Supporting Information for

Hydrothermal Liquefaction of Enzymatic Hydrolysis Lignin – Biomass Pretreatment Severity Affects Lignin Valorization

Mads M. Jensen[1], Demi T. Djajadi[2], Cristian Torri[3], Helene B. Rasmussen[1], René B. Madsen[1], Elisa Venturini[4], Ivano Vassura[4], Jacob Becker[1], Bo B. Iversen[1], Anne S. Meyer[2], Henning Jørgensen[2,5], Daniele Fabbri[3], Marianne Glasius[1]*

[1]Department of Chemistry and iNANO, Aarhus University, Langelandsgade 140, 8000 Aarhus C, Denmark
[2]Department of Chemical and Biochemical Engineering, Technical University of Denmark, Søltofts Plads Building 229, 2800 Kgs. Lyngby, Denmark
[3]Dipartimento di Chimica "Giacomo Ciamician", University of Bologna, Via Selmi 2, 40126 Bologna, Italy
[4]Department of Industrial Chemistry "Toso Montanari", University of Bologna, Viale Risorgimento 4, 40146 Bologna, Italy
[5]Present address: Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark
*corresponding author: glasius@chem.au.dk

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1. Materials and methods

Materials

The solvents diethyl ether (stabilized with 5-8 ppm 2,6-di-*tert*-butyl-4-methylphenol (BHT)), tetrahydrofuran (stabilized with 250 ppm BHT), ethyl acetate, dichloromethane, and dimethyl sulfoxide were of HPLC quality purchased from Sigma-Aldrich. KOH, LiBr, Na_2SO_4 and hydrochloric acid were of reagent grade or better. *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) was of GC derivatization quality (>98.5%) purchased from Sigma-Aldrich. *N,O*-Bis(trimethylsilyl)trifluoroacetamide/trimethylchlorosilane 99:1 (BSTFA/1%TMCS) used for on-fiber silylation was of GC derivatization quality purchased from Sigma-Aldrich. Compounds used as standards for quantitative analysis were purchased from Sigma-Aldrich, Merck or Alfa Aesar in >97% purity and used as received.

Enzymatic hydrolysis residues

The composition of the EnzHR is shown in Table S1. Composition analysis were performed using strong acid hydrolysis procedure according to National Renewable Energy Laboratory (NREL).[1] The enzymatic hydrolysis residues will resemble the residual biomass produced as a side-stream of second generation bioethanol production.

Table S1. Composition of protease treated enzymatic hydrolysis residues used for hydrothermal liquefaction.

Samples[1]	(% w/w DM)							
	Arabinan	Galactan	Glucan	Xylan	Mannan[2]	AIL[3]	ASL[4]	Ash
M3.65	0.51 ± 0.04	0.27 ± 0.04	31.76 ± 0.60	6.43 ± 0.21	ND	50.99 ± 0.99	0.63 ± 0.01	2.64 ± 0.03
M3.97	0.24 ± 0.01	0.22 ± 0.03	11.01 ± 0.21	1.85 ± 0.02	ND	76.30 ± 0.53	0.54 ± 0.04	4.56 ± 0.17
W3.65	0.51 ± 0.01	0.21 ± 0.03	13.14 ± 0.80	4.25 ± 0.15	ND	70.27 ± 0.52	0.59 ± 0.01	4.21 ± 0.02
W3.97	0.15 ± 0.02	0.16 ± 0.01	3.64 ± 0.11	1.19 ± 0.27	ND	83.14 ± 0.25	0.56 ± 0.01	5.18 ± 0.21

[1]M: *Miscanthus* × *giganteus* stalks; W: wheat straw. The numbers denote severity factor used during biomass pretreatment: 3.65 - 190 °C 10 min; 3.97 - 195 °C 15 min.

[2]ND: not determined

[3]AIL: acid insoluble (Klason) lignin

[4]ASL: acid soluble lignin

Hydrothermal liquefaction of lignin

The number of experiments was aimed duplicate HT for each of part I and II. However, a few experiments had to be excluded as outliers due to post-HTL sources of errors. Analytical results that showed outlying values without identifiable sources of errors have been retained to show the uncertainty of the entire procedure.

EnzHR containing 1 g dry matter (DM) was added to an aqueous solution of KOH giving a slurry of 10 wt% DM EnzHR and 5.6 wt% KOH (~1 M). The slurry was homogenized by stirring and transferred to a 20 mL batch reactor made from stainless steel (SAE316) Swagelok parts. The slurries were prepared fresh moments before each experiment. The reactor was heated to the target temperature in an Omega Engineering FSB-4 fluidized sand bath. The reported reaction temperatures are the rounded off maximum temperatures measured using a thermocouple inserted in the reactor. The reaction time was 20 min after heating up for 4 min. The temperature for the start of reaction was defined as $T_{Rstart} = T_I + 0.92(T_{max} - T_I)$, where T_I is the initial temperature of the slurry (ca. 25 °C) and T_{max} is maximum reaction temperature. The reactor content was cooled to below 50 °C in less than 2 min by submersion in a water bath. The reactor was weighed before and after pressure release. However, this gas yield was low (<4 wt%) and just above or within the uncertainty of the balance (readability 0.01 g).

Product fractionation

The liquid and solid products were separated using the procedure outlined in Figure S1. The product mixture was poured out of the reactor, which was then washed three times with 5 mL deionized water,

and the mixture was acidified with 4 M HCl to pH 1.9–2.0 under stirring. Gas evolved from the mixture during acidification. The gas could be CO_2 produced during HTL that has reacted with KOH to yield K_2CO_3, then released as CO_2 during acidification. Acidification resulted in precipitation, and the solids were separated from the water phase (WP) by suction filtration through a cellulose filter (Fisherbrand QL100) on top of a sintered glass filter (por. 2). The reactor was washed 3–5 times with 5 mL diethyl ether (Et_2O) until the solution was colorless. The Et_2O washes were filtered through the solids on the filter and the solids were further washed about 20 times with 5 mL Et_2O until the filtrate appeared colorless. The resulting Et_2O solution was combined with the WP. The WP was liquid-liquid extracted 3 times with Et_2O (~1:1 ratio). The Et_2O extracts were combined and dried over Na_2SO_4 for at least 20 min before suction filtration through a cellulose filter on top of a sintered glass filter. The Na_2SO_4 drying salt was washed about 15 times with 5 mL Et_2O until the filtrate appeared colorless. The liquid product (LP) was obtained as a dark orange/brown viscous oil after concentration (850 mbar, 40 °C), evaporation of the Et_2O at reduced pressure (200 mbar, 40 °C, 5 min) in a rotary evaporator and being left at 22 °C overnight. The chemically modified lignin (CML) fraction was obtained using tetrahydrofuran (THF). The reactor was washed 3–5 times with 5 mL THF until the solution was colorless. The THF washes were filtered through the solids on the filter and the solids were further washed about 8 times with 5 mL THF until the filtrate appeared colorless. The THF dissolved most of the solids. The THF solution resulted in the CML fraction as a dark brown/black brittle solid after concentration (350 mbar, 40 °C), evaporation of the THF under reduced pressure (60 mbar, 40 °C, 5 min) and being left at 22 °C overnight. The solid residue (SR) remaining on the filters was oven dried at 105 °C overnight. SR caught in the sintered glass filter was determined to correspond to an additional 0.7 wt% yield (on dry matter basis) in the W3.97-300-2 experiment. The LP and CML were stored refrigerated and the SR at room temperature when not in use.

The yield of the CML fraction was corrected for the BHT introduced into this fraction by the use of BHT-stabilized THF as described elsewhere in the section.

Control experiments using M3.65 and W3.65 EnzHR were conducted in order to obtain reference points for the effect of the slurry preparation and fractionation procedure on the biomass. EnzHR containing 0.5 g dry matter (DM) was added to an aqueous solution of KOH giving a slurry of 10 wt% DM EnzHR and 5.6 wt% KOH (~1 M). The slurry was then stirred and transferred to a 20 mL stainless steel batch reactor where it was kept at room temperature for 24 min before going through the same fractionation procedure as the HTL products.

Size exclusion chromatography

The molar mass distribution of the samples was determined using size exclusion chromatography, SEC. The analysis was performed on a Dionex Ultimate 3000 HPLC with UV detection at 280 nm. The compounds were separated on a Polymer Standards Service PolarSil column (300 x 8 mm, 5 µm, 100 Å, 100–100.000 Da separation range) at 70 °C according to their hydrodynamic radius in a 9:1 (v/v) dimethyl sulfoxide/water eluent with 0.05 M LiBr. The eluent flow rate was 1 mL min^{-1}. The molar mass scale was calibrated using the retention times of 5 phenolic compounds in the range 152–1701 g/mol and extrapolated using a linear regression curve of time (min) versus log(Mw). This

calibration was chosen because of the lack of commercially available standards that are both UV detectable and soluble in the eluent. The calibration compounds were vanillin, acetosyringone, guaiacylglycerol-β-guaiacyl ether (GGE), polydatin, and tannic acid. The LP, CML, and SR samples were dissolved in the eluent at concentrations of about 1, 3, and 3 g L^{-1}, respectively. The samples were left overnight on a nutating mixer. Samples were then subjected to mild sonication for several minutes in an ultrasound bath. The samples were filtered through a 0.22 µm PTFE syringe filter (Q-Max) before analysis. Prior to filtration the CML samples and the wheat straw LP samples generally contained few undissolved particles, while the SR samples showed very poor solubility and the SR results are thus not reported. The UV response from each sample was treated as a single peak, expect for CML samples where the peak corresponding to BHT was excluded from calculations. The number average (M_n) and mass average molar mass (M_w) were calculated using the GPC template supplied with the Chromeleon 6.80 Extension Pack V2 as:

$$M_n = \frac{\sum_i n_i M_i}{\sum_i n_i} = \frac{\sum_i A_i}{\sum_i A_i / M_i} \quad (1)$$

$$M_w = \frac{\sum_i n_i M_i^2}{\sum_i n_i M_i} = \frac{\sum_i A_i M_i}{\sum_i A_i} \quad (2)$$

where n_i is the number of molecules with a certain molecular mass, M_i, and A_i is the area of the i'th retention time slice of the peak. The polydispersity index (PDI) was calculated as M_w/M_n.

Quantification of BHT in CMLs

Diethyl ether was distilled and stabilized with 2% ethanol before use. The sample of the CML was ground to a fine powder in an agate mortar. CML (10 mg) was transferred to a 2 mL vial and 1 mL of 95/5% (v/v) distilled diethyl ether/ethyl acetate containing 4-bromotoluene as internal standard was added. The samples were placed in an ultrasonic bath for 15 min followed by 1 hour of settling. Then, 250 µL of the supernatant was diluted to 5 mL using ethyl acetate.

Matrix-matched calibration standards were produced using a CML extracted using inhibitor-free THF from the product after HTL at 300 °C of Protobind 1000 lignin.[2] Aliquots of the Protobind 1000 CML (10 mg) were spiked with 75 µL of dichloromethane containing 0–40 g/L BHT (6 calibration levels in duplicate). The calibration samples were left at room temperature until the dichloromethane was evaporated before being subjected to the same sample preparation as the EnzHR CML samples.

