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Qualitative characterization of solid residue from hydrothermal liquefaction of biomass using thermochemolysis and stepwise pyrolysis-gas chromatography-mass spectrometry

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Abstract

Hydrothermal liquefaction (HTL) produces a side-product of solid residue (SR) with an organic fraction of char highly dependent on the feedstock. In this work the char from batch HTL of poplar, *spirulina*, and their 1:1 mixture was characterized for the first time using stepwise thermal desorption and pyrolysis-gas-chromatography-mass spectrometry (py-GC-MS) along with thermochemolysis. Three distinct compound fractions were identified in the form of trapped or strongly adsorbed compounds, residual lignin, and repolymerized phenolics. The trapped or adsorbed fraction resembled the compounds in bio-crude and aqueous phase from both poplar and *spirulina*. Residual lignin was only found from poplar while repolymerized phenolics were predominantly observed from poplar through *ortho* and *para*-directed polymerization. Multiple alkylated aliphatics and some pyrroles were observed from *spirulina*. Co-liquefaction of biomasses led to a markedly different SR from the individual biomasses with multiple alkylated pyrroles and indoles, both volatile and non-volatile, while repolymerized phenolics diminished due to Schiff base

formation. This work demonstrates that potential bio-crude is present in SR from both poplar and *spirulina* while co-liquefaction hinders repolymerization of phenolics but also produces a vast number of volatile and non-volatile pyrroles. The work shows that additional information for the reaction pathways of HTL may be found from characterization of the SR and provides researchers within biomass conversion with a method to evaluate effects on SR formation.

1. Introduction

Reduction of CO₂ emissions is a focus point of attempts to mitigate global climate changes. Combustion of fossil fuels for transport is an important contributor of CO₂ and renewable bio-fuels from conversion of biomass may reduce CO₂ emissions from a life cycle perspective.¹ Many factors influence the overall CO₂ emission and economic viability of biomass conversion including cultivation, harvest, transportation, pretreatment, process type, catalyst type, and product separation.^{2, 3} Hydrothermal liquefaction (HTL) is a promising technique for conversion of wet biomass avoiding energy intensive drying (necessary for other techniques such as pyrolysis), which significantly reduces the overall generation of CO₂.⁴ HTL utilizes the unique properties of hot compressed water and is carried out at elevated temperature (250-400 °C) and pressure (100-250 bar) with biomass in aqueous slurry. The aqueous media makes virtually all biomasses amenable to HTL including residues, waste, aquatics, and lignocellulosics. Many of these feedstocks can be grown on non-arable land or be used in waste water treatment prior to conversion.⁵⁻⁷ Microalgae have been the focus of many HTL studies due to the high yields of bio-crude along with ease of processing from good pumping ability at high pressures and limited char formation.^{8, 9} Lignocellulosics have received increasing attention due to their availability. Many of the studies on lignocellulosics have evaluated char formation and it is generally accepted that addition of alkali salts increase bio-crude yields and reduce solid residue (SR) formation by increasing the pH

value.¹⁰ Pumping of lignocellulosics has long been established as a major challenge for scaling up the process⁹ while the char formation represents a loss of carbon for which an effective utilization is required. Efforts to increase the pumping ability of lignocellulosics include thermal pretreatment, alkali pretreatment, addition of slurry stabilizer, and recirculation of bio-crude.^{11, 12} Recently, co-liquefaction with macroalgae and microalgae has been suggested, which additionally can compensate for cost, seasonal variability, and availability of the feedstocks while microalgae also produce minimum char.^{13, 14} The majority of HTL studies are performed without catalyst while many use alkali salts, as in this study, to decrease char formation. Char is defined as the primary decomposition and secondary polymerization of thermally decomposed biomass whereas coke is due to catalytic polymerization inside catalyst pores.¹⁵ Hence, in this study only char was considered as no catalyst was used during HTL. The char from HTL is often overlooked as a by-product although it may be used as fertilizer.¹⁶ Finding additional markets for the SR require extensive knowledge of the composition, which could also improve understanding of reaction pathways of HTL.

