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Characterizing Semivolatile Organic Compounds of Biocrude from Hydrothermal Liquefaction of Biomass

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Supporting Information

ABSTRACT: Hydrothermal liquefaction of biomass produces a complex biocrude, which can be further upgraded to biofuel or chemicals, but there is a need for improved molecular understanding of product composition and reaction pathways. This study extensively characterizes semivolatile compounds in biocrudes from hydrothermal liquefaction (HTL) of microalgae (*N. gaditana*, *C. vulgaris*), macroalgae (*L. hyperborea*), residue (dried distillers grains with solubles), and lignocellulosic (*M.* × *giganteus*). The biocrudes were analyzed using 2D gas chromatography coupled to time-of-flight mass spectrometry with in situ silylation. A total of 73 fatty acids were detected of which C_{18} and C_{20} compounds were most diverse, while palmitic acid was the single most abundant fatty acid. Multiple fatty acid amides were detected in biocrude from *N. gaditana* while being almost absent in samples from other lipid- and protein-containing feedstocks. Several alkylated indoles and quinolines were observed in biocrudes from protein-containing feedstocks. Monoglycerides, indanones, and alkylated benzenediols and chromen-2-ones, which are typically not reported, were also detected. These results provide new knowledge of a biocrude fraction, which is difficult to characterize.

1. INTRODUCTION

Hydrothermal liquefaction (HTL) is a promising technique for conversion of wet biomasses in particular into biofuels and value-added chemicals.[1−3] The process is carried out at conditions of 300−400 °C and 200−300 bar where the unique properties of hot compressed water are utilized to obtain a biocrude with typically 10−20% oxygen.[4] The process results in four complex product phases: a gas phase, a biocrude, an aqueous phase, and a solid residue.

Research into HTL has expanded in recent years to cover all aspects of the technology from accessibility of feedstock to potential fates of products.[5] The search for new feedstocks continues with tested feedstocks now including residues, lignocellulosics, yeast, bacteria, and algae.[6−9] The desired biocrude product is intended for use as either a heavy fuel or a drop-in fuel upon upgrading.[10] The product will thus require further processing based on comprehensive information of its chemical composition in order to avoid additional expenses.

The biocrude is a highly complex mixture of organic compounds of which most contain heteroatoms.[7,11] A variety of advanced analytical techniques has been applied to investigate the composition of the biocrude, including Fourier-transform ion cyclotron resonance mass spectrometry (FTICR-MS),[12] nuclear magnetic resonance (NMR), size exclusion chromatography (SEC),[7] gas chromatography coupled to mass spectrometry (GC-MS), and pyrolysis-GC-MS (py-GC-MS).[13] Conventional GC-MS is routinely used in molecular characterization of HTL biocrude, often relying on automated library searches of mass spectra. Many studies report relative peak areas of either summed compound classes or single compounds based on the total ion chromatogram (TIC). The complex nature of biocrudes means that coelution often occurs leading to overestimation of peak areas based on TICs. The samples may also have varying fractions amenable to GC introducing variation to the data set based on relative peak areas.

The compound classes reported from GC-MS analysis of biocrudes include hydrocarbons, alcohols, cyclic oxygenates, fatty acids, fatty acid derivatives, nitrogenated organic compounds, oxygenated aromatics, small organic acids, and triterpenoids.[14] Many of these compound classes are known to suffer from matrix effects, discrimination, and poor chromatographic performance in GC-MS analysis which can increase the extent of coelution and lead to poor repeatability, thus complicating analysis.[15] The use of silylating reagents can enhance chromatographic performance, and the use of 2D GC can be used to overcome coelution and poor repeatability as recently applied to crude oils and other complex petroleum mixtures.[16]

Emphasis is extending to include more compounds, in contrast to earlier work which included only the most abundant peaks.[11,17] The interpretations are almost exclusively based on relative peak areas, which potentially lead to misinterpretations from incorrectly identified compounds, coeluting peaks, and normalization with varying total peak area depending on the sample preparation method and GC-MS settings. Recent analytical work is implementing derivatization to enhance chromatographic performance with silylating reagents being the preferred method.[18,19] Performing silylation in solution raises the question of derivatization efficiency and stability, which potentially introduces variation.[20]

The analytical method of GC-MS is particularly valuable for characterization of volatile compounds, while identification

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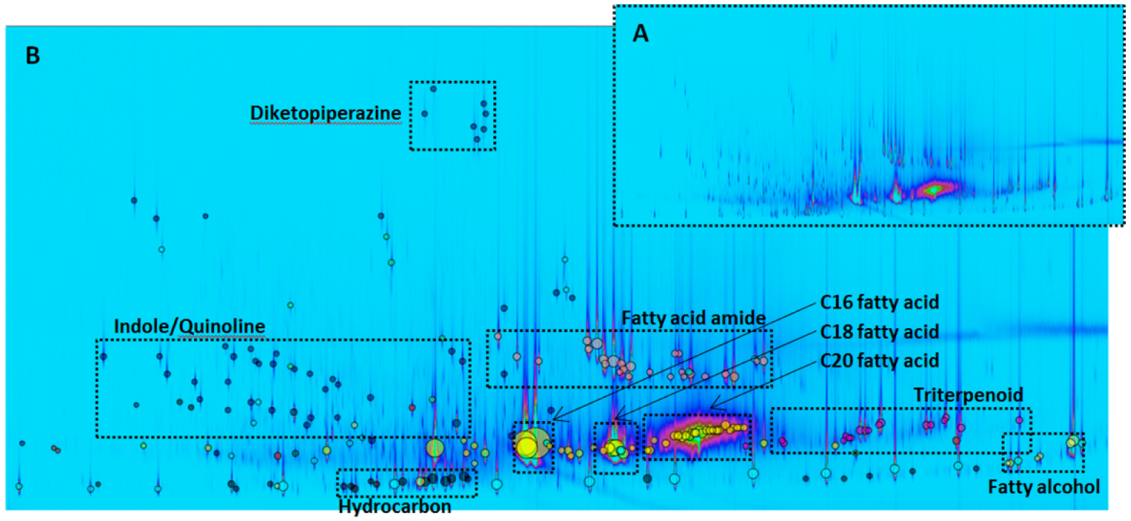

Figure 1. (A) TIC of *N. gaditana* biocrude. (B) TIC of *N. gaditana* biocrude with compound markers. (Dark blue) nitrogen ring structures, (black) hydrocarbons, (pink) fatty acid amides, (yellow) fatty acids, (light yellow) alcohols, (purple) triterpenoids. Primary and secondary axes show retention on the first and second column, respectively. Circles reflect the abundance of the peak.

becomes increasingly difficult with increasing molecular size as chromatographic noise increases and fewer compounds are available in the database. Relatively limited work has been done on characterization of semivolatile compounds in biocrude from HTL of biomass (semivolatile defined as organic compounds with boiling points ranging from 240 to 400 °C).

In this study we present an extensive characterization of semivolatiles in HTL biocrude from five highly varying biomasses, namely, the microalgae *Chlorella vulgaris* (*C. vulgaris*) and *Nannochloropsis gaditana* (*N. gaditana*), the macroalgae *Laminaria hyperborea* (*L. hyperborea*), lignocellulosic *Miscanthus* × *giganteus* (*M.* × *giganteus*), and dried distillers grains with solubles (DDGS), a residue from bioethanol production. The resulting biocrudes represent a wide range of semivolatile compounds being produced at standard HTL process conditions. Biocrudes were analyzed using highly efficient in situ gas-phase derivatization (silylation) coupled to 2D GC with time-of-flight MS detection (GC × GC-TOFMS). Development of the in situ gas-phase derivatization and its application with GC × GC-TOFMS has been described in detail elsewhere.[21,22] The efficiency of the gas-phase derivatization means that compounds which are typically not reported due to insufficient volatility or inefficient derivatization were detected here. The 2D setup furthermore provides separation of most compounds. However, the method is limited by the fact that substantial silylating reagent is trapped, requiring a long solvent delay, meaning that the most volatile organic compounds are not detected. Furthermore, the biocrude contains a heavy fraction, which is not amenable to GC. In order to make the results applicable for future studies, we included information on Kovats retention indices of identified compounds.[23]

2. EXPERIMENTAL SECTION

2.1. Chemicals, Standards, and Biomass. All standards were of GC grade and obtained from Sigma-Aldrich. Potassium carbonate was obtained from Merck Chemicals. Chloroform and methanol were of HPLC grade and obtained from Sigma-Aldrich. *M.* × *giganteus* was from the Department of Agroecology, Aarhus University. DDGS was from Lantmännen Agroetanol AB, Norrköping, Sweden. *C. vulgaris* was from a local health food store. *N. gaditana* was from Lgem, Netherlands, while *L. hyperborea* was obtained from The Scottish Association for Marine Sciences (SAMS). The elemental distribution and biochemical composition of the biomasses are presented in Supporting Information (SI Table 1) along with the analytical methods.