GC-MS analysis of the diluted supernatants from unknowns and calibration samples was performed on an Agilent system (7890B GC, 5977A MSD) equipped with an Agilent VF-5ms (60 m, 0.25 mm, 0.25 µm) column with 5 m integrated guard column. Injection volume was 1.0 µL and the GC inlet was held at 280 °C with a 1:20 split ratio. Helium was used as carrier gas with a column flow of 1 mL/min. The GC oven program was 60 °C (2 min), to 255 °C (10 °C min^{-1}), and finally to 320 °C (40 °C min^{-1}, 5 min). The MS temperatures were 300 °C, 300 °C, and 180 °C for transfer line, ion source, and quadrupole, respectively. The MS was scanning the 35-500 *m/z* range. Quantitative data analysis was performed with Agilent Masshunter Quantitative Analysis (ver. B.07) using extracted ion chromatograms of BHT after signal correction using the internal standard.

Quantification by gas chromatography – mass spectrometry

The GC-MS analyses were performed using an Agilent system (7890B GC, 5977A MSD) equipped with an Agilent VF-5ms (60 m, 0.25 mm, 0.25 µm) column with 5 m integrated guard column. Twenty-three carboxylic acid, phenolic and methoxybenzene compounds (propanoic acid, anisole, phenol, 4-ethylphenol, o-cresol, p-cresol, creosol, guaiacol, 4-ethylguaiacol, catechol, 3-methoxycatechol, 3-methylcatechol, 4-methylcatechol, 4-ethylcatechol, 1,2-dimethoxybenzene, 1,2,3-trimethoxybenzene, syringol, vanillin, vanillic acid, syringaldehyde, acetovanillone, acetosyringone, and hexadecanoic acid) were quantified using calibration standards with 5–7 levels (linear or quadratic $R^2 > 0.999$) spanning a concentration range with up to a factor of 125 within the linear or dynamic range of the instrument. The detection limit (DL) and quantification limit (QL) were defined as 3*s*/*m* and 10*s*/*m*, respectively, where *m* is the slope of the calibration curve in the linear range and *s* is the standard deviation of seven replicate analysis of the lowest calibration level. The LP was dissolved in ethyl acetate at a concentration of about 4 g L^{-1}. 4-Bromotoluene and 4-bromophenol were added as internal standards. The solution was then filtered through a 0.22 µm Q-Max PTFE syringe filter. Aliquots of the standards and LP solutions were silylated by adding 80 µL MSTFA and 2 µL pyridine to 918 µL solution. Silylation was quantitative for the above compounds after one hour on a nutating mixer at room temperature. Injection volume was 1.0 µL and the GC inlet was held at 280 °C with a 1:20 split ratio. Helium was used as carrier gas with a column flow of 1 mL min^{-1}. The GC oven program was 40 °C (5 min), to 100 °C (10 °C min^{-1}), then to 280 °C (4 ˚C min^{-1}), and finally to 320 °C (10 °C min^{-1}, 10 min) giving a total run time of 70 min. The MS temperatures were 300 °C, 300 °C, and 180 °C for transfer line, ion source, and quadrupole, respectively. The MS was running in EI mode at 70 eV and scanning the 35-500 *m/z* range (3.1 scans s^{-1}). Quantitative data analysis was performed with Agilent Masshunter Quantitative Analysis (ver. B.07) using extracted ion chromatograms of the calibrated compounds after signal correction using the internal standards. Additional 105 compounds were semi-quantified using the calibration curve of a chromatographically nearby or structurally similar compound (propanoic acid, anisole 4-ethylguaiacol, 4-ethylcatechol, vanillic acid, or acetosyringone). The signal detection limit of 4-ethylguaiacol was used as a lower area threshold for the semi-quantified compounds. The semi-quantification was performed using deconvoluted total ion chromatogram (TIC) peak areas obtained using the PARAFAC2 based Deconvolution and Identification System (PARADISe) ver. 1.1.1.[3] The deconvolution was performed using the default modelling options and the deconvoluted mass spectra were identified using the NIST11 library and a personal library of phenolic compounds. Results from this semi-quantification procedure are obtained fairly easily and have increased reproducibility compared to using relative peak areas or using a single internal standard. However, the semi-quantitative results are still subject to an unknown error on accuracy.

Principal component analysis

Principal component analysis (PCA) was used for exploring the covariance of the multiple variables in the GC-MS results of Part I. The deconvoluted TIC peak areas from PARADISe without a lower area threshold were used as input. The peak areas were normalized with respect to the signal of the

internal standard and sample concentration to correct for instrumental and sample preparation variations. Data preprocessing and PCA were performed using PLS_toolbox 8.2 in MatLab R2017a. Data for the PCA model were mean centered and Pareto scaled. Pareto scaling, compared to autoscaling, partly retain the relative importance of large peaks while being less sensitive to small peaks with larger relative standard deviation, such as noisy peaks.[4] Pareto scaling appeared to give the best compromise between normalizing variance across variables and minimizing noise for this dataset.

Thermogravimetric analysis

The weight loss from volatilization and pyrolysis of the LP, CML and SR was investigated using thermogravimetric analysis (TGA) on a Mettler Toledo TGA/SDTA 851e. RL and SR samples were ground to a fine powder in an agate mortar prior to analysis. Five to eight mg of sample was placed in a 70 µL alumina crucible with pinhole lid. The TGA program was 25–700 °C (10 °C min^{-1}) under a nitrogen flow of 90 mL min^{-1}. Representative samples were subjected to more detailed TGA using a multistep pyrolysis and combustion program to evaluate extended pyrolysis and quantify carbon black and ash content. The multistep program was 25–900 °C (10 °C min^{-1}) under a nitrogen flow of 90 mL min^{-1} follow by cooling to 600 °C (-40 °C min^{-1}) prior to switching to air and heating to 950 °C (15 °C min^{-1}). Weight loss up to 120 °C was interpreted as moisture and residual solvent.

Stepwise thermal desorption and pyrolysis of Part I samples

Samples of LP, CML, and SR were subjected to stepwise thermal desorption and pyrolysis on a CDS Pyroprobe 1000 prior to GC-MS analysis (TD-Py-GC-MS). Samples were prepared by transferring <0.1 mg to pyrolysis tube. LP was applied as a thin layer and narrow band to a quartz tube using a glass pipette. RL and SR samples were ground to a fine powder in an agate mortar and placed on a quartz wool plug in a quartz tube. Thermal desorption and pyrolysis with SPME sampling was performed using a procedure modified from a previous study.[5] Briefly, samples were heated in steps to 200, 280, 350, 420 and 500 °C (calibrated using a thermocouple) at a heating rate of 0.01 °C ms^{-1} under a nitrogen flow of 100 mL min^{-1} (20 mL min^{-1} for CML and SR at 350, 420 and 500 °C due to low response). The temperature of each step was held for 5 min. Pyrolysates were sampled using a carboxen/polydimethylsiloxane SPME fiber (Supelco) and the pyrolysates were subjected to on-fiber derivatization using a procedure modified from a previous study.[6] The SPME fiber was exposed for 15 min in the headspace of a 2 mL vial, closed with a PTFE/silicone septum, containing 50 µL BSTFA/1% TMCS and 5 µL pyridine. The SPME fiber was desorbed in the GC-MS (Agilent 6851 GC, 5668 MSD) inlet at 300 °C in splitless mode. The desorbed compounds were separated on an Agilent HP-5ms (30 m, 0.25 mm, 0.25 µm) column using helium as carrier gas at a flow of 1 mL min^{-1}. The oven program of the GC-MS was 50 °C (5 min) to 325 °C (10 ˚C min^{-1}, 10 min) giving a run time of 42.5 min. The MS temperatures were 280 °C, 230 °C, and 150 °C for transfer line, ion source, and quadrupole, respectively. The MS was running in EI mode at 70 eV and scanning the 8–650 *m*/*z* range.

Single-step pyrolysis of Part II samples

Single-step Py-GC-MS was performed using a Agilent GC-MS system (7890B GC, 5977A MSD) equipped with a Gerstel automated pyrolysis setup as described elsewhere.[2] Samples of CML for pyrolysis were prepared by transferring 70–150 µg to a pyrolysis tube. Flash pyrolysis was performed under a helium flow of 50 mL min^{-1} at 500 °C (calibrated as sample-received temperature). Peak areas were obtained by deconvolution of the TIC using PARADISe as described in "Quantification by gas chromatography – mass spectrometry". Peak areas were normalized to sample mass (corrected for BHT content). Peak identification was performed using authentic standards, published mass spectra or the NIST11 libraries.[7] Peaks that could not be identified using standards or published mass spectra were only included if they had a match factor above 800 in NIST MS Search 2.0. A solution of a homologous series of straight-chained alkanes was analyzed to provide Kovats retention index (RI) in the C_7-C_{34} range. Aliphatic compounds and structural isomers of aromatic compounds were identified using the NIST RI library where possible. The RI for each identified compound is reported in Table S11. The RIs can be used for future lignin HTL studies to correct the erroneous identifications that occur if relying purely on the NIST11 mass spectral library.

2. Correcting CML yields for BHT content

The use of tetrahydrofuran (THF) stabilized with 250 ppm 2,6-di-*tert*-butyl-4-methylphenol (BHT) for fractionation of the products from hydrothermal liquefaction (HTL) of the enzymatic hydrolysis residues (EnzHR) resulted in that the chemically modified lignin (CML) fraction contained a large and varying mass fraction of BHT. Consequently, the BHT content of the CML was quantified and subtracted from the measured mass of CML. Matrix-matched calibration standards were used since a matrix effect was observed in an initial spike-recovery test; the calibration slopes, when treating the spiked samples as one-point standard addition, were similar for CML from HTL of *Miscanthus* EnzHR, wheat straw EnzHR and Protobind 1000 lignin, yet they were distinctly lower than the slope of the pure-solvent calibration standards. Consequently, matrix-matched calibration using the Protobind 1000 CML was determined to give more accurate calibration than pure-solvent standards. The latter would have resulted in an underestimation of the BHT content.
The results of the quantification of BHT in the EnzHR CML are shown in Table S2. The results were used to correct the yield of the CML.

Table S2. BHT content in the EnzHR CML (% w/w).

	W3.65_20_1	W3.65_20_2	M3.65_20_1	M3.65_255_1	W3.65_255_1	M3.65_300_1	M3.65_300_2	M3.65_300_3	W3.65_300_1	W3.65_300_2
BHT	4.4	4.1	4.1	9.3	6.6	9.4	9.8	13.0	7.1	7.5
	W3.65_300_3	M3.65_345_1	M3.65_345_2	W3.65_345_1	W3.65_345_2	M3.97_300_1	M3.97_300_2	W3.97_300_1	W3.97_300_2	
BHT	7.6	13.1	13.9	9.4	8.7	6.6	5.9	5.7	6.2	

3. Supplementary figures for Fraction yields

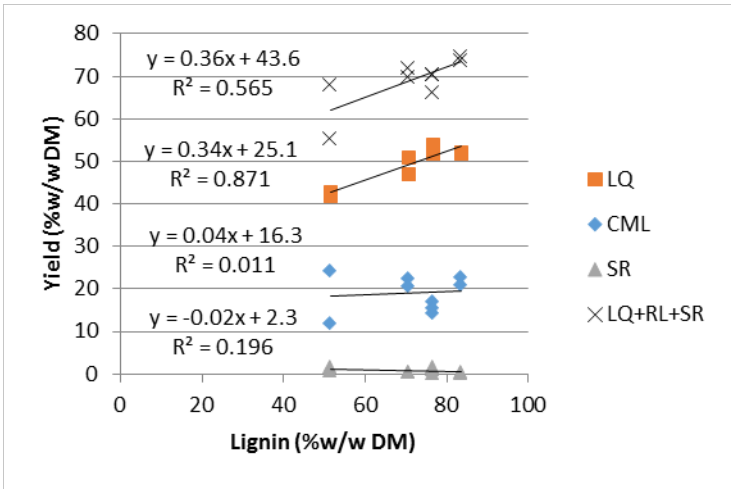


Figure S1. Correlation between HTL fraction yield at 300 °C and lignin content of feedstock EnzHRs. Linear least squares regressions on LP yield, CML yield, SR yield, and the sum of the three fraction yields as functions of the lignin content (acid insoluble lignin (AIL) and acid soluble lignin (ASL)) in the feedstock EnzHRs.

