switzerland). The temperature of the column was 50 °C. Atmospheric pressure chemical ionization (APCI) in positive and negative mode was used for the ionization. Data acquisition was performed using Thermo ScientificTM Q-ExtractiveTM hybrid quadrupole-Orbitrap mass spectrometer controlled by Xcalibur 4.1 software. Mass spectra were acquired in data dependent scan mode with an isolation window of 1 m/z from 70-100 m/z. The resolution was set to 70000. Raw mass spectral data files were collected in triplicate including a blank between each run. The data

were imported into Compound DiscovererTM 2.0 software (Thermo, Switzerland) and processed according to the work flow shown in Figure ???. Standard settings were used except for the following variables: 10^5 counts intensity threshold for precursors, 0.1 min maximum shift alignment, and 2.5 ppm mass tolerance. Chromatographic peaks detected in one of the input files but missing in others were checked by Fill Gaps option. The composition (of a general formula $C_cH_hO_oN_nS_s$) was predicted based on exact mass and isotopic patterns. The predicted sum formulas were searched against all ChemSpider databases.²⁴ The mass spectrometric features leading to unique sum formulae within limitations described above and present in ChemSpider database were used for further analysis. The results were summarized by descriptive statistics and were used as an input to differential analysis. Project specific data evaluation was performed using *in-house* Python scripts. Kernel density estimation (KDE) is a non-parametric technique for estimating probability density function of a random variable. Here we use Gauss function as density function as implemented in Scipy.²⁵ It includes automatic bandwidth estimation using Scotts rule.²⁶ Additionally, we have implemented weighting of the density function in order to account for different peak areas as observed during mass spectrometry measurements. Applying this analysis strategy, no cut offs are needed, all measured data were used. The Python libraries SciPy, Numpy, Pandas, and Matplotlib were used for data processing and plotting, respectively.²⁷

Results and discussion

In this study, we have used bio-crude obtained from HTL process²¹ as well as C5 extract and asphaltene fraction after catalytic hydrotreating.¹² The bio-crude examined in this study exhibited an asphaltene fraction of around 60 wt.% and had an appearance of black, solid material at room temperature (see Figure 1). This value was comparable to the fraction of distillation residue (> 374 °C) obtained from bio-crude produced from a mixture of hard wood and glycerol (residue 60%) and a petroleum crude (60%),⁹ but was higher than a

typical asphaltene fraction from hard wood bio-crude (43%).⁸ The asphaltene content after hydrotreating was consistently lower (34 wt.%) compared to the crude, showing that the hydrotreating catalysts were indeed able to convert some of this fraction and reduce its amount. The asphaltenes of the upgraded fuels could be obtained as a solid material upon extraction (see Figure ??, whereas the extracts were characterized by a reduced viscosity and lighter color (see Figure 1). It is likely that some volatile components of the extracts were evaporated during the step of pentane removal.

Table 1: CHNSO composition of bio-crude and the fractions of the upgraded bio-crude.

	C (wt.%)	H (wt.%)	N (wt.%)	S (wt.%)	O (wt.%)	HHV (MJ/kg)	H/C	O/C
Bio-crude	80.5	8.4	0.4	0.08	10.7	37.3	1.25	0.1
Asphaltene	90.5	5.8	0.4	0.1	3.2	38.4	0.77	0.03
C5 Extract	86.3	9.5	0.4	0	3.8	42.1	1.32	0.03