2.2. Hydrothermal Liquefaction. HTL reactions were carried out in 20 mL Swagelok batch reactors. Reactors were conditioned with demineralized water and loaded with 10 mL of biomass slurry containing 10 w/w % biomass, 2 w/w % potassium carbonate (no catalyst added to *N. gaditana*), and 88 w/w % demineralized water. All HTL reactions were carried out at 338 °C for 20 min in a fluidized sand bath, then quenched by rapid cooling in a water bath, and subsequently vented. The aqueous phase was decanted into a centrifuge tube. The tube was centrifuged at 6500 rpm for 5 min, and the aqueous phase was decanted into a preparative vial. The remaining content of the reactor was extracted with dichloromethane. This organic phase was vacuum filtrated, and subsequently, the dichloromethane was evaporated under a stream of nitrogen.

2.3. GC × GC-TOFMS. Biocrudes were dissolved in 1:1 chloroform and methanol solution at approximately 15 mg mL^{-1}. The sample solution (0.5 μL) was deposited on a quartz fiber filter together with 2 μL of internal standard solution of deuterated even-numbered *n*-alkanes (C_{12}−C_{36}). The filter was thermally desorbed at 320 °C (in helium) using a thermal desorption system (TDS 3, Gerstel) with in situ gas-phase derivatization by *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA).[21,22] Thus, compounds were either derivatized as they evaporated from the filter or made volatile by derivatization on the filter. Only compounds less volatile than MSTFA can be analyzed due to final purging and solvent delay. Desorbed compounds were trapped in a focusing trap held at 30 °C. The compounds were then desorbed onto a first-dimension nonpolar column (60 m × 0.25 mm × 0.25 μm, Rxi-5Sil MS, Restek Corp.) coupled to a second-dimension polar column (1 m × 0.25 mm × 0.25 μm, Rtx-200MS, Restek Corp.) by a dual-loop modulator (1.5 m × 0.25 mm, Rxi guard column) that is cooled with refrigerated air and periodically heated by a pulse of hot air every 2 s. The oven temperature was initially set at 40 °C, increasing at 3.5 °C min^{-1} to 320 °C (hold time 5 min). The MS was operated in electron ionization mode (70 eV), and data was collected at 100 Hz.

The data was acquired with Chemstation, and chromatograms were processed using GC Image. Analytes were tentatively identified using NIST2011 based on fragmentation patterns.

Results were evaluated based on normalized peak responses across the five different samples as responses are expected to be within the linear range of the TOF-mass spectrometer.[24] This means that double-peak response is equal to twice as high concentration for the single compound; however, it does not extend across individual compounds, and the peak response for two different compounds is not necessarily

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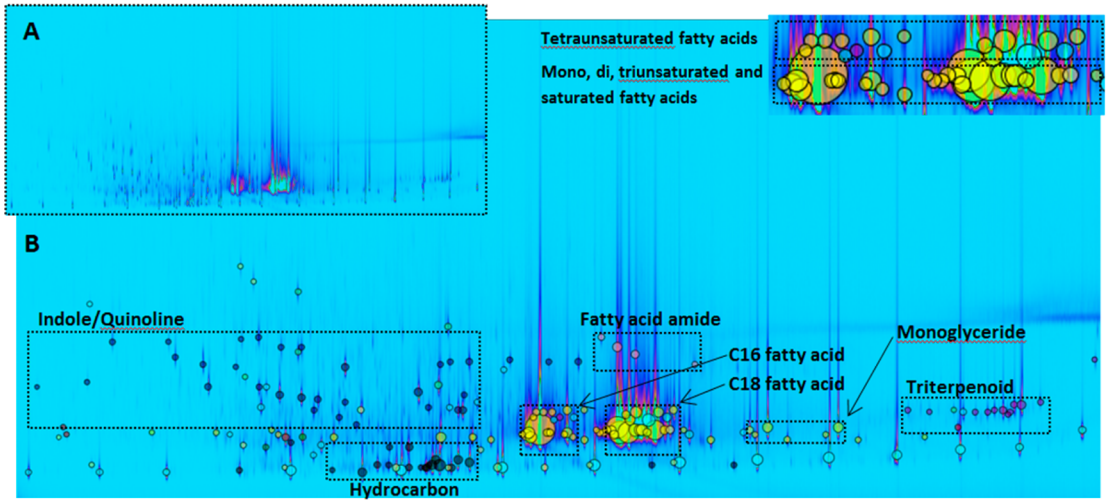

Figure 2. (A) TIC of *C. vulgaris* biocrude. (B) TIC of *C. vulgaris* biocrude with compound class markers. (Dark blue) nitrogen ring structures, (black) hydrocarbons, (pink) fatty acid amides, (yellow) fatty acids, (light yellow) alcohols, (purple) triterpenoids. Primary and secondary axes show retention on the first and second column, respectively. Circles reflect the abundance of the peak.

comparable, which is especially true for derivatized and nonderivatized compounds. Furthermore, it is our experience that compounds with increasing retention on the second dimension (polar column) have decreasing response factors, leading to lower peak responses for more polar compounds such as carbonyls and diketopiperazines (DKPs). Hence, peak abundance does not necessarily represent relative mass concentrations, and comparisons across compound classes should be especially cautious.

3. RESULTS AND DISCUSSION

3.1. Compound Group Analysis of Biocrude of Algae.

Microalgae have been extensively investigated as feedstocks for HTL because of the wet nature of the biomass and the often high lipid content. Furthermore, microalgae do not need size reduction to obtain stable slurries, making the feedstock easily amenable for continuous processing.[5] The vast majority of characterization studies of biocrude from microalgae have relied on conventional one-dimensional GC-MS identifying the most abundant peaks, typically palmitic acid, oleic acid, or hexadecanamide along with smaller peaks of indoles, phytane, pyrazines, pyrroles, and triterpenoids depending on the feedstock and process conditions.[7,25,26]

Figure 1 shows the GC × GC-TOFMS chromatogram of biocrude from *N. gaditana*, a high-protein, high lipid-containing microalgae in which the unsaturated fatty acid content is especially high.

The chromatogram displayed several regions of interest, which are seldomly reported for *N. gaditana* biocrude. The high lipid content is converted by HTL to saturated and unsaturated fatty acids of C_{14}, C_{16}, C_{18}, and C_{20}, seen as four specific regions in the chromatograms. While the regions of C_{16} and C_{18} fatty acids are consistently reported in the literature, they are often identified as only palmitic acid and oleic acid or just as fatty acids. This work identified the presence of myristic acid (C_{14}), 2 C_{15} fatty acids, 4 C_{16} fatty acids, 3 C_{17} fatty acids, 9 C_{18} fatty acids, 25 C_{20} fatty acids, and lignoceric acid (C_{22}). The presence of C_{20} fatty acids is typically not reported in the literature because without derivatization the C_{20} fatty acids are nonamenable for GC analysis. Instead, they condense in the inlet with subsequent decomposition and potential artifacts in later runs.

Fatty acid amides, formed from condensation of free fatty acids with ammonium or amines,[27] constitute another region of interest. Most studies report only the presence of hexadecanamide,[6,28] while more recent work on model compounds has shown the presence of over 40 different fatty acid amides.[27] Barreiro et al.[29] reported the presence of six different fatty acid amides in biocrude from *N. gaditana* while finding only four different fatty acids. This study identified the presence of 26 different fatty acid amides from biocrude of *N. gaditana*. The fatty acid amides ranged from tetradecanamide to octadecanamide and were primary amides, methylated, dimethylated, or pyrrolidine amides.

Diketopiperazines (DKP) have been reported from HTL of amino acids[30] and are formed from dimerization of amino acids; however, they seem to form only at low-temperature HTL and to be further degraded at temperatures above 300 °C.[31] DKPs were only found in minor amounts in biocrude of *N. gaditana*, and it is likely that they are initially formed and then degraded.

Indoles constitute another class of nitrogen-containing compounds which is often found in biocrude of protein-rich feedstocks.[14,26] For biocrude of *N. gaditana*, indoles were present only to a minor extent in a region encompassing also quinolines. Indole is often reported as a major component from protein-rich feedstock and is proposed to form from degradation of tryptophan or from reaction between phenol and glycine. In this work multiple alkylated indoles were detected, which indicates that they are likely to form from secondary reactions between phenol and amino acids as they do not occur naturally in biomass, and alkylation from indole is likely to occur at the 1 and 3 positions.