4. Supplementary figures for Part I – HTL temperature study of EnzHR conversion

Table S3. The number average (M_n), mass average (M_w) molar mass (g/mol) and polydispersity index (PDI) ± standard deviation of the aromatic compounds (UV 280 nm) in the product fractions from the HTL and control experiments.

	M_n		M_w		PDI	
	LP	CML	LP	CML	LP	CML
M3.65-20°C	96	2943	167	30732	1.75	10.44
M3.65-255°C	286	1214	611	3645	2.14	3.00
M3.65-300°C	281 ± 13	1228 ± 74	649 ± 9	6677 ± 1352	2.31 ± 0.14	5.48 ± 1.43
M3.65-345°C	283 ± 5	1287 ± 23	672 ± 43	7408 ± 986	2.37 ± 0.11	5.75 ± 0.67
M3.97-300°C	311 ± 5	1558 ± 22	746 ± 28	6607 ± 21	2.39 ± 0.05	4.24 ± 0.05
W3.65-20°C	108	2917	207	36892	1.91	12.65
W3.65-255°C	275	1513	578	6892	2.10	4.56
W3.65-300°C	281 ± 8	1488 ± 98	681 ± 60	9086 ± 3658	2.42 ± 0.19	6.03 ± 2.04
W3.65-345°C	273 ± 3	1205 ± 4	620 ± 12	7057 ± 247	2.27 ± 0.02	5.86 ± 0.23
W3.97-300°C	290 ± 1	1585 ± 122	647 ± 26	7923 ± 1610	2.23 ± 0.08	4.98 ± 0.63

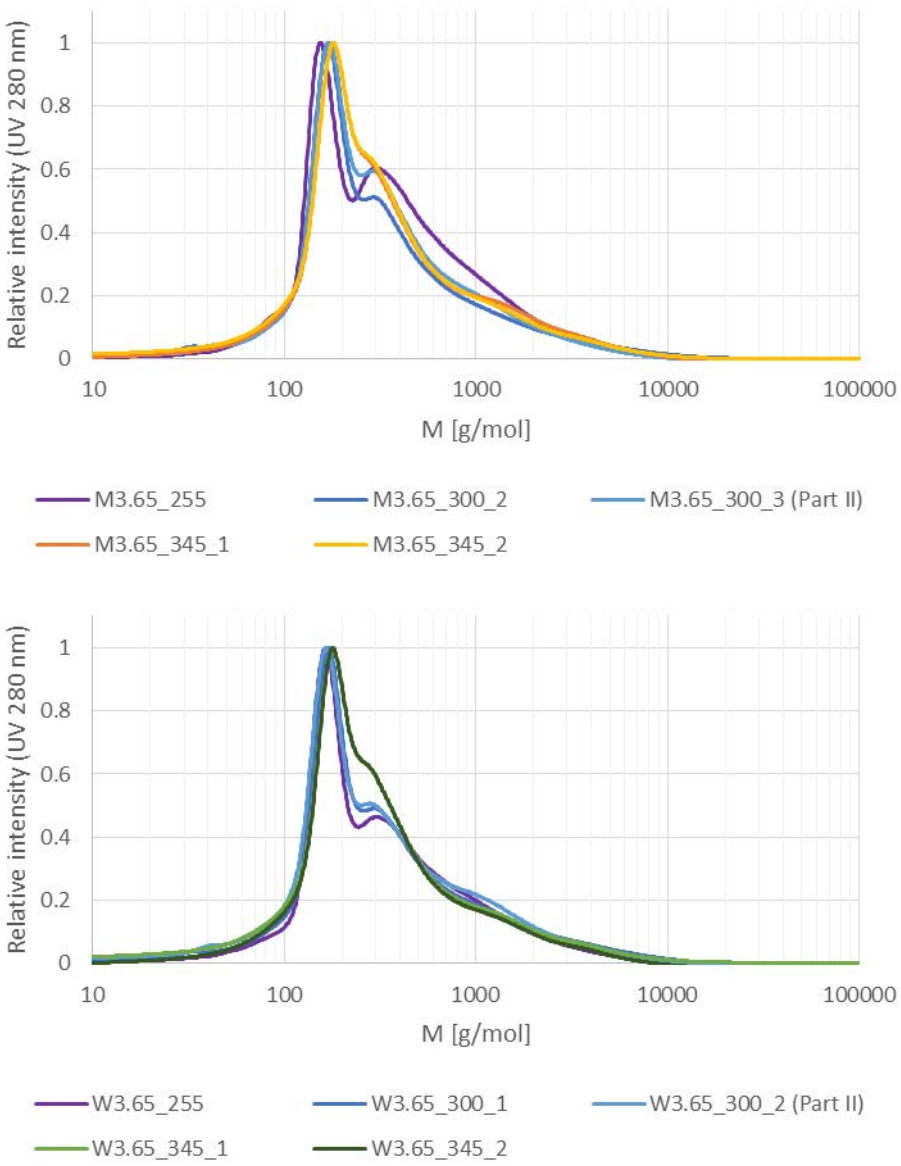


Figure S2. SEC molar mass distributions of UV 280 nm detectable compounds in the LPs from HTL of wheat straw and *Miscanthus* EnzHR. Note how the relative abundance of dimers of M3.65-300 was different for two replicates produced and analyzed 8 months apart. This shows that either the product distribution is sensitive to small variations of HTL parameters (temperature or heating rate) or it reflects the uncertainty associated with product fractionation and sample preparation. Despite this, it appears at least in the case of the wheat straw molar mass distributions that the dimer peak at about 300 g/mol has increased at 345 °C.

Table S4. Yield ± standard deviation (% w/w dry matter of EnzHR) of compounds in the HTL temperature study. <DL denotes a concentration below the instrument detection limit, <QL denotes a concentration below the instrument quantification limit, and <Cal denotes a concentration above the instrument quantification limit, though below the lowest calibration level. Compounds marked with asterisk (*) were estimated using a calibration curve of a similar compound. The number of compounds (*n*) is given for groups of compounds.

		Yield (% w/w DM EnzHR)					
	n	M3.65-255°C	M3.65-300°C	M3.65-345°C	W3.65-255°C	W3.65-300°C	W3.65-345°C
Aliphatic carbonyls							
Propanoic acid		0.06	0.13 ± 0.01	0.33 ± 0.01	0.12	0.16 ± 0.01	0.32 ± 0.01
Other aliphatic acids*	11	0.62	0.72 ± 0.07	1.02 ± 0.02	0.85	0.98 ± 0.14	0.96 ± 0.02
Hydroxy acids*	17	2.60	2.91 ± 0.23	2.45 ± 0.23	1.52	2.08 ± 0.19	1.53 ± 0.15
Keto acids*	11	0.12	0.10 ± 0.00	0.09 ± 0.01	0.03	0.08 ± 0.02	0.10 ± 0.01
Diacids*	8	0.23	0.32 ± 0.02	0.69 ± 0.09	0.29	0.43 ± 0.06	0.59 ± 0.09
Hexadecanoic acid		0.45	0.46 ± 0.03	0.50 ± 0.00	1.00	1.04 ± 0.06	0.97 ± 0.03
C14, C18, C22 fatty acids*	3	0.02	0.03 ± 0.00	0.03 ± 0.00	0.26	0.27 ± 0.02	0.25 ± 0.05
Aliphatic ketones*	5	0.15	0.03 ± 0.00	0.05 ± 0.00	0.22	0.13 ± 0.06	0.21 ± 0.03
Aromatics							
Hydroxynapthalenes*	2	0.01	0.13 ± 0.03	0.08 ± 0.01	0.05	0.18 ± 0.01	0.09 ± 0.02
1,2,3-Benzenetriol*		-	0.02 ± 0.02	-	-	0.07 ± 0.02	-
Unknown catechol/benzenetriol*	2	0.02	0.09 ± 0.01	-	0.21	0.64 ± 0.07	0.01 ± 0.00
Methoxybenzenes							
Anisole		<QL	0.07 ± 0.01	0.02 ± 0.00	<QL	0.03 ± 0.01	0.02 ± 0.00
1,2-Dimethoxybenzene		0.03	0.09 ± 0.02	<QL	0.05	0.09 ± 0.01	<Cal
1,2,3-Trimethoxybenzene		0.02	0.01 ± 0.00	<DL	0.04	0.01 ± 0.00	<DL
Other methoxybenzenes*	2	0.02	0.15 ± 0.02	0.07 ± 0.01	0.01	0.04 ± 0.01	0.03 ± 0.01
Phenols							
Phenol		0.63	0.83 ± 0.06	0.81 ± 0.00	0.33	0.46 ± 0.05	0.59 ± 0.02
o-Cresol		0.01	0.04 ± 0.00	0.15 ± 0.00	0.01	0.03 ± 0.00	0.13 ± 0.00
p-Cresol		0.01	0.06 ± 0.01	0.22 ± 0.00	0.00	0.03 ± 0.00	0.15 ± 0.00
4-Ethylphenol		0.06	0.11 ± 0.01	0.15 ± 0.01	0.02	0.04 ± 0.00	0.07 ± 0.00
Guaiacol		1.25	0.69 ± 0.05	0.03 ± 0.00	1.83	0.98 ± 0.04	<Cal
4-Methylguaiacol		0.04	0.13 ± 0.01	<QL	0.06	0.15 ± 0.01	<QL
4-Ethylguaiacol		0.11	0.12 ± 0.01	0.01 ± 0.00	0.12	0.11 ± 0.01	0.01 ± 0.00
Syringol		0.92	0.06 ± 0.00	<DL	1.49	0.12 ± 0.02	<DL
Other phenols*	18	0.16	0.22 ± 0.01	0.28 ± 0.01	0.20	0.24 ± 0.02	0.27 ± 0.01
Catechols							
Catechol		0.13	1.68 ± 0.03	0.92 ± 0.06	0.15	2.47 ± 0.17	1.58 ± 0.07
4-Methylcatechol		<QL	0.77 ± 0.01	1.15 ± 0.05	<QL	1.00 ± 0.07	1.59 ± 0.07
3-Methylcatechol		0.02	0.13 ± 0.00	0.37 ± 0.01	0.01	0.17 ± 0.02	0.55 ± 0.02
4-Ethylcatechol		<QL	0.52 ± 0.03	0.54 ± 0.01	<QL	0.54 ± 0.04	0.58 ± 0.01
3-Methoxycatechol		0.12	0.24 ± 0.02	<DL	0.22	0.47 ± 0.04	<DL
Other catechols*	13	0.07	0.50 ± 0.03	1.98 ± 0.03	0.07	0.53 ± 0.09	2.22 ± 0.17