The elemental composition of the bio-crude and the fractions of the catalytically upgraded bio-crude is shown in Table 1. Catalytic hydrotreating decreased the oxygen levels down to a minimum of 0.7 wt.% at the optimum upgrading conditions.¹² Fractionation of the bio-crudes with pentane led to two fractions with distinct differences; most remarkable is the high carbon content of the asphaltene residues from upgraded bio-crudes which were found in the range of 90 wt.% compared to around 85 wt.% for the extracts. The asphaltenes therefore represent a valuable resource of carbon which should utilize in order to increase the carbon efficiency of a HTL fuel system. While the carbon contents were high for the asphaltene fraction, the hydrogen contents were relatively low at around 6 wt.%, which results in a hydrogen deficient fuel with H/C ratio <1 . The H/C ratio of the pentane extracts on the other hand was around 1.3, which results in a higher HHV of the extracts compared to the residues. The oxygen contents were found to be slightly higher for C5 extracts apart from the original bio-crude. This is contrary to the observation of Biller et al. who showed that pentane extraction of bio-crude and catalytically upgraded bio-crude from microalgae resulted in reduced oxygen and nitrogen contents.¹¹ Hoffman et al. performed a thermal separation via

vacuum distillation and found that oxygen was present in all distillate fractions, but higher in the solid distillation residue (10 wt.%) compared to 1.5-2 wt.% in the lower boiling point fractions.⁸ Petersen et al. fractionated bio-crude from co-liquefaction of wood and glycerol and found an equal distribution of oxygen between the distillates and distillation residue.⁹ Thus, the heteroatom distribution in HTL bio-crudes is distinct to specific bio-crudes and cannot be universally fractionated by thermal or chemical separation via e.g. distillation or solvent extraction.

In this study, comprehensive evaluation of the bio-crude, the C5 extract and the asphaltene fraction of the catalytically upgraded fuel by the means of elemental composition, GC-MS, NMR and UHPLC-HRMS analysis is presented. Figure 2 shows the GC-MS chromatograms of the bio-crude, C5 extract and asphaltene fraction of the catalytically upgraded fuel. The upgraded fuel shows a large abundance of saturated hydrocarbons such as alkanes in the range of C7-C16 and polyaromatic hydrocarbons with three rings and a large peak for retene ($C_{18}H_{18}$, MW 234 Da, 53 000 ppm). The asphaltene chromatogram very clearly shows a lower abundance of compounds compared to the other two chromatograms and an overall much lower total peak area (see Figure ?? with scaling by the most abundant peak in each individual chromatogram). This is due to the compounds in the asphaltene material not being volatile enough for a standard liquid injection GC-MS analysis. The only peaks visible at the same scale as the other chromatograms are retene (8838 ppm) and the peak at 18 minutes which is the internal standard. The concentration of heptadecane has decreased from 11550 ppm in the original sample down to below detection limit in the asphaltene sample and makes up 25400 ppm in the pentane extract. The GC-MS data shows a concentration of the majority of compounds visible via GC-MS in the pentane extract and therefore suggests a concentration of compounds in the asphaltene fraction which are not detectable via liquid injection GC-MS. The characterization of asphaltene is therefore not suitable using standard GC-MS analysis due to its low volatility. Additional information might be accessed by applying pyrolysis of asphaltene fraction coupled to GC-MS.^{16,28}

Thermogravimetric analysis of the bio-crude, C5 extract and asphaltene residue in argon is depicted in Figure 3. The asphaltene trace supports the observations from the GC-MS analysis which showed the low volatility of this material. The data shows that at 280 °C, which is the typical injection temperature (280 °C) and the maximum temperature (300 °C) of the typical GC-MS program, only around 4 wt.% of the sample has volatilized. Around 33% was volatile at a temperature of 500 °C. At the final temperature of 900 °C, only 50% of the asphaltene sample has volatilized, suggesting the majority of the carbon in the sample was present as fixed carbon. The bio-crude as well as C5 extract showed sigmoidal behavior and maximal liquid injection GC-MS observable fraction (at 280 °C) of 33 and 49 %, respectively. After reaching pyrolysis temperature, only small fraction (less than 5 %) of C5 extract was left. A slightly higher portion (around 15 %) of original bio-crude was not being volatilized under the same conditions.