Characterizations of gas, aqueous phase (AqP), and bio-crude are common with many suggestions made for reaction networks based on analysis by gas chromatography coupled to mass spectrometry (GC-MS). Despite the efforts to minimize char, analyses of the SR have been limited with focus on elemental analysis, and more specific measurements for its application as an adsorbent.¹⁷

Standardized methods for molecular characterization of the SR are challenging due to the low solubility in organic solvents. Characterization of SR from HTL (220-260 °C) of cypress wood in ethanol has previously been reported using a combination of Fourier transform infrared spectroscopy (FT-IR), gel permeation chromatography (GPC), and nuclear magnetic resonance (NMR) identifying that the SR consisted predominantly of decomposed and repolymerized lignin.¹⁸

Recently, SR from HTL (220-300 °C) of cypress wood in water was characterized using advanced

72 methods such as liquid 2D NMR and pyrolysis-GC-MS (py-GC-MS).¹⁹ Both studies involved prior
73 fractionation including milling and multiple extractions.

74 The purpose of this work was to comprehensively characterize the SR from HTL of biomass. To the
75 best of our knowledge no detailed studies have been presented on the molecular composition of
76 char present in the SR without extensive fractionation. In this work we present a stepwise thermal
77 desorption and py-GC-MS analysis of unprocessed SR from batch HTL of poplar (lignocellulosic),
78 *spirulina* (microalgae) and a 1:1 mixture of them. Furthermore, thermochemolysis in the presence
79 of tetramethylammonium hydroxide (TMAH) was carried out to identify additional compounds.

80 **2. Materials and methods**

81 **2.1 Chemicals and reagents**

82 Potassium carbonate, dichloromethane (HPLC grade), and tetramethylammonium hydroxide (25
83 wt.% in water) were purchased from SigmaAldrich. Poplar was supplied by Department of
84 Agroecology, Aarhus University (Denmark). Poplar was separated into stems and leaves. The stems
85 were chopped in coarse pieces of 5-10 cm and dried overnight at 105°C. The dried pieces were
86 knife milled and further cyclone milled to a particle size of < 100 µm. *Spirulina* was obtained as a
87 powder from commercial sources and used as received. The ash content and elemental distribution
88 of the feedstocks, on dry and ash free basis, is presented in Table 1 along with the biochemical
89 composition.

90 **2.2 Feedstock analysis**

91 Ash content was determined by incineration at 575 °C for 5 h following evaporation of moisture.
92 The elemental distribution was measured using a CHNS Elementar Vario Macro Cube analyzer by
93 Elementar Analysensysteme GmbH, Hanau, Germany. The oxygen content was determined by
94 difference. Total carbohydrate content was determined from reaction of phenol and sulfuric acid
95 with carbohydrate, followed by colorimetric determination at 420 nm.²⁰ The carbohydrate content

of poplar was determined by difference as the lignin content interferes with the method. Total protein content was determined according to the Lowry protein assay, with reduction of folin reagent and subsequent colorimetric determination at 720 nm.²¹ Total lipid content was determined gravimetrically following extraction in 2:1 chloroform and methanol aided by sonication. Total lignin content was determined with the acetyl bromide method upon extensive washing to remove proteins.²² The biochemical composition of the 1:1 mixture was calculated as the averages from poplar and *spirulina*.

2.3 Hydrothermal liquefaction

Biomass slurries were prepared by mixing 10 wt.% biomass, 2 wt.% potassium carbonate, and 88 wt.% demineralized water. HTL experiments were performed in 20 ml batch reactors from Swagelok. Experiments were initiated by loading 10 ml of 10 wt.% biomass slurry into the reactor. Reactors were sealed and lowered into an Omega Engineering FSB-4 fluidized sand bath preheated to 350 °C. A reaction time of 20 min was applied, after which the reactors were cooled to ambient temperature in a water bath. The reactors were vented and the AqP was decanted into a centrifuge tube. The centrifuge tube was washed with 2 ml of dichloromethane and the reactor was extracted with 4 x 3 ml of dichloromethane, which were combined. Prior to the last extraction the walls and bottom of the reactor were scratched with a spatula. The dichloromethane phase was vacuum filtrated, and the residue washed thoroughly with dichloromethane until the filtrate appeared clear. The organic phase was dried under a stream of nitrogen giving the resulting bio-crude. The filter paper with the SR was dried overnight at 105 °C. The SR was carefully wiped off the filter paper into a sample preparation glass.