Several hydrocarbons were detected which included both saturated branched aliphatics and unsaturated aliphatics. Unsaturated aliphatics were mainly C_{16}, C_{18}, or C_{20} with either double bonds or triple bonds. Microalgae are rich in phytol, and previous studies have shown that unsaturated hydrocarbons are produced during HTL, while fatty acids have been shown to partially decarboxylate especially in alkaline HTL.[32,33] The final quarter of the chromatogram was characterized by 18 different triterpenes and triterpenoids in the form of choletane and ergostane derivatives. Typically, only a few of these compounds are reported in the literature despite their apparent abundance in the chromatogram here, where most of these are not even derivatized. The presence of six long-chain unsaturated fatty

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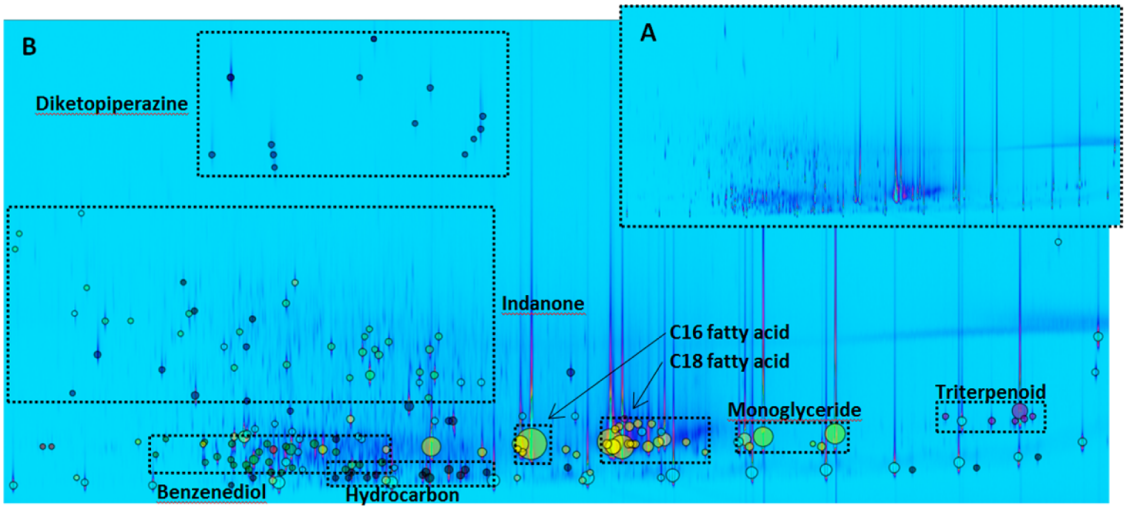

Figure 3. (A) TIC of *L. hyperborea* biocrude. (B) TIC of *L. hyperborea* biocrude with compound markers. (Dark blue) nitrogen ring structures, (black) hydrocarbons, (yellow) fatty acids, (light yellow) alcohols, (purple) triterpenoids, (dark green) benzenediols, (light green) carbonyls. Primary and secondary axes show retention on the first and second column, respectively. Circles reflect the abundance of the peak.

alcohols was unique for biocrude of *N. gaditana*. These were highly abundant C_{30}, C_{31}, and C_{32} compounds.

C. vulgaris is a protein-rich and moderate lipid-containing microalgae, extensively investigated in HTL.[17,34] The biocrude of *C. vulgaris* gave only a small peak for arachidic acid (C_{20}) compared to the massive number of C_{20} fatty acids of *N. gaditana* (Figure 2). Instead, a substantial number of unsaturated C_{16} and C_{18} fatty acids were observed which included 16 different C_{16} fatty acids and 21 different C_{18} fatty acids (enlargement Figure 2) along with palmitic acid and stearic acid. Several of these were tetraunsaturated fatty acids, which have previously not been reported for biocrude of HTL even though they are naturally present in microalgae.[35] Most publications on *C. vulgaris* identify these regions as being the most abundant ones. However, the entire regions are in most cases assigned to one or two fatty acids, such as palmitic acid or oleic acid.

Fatty acid amides were almost completely absent (only four minor peaks) despite the high protein and moderate lipid content of *C. vulgaris* and the high abundance of C_{16} and C_{18} fatty acids of the biocrude. Madsen et al.[36] showed that high concentrations of pyrazines are formed in the aqueous phase from HTL of *C. vulgaris* when the carbohydrate to protein ratio of the feedstock is close to 1, as the degradation products of carbohydrate and protein react instead of forming fatty acid amides. This also implies that Strecker degradation and Amadori rearrangement occurs faster than fatty acid amide formation.

Partially degraded triglycerides in the form of monoglycerides of hexadecanoic acid and octadecanoic acid constitute a group of compounds which are rarely reported in HTL biocrude.[37] The main reason for lack of detection of these compounds is that they are not amenable to conventional GC-MS due to low vapor pressures, and the lower efficiency of silylation in solvents means that they would likely not be detected without optimization of silylation conditions. It is interesting to note that monoglycerides were not detected in biocrude of *N. gaditana* even though it has similar abundances of C_{16} and C_{18} fatty acids.

Biocrude of *C. vulgaris* contained a region of indole/quinoline similar to *N. gaditana*, while the region of hydrocarbons was more abundant in aliphatic hydrocarbons with triple bonds.

Few characterizations based on GC-MS of biocrude from HTL of macroalgae have been reported. Yang et al.[38] showed that biocrude from *Enteromorpha prolifera* consisted of palmitic acid, cyclic oxygenates, alkenes, and pyrazines. Anastasakis and Ross (2011) found that biocrude of *L. saccharina* consisted mainly of cyclic oxygenates, phenolics, indoles, and fatty acids.

To our knowledge, extensive molecular characterization of HTL biocrude of macroalgae has not been published and this is the first study to report DKPs, indanones, benzenediols, monoglycerides, and triterpenoids. *L. hyperborea* has a high carbohydrate content which typically leads to formation of a number of cyclic oxygenates not detected with the present analytical method.[39,40]

The most notable regions were C_{14}, C_{16}, and C_{18} saturated and unsaturated fatty acids (Figure 3). However, the biocrude also contained saturated pelargonic acid (C_9), capric acid (C_{10}), lauric acid (C_{12}), pentadecyclic acid (C_{15}), margaric acid (C_{17}), and arachidic acid (C_{20}). A total of 14 different C_{18} fatty acids were detected including four tetraunsaturated. High abundances of monoglycerides of C_{16} and C_{18} were observed along with a minor peak of C_{14} monoglyceride. The monoglyceride abundance was even higher than for biocrude of *C. vulgaris*, despite the significantly lower lipid content of *L. hyperborea*. Thus, the triglycerides of macroalgae seem to be significantly more resistant to hydrolysis than those of microalgae.

Several minor peaks of DKPs were observed in biocrude of *L. hyperborea* (no silylated DKPs were observed), which were different from the DKPs of *N. gaditana* biocrude. It has been proposed that DKPs are formed from degradation of protein and recombination of amino acids.[33] However, the high-protein feedstock of *C. vulgaris* did not yield DKPs, while the high-protein feedstock of *N. gaditana* and low-protein feedstock of *L. hyperborea* did, which indicates that competitive reactions occur affecting the formation or degradation of DKPs.

A number of carbonyl compounds were detected for biocrude of *L. hyperborea* including acetophenones and indanones which are formed by degradation of lignin and carbohydrates.[41,42] Biocrude of *L. hyperborea* contained 23 different benzenediols in the form of alkylated catechol and hydroquinone, while microalgae had almost no benzenediols.

The sample also contained a number of hydrocarbons which compared to other samples consisted mostly of saturated

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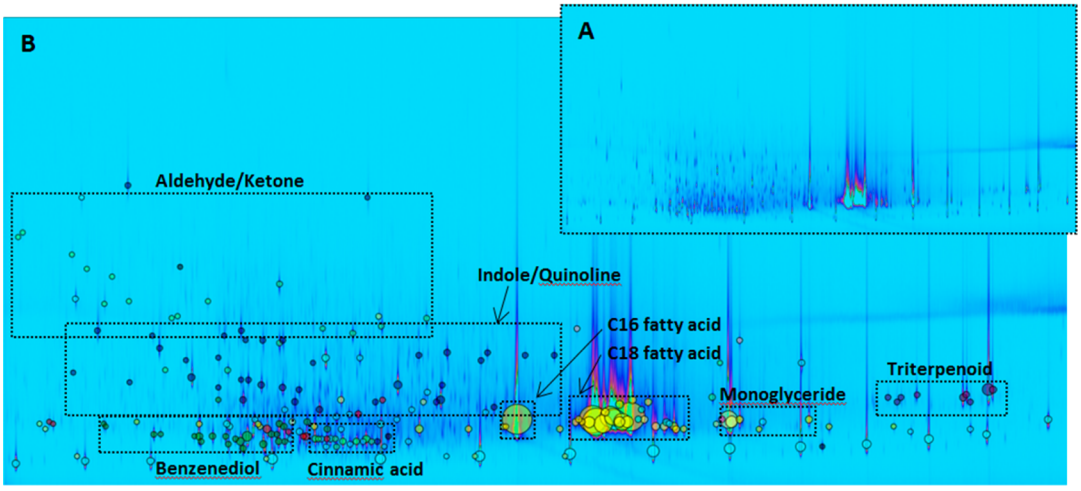

Figure 4. (A) TIC of DDGS biocrude. (B) TIC of DDGS biocrude with compound markers. (Dark blue) nitrogen-containing ring structures, (black) hydrocarbons, (yellow) fatty acids, (light yellow) alcohols, (purple) triterpenoids, (dark green) benzenediols, (light green) carbonyls, (red) phenolics. Primary and secondary axes show retention on the first and second column, respectively. Circles reflect the abundance of the peak.