Proton NMR spectra (see Figure 4 top panel) were recorded for the bio-crude, C5 extract and asphaltene residue. The figure shows that the amount of hydrogen in the aromatics and heteroaromatics chemical shift range has increased upon hydrotreating while saturation was reduced slightly. The main objective of this analysis was to shed light on the difference in composition of the pentane extract and the residue. The data showed a distinctly reduced amount of protons in the alkane range of 0.5-1.5 ppm. The aromatic, heteroaromatic and the α - to hetero-unsaturated aliphatic fractions increased in the asphaltene fraction. The asphaltene fraction also exhibited hydrogen in the shift range of 3-4.4 where alcohol, methylene and dibenzene functional groups are found. Overall, asphaltene was less saturated, more aromatic and contained less alkane groups compared to its pentane extract counterpart. Chemical classes observed by LC-MS in bio-crude C5 extract and asphaltene fraction are depicted in the bottom panel of the Figure 4.

Using UHPLC-HRMS,^{29,30} 955 different organic species were detected, ranging from 96 Da ($\text{C}_6\text{H}_8\text{O}$) till 662 Da ($\text{C}_{33}\text{H}_{58}\text{N}_8\text{O}_6$). There were 845, 440 and 209 individual molecules detected in the bio-crude, C5 extract and the asphaltene from the upgraded biofuel, respectively. According to the aromaticity index (as proposed by Koch et. al.³¹), there were 621, 207 and 17 molecules in bio-crude classified as aliphatic, aromatic and condensed aromatics (polyaromatics), respectively. In C5 extract the corresponding ratio was 254/164/22, and for asphaltene fraction the ratio 90/99/20 held true. Two trends are observable: *i*) the number of observed compounds decreased from bio-crude over C5 extracts to asphaltene fraction; *ii*) along the same line the relative abundance of aromatics and polyaromatics increased. It is worth noting that we have applied APCI as ionization method in both positive and negative mode. The APCI ionization is known to be less prone to matrix effects.^{32,33} This was done in order to obtain as complete picture as possible.^{34,35} In opposite to the electron spray ionization (ESI), the APCI is also able to ionize non-polar molecules, whereas ESI typically require an heteroatom, e.g. oxygen or nitrogen, for ionization. Nevertheless, none of the ionization methods is perfect, thus slightly different results might be obtained by applying another method.³⁶

Van Krevelen plot firstly introduced in the 1950s³⁷ as molar ratio of H/C vs O/C for the chemical makeup of coals and adapted firstly in 2003 for high resolution mass spectrometry.³⁸ It is a valuable tool for investigation of complex mixtures from environmental till energy science.³⁹⁻⁴³ Van Krevelen plots of the bio-crude, the pentane extract and the asphaltene fraction of the upgraded bio-crude are shown in Figure 5. The color coding is done according to the aromaticity index: the colors blue, orange, and red represent aliphatic, aromatic, and condensed aromatics, respectively. The size of the dots is normalized by the total intensity observed within individual mass spectrometry measurements. The bio-crude samples exhibited a wide range of aliphatic hydrocarbons including oxygen-free compounds as well as compounds with an O/C ratio of 0.3 (see 5 - left panel). The majority of com-

pounds were aliphatics with a H/C ratio >1 while there were also aromatics present with H/C ratios around or below 1. A small amount of polyaromatics was present with low H/C ratios and a range of different oxygen ratios. Oxygen-free hydrocarbons were found for all three types of structural compounds (aliphatic, aromatics and polyaromatics) as indicated on the first vertical line, having O/C ratio of 0.0.