2.4 Py-GC-MS and thermochemolysis

Analysis was performed using a Gerstel multipurpose sampler coupled to a thermal desorption unit (TDU, Gerstel) with a pyrolysis unit (PYRO, Gerstel) inside. The TDU was connected to a cooled

120 injection system (CIS4, Gerstel) mounted on an Agilent 7890B GC coupled to a quadrupole mass
121 filter MS (Agilent, 5977A). Approximately 100 µg of sample was placed inside a 100 µl vial
122 connected to a transport adaptor. The vial was transferred to the TDU and thermally desorbed at
123 280 °C. The same sample was then stepwise pyrolyzed at 400, 500, and 600 °C. The TDU initial
124 temperature was 40 °C and increased at 12 °C sec⁻¹ to 280 for thermal desorption (TD) and to 300
125 °C for pyrolysis. For pyrolysis the temperature was rapidly increased to the desired temperature and
126 the TDU temperature was held at 300 °C for 1 min after pyrolysis. The helium desorption flow of
127 the TDU was 50 ml min⁻¹. The transfer line was held at 320 °C and the desorbed analytes were
128 trapped in the CIS on a quartz wool liner at -60 °C using liquid N₂. The trapped analytes were
129 released onto the column when heating the CIS to 150 °C at a rate of 16 °C sec⁻¹ and to 300 °C at a
130 rate of 12 °C sec⁻¹. A total split of 100:1 was applied through the TDU and CIS.
131 For thermochemolysis the sample was mixed with 2 µl of TMAH solution before it was pyrolyzed
132 at 500 °C following the settings outlined above.
133 The GC was equipped with a VF-5ms column (60m x 0.25mm x 0.25µm, 5m EZ-guard, Agilent)
134 with a 5% phenyl, 95% dimethylpolysiloxane stationary phase. The carrier gas was helium (1 ml
135 min⁻¹). The column oven program started at 50 °C (held for 2 min), increased to 160 °C at a rate of
136 8 °C min⁻¹, increased to 230 °C at a rate of 5 °C min⁻¹, and finally increased to 320 °C at a rate of 10
137 °C min⁻¹ (held for 10 min) giving a total run time of 46.75 min. The ion source was maintained at
138 300 °C and the quadrupole at 180 °C. The MS was scanning the 35-500 m/z range. Data acquisition
139 was performed using Agilent Masshunter software.

140 **3. Results**

141 **3.1 Poplar**

142 The product separation applied means that volatile compounds should either be soluble in the
143 extraction solvent ending up in the bio-crude or be evaporated when drying the SR. The SR was

thermally desorbed to evaluate the presence of volatile compounds showing that it contains many of the small organic compounds typically identified in the bio-crude and AqP from HTL of lignocellulosics including small organic acids, cyclopent-2-enones, and phenolics (Fig. 1, green). Small organic acids in the form of acetic acid, propionic acid, and lactic acid were detected from TD, which have been shown to be highly abundant compounds of the AqP.²³ The AqP was decanted in this experiment and some AqP may have been trapped in the bio-crude ending up in the SR after extraction and filtration. Several cyclopent-2-enones and phenolics (phenol, guaiacol, and syringol) that are abundant in the bio-crude from lignocellulosics²⁴ were detected from TD despite extensive washing with dichloromethane and subsequent drying. The presence of especially volatile cyclopent-2-enones could indicate that bio-crude is trapped inside the SR or they are strongly adsorbed to the surface of the SR. The ability to adsorb organic compounds from water has previously been demonstrated for SR from HTL of biomass.¹⁷ Additionally some indanones and chrom-2-ones were identified along with 1-monopalmitate, which have also been observed in the bio-crude.²⁵ The presence of trapped or adsorbed bio-crude in the SR means that potentially increased yields are possible for batch HTL with improved product separation. The majority of continuous HTL systems take advantage of in-line filtering²⁶ and bio-crude is less likely to end up in the SR, which apart from the solvent extraction could explain some of the difference in yields between batch and continuous HTL.²⁷