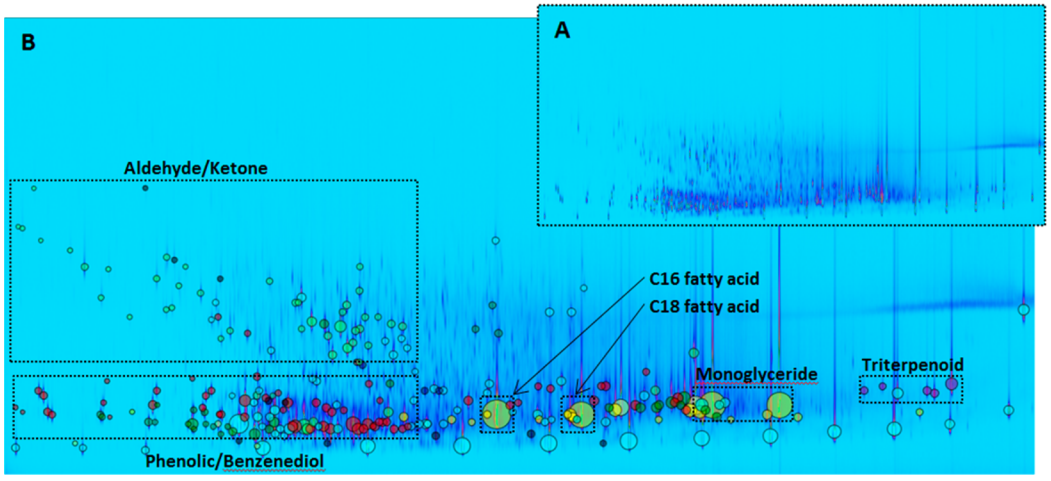

Figure 5. (A) TIC of biocrude of *M.* × *giganteus* biocrude. (B) TIC of biocrude of *M.* × *giganteus* biocrude with compound markers. (Dark blue) nitrogen ring structures, (black) hydrocarbons, (yellow) fatty acids, (light yellow) alcohols, (purple) triterpenoids, (dark green) benzenediols, (light green) carbonyls, (red) phenolics. Primary and secondary axes show retention on the first and second column, respectively. Circles reflect the abundance of the peak.

aliphatics. Also, the triterpenoid composition was different as it consisted of pregnenane derivatives for biocrude of *L. hyperborea*.

3.2. Compound Group Analysis of Biocrude of DDGS. The DDGS used in this work is a residue from bioethanol production. Residues are often found in large quantities as wet streams, making them highly attractive for HTL processing.

The most notable region in the chromatogram of this sample is the dense region of C_{18} fatty acids (Figure 4). A total of 18 peaks were detected here, including stearic acid (C_{18}) and several mono-, di-, tri-, and tetraunsaturated C_{18} fatty acids. The sample contained only minor amounts of palmitoleic acid (C_{16}) compared to biocrude of *C. vulgaris*. The analysis also showed pelargonic acid (C_9), capric acid (C_{10}), lauric acid (C_{12}), myristic acid (C_{14}), pentadecylic acid (C_{15}), margaric acid (C_{17}), arachidic acid (C_{20}), behenic acid (C_{22}), lignoceric acid (C_{24}), and cerotic acid (C_{26}), similar to biocrude of *C. vulgaris*. The abundance of monoglycerides was also analogous to *C. vulgaris*.

Furthermore, the region of fatty acid amides was almost completely absent, as for *C. vulgaris*, even though the feedstock consists of 33% protein. DDGS contains approximately the same ratio of carbohydrate to protein as *C. vulgaris*, and similar reaction pathways for pyrazine formation were observed in the aqueous phase.[36] This supports our previous notion that degradation products of carbohydrates react with degradation products of proteins, leading to an absence of fatty acid amides. Additional similarity of biocrudes from DDGS and *C. vulgaris* was observed for the indole/quinoline region which was almost identical in terms of compounds.

The samples of DDGS and *L. hyperborea* contained comparable aldehyde/ketone and benzenediol regions. However, for DDGS the aldehyde/ketone region only showed indenones, acetophenones, and chromenones and thus no indanones. Instead, an additional region with aromatic acids containing eight cinnamic acids was present in the biocrude from DDGS.

Furthermore, this sample only showed eight triterpenoids which were ergostane, cholestane, and stigmastane derivatives, similar to *C. vulgaris*.

3.3. Compound Group Analysis of Biocrude of *M.* × *giganteus*. Lignocellulosics are less often used for HTL for a

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number of reasons which include necessary size reduction, need for use of process water (can be overcome with water recycling), difficulties with pumping, low growth rates, and high lignin contents leading to higher amounts of solid residue.[5,43]

However, their widespread abundance means that they are an accessible biomass with low demands for nutrients. Energy crops, such as *M.* × *giganteus*, are of specific interest as they can grow on nonarable land due to low nutrient requirements. Despite the abundance of grass feedstocks only few have been subjected to HTL.[44,45]

Biocrude of *M.* × *giganteus* was the most complex sample analyzed here (Figure 5). Despite the low lipid content of *M.* × *giganteus*, the most abundant peaks detected were for palmitic acid and oleic acid, although they were far less abundant than in other samples. Only three other C_{18} fatty acids were detected. The third and fourth most abundant peaks were monoglycerides of C_{16} and C_{18} fatty acids. This is despite the fact that the feedstock was grinded to <90 μm, making them more accessible to degradation at typical HTL process parameters. This indicates that the glycerides of lignocellulosics are far more resistant to hydrolysis than in other feedstocks and process conditions can be optimized. Phenolics and benzenediols are typically reported as the main components of biocrude from lignocellulosics. The responses of single compounds of this class were considerably lower than fatty acids; however, the number of different compounds was the highest for all compound classes with 62 different phenolics and 30 different benzenediols.

The region of aldehyde/ketone (indanones, acetophenones, and chromenones) is typically not reported for lignocellulosics. While it is the least abundant group of peaks in this study it represents one of the most diverse ones, with 38 different compounds of cyclic oxygenates, indanones, indenones, acetophenones, propriophenones, and chromenones.

3.4. Comparative Characterization of Biocrude. In the following sections the most abundant compounds, based on normalized peak response, of each compound class are evaluated across the five different samples. A threshold peak response was used in order to evaluate only the most abundant compounds within each compound class of fatty acids (>25), monoglycerides (>25), alcohols (>3), phenolics (>15), benzenediols (>15), nitrogen-containing compounds (>10), hydrocarbons (>5), and carbonyls (>5).

Figure 6 shows the sum of normalized peak responses for the compound classes highlighted in sections 3.1−3.3. Fatty acids are identified as the most abundant semivolatile compound class in all samples, which is coherent with previous studies on HTL of microalgae, macroalgae, and DDGS, while phenolics and benzenediols are often considered the most abundant compound classes in biocrudes of lignocellulosics. However, it is well known that analysis of fatty acids suffers from matrix effects, active site interactions, and poor chromatographic performance without derivatization. Alcohols are the second most abundant semivolatile compound class of *N. gaditana* and DDGS, while nitrogen-containing compounds are equally abundant in *N. gaditana*; however, most of the nitrogen-containing compounds are nonderivatized, and it is likely that their concentrations are actually higher than those of alcohols. It is interesting to note that the protein contents of *N. gaditana* (46%), *C. vulgaris* (49%), and DDGS (33%) are similar, while the sum of peak responses for semivolatile nitrogen-containing compounds is 3−4 times higher for *N. gaditana*, indicating that a change in reaction pathways occurs based on the relative content of carbohydrates, proteins, and lipids of the feedstock.

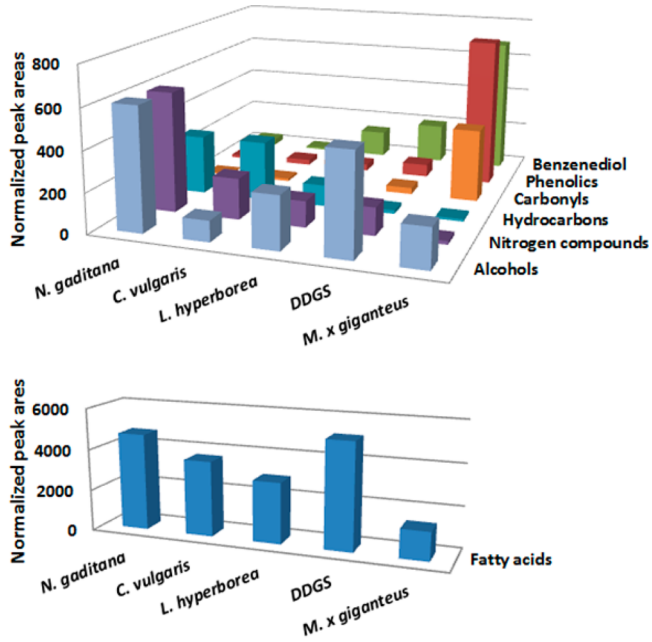

Figure 6. Normalized peak responses of fatty acids, alcohols, nitrogen-containing compounds, hydrocarbons, carbonyls, phenolics, and benzenediols detected in biocrudes of *N. gaditana*, *C. vulgaris*, *L. hyperborea*, DDGS, and *M.* × *giganteus*.