Figure 5 - middle panel depicts the pentane extract after hydrotreating. There was an apparent increase in the pure hydrocarbon fraction on the first vertical line for aliphatics (O/C = 0), aromatics and polyaromatics compared to bio-crude. This was also supported by the GC-MS data in Figure 2. It is clear that the total amount of different compounds was reduced drastically - the compounds showing O/C ratio greater than 0.15 were virtually absent. There were, however, some abundant individual oxygen containing aliphatic compounds still present, which could not be observed in the GC-MS data and were therefore assumed to be of higher boiling points than what can be seen via GC-MS. The compounds with the largest peak area observed via UHPLC-HRMS for the extract were C12, C9, C10 and C14 compounds with a single oxygen atom. Only the fifth largest peak area compound was a pure hydrocarbon ($C_{16}H_{18}$). In a bio-fuel context, those highly abundant oxygen containing compounds remain a challenge and should be removed, either by catalytic hydrotreatment, distillation or solvent extraction. Figure 5 - right panel shows the Van Krevelen diagram of the asphaltene residue. It is apparent that there were less pure hydrocarbons present, compared with C5 extract, and the majority of compounds contained oxygen. There was also a cluster of compounds, both aliphatic and aromatic, present in the 0.05 O/C and 1 H/C region.

The changes in the bio-crude upon hydrothermal upgrading are depicted in the Figures 6 and 7. The relative Van Krevelen plots of the C5 extract and asphaltene fraction against bio-crude showed a large increase of the oxygen-free substances (on O/C = 0), especially for the

C5 extracts (see Figure 6 left and middle panel). Analogously, the majority of the molecules having O/C ratio above 0.2 were significantly diminished. An important observation about the asphaltene fraction is depicted on the right panel of Figure 6: a considerable fraction of the 201 molecules out of total 209 were not significantly changed upon hydro-treatment and extraction, when applying 5-fold change cut-off. Half of the molecules have not changed the observed peak area within experimental error. Those molecules are forming core of the absolutely hydrotreatment resistant molecules, mainly belonging to the class of aromatics with an O/C fraction around or below 0.5.

After the hydro-treatment, we observe an expected decrease in the abundance of oxygenated species, but also an increase in high molecular weight species implying the presence of polyaromatics. While the former is expected, the latter is counter-intuitive to the upgrading concept. Thus, a deeper insight into molecular composition is needed. A conclusive way of performing such evaluation is by applying kernel density (KDE) analysis. In the KDE plots presented (see Figure 7), no cut off was applied, rather all data are used and weighted by their peak area as observed by mass spectrometry measurements. The 3x3 matrix plot of double bound equivalents (DBE) against molecular weight (MW) of the bio-crude, asphaltene and C5 extract are depicted in top, middle and bottom row, respectively. The corresponding columns are separated according to the chemical classes: all observed molecules, oxygen-free and oxygen containing molecules are separated in the left, middle and right columns, respectively. The total bio-crude could be characterized by two double maxima for both DBE and MW around 4 and 6 as well as 170 and 250 Da, respectively. The higher values for DBE and MW are majorly contributed by the oxygen-free fraction. Accordingly, oxygen containing fraction is shifted towards lower MW and DBE. Asphaltene fraction, on the other hand, shows expected behavior by increasing both DBE and MW independently of the oxygen content to the maxima between 10 and 15 as well as around 300 Da for DBE and MW, respectively. Finally, the liquid C5 extracts did not show the

expected behavior after hydro-treatment: there was a clear shift of the second maximum at higher DBE from 6 in bio-crude to around 10. Also, an overall maximum of MW was increased with apparently diminished maximum around 170 Da compared to the bio-crude fraction. When the components are separated according to the expected mode of action of the catalytic hydro-treatment into oxygen containing and oxygen-free molecules, there was a clear decrease in DBE and MW for the oxygenated species. In contrast, the oxygen-free species showed a clear increase in both MW and DBE. The maximum around 280 Da for MW as well as a double maxima at 9 and 13 for DBE clearly distinguished the oxygenated from the oxygen-free molecules in the C5 extract.