At 400 °C syringol and alkyl derivatives of phenol, guaiacol, catechol, and syringol dominate the chromatogram indicating that residual and repolymerized lignin forms this part of the SR (Fig. 1, pink). A previous study has shown that pyrolysates of unprocessed lignin are characterized by a higher degree of unsaturated side chains, whereas the liquid product and solid product are a combination of the compounds observed in this study even though markedly different product separations were employed. Generally, it is observed that the alkyl group of the phenolic derivatives

168 is placed in the *ortho* or *para* position. The importance of *ortho*-directed repolymerization from
169 HTL of lignin has previously been shown²⁸ while para-repolymerization may also occur when the
170 position is not substituted. The complexity of the biomass degradation means that many aldehydes
171 and ketones may be formed from protein, carbohydrates, and lignin. The phenolics may
172 repolymerize at alkaline conditions from nucleophilic attack of the phenolate ion at the *ortho* or
173 *para* position with aldehydes as shown in Fig. 2.

174 Another important observation from the pyrolysate at 400 °C is the absence of hydrocinnamic acid,
175 which facilitates the bonding between lignin and carbohydrates. Hence, residual lignin from
176 alkaline HTL may be better described as chemically modified lignin.²⁸ Even with thermochemolysis
177 it was not possible to detect hydrocinnamic acids (see section 3.3).

178 Increasing the temperature to 500 °C leads to a shift towards alkylated phenols while derivatives of
179 guaiacol, catechol, and syringol diminishes or disappear completely (Fig. 1, red). Hence, this part
180 likely displays the less sterical hindrance of phenols compared to guaiacols, catechols, and syringols
181 leading to more extensive polymerization especially when reacting with aldehydes. The more
182 thermally stable part of the SR may therefore derive from repolymerization of phenol derivatives
183 from lignin breakdown but also from recondensation of small organic acids.²⁴ Additionally this
184 fraction also showed the presence of several methylated naphthols in low abundance whose
185 formation requires further investigation.

186 Further increasing the temperature to 600 °C shows the presence of predominantly alkylated
187 benzenes, phenolics, and naphthalenes from repolymerized biomass (Fig. 1, purple). Both fractions
188 at 500 °C and 600 °C displayed several aromatic hydrocarbons, which were not considered as they
189 are typical secondary pyrolysis products and may not display the actual composition of the SR.

190 Based on these results it seems that the SR consists of four fractions; 1) trapped or strongly
191 adsorbed compounds from AqP and bio-crude 2) residual lignin and repolymerized phenols,

192 guaiacols, catechols, and syringols, 3) repolymerized phenols from carbohydrate and lignin
193 degradation, 4) repolymerized polyaromatic hydrocarbons and phenols. The majority of the total
194 peak area from the pyrolysates of fractions 2-4 was made up of only 12 different phenolic
195 compounds. This is in contrast to most studies of py-GC-MS of lignin where 30-80 compounds are
196 used to describe the pyrolysates.^{29, 30} High yields of value-added aromatics could potentially be
197 obtained through catalytic pyrolysis of the dry SR from HTL of lignocellulosics and further
198 investigations should be made regarding the potential revenue.