Hydrocarbons we found are mainly from algae feedstocks, but they are not derivatized and are therefore expected to be found in higher concentrations than indicated by the peak responses presented in this work. Semivolatile carbonyls are a mixture of mainly nonderivatized indanones/indenones and smaller abundances of oxygenated ketonized aromatics, which are derivatized. These are almost solely detected for *L. hyperborea* and *M.* × *giganteus*. Phenolics and benzenediols are predominantly detected in biocrude from *M.* × *giganteus*, while smaller amounts are found from DDGS and *L. hyperborea*.

In the following, individual compounds from fatty acid, monoglycerides, alcohols, phenolics, benzenediols, nitrogen-containing compounds, and carbonyls are investigated. Hydrocarbons are listed in the Supporting Information (SI Table 2).

3.4.1. Fatty Acids and Monoglycerides. Fatty acids are the main components of most HTL biocrudes since high-lipid feedstocks result in a high biocrude yield, and further upgrading is accomplished in high yield as well. Experiments with model compounds (e.g., sunflower oil) have shown almost 100% conversion to biocrude.[46]

The largest peak for all samples was observed for palmitic acid (hexadecanoic acid), and the largest value was observed for biocrude of *N. gaditana* (Table 1). Fatty acids with molecular weights less than palmitic acid were generally at least three times more abundant in the sample of *N. gaditana*, except myristic acid and lignoceric acid, which were also highly abundant for *L. hyperborea*. The sample of *M.* × *giganteus* contained 4−7 times less palmitic acid and equal amounts of stearic acid compared to the other samples. However, it contained almost none of the otherwise highly abundant fatty acids of *cis*-9-hexadecenoic acid, palmitoleic acid ((*Z*)-9-hexadecenoic acid), oleic acid, and *trans*-9-octadecenoic acid. The biocrude of *C. vulgaris* and *L. hyperborea* had similar abundances of *cis*-9-hexadecenoic acid, palmitic acid, linoleic acid, oleic acid, and stearic acid; however, the diversity of the unsaturated C_{18} fatty acids was far larger for *C. vulgaris*. Abundances of these unsaturated C_{18} fatty acids were

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Table 1. Normalized Peak Responses of the Most Abundant Fatty Acids (only peak responses > 25)[a]

	RI	*N. gaditana*	*C. vulgaris*	*L. hyperborea*	DDGS	*M.* × *giganteus*
capric acid (C_{10})	1480	40	15	14	14	ND
lauric acid (C_{12})	1678	36	11	17	8	ND
myristic acid (C_{14})	1879	523	50	255	16	15
pentadecylic acid (C_{15})	1941	29	7	18	7	8
?-hexadecenoic acid (C_{16})	2051	ND	71	6	4	ND
?-hexadecenoic acid (C_{16})	2060	873	68	142	3	5
palmitelaidic acid (C_{16})	2065	581	71	10	ND	ND
palmitic acid (C_{16})	2084	1500	1098	778	1098	302
unknown fatty acid (C_{17}) (branched)	2140	7	45	ND	ND	ND
?,?-octadecadienoic acid (C_{18})	2240	ND	29	ND	ND	ND
linoleic acid (C_{18})	2246	53	23	17	67	6
oleic acid (C_{18})	2254	271	479	443	841	29
trans-9-octadecenoic acid (C_{18})	2260	255	505	ND	816	13
stearic acid (C_{18})	2280	62	439	357	289	224
?,?-octadecadienoic acid (C_{18})	2297	ND	95	10	410	ND
?,?-octadecatetraenoic acid (C_{18})	2298	ND	63	16	ND	ND
?,?-octadecadienoic acid (C_{18})	2301	5	ND	5	194	ND
?,?-octadecadienoic acid (C_{18})	2306	ND	61	9	310	ND
?,?-octadecadienoic acid (C_{18})	2311	ND	59	6	ND	ND
?,?-octadecadienoic acid (C_{18})	2319	ND	ND	ND	42	ND
?,?-octadecadienoic acid (C_{18})	2324	ND	18	ND	184	ND
?,?-octadecadienoic acid (C_{18})	2336	12	ND	ND	64	ND
?,?-octadecadienoic acid (C_{18})	2342	23	300	37	736	ND
?,?-octadecatetraenoic acid (C_{18})	2352	10	35	ND	11	ND
1,3-propanediol palmitate	2366	ND	ND	29	ND	28
1,3-propanediol stearate	2567	ND	ND	ND	ND	52
lignoceric acid (C_{24})	2886	2	4	67	8	11
α-glyceryl myristate	2433	ND	ND	8	ND	ND
β-glyceryl palmitate	2593	ND	4	16	ND	44
α-glyceryl palmitate	2628	20	44	322	16	316
β-glyceryl stearate	2791	ND	3	14	ND	15
α-glyceryl stearate	2826	12	58	356	18	313

[a]? means that the configuration is unknown.

even higher for DDGS. A number of eicosatetraenoic acids and eicosapentaenoic acids were only present for *N. gaditana* and not included in this analysis.

Monoglycerides are rarely reported from HTL biocrudes. Biocrudes from the high-lipid feedstocks of *N. gaditana* and DDGS had the lowest abundances of all monoglycerides, while it was three times higher for *C. vulgaris* biocrude. Biocrudes of *L. hyperborea* and *M.* × *giganteus* had up to 26 times higher concentrations of α-glyceryl stearate compared to *N. gaditana* biocrude, which may be due to less accessible cell structures. Furthermore, the concentrations of oleic acid and stearic acid are comparable for *L. hyperborea* biocrude; however, neither α- nor β-glyceryl oleate (RI 2788 and 2744) could be detected. A very small peak of β-glyceryl oleate was detected for *C. vulgaris*. This indicates that the glycerides containing saturated fatty acids may be less susceptible to hydrolysis during the HTL process than those containing unsaturated fatty acids.

3.4.2. Alcohols. Alcohols are rarely reported from HTL studies because single alcohols are found in small amounts with vapor pressures coinciding with a significant fraction of the biocrude leading to coelution. The alcohols detected in this study were primarily derived from pigments or wax.

The most abundant alcohols were straight long chain and branched long chain alcohols (Table 2). The biocrude of *C. vulgaris* was the most diverse with regard to simple long chain primary alcohols, albeit none of these were highly abundant. The biocrudes of *M.* × *giganteus* and *L. hyperborean* had the highest abundances of the phytol-like compounds of 3,7,11,15-tetramethylhexadecan-1-ol and 3,7,11,15-tetramethyloctadecan-1-ol. Biocrude of *L. hyperborea* also showed two abundant glycols.

The biocrude of DDGS contained several different unknown saccharides, which in one case was highly abundant. However, saccharides were not detected for biocrudes of high-carbohydrate feedstock such as *L. hyperborea* and *M.* × *giganteus*. In general, it would be expected that saccharides are displaced to the aqueous phase.

The biocrude of *N. gaditana* was the only one to have a specific alcohol region appointed in section 3.1. Dotricontenol was the most abundant of the six fatty alcohols determined and was the compound with the highest response for all alcohols across the samples.

Glycerol was detected in equally low abundance for all samples. However, it was not detected in *N. gaditana*.

3.4.3. Phenolics and Benzenediols. Phenolics and benzenediols are highly abundant compounds from HTL of especially carbohydrate- and lignin-rich feedstocks and represent compounds of potentially high value. They show medium to high activity toward hydrodeoxygentation and are a source of oxygen in the final biofuels.[47,48]

The biocrudes of *N. gaditana* and *C. vulgaris* contained almost no phenolics or benzenediols, although two long chain alkyl

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Table 2. Normalized Peak Responses of the Most Abundant Alcohols (only peak responses > 3)[a]

	RI	*N. gaditana*	*C. vulgaris*	*L. hyperborea*	DDGS	*M.* × *giganteus*
phenylethanol	1242	ND	4	ND	4	ND
glycerol	1290	ND	5	6	5	4
glycol compound	1530	ND	ND	10	ND	ND
glycol compound	1545	ND	ND	56	ND	ND
dodecan-1-ol	1591	1	2	8	1	1
levoglucosan	1727	3	6	10	6	ND
tetradecan-1-ol	1790	ND	16	14	ND	ND
3,7,11,15-tetramethylhexadec2-en-1-ol	1850	42	ND	ND	ND	ND
(1*E*)-*o*-methyloxyme-D-glucose	1957	3	2	ND	ND	ND
hexadecan-1-ol	1991	ND	18	ND	1	ND
heptadecan-1-ol	2092	ND	5	ND	ND	ND
octadecan-1-ol	2190	ND	2	3	1	ND
3,7,11,15-tetramethylhexadecan-1-ol	2379	ND	17	67	8	66
saccharide	2407	ND	ND	ND	14	ND
saccharide	2429	ND	ND	ND	13	ND
3,7,11,15-tetramethyloctadecan-1-ol	2581	ND	14	74	ND	120
saccharide	2597	ND	ND	ND	325	ND
saccharide	2606	ND	ND	ND	109	ND
?-triacontenol	3366	10	ND	ND	ND	ND
?-triacontenol	3375	50	ND	ND	ND	ND
?-hentriacontenol	3430	7	ND	ND	ND	ND
?-hentriacontenol	3434	15	ND	ND	ND	ND
?-dotriacontenol	3482	135	ND	ND	ND	ND
?-dotriacontenol	3500	344	ND	ND	ND	ND