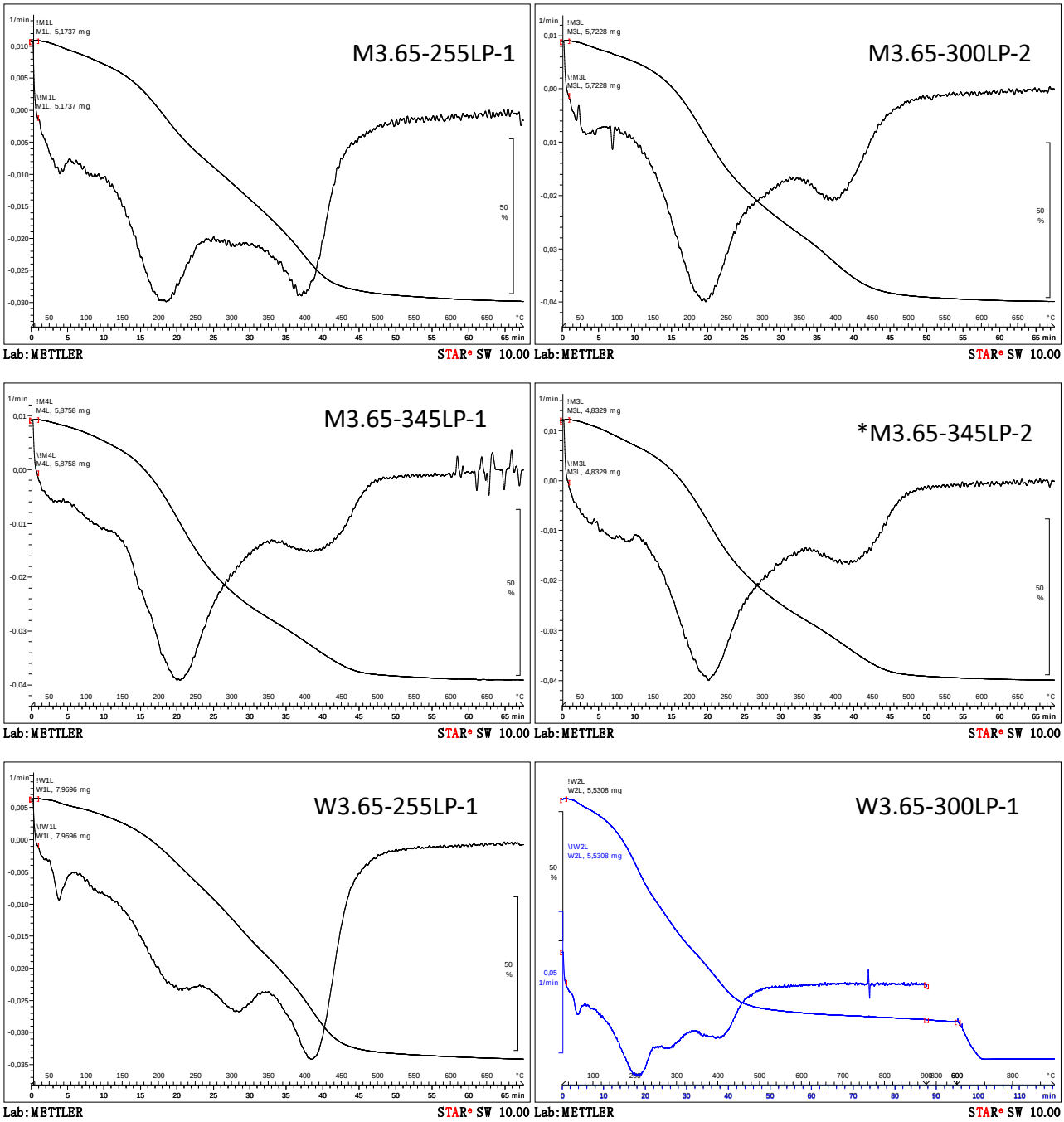
Phenolic aryl carbonyls							
4-Hydroxybenzaldehyde*		-	0.04 ± 0.00	0.01 ± 0.00	-	0.03 ± 0.01	0.01 ± 0.00
Vanillin		0.30	0.13 ± 0.00	0.02 ± 0.00	0.37	0.18 ± 0.02	0.02 ± 0.00
Syringaldehyde		0.23	0.06 ± 0.00	<DL	0.30	0.09 ± 0.01	<DL
Acetovanillone		0.26	0.19 ± 0.00	0.02 ± 0.00	0.34	0.28 ± 0.02	0.02 ± 0.00
Acetosyringone		0.43	0.20 ± 0.00	<DL	2.36	0.97 ± 0.08	<DL
Vanillic acid		<Cal	<Cal	<DL	<Cal	<Cal	<DL
Vanillactic acid*		0.12	-	-	0.18	-	-
Other aromatic acids*	10	0.07	0.18 ± 0.03	0.10 ± 0.00	0.12	0.24 ± 0.03	0.10 ± 0.02
Compound group totals							
Small aliphatic acids	31	1.02	1.27 ± 0.06	2.12 ± 0.09	1.30	1.65 ± 0.20	1.98 ± 0.14
Hydroxy acids	17	2.60	2.91 ± 0.23	2.45 ± 0.23	1.52	2.08 ± 0.19	1.53 ± 0.15
Fatty acids	4	0.47	0.48 ± 0.04	0.53 ± 0.00	1.26	1.31 ± 0.06	1.22 ± 0.07
Aliphatic ketones	5	0.15	0.03 ± 0.00	0.05 ± 0.00	0.22	0.13 ± 0.06	0.21 ± 0.03
Methoxybenzenes	5	0.07	0.31 ± 0.04	0.09 ± 0.02	0.10	0.17 ± 0.02	0.05 ± 0.01
Phenols	26	3.20	2.27 ± 0.15	1.67 ± 0.02	4.06	2.16 ± 0.08	1.22 ± 0.04
Catechols	18	0.34	3.83 ± 0.12	4.95 ± 0.10	0.45	5.18 ± 0.32	6.52 ± 0.01
Phenolic aldehydes	3	0.53	0.23 ± 0.01	0.03 ± 0.00	0.67	0.29 ± 0.03	0.03 ± 0.00
Phenolic ketones	2	0.69	0.39 ± 0.00	0.02 ± 0.00	2.71	1.25 ± 0.09	0.02 ± 0.00
Aromatic acids	12	0.19	0.18 ± 0.03	0.10 ± 0.00	0.30	0.24 ± 0.03	0.10 ± 0.02
Other aromatics	5	0.03	0.24 ± 0.04	0.09 ± 0.01	0.25	0.90 ± 0.04	0.10 ± 0.02
Total monomer yield		9.3	12.2 ± 0.5	12.1 ± 0.3	12.8	15.4 ± 0.8	13.0 ± 0.5

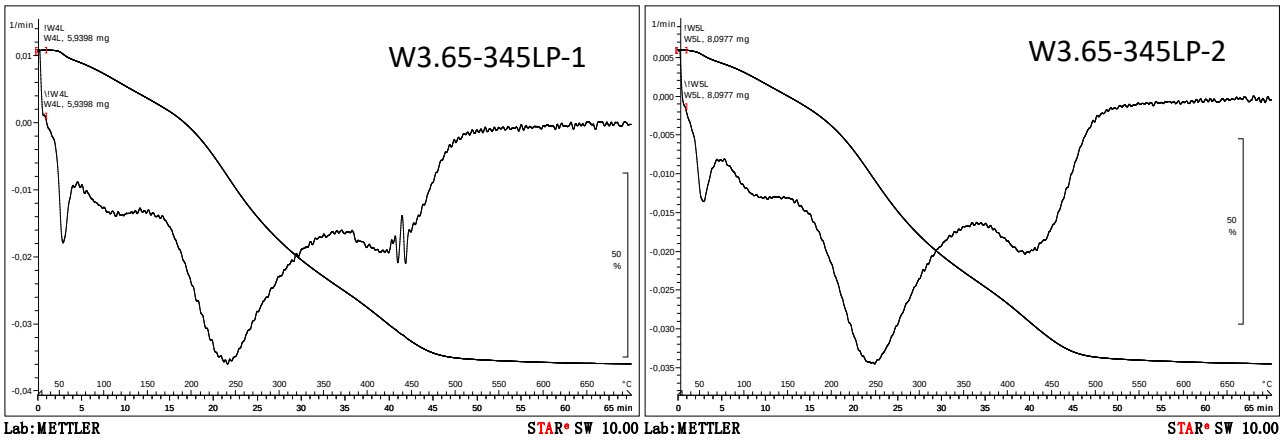
Table S5. Yield ± standard deviation (% w/w lignin (acid insoluble lignin (AIL) + acid soluble lignin (ASL)) of EnzHR) of aromatic compounds in the HTL temperature study.

	Yield (% w/w lignin (AIL+ASL))						
	n	M3.65-255°C	M3.65-300°C	M3.65-345°C	W3.65-255°C	W3.65-300°C	W3.65-345°C
Aromatics							
Hydroxynapthalenes*	2	0.03	0.26 ± 0.06	0.16 ± 0.01	0.07	0.26 ± 0.01	0.13 ± 0.02
1,2,3-Benzenetriol*		-	0.04 ± 0.03	-	-	0.10 ± 0.03	-
Unknown catechol/benzenetriol*	2	0.04	0.16 ± 0.02	-	0.29	0.91 ± 0.09	0.01 ± 0.00
Methoxybenzenes							
Anisole		<QL	0.13 ± 0.01	0.04 ± 0.01	<QL	0.04 ± 0.01	0.02 ± 0.00
1,2-Dimethoxybenzene		0.06	0.17 ± 0.03	<QL	0.07	0.13 ± 0.01	<Cal
1,2,3-Trimethoxybenzene		0.04	0.02 ± 0.00	<DL	0.06	0.02 ± 0.00	<DL
Other methoxybenzenes*	2	0.04	0.28 ± 0.04	0.14 ± 0.03	0.01	0.05 ± 0.01	0.05 ± 0.01
Phenols							
Phenol		1.23	1.61 ± 0.11	1.57 ± 0.00	0.46	0.65 ± 0.07	0.84 ± 0.02
o-Cresol		0.02	0.08 ± 0.00	0.30 ± 0.01	0.01	0.05 ± 0.01	0.18 ± 0.00
p-Cresol		0.01	0.12 ± 0.01	0.43 ± 0.01	0.01	0.04 ± 0.01	0.22 ± 0.01
4-Ethylphenol		0.11	0.21 ± 0.02	0.30 ± 0.01	0.03	0.05 ± 0.01	0.10 ± 0.01
Guaiacol		2.43	1.34 ± 0.11	0.06 ± 0.00	2.58	1.38 ± 0.06	<Cal
4-Methylguaiacol		0.08	0.26 ± 0.01	<QL	0.09	0.22 ± 0.01	<QL
4-Ethylguaiacol		0.22	0.23 ± 0.01	0.03 ± 0.00	0.17	0.15 ± 0.01	0.02 ± 0.00
Syringol		1.79	0.12 ± 0.00	<DL	2.10	0.17 ± 0.03	<DL
Other phenols*	18	0.32	0.43 ± 0.01	0.54 ± 0.02	0.28	0.34 ± 0.02	0.38 ± 0.02
Catechols							
Catechol		0.25	3.26 ± 0.06	1.79 ± 0.12	0.21	3.48 ± 0.24	2.23 ± 0.10
4-Methylcatechol		<QL	1.50 ± 0.01	2.22 ± 0.09	<QL	1.42 ± 0.10	2.24 ± 0.10
3-Methylcatechol		0.03	0.25 ± 0.00	0.71 ± 0.02	0.01	0.24 ± 0.03	0.78 ± 0.03
4-Ethylcatechol		<QL	1.00 ± 0.06	1.04 ± 0.02	<QL	0.76 ± 0.06	0.82 ± 0.01
3-Methoxycatechol		0.23	0.46 ± 0.04	<DL	0.31	0.67 ± 0.06	<DL
Other catechols*	13	0.14	0.96 ± 0.06	3.83 ± 0.06	0.10	0.75 ± 0.13	3.13 ± 0.24
Phenolic aryl carbonyls							
4-Hydroxybenzaldehyde*		-	0.08 ± 0.00	0.02 ± 0.00	-	0.04 ± 0.01	0.01 ± 0.00
Vanillin		0.59	0.26 ± 0.01	0.04 ± 0.00	0.53	0.25 ± 0.03	0.03 ± 0.00
Syringaldehyde		0.45	0.11 ± 0.01	<DL	0.43	0.12 ± 0.01	<DL
Acetovanillone		0.50	0.37 ± 0.00	0.03 ± 0.00	0.49	0.40 ± 0.02	0.03 ± 0.00
Acetosyringone		0.83	0.38 ± 0.00	<DL	3.33	1.36 ± 0.11	<DL
Vanillic acid		<Cal	<Cal	<DL	<Cal	<Cal	<DL
Vanillactic acid*		0.23	-	-	0.25	-	-
Other aromatic acids*	10	0.14	0.35 ± 0.06	0.18 ± 0.01	0.17	0.34 ± 0.05	0.14 ± 0.03
Compound group totals							
Methoxybenzenes	5	0.14	0.61 ± 0.08	0.18 ± 0.03	0.14	0.24 ± 0.03	0.07 ± 0.01
Phenols	26	6.20	4.40 ± 0.29	3.23 ± 0.04	5.73	3.05 ± 0.11	1.73 ± 0.06
Catechols	18	0.66	7.43 ± 0.24	9.59 ± 0.20	0.63	7.32 ± 0.46	9.20 ± 0.01