199 **3.2 *Spirulina***

200 Generally microalgae produce limited char in HTL due to the absence of lignin and the high protein
201 content. TD of the SR showed almost exclusively palmitic acid, methyl palmitate and palmitamide
202 (Fig. 3, green). Neither small organic acids, cyclic oxygenates, or phenolics were detected in this
203 fraction despite their abundance in especially the AqP.³¹ Hence, none of the compounds
204 hypothesized to be trapped or adsorbed to the SR from poplar were detected from *spirulina*. The
205 lack of volatiles is simply due to the fact that very little char was formed and the SR was mostly
206 made up of ash from *spirulina* without adsorption ability. Instead the high ash content means that
207 palmitic acid precipitates to some extent. The almost exclusive precipitation of palmitic acid is in
208 contrast to the fatty acid composition of the bio-crude where the concentration of palmitic acid has
209 previously been reported.²⁵ The bio-crude contained additional fatty acids such as palmitoleic acid,
210 oleic acid, and stearic acid in concentrations that were approximately 10-20% of palmitic acid. The
211 selective precipitation of palmitic acid during batch HTL of microalgae is unlikely to occur for
212 model compounds and during continuous HTL leading to slightly improved bio-crude yields.
213 Increasing the temperature to 400 °C predominantly showed the presence of hexadecanitrile and
214 increasing amounts of palmitamide while the most abundant compound remained palmitic acid
215 (Fig. 3, pink). At 400 °C palmitic acid is likely obtained from pyrolysis of 1-monopalmitate and 2-

monopalmitate, which have previously been observed from bio-crudes of microalgae²⁵ and was also observed in the bio-crude of this sample. Palmitamide may arise from two sources; formation during HTL or as a secondary pyrolysis product. In previous experiments using the same processing conditions we have shown that bio-crude from *C. vulgaris* contains limited fatty amides while bio-crude of *N. gaditana* contains numerous fatty amides. The biochemical content of *spirulina* is between *C. vulgaris* and *N. gaditana*. However, we did not observe formation of fatty amides in the bio-crude. Instead the high protein content of *spirulina* leads to formation of ammonium, which may precipitate with anions of the ash potentially reacting with palmitate during pyrolysis. Another notable difference from SR of poplar is the absence of residual lignin and repolymerized guaiacols, catechols, and syringols as lignin is not found in *spirulina*. Instead small amounts of phenol pyrolysates, which could stem from repolymerized phenols formed from carbohydrate degradation products²⁴ or potentially aromatic amino acids³² of residual protein, were detected along with a few alkylated pyrroles, benzofuran, and indole. At 500 °C the same phenol pyrolysates (phenol, *o*-methylphenol, and *p*-methylphenol) increased in abundance while a series of aliphatic hydrocarbons (C₁₀₋₁₅) appeared (marked by plus signs in Fig. 3, red). In previous work we have argued for the presence of alkylated phenols and catechols, and similar compounds have been found from pyrolysis of biomass.^{25, 33} Hence, these aliphatics may derive from predominantly long chain alkylated phenolics. The low abundance of repolymerized phenols at both 400 °C and 500 °C could indicate either that phenols from carbohydrate degradation products contribute very little to SR formation in general, although it has been shown for model compounds that carbohydrates produce up to 10 % SR from HTL.²⁴ Another possibility is that the ammonium produced limits either the formation of phenolics or the repolymerization (further explanation is proposed in section 3.3). In previous work it has been observed that the bio-crude did

not contain less phenol than expected³¹ meaning that it does not limit the formation of phenol, and ammonia may in fact be a way to minimize repolymerization of phenolics.

Increasing the temperature to 600 °C did not result in anymore pyrolysates. Instead thermochemolysis was carried out to further investigate the presence of fatty acids (Fig. 3, blue). Thermochemolysis leads to breakage of the labile ester bonds along with methylation of the resulting carboxylic acids while also turning free carboxylic acids into methyl esters. Additional fatty acids were detected compared to TD and they were similar to the ones previously observed in the bio-crude including myristic acid, palmitoleic acid, palmitelaidic acid, oleic acid, and stearic acid.³¹ Furthermore, the relative abundance of the fatty acids resembled the bio-crude. Even though the formation of methyl esters does improve chromatographic performance, the absence of these fatty acids in the TD analysis shows that only palmitic acid is present as a free fatty acid while the remaining fatty acids are likely present as diglycerides or triglycerides adsorbing to the precipitated palmitic acid.