[a]? means that the configuration is unknown.

benzenediols (3-((1*E*,3*E*)-pentdeca-1,3-dienyl)-1,2-benzenediol and ((3-((1*E*,3*E*)-hexadeca-1,3-dienyl)-1,2-benzenediol) were detected for *N. gaditana* (Table 3). These compounds were not part of the NIST library and are identified based on retention times and fragmentation patterns. It has previously been proposed that phenolics and benzenediols are partly formed from degradation of carbohydrates, which supports the absence of these compounds in the sample made from *N. gaditana*. On the other hand, *C. vulgaris* contains 26.5% carbohydrates (dry weight); however it is proposed that the majority of intermediate products from carbohydrate degradation react with degradation products from protein to produce nitrogen-containing aromatics. DDGS (35.0% carbohydrate) has approximately the same biochemical composition as *C. vulgaris*; however, the biocrude from DDGS has a much higher abundance of especially benzenediols (catechols and hydroquinones). Instead, the carbohydrates are degraded to indanones. The formation of phenolics is clearly favored by high lignin contents with *M.* × *giganteus* showing high abundances of several simple phenolics, such as 3-methoxyphenol, 2-ethylphenol, 3-ethylphenol, and ethylguaiacol. A series of phenolics containing the structure of 3-phenol-prop-2-en-1-ol was detected for *M.* × *giganteus* biocrude and expected to be degradation products from lignin. These compounds were not part of the NIST database.

Benzenediols can be found in three different forms, catechol (benzene-1,2-diol), hydroquinone (benzene-1,4-diol), and resorcinol (benzene-1,3-diol). They are easily distinguished by their approximate ratios of M^+ to 73 m/z, which are 1:4 for catechol, 4:1 for hydroquinone, and 1:1 for resorcinol. In this work, only catechols and hydroquinones were detected. The most abundant benzenediol detected was 4-ethylcatechol, which was only detected in *M.* × *giganteus*. Trimethylsilylated 4-ethylcatechol was not present in the NIST library and has been identified from comparison with the literature.[49] None of the series of alkylated catechols and hydroquinones were present in the NIST library, and their identification is based on comparison of retention times and fragmentation patterns with 4-ethylcatechol (see SI Figure 1).

3.4.4. Nitrogen-Containing Compounds. The nitrogen-containing compounds are an important compound class as combustion of nitrogen-containing biocrude may produce nitrogen oxides. Fossil crude oil contains only small amounts of nitrogen, and commercial catalysts are therefore unsuitable for denitrogenation, making knowledge of nitrogen-containing compounds important.[47]

The nitrogen-containing compounds were mainly ring structures of either aromatics (indoles, quinolones, imidazoles, or indolizines) or combined with amide bonds (pyrrolidinones or fatty acid amide), and free amines were not present. By far the highest diversity and in most cases also the highest abundance of nitrogen-containing compounds was found for biocrudes of the high-protein feedstock of *N. gaditana* (Table 4). The ring structures were mostly made up of indoles which were generally observed in all samples apart from *M.* × *giganteus*. The level of the most abundant indoles was approximately equal in the biocrudes from *N. gaditana*, *C. vulgaris*, *L. hyperborea*, and DDGS, whereas the remaining compounds were either not detected or in significantly lower abundance.

While several fatty acid amides have previously been detected from HTL of model compounds,[27] they are rarely reported from biomasses with the exception of hexadecanamide or *N*-methylhexadecanamide. These compounds were also the most abundant fatty acid amides detected, and generally fatty acid amides were almost exclusively detected in *N. gaditana* biocrude. It is noteworthy that the feedstocks of *C. vulgaris* and DDGS contain approximately the same protein concentration as *N. gaditana*, while the lipid concentration is one-half, and the carbohydrate concentration is double. This implies that the

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Table 3. Normalized Peak Responses of the Most Abundant Phenolics and Benzenediols (only peak responses > 15)[a]

phenolics	RI	*N. gaditana*	*C. vulgaris*	*L. hyperborea*	DDGS	*M.* × *giganteus*
2-methoxyphenol	1234	ND	ND	ND	ND	51
3-methoxyphenol	1242	ND	ND	ND	ND	86
2-ethylphenol	1248	2	6	ND	5	77
3-ethylphenol	1254	5	4	4	7	72
2-methoxy-5-methylphenol	1333	ND	ND	ND	ND	27
ethylguaiacol	1412	ND	ND	ND	ND	125
2,6-dimethoxyphenol	1419	ND	ND	ND	ND	33
4-ethyl-3-methylguaiacol	1497	ND	ND	ND	ND	38
ethylsyringol	1570	ND	ND	ND	ND	34
4-hydroxyphenylethanol	1592	8	ND	9	15	ND
4-propansyringol	1648	ND	ND	ND	ND	19
(*Z*)-3-(*p*-phenol)-prop-2-en-1-ol	1658	ND	ND	ND	4	22
(*E*)-3-(*p*-phenol)-prop-2-en-1-ol	1663	ND	ND	ND	11	47
(*E*)-3-(*p*-phenol)-prop-2-en-1-ol + C_1	1716	ND	ND	ND	ND	21
(*E*)-3-(*p*-phenol)-prop-2-en-1-ol + C_2	1730	ND	ND	ND	ND	24
coniferyl alcohol moiety	1782	ND	ND	ND	ND	33
coniferyl alcohol moiety	1800	ND	ND	ND	ND	25
4-(3-hydroxypropyl)-2-methoxyphenol	1843	ND	ND	ND	ND	16
benzenediols						
1,2-benzenediol	1328	ND	ND	ND	8	77
1,2-benzenediol	1331	ND	ND	ND	6	30
4-methylcatechol	1405	ND	ND	6	17	55
3-methylcatechol	1415	ND	ND	0	5	11
hydroquinone	1420	ND	ND	7	ND	3
3-ethylcatechol	1472	ND	ND	ND	3	13
4-ethylcatechol	1480	ND	ND	ND	57	256
?,?-dimethylcatechol	1482	ND	ND	ND	9	12
methylhydroquinone	1482	ND	ND	17	ND	28
catechol + C_2	1483	ND	ND	22	ND	ND
catechol + C_3	1549	ND	ND	8	15	9
3-propylcatechol	1559	ND	ND	21	42	ND
catechol + C_4	1587	ND	ND	ND	12	28
catechol + C_4	1612	ND	ND	ND	8	10
hydroquinone + C_3	1613	ND	ND	ND	7	ND
5-ethyl-3-methoxy-1,2-benzenediol	1622	ND	ND	ND	ND	23
1,2,3-benzenetriol	1622	3	10	40	6	ND
hydroquinone + C_3	1664	ND	ND	13	5	ND
3-((1*E*,3*E*)-tetradeca-1,3-dienyl)-1,2-benzenediol (A)	2431	ND	ND	ND	ND	11
3-((1*E*,3*E*)-pentdeca-1,3-dienyl)-1,2-benzenediol (B)	2476	9	ND	ND	ND	15
(*E*)-((3-(hexadec-1-enyl)-1,2-benzenediol	2516	ND	ND	ND	ND	31
((3-((1*E*,3*E*)-hexadeca-1,3-dienyl)-1,2-benzenediol (C)	2541	20	ND	ND	ND	10
(*Z*)-((3-(hexadec-1-enyl)-1,2-benzenediol (A)	2551	ND	ND	ND	ND	40
(*E*)-((3-(octadec-1-enyl)-1,2-benzenediol (B)	2609	ND	ND	ND	ND	13
3-docosyl-1,2-benzenediol	2643	ND	ND	ND	ND	12

[a]? means that the configuration is unknown.

proteins are degraded to amino acids at a sufficient rate and able to react with reducing saccharides before the amino acids are deaminated or decarboxylated to form pyrazines. However, a considerable amount of amino acids is also deaminated to form small organic acids dissolved in the aqueous phase. When proteins are in excess the released ammonia, methylamine, or dimethylamine is able to react with fatty acids to form fatty acid amides. It is interesting to note that only (*Z*)-octadec-9-enamide was detected even though (*E*)-octadec-9-enoic acid was found in the same abundance as (*Z*)-octadec-9-enoic acid. Furthermore, a total of only 26 fatty acid amides were detected even though 45 different ones should be possible.