Phenolic aldehydes	3	1.03	0.44 ± 0.01	0.06 ± 0.00	0.95	0.41 ± 0.04	0.04 ± 0.00
Phenolic ketones	2	1.33	0.75 ± 0.01	0.03 ± 0.00	3.82	1.77 ± 0.13	0.03 ± 0.00
Aromatic acids	12	0.37	0.35 ± 0.06	0.18 ± 0.01	0.42	0.34 ± 0.05	0.14 ± 0.03
Other aromatics	5	0.07	0.47 ± 0.07	0.17 ± 0.01	0.36	1.27 ± 0.05	0.14 ± 0.03
Total aromatic monomer yield		9.8	14.4 ± 0.6	13.5 ± 0.1	12.1	14.4 ± 0.6	11.4 ± 0.1

Figure S3. Thermogravimetric and differential thermogravimetric curves for the liquid product (LP) fraction from hydrothermal liquefaction (HTL) of *Miscanthus x giganteus* (M3.65) and wheat straw (W3.65) enzymatic hydrolysis residue at 255, 300 and 345 °C. The key to naming of samples is "biomass, severity factor, -HTL temperature, fraction-, replicate number", i.e. M3.65-255LP-1 is the LP fraction from HTL at 255 °C of enzymatic hydrolysis residue from *Miscanthus* pretreated at a severity factor of 3.65. *Some samples were mislabeled in the TGA software.





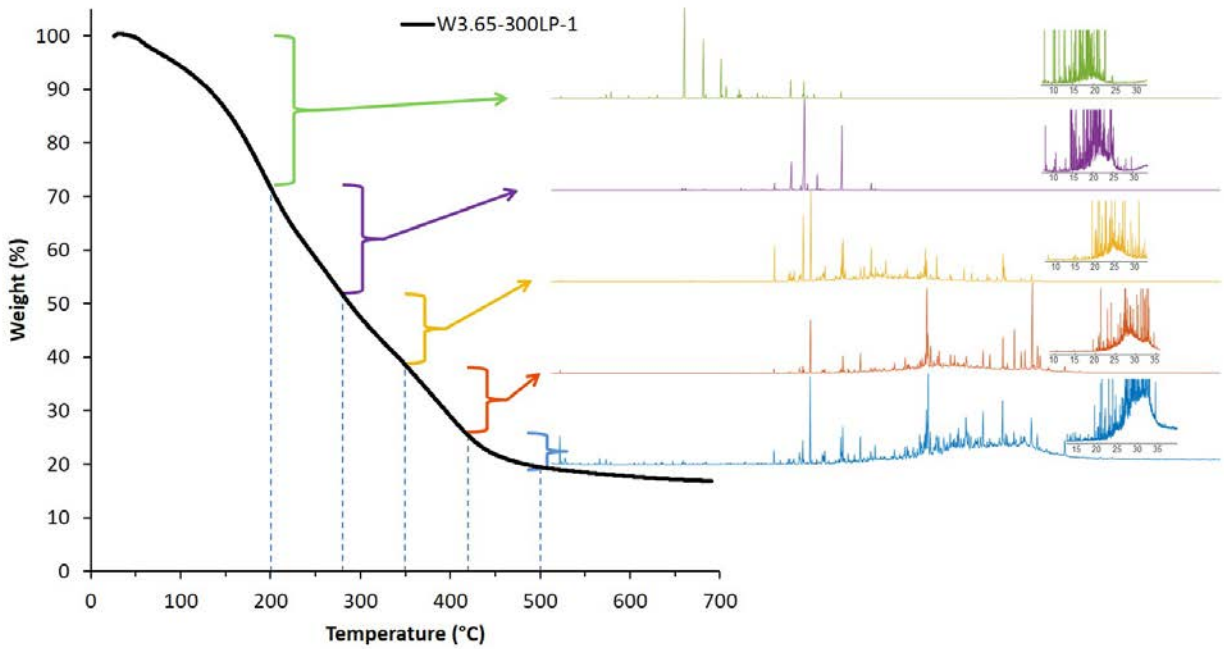
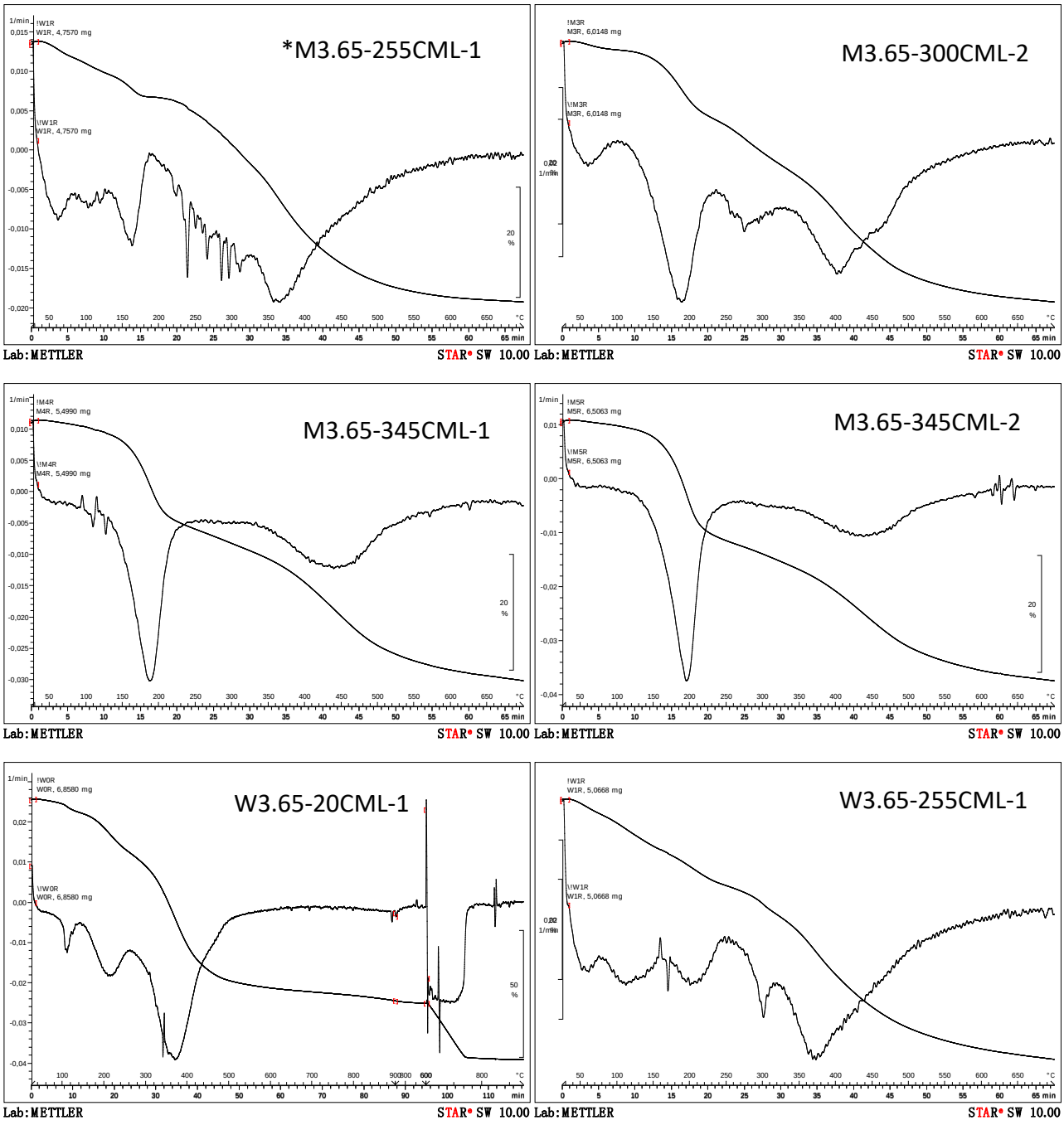
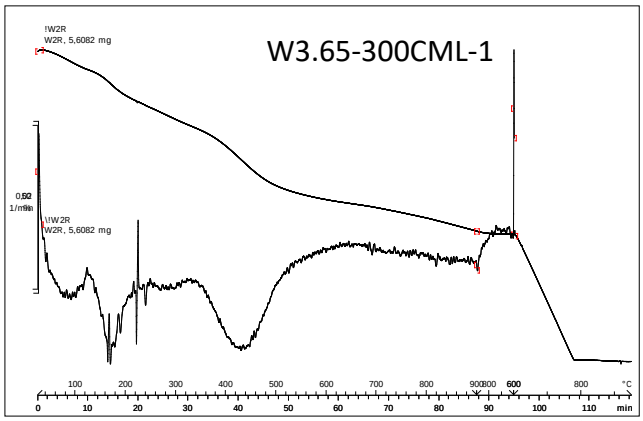


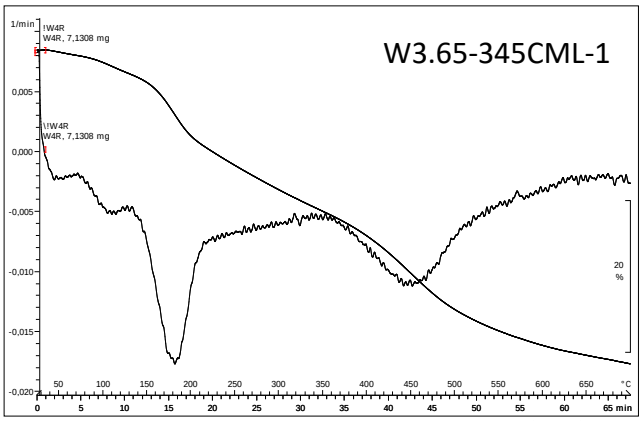
Figure S4. Thermogravimetric curve and chromatograms from stepwise thermal desorption and pyrolysis for the liquid product (LP) fraction from hydrothermal liquefaction (HTL) of wheat straw enzymatic hydrolysis residue (W3.65) at 300 °C. Segments of the chromatograms show a moving hump of decreasingly volatile compounds. The retention time of trimethylsilylated guaiacylglycerol-β-guaiacyl ether (a β-O-4 dimer) was 27.2 min.