Conclusions

Detailed understanding of the molecular level composition of the produced HTL biocrudes as well as their upgraded counterparts is needed for optimal strategy in upgrading and valorization of bio-crudes as fuels. Classical analytical methods such as liquid injection GC-MS or TGA are either lacking coverage or depth in detail description. NMR shows a valuable alternative providing more details about the overall composition of the substances, but detailed characterization is bound to several time consuming steps such as fully relaxed spectra as well as the completeness and consistency in recording of chemical shifts within large data sets. High resolution mass spectrometry together with the advantages of ultra high pressure liquid chromatography offers detailed investigation of bio-oils and the upgrading products thereof. Accessibility of the chemical information at molecular level through sophisticated data analysis is required in order to generate detailed understanding of the processes of interest, such as upgrading and isolation of valuable fractions of bio-crudes. Moreover, the impact of such detailed investigations should never be for its own sake, but as a tool for guiding the desired process to the optimal product. The identification of the hydro-treatment resistant fraction in bio-crude is the major discovery in this study. Thus, this observation has

direct impact on the strategy for upgrading of the bio-crudes: separation of the C5 insoluble fraction (asphaltene) prior to the hydro-treatment would preserve the energy as well as the catalyst and ease the following steps.

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Figure 1: Visual appearance of bio-crude (left), C5 extract (middle) and asphaltene fraction (right). It is worth noting that the asphaltene fraction is solid at ambient conditions.

Figure 2: GC-MS chromatograms of the bio-crude, C5 extract and asphaltene fraction are depicted in the top, middle and bottom panel, respectively. α -Bromotoluene ($C_6H_5CH_2Br$), eluting at 18.8 min is used as internal standard. The scaling for all three samples is identical, in order to facilitate identification capabilities of GC-MS.

Figure 3: Thermogravimetric analysis of bio-crudes fractions. Filled squares represent the maximum mass fraction observable by liquid injection GC-MS for different fractions (280 °C). The mass fractions are 4, 33 and 49 wt% for asphaltene, bio-crude and C5 extract, respectively. After reaching 900 °C, isothermal mode applying oxygen as reactive gas was utilized for 20 min: in all fractions virtually no residue was observed.

Figure 4: Relative abundances of different chemical classes observed by NMR and UHPLC-HRMS. (top panel) Proton NMR spectra of bio-crude, the C5 extract and asphaltene fraction. The amount of hydrogen in the aromatics and heteroaromatics is increased upon hydrotreating while unsaturation is slightly reduced. The data show a distinctly reduced amount of protons in the alkane range. The aromatic, heteroaromatic and the α - to hetero-unsaturated aliphatics are increased in the asphaltene fraction compared to bio-crude and C5 extract. Additionally, alcohols, methylene and dibenzene functional groups are increased in asphaltene fraction. Overall asphaltene is less saturated, more aromatic and contains less alkane groups compared to its pentane extract counterpart. (bottom panel) Donut plot of relative abundances of different chemical classes for bio-crude, C5 extract and asphaltene fraction obtained from UHPLC-HRMS analysis. The relative amounts of oxygen containing species (CHO, CHNO and CHNOS) decreased for bio-crude to C5 extract, and amount of heteroatom species increased. In contrast to this difference between bio-crude and asphaltene fraction in regard to total amount of heteroatom containing species was not altered.

Figure 5: Van Krevelen plots of bio-crude, C5 extract and asphaltene fraction. Size of the dots is proportional to the area detected by LC-MS, normalized by sum. The dots are colored according to the aromaticity index: blue aliphatic, orange aromatic, red condensed aromatics.

Figure 6: Van Krevelen diagram of relative changes between different samples. The relative size indicates the ratio between different samples. The ratio of one is indicated as green dot in origin of van Krevelen plot. Left panel: relative difference of C5 extract versus bio-crude; middle panel: relative change of asphaltene versus bio-crude; right panel: hydrotreatment resistant molecules found in asphaltene fraction with less than 5-fold change. The dots are colored according to the aromaticity index: blue aliphatic, orange aromatic, red condensed aromatics.

Figure 7: Kernel density plots of DBE against molecular weight for bio-crude(orange - upper), asphaltene fraction(red - middle) and C5 extract(green - bottom). The column are separated as following: all molecules (left), oxygen-free species (middle), oxygen-containing species (right).

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