3.4.5. Aldehydes and Ketones. Carbonyls are typically reported as highly abundant in most HTL biocrudes; however, they are nearly always presented as cyclopentenones and mainly occur from lignocellulosic feedstocks. To our knowledge, no other study has reported the presence of semivolatile carbonyls in biocrudes from HTL of biomass. The most abundant carbonyls were largely indenones, acetophenones, and a series of alkylated chromenones (Table 5). Indenones and chromenones are typically not reported from studies of HTL, which is mainly due to the fact that most studied feedstocks have been microalgae, macroalgae, and residues where lignin is either absent or found in only small amounts. The biocrudes from microalgae contained only small amounts of a single carbonyl in the form of 6,10,14-trimethylpentadecan-2-one, while DDGS biocrude contained small amounts of different indenones, acetophenones, and alkylated chromenones. The diversity and

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Table 4. Normalized Peak Responses of the Most Abundant Nitrogen-Containing Compounds (only peak responses > 10)

		N. gaditana	*C. vulgaris*	*L. hyperborea*	DDGS	*M.* × *giganteus*
n-butyl-pyrrolidin-2-one	1365	15	6	ND	8	0
n-pentylsuccinimide	1398	15	ND	ND	ND	ND
3-methylquinoline	1403	21	9	ND	5	ND
7-methylindole	1414	28	21	20	16	ND
indole	1459	18	25	ND	5	ND
1*H*-pyrido[3,4-*b*]indole, 2,3,4,9-tetrahydro-1-(1-methylethyl)-	1468	23	45	60	53	ND
5-isopropyl-2,4-imidazolidinedione	1494	ND	ND	14	ND	ND
(5*R*,8*R*,8a*S*)-8-methyl-5-butyl-1,2,3,5,8,8a-hexahydroindolizine	1494	18	ND	ND	ND	ND
2,5-dimethylindole	1620	9	11	3	4	ND
3-ethan-2-olindole	1921	16	13	12	19	3
N-methyltetradecanamide	2044	13	ND	ND	ND	ND
(*E*)-9-hexadecenamide	2196	23	ND	ND	ND	ND
(*Z*)-9-hexadecenamide	2199	13	ND	ND	ND	ND
hexadecanamide	2218	68	5	ND	2	ND
(*E*)-*N*-methyl-hexadec-9-enamide	2232	34	ND	ND	ND	ND
(*Z*)-*N*-methyl-hexadec-9-enamide	2237	17	ND	ND	ND	ND
N-methylhexadecanamide	2256	92	34	ND	ND	ND
(*Z*)-9-octadecenamide	2275	19	ND	ND	ND	ND
octadecanamide	2298	40	18	ND	ND	ND
eicosanamide	2440	28	ND	ND	ND	ND
unknown fatty acid amide	2478	9	ND	ND	ND	15
unknown eisosenamide	2529	20	ND	ND	ND	ND
unknown fatty acid amide	2551	33	ND	ND	ND	ND
hexadecanoic acid, pyrrolidide	2628	26	ND	ND	4	ND

Table 5. Normalized Peak Responses of the Most Abundant Carbonyls (only peak responses > 5)[a]

		N. gaditana	*C. vulgaris*	*L. hyperborea*	DDGS	*M.* × *giganteus*
2,2,5,5-tetramethylcyclopent-3-en-1-one	1210	ND	ND	ND	2	7
2,3,4,5,6,7-hexahydro-1*H*-inden-1-one	1282	ND	ND	ND	ND	7
2,3-dihydro-1*H*-inden-1-one	1303	ND	ND	11	3	57
2,3-dihydro-2-methyl-1H-Inden-1-one	1329	ND	ND	31	9	23
2,3-dihydro-3-methyl-1*H*-inden-1-one	1341	ND	ND	ND	4	8
7-methylindan-1-one	1419	ND	ND	ND	ND	20
2-hydroxyacetophenone	1435	ND	ND	ND	2	8
6-methylindan-1-one	1436	ND	ND	ND	ND	17
2,3-dihydro-3,3-dimethyl-1*H*-inden-1-one	1445	ND	ND	8	4	8
2,3-dihydro-2,2-dimethyl-1*H*-inden-1-one	1457	ND	ND	21	ND	12
4-hydroxyacetophenone	1494	ND	ND	ND	4	25
7-hydroxychromen-2-one	1693	ND	ND	6	1	16
homovanillic acid	1697	ND	ND	ND	ND	13
2,5-dihydroxyacetophenone	1718	ND	ND	ND	ND	12
2,4-dihydroxyacetophenone	1733	ND	ND	ND	ND	8
unknown 7-hydroxychromen-2-one	1748	ND	ND	ND	ND	32
?-methyl-7-hydroxychromen-2-one	1760	ND	ND	4	ND	8
?-methyl-7-hydroxychromen-2-one	1761	ND	ND	20	ND	17
?-methyl-7-hydroxychromen-2-one	1777	ND	ND	8	ND	7
unknown carbonyl	1785	ND	ND	ND	ND	8
6,10,14-trimethylpentadecan-2-one	1873	6	13	5	ND	ND
unknown chromen-2-one	1874	ND	ND	8	ND	8
unknown chromen-2-one	1884	ND	ND	11	ND	ND
methyl vanillactate moiety	2473	ND	ND	ND	ND	8
methyl vanillactate moiety	2534	ND	ND	ND	ND	20

[a]? means that the configuration is unknown.

abundance of these compounds were significantly higher for *L. hyperborea*, indicating that some of these compounds are also formed from degradation of carbohydrates as the lignin content of *L. hyperborea* is lower than in DDGS. By far the highest number and the greatest abundance of most carbonyls were observed for biocrudes of *M.* × *giganteus*. Indenones are not silylated, and therefore, the response is not enhanced from derivatization, making their responses even more significant, and 2,3-dihydro-1*H*-inden-1-one is especially abundant.

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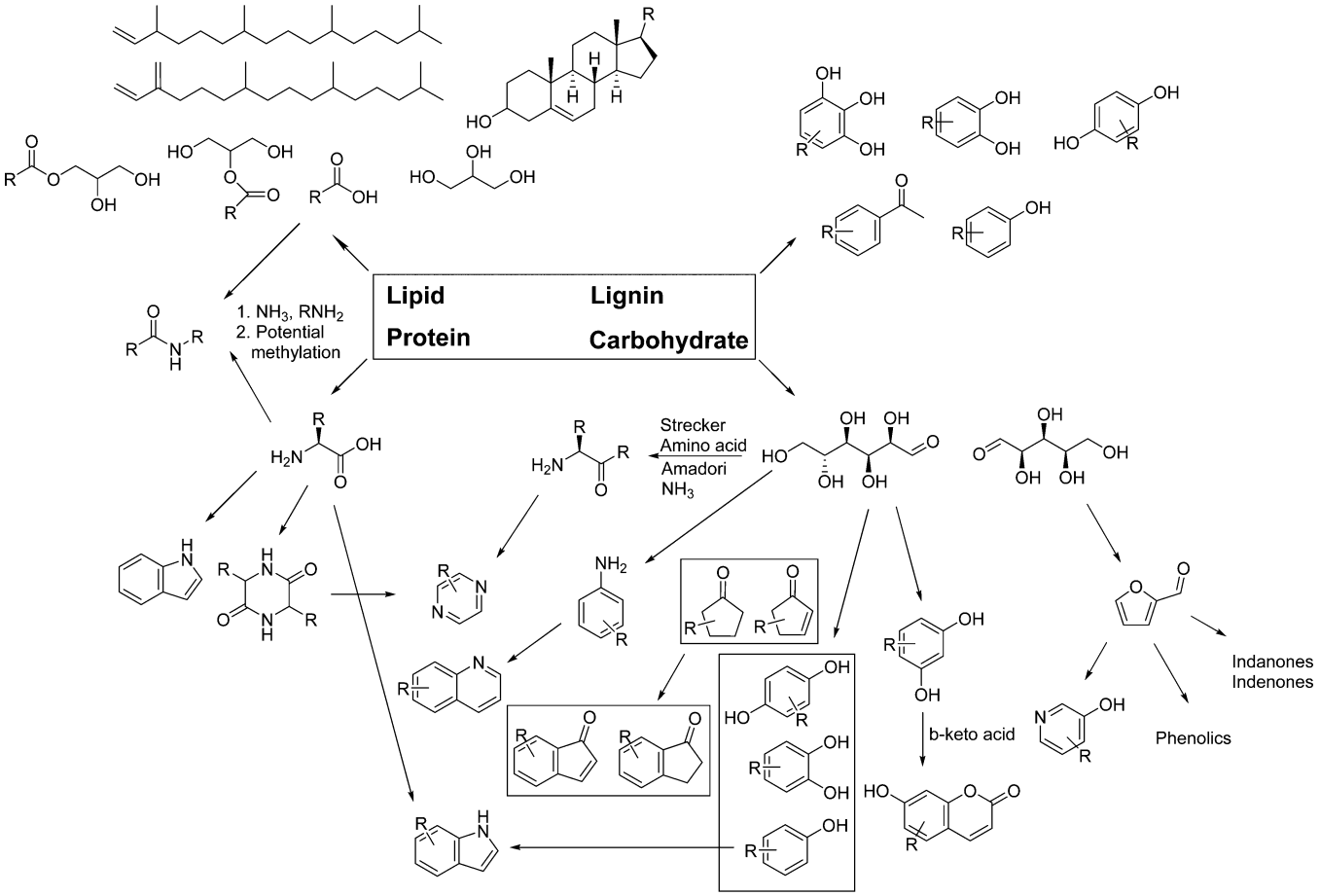

Figure 7. Reaction pathways for degradation of lipids, proteins, carbohydrates, and lignin during HTL.