Figure S5. Thermogravimetric and differential thermogravimetric curves for the chemically modified lignin (CML) fraction from hydrothermal liquefaction (HTL) of *Miscanthus x giganteus* (M3.65) and wheat straw (W3.65) enzymatic hydrolysis residue at 255, 300 and 345 °C and control sample at 20 °C. *Some samples were mislabeled in the TGA software.

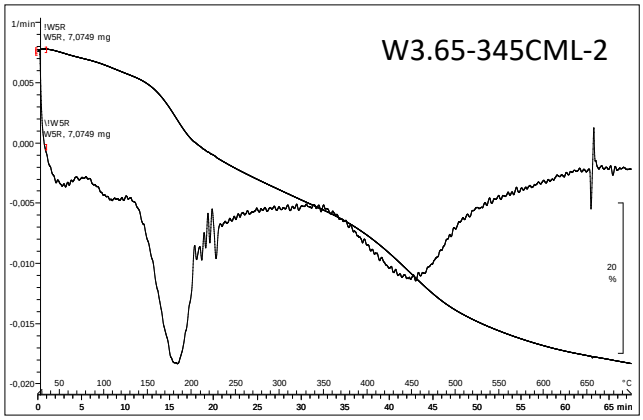




Lab:METTLER STAR° SW 10.00

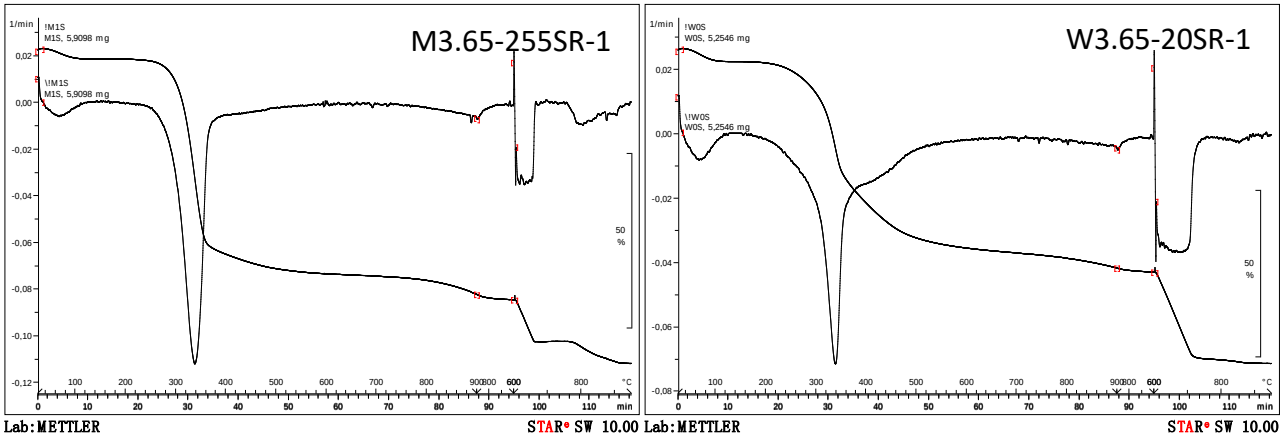


Lab:METTLER STAR° SW 10.00



Lab:METTLER STAR° SW 10.00

Figure S6. Thermogravimetric and differential thermogravimetric curves for the solid residue (SR) fraction from hydrothermal liquefaction (HTL) of *Miscanthus x giganteus* (M3.65) and wheat straw (W3.65) enzymatic hydrolysis residue at 255 °C and control sample at 20 °C.



5. Supplementary figures for Part II – Effects of biomass pretreatment severity on HTL of EnzHR

Table S6. Yield ± standard deviation (% w/w dry matter of EnzHR) of compounds in the HTL study of biomass pretreatment effects. <DL denotes a concentration below the instrument detection limit, <QL denotes a concentration below the instrument quantification limit, and <Cal denotes a concentration above the instrument quantification limit, though below the concentration of the lowest calibration level. Compounds marked with asterisk (*) were estimated using a calibration curve of a similar compound.

	Yield (% w/w DM EnzHR)				
	n	M3.65-300°C	M3.97-300°C	W3.65-300°C	W3.97-300°C
Aliphatic carbonyls					
Propanoic acid		0.13 ± 0.01	0.12 ± 0.02	0.16 ± 0.01	0.16 ± 0.01
Other aliphatic acids*	11	0.72 ± 0.07	0.82 ± 0.14	0.98 ± 0.14	0.92 ± 0.04
Hydroxy acids*	17	2.91 ± 0.23	1.28 ± 0.30	2.08 ± 0.19	0.61 ± 0.03
Keto acids*	11	0.10 ± 0.00	0.09 ± 0.01	0.08 ± 0.02	0.07 ± 0.03
Diacids*	8	0.32 ± 0.02	0.31 ± 0.07	0.43 ± 0.06	0.28 ± 0.03
Hexadecanoic acid		0.46 ± 0.03	0.74 ± 0.01	1.04 ± 0.06	1.30 ± 0.00
C14, C18, C22 fatty acids*	3	0.03 ± 0.00	0.05 ± 0.00	0.27 ± 0.02	0.40 ± 0.05
Aliphatic ketones*	2	0.03 ± 0.00	0.01 ± 0.00	0.13 ± 0.06	0.11 ± 0.00
Aromatics					
Hydroxynapthalenes*	2	0.13 ± 0.03	0.22 ± 0.01	0.18 ± 0.01	0.20 ± 0.01
1,2,3-Benzenetriol*		0.02 ± 0.02	0.07 ± 0.00	0.07 ± 0.02	0.11 ± 0.02
Unknown catechol/benzenetriol*	2	0.09 ± 0.01	0.12 ± 0.01	0.64 ± 0.07	0.73 ± 0.08
Methoxybenzenes					
Anisole		0.07 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.01 ± 0.00
1,2-Dimethoxybenzene		0.09 ± 0.02	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.00
1,2,3-Trimethoxybenzene		0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Other methoxybenzenes*	2	0.15 ± 0.02	0.08 ± 0.01	0.04 ± 0.01	0.01 ± 0.00
Phenols					
Phenol		0.83 ± 0.06	1.21 ± 0.07	0.46 ± 0.05	0.52 ± 0.02
o-Cresol		0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
p-Cresol		0.06 ± 0.01	0.06 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
4-Ethylphenol		0.11 ± 0.01	0.10 ± 0.01	0.04 ± 0.00	0.03 ± 0.00
Guaiacol		0.69 ± 0.05	1.18 ± 0.03	0.98 ± 0.04	1.17 ± 0.00
4-Methylguaiacol		0.13 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
4-Ethylguaiacol		0.12 ± 0.01	0.11 ± 0.00	0.11 ± 0.01	0.09 ± 0.00
Syringol		0.06 ± 0.00	0.14 ± 0.02	0.12 ± 0.02	0.18 ± 0.01
Other phenols*	18	0.22 ± 0.01	0.23 ± 0.01	0.24 ± 0.02	0.22 ± 0.01
Catechols					
Catechol		1.68 ± 0.03	2.23 ± 0.13	2.47 ± 0.17	2.41 ± 0.05
4-Methylcatechol		0.77 ± 0.01	0.84 ± 0.04	1.00 ± 0.07	0.84 ± 0.00
3-Methylcatechol		0.13 ± 0.00	0.14 ± 0.01	0.17 ± 0.02	0.15 ± 0.00
4-Ethylcatechol		0.52 ± 0.03	0.47 ± 0.03	0.54 ± 0.04	0.41 ± 0.01

3-Methoxycatechol		0.24 ± 0.02	0.47 ± 0.04	0.47 ± 0.04	0.61 ± 0.00
Other catechols*	13	0.50 ± 0.03	0.50 ± 0.04	0.53 ± 0.09	0.46 ± 0.06
Phenolic aryl carbonyls					
4-Hydroxybenzaldehyde*		0.04 ± 0.00	0.08 ± 0.00	0.03 ± 0.01	0.03 ± 0.01
Vanillin		0.13 ± 0.00	0.23 ± 0.01	0.18 ± 0.02	0.20 ± 0.01
Syringaldehyde		0.06 ± 0.00	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01
Acetovanillone		0.19 ± 0.00	0.28 ± 0.00	0.28 ± 0.02	0.30 ± 0.01
Acetosyringone		0.2 ± 0.00	0.31 ± 0.01	0.97 ± 0.08	1.15 ± 0.02
Vanillic acid		<Cal	<Cal	<Cal	<Cal
Vanillactic acid*		-	-	-	-
Other aromatic acids*	10	0.18 ± 0.03	0.24 ± 0.02	0.24 ± 0.03	0.27 ± 0.05
Compound group totals					
Small aliphatic acids	31	1.27 ± 0.06	1.35 ± 0.24	1.65 ± 0.20	1.42 ± 0.01
Hydroxy acids	17	2.91 ± 0.23	1.28 ± 0.30	2.08 ± 0.19	0.61 ± 0.03
Fatty acids	4	0.48 ± 0.04	0.79 ± 0.01	1.31 ± 0.06	1.70 ± 0.05
Aliphatic ketones	5	0.03 ± 0.00	0.01 ± 0.00	0.13 ± 0.06	0.11 ± 0.00
Methoxybenzenes	5	0.31 ± 0.04	0.22 ± 0.02	0.17 ± 0.02	0.11 ± 0.00
Phenols	26	2.27 ± 0.15	3.22 ± 0.03	2.16 ± 0.08	2.40 ± 0.01
Catechols	18	3.83 ± 0.12	4.65 ± 0.21	5.18 ± 0.32	4.89 ± 0.03
Phenolic aldehydes	3	0.23 ± 0.01	0.41 ± 0.01	0.29 ± 0.03	0.34 ± 0.03
Phenolic ketones	2	0.39 ± 0.00	0.60 ± 0.01	1.25 ± 0.09	1.45 ± 0.02
Aromatic acids	12	0.18 ± 0.03	0.24 ± 0.02	0.24 ± 0.03	0.27 ± 0.05
Other aromatics	5	0.24 ± 0.04	0.40 ± 0.03	0.90 ± 0.04	1.03 ± 0.09
Total monomer yield		12.2 ± 0.5	13.2 ± 0.8	15.4 ± 0.8	14.3 ± 0.3

Table S7. Yield ± standard deviation (% w/w lignin (AIL+ASL) of EnzHR) of aromatic compounds in the HTL study of biomass pretreatment effects.