Compared to SR from poplar it is noteworthy that SR from *spirulina* did not contain any small organic acids in either TD, pyrolysis, or thermochemolysis even though they are abundant in AqP from HTL of microalgae.³⁴ The absence further point towards selective precipitation of palmitic acid and adsorption of predominantly palmitate derivatives to the precipitated palmitic acid.

3.3 Co-liquefaction of poplar and *spirulina*

Mixing *spirulina* and poplar led to a SR that was markedly different from that of either feedstock. TD showed the presence of some small organic acids (acetic acid and propionic acid) at approximately the same abundances as for poplar, while palmitic acid was observed at approximately half the abundance as for *spirulina* (Fig. 4, green). Similar to SR from *spirulina*, palmitic acid was found to selectively precipitate as no other fatty acid was observed. Very few of the volatile compounds in SR from poplar were detected including cyclopent-2-enones and

263 guaiacol. Their absence is mostly explained from analysis of the bio-crude, which has shown that
264 significantly less of the amounts of expected cyclopent-2-enones and guaiacol are formed when
265 mixing poplar and *spirulina* due to Schiff base formation.³¹ At the same time the lowered char
266 formation also limits the adsorption. Instead multiple alkylated pyrroles and indoles were present in
267 the SR along with 3-hydroxypyridine. Pyrroles are typically present in the bio-crude from
268 feedstocks with similar biochemical composition to the mixture but are lost with solvent
269 evaporation.³⁵ This further indicates that bio-crude is either trapped in the SR or the volatile
270 compounds are strongly adsorbed to the surface. Previous experiments have shown that formation
271 of 3-hydroxypyridines and pyrazines is strongly favored in the bio-crude and AqP of this mixture.³¹
272 Yet pyrazines were not observed in the SR despite having approximately the same vapor pressures
273 as pyrroles. This shows that the SR of the mixture preferentially adsorbs pyrroles over pyrazines
274 and a selective displacement of nitrogenated compounds occurs where pyrazines almost exclusively
275 appear in AqP while pyrroles almost exclusively occur in SR when performing batch HTL when
276 mixing these biomasses. Hence, to fully study the reaction pathways with batch HTL it is required
277 to characterize at least the bio-crude, AqP, and SR.

278 Pyrolysis at 400 °C showed upwards of 20 different alkylated pyrroles along with predominantly
279 alkylated phenols and indoles (Fig. 4, pink). The pyrroles and indoles may be formed from
280 interaction of carbohydrate and protein degradation products as proposed in previous work where
281 the diversity of alkyl side chains is linked to the reaction between amino acids and carbonyls.³¹ The
282 repolymerization of pyrroles occurs rapidly under acidic conditions or in the presence of oxidants
283 and the diversity of these polymers is increased further by reactivity in either position of the carbon
284 ring depending on sterical hindrance.³⁶ Figure 5 shows the potential polymerization through radicals
285 and the diversification that is possible.

286 The absence of guaiacols, catechols, and syringols shows that repolymerized lignin is minimized
287 and the presence of *spirulina* may assist in this process. This effect is likely the result of several
288 factors where Schiff bases are formed with ammonia from amino acid deamination and ketones and
289 aldehydes from degradation of carbohydrates and lignin (Fig. 2). These interactions are complex
290 and require further investigation. Furthermore, palmitic acid was not observed in the pyrolysate
291 showing that co-liquefaction may aid in the degradation of glycerides.