A number of alkylated chromen-2-ones were detected which were not present in the NIST library. Chromenones are widely distributed in plant material where they have a protective function following stress events and antimicrobial activity,[50] and they are therefore expected to be naturally present in *M.* × *giganteus*; however, their abundance should also be highly dependent on the growth conditions of the feedstock. Chromenones were observed with the same abundance in biocrudes of both *L. hyperborea* and *M.* × *giganteus*, and while they are naturally present in plant material they have not been extensively reported for macroalgae. Furthermore, since *L. hyperborea* contains only minor amounts of lignin, it indicates that chromenones in HTL biocrude are formed from carbohydrate degradation. The alkylated chromen-2-ones were identified from umbelliferone and a series of mass spectra with increments of 14 m/z and characteristic fragmentation patterns (SI Figure 2).

3.5. Reaction Pathways. A generalized scheme of reaction pathways for biomasses containing carbohydrates, lipids, proteins, and lignin based on the most abundant compounds is presented in Figure 7. The limited fraction characterized in this work means that several secondary products are likely to be formed through additional pathways observed by other studies, which are referenced throughout the section.

Lipids include fatty acids, monoglycerides, diglycerides, triglycerides, phospholipids, waxes, steroids, and hydrophobic vitamins. Hydrolysis of glycerides leads to the formation of glycerol, free fatty acids, and monoglycerides observed in this work and previously reported in other studies.[37] Unsaturated fatty alcohols (C_{30-32}) were also observed, likely formed from hydrolysis of waxes.[51] Triterpenoid derivatives detected in this work and other studies are naturally occurring compounds in algae and plants released from the cells during HTL.[26,51,52] In the reaction pathways outlined, chlorophyll is considered a lipid as it is extracted during the determination of the total lipid of the feedstock (other pigments were not considered). Hydrolysis of chlorophyll leads to the release of phytol, which has been reported to be reduced to several pristene, phytene, and neophytadiene isomers, which were also observed during this work.[32]

Proteins are initially hydrolyzed to amino acids by cleavage of peptide bonds. Studies on HTL of pure protein and amino acids show rapid degradation through deamination and decarboxylation producing ammonia, carboxylic acids, carbon dioxide, and amines, which are predominantly water-soluble compounds and not observed in this work.[53] Depending on the side chain of the amino acids a number of additional degradation and recombination products may be produced of which only a fraction are accounted for in these reaction pathways.[54]

In the presence of fatty acids, ammonia and amines can react through condensation to form fatty acid amides. Previous studies focused specifically on fatty acid amides identified 39 amides from HTL of municipal solid waste in many of which the amine was longer than the methylamine.[27] Most other studies report only the presence of hexadecanamide, while this work reports 26 different fatty acid amides predominantly as primary, methyl, or pyrrolidine amides in biocrude from *N. gaditana*. These amides may be formed from ammonia, methylamine, and pyrrolidine,

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while the remaining amines most likely react faster with other products.

Prior to deamination or decarboxylation, amino acids may condensate to form DKPs, which can decompose to pyrazines at temperature > 300 °C.[14,37] Pyrazines are likely displaced to the aqueous phase and are too volatile to be detected with the method used in this work.[36]

The interaction of carbohydrates and proteins is often mentioned in HTL studies. Amino acids and ammonia may react with saccharides in Strecker degradation or Amadori rearrangements ultimately leading to formation of pyrazines.[36] It is also well established that hemicellulose initially degrades to furfurals and furans which can react with ammonia to form hydroxypyridines.[37] However, these compounds were not detected in this work. Another potential interaction is the reduction of Schiff bases formed between ammonia and saccharides to form aniline. Both the amine and the ortho position of aniline are nucleophilic, and reaction with, for example, β-keto acids would lead to favorable six-membered ring formation and subsequent reduction to form quinolines. However, it should be noted that the formation of aniline has not been shown in the literature.

Formation of stable five- and six-membered rings with subsequent reduction to obtain conjugated double bonds or in many cases aromaticity is a common theme for products of carbohydrate and protein degradation in HTL. Carbohydrates are especially noted for the degradation products of alkylated cyclopentanones and cyclopent-2-enones, which are too volatile to be detected with the method used, while oxygenated aromatics in the form of phenolics, catechols, and hydroquinones are also abundant.[55] Cyclopent-2-enones can further react in either two consecutive aldol condensations or one aldol condensation followed by a nucleophilic enol reaction and subsequent reduction to form indenones. Cyclopentanones can also undergo aldol condensation, and the α-position is stabilized by the sp^2 orbitals of the ketone, making the 3-position of the cyclopentanone nucleophilic, leading to ring closure and reduction forming indanones. Indanones and indenones may also be formed from condensation of furfurals.[41]

Nucleophilic reaction of the ortho position of phenol with amino acids would add an electron-withdrawing ketone, making the already favorable 5-membered ring formation possible through nucleophilic attack of the amine and subsequent elimination of the hydroxyl group. Finally, reduction would lead to formation of alkylated indoles, which are observed with a large alkyl variation. Both amino acids and saccharides also degrade to multiple small carboxylic acids which are mostly water soluble or too volatile to be detected with the method used and not included in the pathways.[30,55]

Chromen-2-ones are observed naturally in plant material, being formed from cinnamic acid, and it is likely that these are simply released from the biomass during the HTL process. Another possible source of formation could be the nucleophilic reaction between the hydroxyl group of resorcinols and β-keto acids (present in aqueous phase) followed by favorable six-membered ring formation through the para-directing nucleophilic attack of phenol and subsequent reduction to form chromen-2-ones. While a number of alkylated benzenediols were observed none of these were from resorcinol; thus, the pathway is questionable.

The most notable difference between biocrude of lignocellulosic and other high carbohydrate feedstocks is the presence of multiple alkylated phenolics, catechols, and hydroquinones from which the diversity is much higher than reported from other studies.[56] The reaction network of lignin is complex, consisting of breakages of polymeric ether bonds and demethoxylation, producing a multitude of phenolic compounds which to a large extent are similar to those of cellulose and hemicellulose.[55] These compounds consist of phenolics, catechols, hydroquinones, and acetophenones.

4. CONCLUSION

The composition of semivolatile compounds in biocrude from HTL of microalgae, macroalgae, DDGS, and *M.* × *giganteus* was analyzed by GC × GC-TOFMS with in situ silylation. Numerous C_{16}, C_{18}, and C_{20} fatty acids were detected, a complexity which has previously not been reported. Several monoglycerides were observed, especially in biocrudes from low-lipid-containing feedstocks highlighting the differences in accessibility of cell structures. In the presence of protein, fatty acid amides may be obtained, which included 24 different compounds; most were primary, methyl, and pyrrolidine amides present almost solely for protein- and lipid-rich feedstocks (*N. gaditana*).

Alkylated indoles and quinolines were abundant in biocrudes from protein-rich feedstocks. Indoles are likely formed from phenolics and amino acids, while quinolines may derive from reactions between ammonia, saccharides, and β-keto acids.

Several diverse carbonyls were observed which included acetophenones, indanones/indenones, and chromen-2-ones. Acetophenones were formed from lignin in *M.* × *giganteus*, indanones/indenones were derived from further reaction of carbohydrate degradation products, which may involve cyclic oxygenates, while chromen-2-ones most likely occur naturally in the feedstock.

A diversity of alkylated benzenediols was observed from carbohydrate-rich feedstocks (DDGS and *L. hyperborea*), while their abundances were increased along with the presence of alkylated phenolics when processing a lignin- and carbohydrate-rich feedstock (*M.* × *giganteus*).

This work sheds additional light on the complexity of a part of HTL biocrude, which is difficult to analyze and provides useful information on characterization of semivolatile compounds of HTL biocrude.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.energyfuels.7b00160.

Elemental distribution and biochemical composition on dry and ash free basis of feedstock used for HTL; normalized peak responses of the most abundant hydrocarbons; mass spectra of silylated 4-ethylcatechol, catechol + C_3, methylhydroquinone, and hydroquinone + C_3; mass spectra of silylated 7-hydroxychromen-2-one, 5-hydroxychromen-2-one, 6- methyl-7-hydroxychromen-2-one, and 5,6-dimethyl-7-hydroxychromen-2-one, (PDF)

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Notes
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