	Yield (% w/w lignin (ASL+AIL))				
	n	M3.65-300°C	M3.97-300°C	W3.65-300°C	W3.97-300°C
Aromatics					
Hydroxynapthalenes*	2	0.26 ± 0.06	0.28 ± 0.02	0.26 ± 0.01	0.24 ± 0.01
1,2,3-Benzenetriol*		0.04 ± 0.03	0.09 ± 0.01	0.10 ± 0.03	0.13 ± 0.02
Unknown catechol/benzenetriol*	2	0.16 ± 0.02	0.16 ± 0.01	0.91 ± 0.09	0.87 ± 0.09
Methoxybenzenes					
Anisole		0.13 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	0.01 ± 0.00
1,2-Dimethoxybenzene		0.17 ± 0.03	0.10 ± 0.01	0.13 ± 0.01	0.08 ± 0.00
1,2,3-Trimethoxybenzene		0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Other methoxybenzenes*	2	0.28 ± 0.04	0.10 ± 0.02	0.05 ± 0.01	0.02 ± 0.00
Phenols					
Phenol		1.61 ± 0.11	1.58 ± 0.08	0.65 ± 0.07	0.63 ± 0.02
o-Cresol		0.08 ± 0.00	0.06 ± 0.00	0.05 ± 0.01	0.04 ± 0.00
p-Cresol		0.12 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	0.03 ± 0.00
4-Ethylphenol		0.21 ± 0.02	0.13 ± 0.01	0.05 ± 0.01	0.04 ± 0.00
Guaiacol		1.34 ± 0.11	1.54 ± 0.03	1.38 ± 0.06	1.40 ± 0.00
4-Methylguaiacol		0.26 ± 0.01	0.20 ± 0.01	0.22 ± 0.01	0.16 ± 0.01
4-Ethylguaiacol		0.23 ± 0.01	0.14 ± 0.00	0.15 ± 0.01	0.10 ± 0.01
Syringol		0.12 ± 0.00	0.18 ± 0.03	0.17 ± 0.03	0.21 ± 0.01
Other phenols*	18	0.43 ± 0.01	0.30 ± 0.01	0.34 ± 0.02	0.26 ± 0.01
Catechols					
Catechol		3.26 ± 0.06	2.91 ± 0.17	3.48 ± 0.24	2.88 ± 0.06
4-Methylcatechol		1.50 ± 0.01	1.09 ± 0.05	1.42 ± 0.10	1.01 ± 0.00
3-Methylcatechol		0.25 ± 0.00	0.19 ± 0.01	0.24 ± 0.03	0.18 ± 0.00
4-Ethylcatechol		1.00 ± 0.06	0.61 ± 0.04	0.76 ± 0.06	0.50 ± 0.01
3-Methoxycatechol		0.46 ± 0.04	0.61 ± 0.05	0.67 ± 0.06	0.73 ± 0.00
Other catechols*	13	0.96 ± 0.06	0.65 ± 0.05	0.75 ± 0.13	0.55 ± 0.07
Phenolic aryl carbonyls					
4-Hydroxybenzaldehyde*		0.08 ± 0.00	0.10 ± 0.00	0.04 ± 0.01	0.04 ± 0.01
Vanillin		0.26 ± 0.01	0.30 ± 0.01	0.25 ± 0.03	0.24 ± 0.01
Syringaldehyde		0.11 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
Acetovanillone		0.37 ± 0.00	0.37 ± 0.00	0.40 ± 0.02	0.36 ± 0.01
Acetosyringone		0.38 ± 0.00	0.41 ± 0.01	1.36 ± 0.11	1.37 ± 0.03
Vanillic acid		<Cal	<Cal	<Cal	<Cal
Vanillactic acid*		-	-	-	-
Other aromatic acids*	10	0.35 ± 0.06	0.31 ± 0.02	0.34 ± 0.05	0.33 ± 0.06
Compound group totals					
Methoxybenzenes	5	0.61 ± 0.08	0.29 ± 0.02	0.24 ± 0.03	0.13 ± 0.00
Phenols	26	4.40 ± 0.29	4.19 ± 0.04	3.05 ± 0.11	2.87 ± 0.02
Catechols	18	7.43 ± 0.24	6.05 ± 0.27	7.32 ± 0.46	5.84 ± 0.04
Phenolic aldehydes	3	0.44 ± 0.01	0.53 ± 0.02	0.41 ± 0.04	0.40 ± 0.03

Phenolic ketones	2	0.75 ± 0.01	0.78 ± 0.01	1.77 ± 0.13	1.73 ± 0.02
Aromatic acids	12	0.35 ± 0.06	0.31 ± 0.02	0.34 ± 0.05	0.33 ± 0.06
Other aromatics	5	0.47 ± 0.07	0.52 ± 0.04	1.27 ± 0.05	1.24 ± 0.11
Total aromatic monomer yield		14.4 ± 0.6	12.7 ± 0.4	14.4 ± 0.6	12.5 ± 0.2

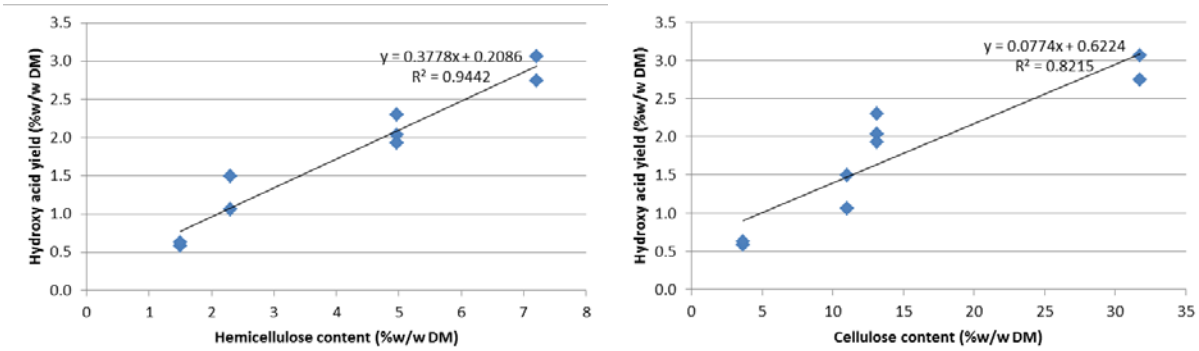


Figure S7. Correlation between 300 °C HTL hydroxy acid yield and (left) hemicellulose content (sum of arabinan, galactan and xylan) and (right) cellulose content (glucan) of the feedstock EnzHRs.

Table S8. Sample mass normalized peak areas ± standard deviation of pyrolysates from single-step Py-GC-MSof CML fractions of the HTL study of biomass pretreatment effects. SR fractions of the control experiments are included here due to their large contribution to the mass balance of the control experiments. The Kovats retention indexes (RI) of the pyrolysates were calculated from the retention times of C_7 and C_9-C_{34} linear alkanes.

	RI	Category	M3.65-20°C-SR	W3.65-20°C-SR	M3.65-20°C-CML	W3.65-20°C-CML	M3.65-300°C-CML	M3.97-300°C-CML	W3.65-300°C-CML	W3.97-300°C-CML
$n_{analyses}$			2	2	2	2	2	4	4	4
1-Nonene	891	Extractive	1530 ± 235	2134 ± 277	3819 ± 296	6580 ± 3206	4122 ± 46	5044 ± 984	2654 ± 545	3842 ± 644
Styrene	898	*p*	3405 ± 557	3894 ± 671	2702 ± 29	2018 ± 683	4336 ± 79	2829 ± 843	2403 ± 353	3270 ± 1392
2(5H)-Furanone	918	Carbohydrate	19851 ± 2020	10378 ± 370	1255 ± 32	716 ± 88	327 ± 57	71 ± 137	124 ± 58	20 ± 159
Phenol	980	H	21292 ± 3761	21686 ± 1292	46426 ± 1127	26857 ± 1285	32603 ± 2625	41234 ± 3238	22508 ± 4031	30176 ± 7430
1-Decene	991	Extractive	1330 ± 339	1440 ± 91	5078 ± 125	8704 ± 3407	4735 ± 202	5477 ± 1104	2675 ± 668	3427 ± 419
Decane	999	Extractive	1833 ± 184	1202 ± 99	649 ± 72	942 ± 41	2319 ± 151	2727 ± 389	2644 ± 181	4373 ± 1164
1-Methoxy-2-methylbenzene	1014	*o* / H	-8 ± 120	17 ± 124	375 ± 2	254 ± 20	1418 ± 41	1708 ± 145	836 ± 67	926 ± 200
1-Methoxy-4-methylbenzene	1028	*p* / H	1739 ± 242	1923 ± 179	4557 ± 185	2502 ± 206	8490 ± 112	9220 ± 720	5327 ± 377	5698 ± 1118
Limonene	1038	Extractive	-51 ± 2	742 ± 100	549 ± 71	7643 ± 758	427 ± 55	635 ± 68	712 ± 73	857 ± 144
o-Cresol	1054	*o* / H	6127 ± 1278	6950 ± 379	11364 ± 575	8798 ± 1651	10782 ± 934	13249 ± 1211	8175 ± 1315	9833 ± 2071
p-Cresol	1076	*p* / H	10751 ± 2335	11352 ± 391	27393 ± 5273	15784 ± 4668	21477 ± 3148	25718 ± 1767	21375 ± 2818	24226 ± 6407
1-Undecene	1092	Extractive	960 ± 247	1158 ± 14	3345 ± 117	6355 ± 2122	3244 ± 72	3778 ± 775	2079 ± 444	2779 ± 368
Guaiacol	1097	G	40785 ± 6392	56755 ± 1598	132108 ± 12863	116853 ± 2285	11411 ± 572	12816 ± 1729	9532 ± 2183	11772 ± 763
1-Ethyl-4-methoxybenzene	1122	*p* / H	500 ± 78	544 ± 51	1676 ± 121	863 ± 59	7189 ± 64	7664 ± 502	3115 ± 267	3229 ± 544
2-Ethylphenol	1139	*o* / H	866 ± 249	1124 ± 72	2921 ± 602	3046 ± 1062	2332 ± 151	2653 ± 198	1464 ± 275	1646 ± 322
?,?-Dimethylphenol	1151	H	2257 ± 586	2797 ± 43	4483 ± 328	3449 ± 784	5000 ± 27	5557 ± 455	4798 ± 653	5407 ± 1160
4-Ethylphenol	1169	*p* / H	7875 ± 2692	5986 ± 562	21608 ± 1703	8447 ± 1063	11281 ± 458	11766 ± 747	6182 ± 887	7466 ± 1713
2-Methoxy-5-methylphenol	1188	*m* / G	1747 ± 314	2495 ± 96	8247 ± 470	6739 ± 8	1817 ± 102	2086 ± 233	1654 ± 331	1853 ± 178
1-Dodecene	1192	Extractive	465 ± 185	833 ± 49	2230 ± 151	6488 ± 1699	2226 ± 53	2599 ± 627	1431 ± 317	1950 ± 247