292 Pyrolysis at 500 °C shows substantially fewer and less abundant pyrroles. However, the variety of
293 pyrroles and their presence from TD and in both pyrolysates shows that they react in no specific
294 pattern creating multiple different polymers of alkylated pyrroles. These polymeric pyrroles are
295 more likely to end up in the bio-crude during continuous HTL with in-line filtering increasing the
296 nitrogen content of the heavy oil with potential impact on upgrading that may not be encountered
297 from bio-crude of batch HTL. In contrast, pyrolysis at 500 °C also showed multiple and abundant
298 alkylated phenols (Fig. 4, red). The diversity of alkylated phenols was even greater than for SR
299 from poplar while guaiacols and catechols were still absent. This further supports our hypothesis
300 that residual lignin is minimized and repolymerization (mainly *ortho*- and *para* directed) of only
301 phenols from lignin and carbohydrate products is the main contributor of the char formation from
302 the mixture.

303 The chromatogram at 600 °C did not show any peaks. Instead thermochemolysis showed a similar
304 group of fatty acids as for SR of *spirulina* with approximately half of the abundance (Fig. 4, blue).
305 This shows that the precipitation of palmitic acid and adsorption of other fatty acids and glycerides
306 hereto is only dependent on the amount of ash from *spirulina*, even though analysis of the
307 concentration of palmitic acid in the bio-crude showed less than expected concentrations.³¹ A few
308 other small organic acids were observed, which included succinic acid and methylsuccinic acid that
309 are both abundant in the AqP from HTL of carbohydrates and protein.³⁷

4. Conclusion

Detailed characterization of SR from batch HTL of biomass suggests that both volatile and semi-volatile compounds of bio-crude and AqP are trapped or strongly adsorbed to the SR. Residual lignin and repolymerized guaiacol, catechols, and syringols constituted a fraction from poplar, absent from co-liquefaction with *spirulina*. Co-liquefaction leads to formation of multiple pyrroles and indoles, both volatile and non-volatile. The presence of pyrroles across all fractions shows the extensive and non-selective polymerization. Co-liquefaction also hindered the formation and subsequent repolymerization of guaiacols, catechols, and syringols through Schiff base formation. The most thermally resistant fraction consisted predominantly of repolymerized phenols in all samples, although SR from *spirulina* also showed aliphatic hydrocarbons, which may derive from phenolics with long aliphatic chains. Generally the repolymerization of phenolics was found to be predominantly *ortho*- and *para* directed.

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387 Table 1: Ash content and elemental distribution presented in percentage on dry and ash free basis
 388 and biochemical composition of feedstocks presented in percentage.

	Ash content	C	H	N	S	O ^a	Carbohydrate	Protein	Lipid	Lignin
Poplar	0.79	47.65	6.27	0.22	0.01	45.06	80.9 ^b	1.3	5.2	12.7
<i>Spirulina</i>	18.58	48.09	6.51	10.91	0.89	15.02	14.0	68.2	20.0	ND
<i>Mixture (1:1)</i>	9.69	47.87	6.39	5.57	0.45	30.04	47.5	34.8	12.6	6.4

389 ^{a-b} calculated by difference.

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Fig. 1: Total ion chromatogram of solid residue (poplar) with stepwise thermal desorption and py-GC-MS at 280 °C (green), 400 °C (pink), 500°C (red), and 600 °C (purple).

Fig. 2: Proposed *ortho* and *para*-directed repolymerization of phenolics with aldehyde in HTL of poplar and proposed change of reaction pathway when mixing poplar with *spirulina*.

Fig. 3: Total ion chromatogram of solid residue (spirulina) with stepwise thermal desorption and py-GC-MS at 280 °C (green), 400 °C (pink), and 500°C (red), and thermochemolysis at 500 °C (blue). The fatty acids detected from chemolysis were all methyl esters. + indicates alkylated aliphatics and * indicates pyrroles.

Fig. 4: Total ion chromatogram of solid residue (mixture) with stepwise thermal desorption and py-GC-MS at 280 °C (green), 400 °C (pink), and 500°C (red), and thermochemolysis at 500 °C (blue). The fatty acids detected from chemolysis were all methyl esters. * indicates pyrroles.

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428 Fig. 5: Proposed reaction for polymerization of pyrroles based on radical formation. The R group of the
 429 reacting carbon is required to be an H group.