Catechol	1193	G	21529 ± 6153	25829 ± 292	30503 ± 3287	31179 ± 2212	33494 ± 312	46034 ± 6862	36773 ± 8067	55531 ± 14053
4-Methylguaiacol	1201	*p* / G	12641 ± 2096	16478 ± 125	46019 ± 5919	33214 ± 3101	5990 ± 451	7465 ± 709	5846 ± 973	6988 ± 487
4-Vinylphenol	1223	*p* / *p*-Coumaric acid	77577 ± 12948	33689 ± 2444	176811 ± 6935	72772 ± 1709	4747 ± 81	3110 ± 180	1683 ± 189	2486 ± 967
3-Methylcatechol	1260	*o* / G	1414 ± 447	1877 ± 46	3504 ± 374	3276 ± 132	4303 ± 19	5934 ± 1295	4278 ± 795	5789 ± 1489
3-Methoxycatechol	1270	S	11460 ± 3019	17536 ± 676	30399 ± 687	31613 ± 361	2478 ± 17	2995 ± 363	3109 ± 699	4663 ± 451
4-Ethylguaiacol	1285	*p* / G	6344 ± 1257	10091 ± 1308	32278 ± 6548	25060 ± 3487	3809 ± 99	3600 ± 294	2864 ± 601	3372 ± 238
4-Methylcatechol	1289	*p* / G	3050 ± 1200	5059 ± 267	9380 ± 1047	9408 ± 689	12226 ± 103	14788 ± 3146	10794 ± 1751	14962 ± 3628
Tridecane	1299	Extractive	1 ± 230	529 ± 132	377 ± 31	1329 ± 108	1720 ± 253	1800 ± 199	2048 ± 161	3388 ± 821
4-Vinylguaiacol	1324	*p* / Ferulic acid	50900 ± 7711	65544 ± 4389	118741 ± 11155	109146 ± 10713	1408 ± 87	1004 ± 141	1004 ± 145	1360 ± 477
Syringol	1359	S	24292 ± 3465	29593 ± 1843	81443 ± 12053	69193 ± 9673	542 ± 42	684 ± 156	556 ± 204	888 ± 73
Eugenol	1365	*p* / G	2422 ± 488	3003 ± 269	5351 ± 418	3591 ± 343	738 ± 53	456 ± 27	499 ± 42	595 ± 263
Pentamethylbenzene	1374		500 ± 220	1280 ± 83	4635 ± 1483	5333 ± 1692	252 ± 38	179 ± 19	167 ± 29	226 ± 52
4-Propylguaiacol	1375	*p* / G	1657 ± 397	2237 ± 256	5366 ± 691	3803 ± 260	712 ± 4	643 ± 41	496 ± 108	576 ± 46
4-Ethylcatechol	1384	*p* / G	1357 ± 582	2391 ± 241	4515 ± 930	5443 ± 1038	6639 ± 79	5186 ± 1669	3425 ± 568	4334 ± 1285
1-Tetradecene	1392	Extractive	463 ± 127	823 ± 19	2402 ± 225	6923 ± 1276	1827 ± 35	2290 ± 607	1160 ± 269	1534 ± 185
Tetradecane	1399	Extractive	234 ± 69	553 ± 123	298 ± 19	740 ± 80	1348 ± 157	1463 ± 173	1699 ± 148	2853 ± 630
4-MethyLPuinoline	1403	Aromatic N	-51 ± 10	-33 ± 1	-46 ± 5	-9 ± 15	1641 ± 52	1177 ± 55	1636 ± 200	1226 ± 513
3-Methyl-1H-indole (skatole)	1405	Aromatic N	951 ± 295	1571 ± 148	663 ± 4	488 ± 6	2270 ± 98	2057 ± 182	2054 ± 272	2600 ± 488
Vanillin	1413	*p* / G	11381 ± 1614	9566 ± 1117	24007 ± 1055	15704 ± 949	440 ± 3	569 ± 215	521 ± 58	544 ± 107
cis-Isoeugenol	1417	*p* / G	1997 ± 452	2900 ± 252	4850 ± 221	3556 ± 213	67 ± 0	49 ± 7	56 ± 6	69 ± 22
4-Methylsyringol	1453	*p* / S	5842 ± 835	7266 ± 174	24657 ± 4360	18619 ± 3791	328 ± 11	439 ± 82	321 ± 101	496 ± 27
3-Methylsyringol?	1457	*m* / S	1518 ± 522	2810 ± 125	5519 ± 291	6501 ± 460	269 ± 21	211 ± 17	223 ± 46	296 ± 40

trans-Isoeugenol	1462	*p* / G	11026 ± 1853	14180 ± 1138	22565 ± 2143	15960 ± 2011	465 ± 168	323 ± 29	372 ± 75	416 ± 76
1-Pentadecene	1493	Extractive	612 ± 174	683 ± 76	1949 ± 147	4741 ± 742	1638 ± 29	2007 ± 540	899 ± 211	1141 ± 176
Acetovanillone	1499	*p* / G	5532 ± 961	7050 ± 505	10197 ± 414	9743 ± 487	1309 ± 15	850 ± 134	874 ± 101	1135 ± 155
Pentadecane	1500	Extractive	185 ± 50	784 ± 238	360 ± 9	1540 ± 212	1219 ± 28	1110 ± 137	1179 ± 88	1843 ± 417
Vanillic acid methyl ester	1528	*p* / G	816 ± 151	943 ± 49	1735 ± 66	1372 ± 35	80 ± 17	59 ± 19	79 ± 26	82 ± 41
4-Ethylsyringol	1530	*p* / S	1873 ± 277	2911 ± 82	14162 ± 3177	12965 ± 2712	162 ± 6	122 ± 27	78 ± 70	175 ± 32
1-Naphthalenol	1531		243 ± 69	289 ± 3	202 ± 1	167 ± 11	1203 ± 36	1126 ± 125	1168 ± 237	1659 ± 327
Guaiacylacetone	1539	*p* / G	7057 ± 944	7820 ± 496	11445 ± 1152	11059 ± 1355	186 ± 13	188 ± 24	160 ± 36	299 ± 30
Vanillic acid	1565	*p* / G	1918 ± 355	1527 ± 220	2640 ± 3	2038 ± 125	357 ± 24	270 ± 23	319 ± 55	356 ± 48
4-Vinylsyringol	1573	*p* / Sinapinic acid	12842 ± 1720	17459 ± 1044	33449 ± 3102	35522 ± 6660	73 ± 38	55 ± 15	45 ± 20	93 ± 60
Cetene	1593	Extractive	476 ± 155	490 ± 4	1647 ± 140	2171 ± 347	1546 ± 101	1904 ± 517	756 ± 180	970 ± 164
Propiovanillone	1596	*p* / G	1973 ± 295	2057 ± 207	3865 ± 275	5095 ± 504	207 ± 7	120 ± 22	120 ± 14	143 ± 17
4-Allyl-2,6-dimethoxyphenol	1607	*p* / S	1507 ± 237	1724 ± 6	4588 ± 665	2979 ± 691	44 ± 14	10 ± 20	38 ± 14	28 ± 33
2,6-Dimethoxy-4-propylphenol	1614	*p* / S	459 ± 77	581 ± 45	2740 ± 619	1915 ± 329	71 ± 12	31 ± 9	66 ± 11	73 ± 11
cis-2,6-Dimethoxy-4-propenylphenol	1659	*p* / S	1329 ± 265	1677 ± 56	4593 ± 358	3226 ± 515	55 ± 20	17 ± 28	41 ± 15	25 ± 43
Dihydroconiferyl alcohol	1661	*p* / G	1628 ± 237	651 ± 58	3734 ± 364	774 ± 69	61 ± 16	5 ± 25	18 ± 25	3 ± 34
Syringaldehyde	1672	*p* / S	4713 ± 738	4507 ± 591	13792 ± 784	11309 ± 861	82 ± 9	3 ± 36	30 ± 31	28 ± 13
cis-Coniferyl alcohol	1685	*p* / G	1581 ± 347	1486 ± 300	2182 ± 137	1527 ± 50	51 ± 6	24 ± 2	30 ± 7	26 ± 7
Unknown aromatic (3-Methoxy-5-propylcatechol?)	1689		292 ± 94	539 ± 11	519 ± 72	1194 ± 146	793 ± 111	407 ± 102	1407 ± 312	2369 ± 1660
3-Methoxy-2-naphthalenol	1690		628 ± 152	692 ± 24	1966 ± 33	1329 ± 50	541 ± 19	525 ± 55	334 ± 72	436 ± 62
Heptadecane	1698	Extractive	288 ± 80	390 ± 81	328 ± 23	301 ± 9	1168 ± 30	1075 ± 175	610 ± 82	1020 ± 248
2-Pentadecanone	1701	Extractive	50 ± 25	200 ± 34	80 ± 4	1671 ± 37	8 ± 5	4 ± 28	370 ± 246	253 ± 50
trans-2,6-Dimethoxy-4-propenylphenol	1712	*p* / S	6437 ± 791	7314 ± 53	20212 ± 3195	13515 ± 3280	33 ± 11	23 ± 7	27 ± 19	70 ± 52
Acetosyringone	1744	*p* / S	3385 ± 519	12396 ± 836	10965 ± 800	26570 ± 3077	1370 ± 105	661 ± 396	2628 ± 575	2459 ± 1536

trans-Coniferaldehyde	1753	*p* / G	2871 ± 308	2197 ± 162	2678 ± 229	3206 ± 12	457 ± 20	210 ± 80	396 ± 74	461 ± 82
trans-Coniferyl alcohol	1755	*p* / G	7872 ± 1149	5775 ± 2150	10165 ± 59	2791 ± 69	68 ± 20	6 ± 28	50 ± 17	38 ± 40
Syringylacetone	1776	*p* / S	3424 ± 373	4162 ± 193	7574 ± 1204	8347 ± 1661	6 ± 2	12 ± 6	26 ± 9	48 ± 16
p-Coumaric acid	1792	*p* / *p*-Coumaric acid	246 ± 41	106 ± 19	4669 ± 229	1200 ± 144	48 ± 19	8 ± 15	34 ± 13	23 ± 25
?-Octadecene	1794	Extractive	454 ± 157	455 ± 15	1704 ± 131	2268 ± 259	1528 ± 134	1840 ± 548	604 ± 160	764 ± 170
Propiosyringone (+ 3-(3,5-dimethoxy-4-hydroxyphenyl)-3-oxopropanal)	1836	*p* / S	1086 ± 189	1117 ± 97	3145 ± 287	2824 ± 414	112 ± 4	44 ± 16	58 ± 7	67 ± 13
Ferulic acid methyl ester	1855	*p* / Ferulic acid	831 ± 153	1088 ± 104	4505 ± 21	3267 ± 270	33 ± 11	16 ± 8	30 ± 12	23 ± 19
1-Nonadecene	1892	Extractive	344 ± 129	301 ± 6	1495 ± 122	1854 ± 241	1438 ± 138	1837 ± 569	545 ± 161	696 ± 164
2-Heptadecanone	1905	Extractive	50 ± 21	402 ± 55	95 ± 7	5183 ± 56	42 ± 1	35 ± 5	546 ± 322	380 ± 49
n-Hexadecanoic acid	1963	Extractive	183 ± 88	371 ± 29	14949 ± 13	16927 ± 1726	277 ± 5	91 ± 5	239 ± 159	211 ± 37
N-methylnorharmane	1974	Aromatic N	93 ± 24	241 ± 3	260 ± 22	279 ± 57	2764 ± 342	3944 ± 176	4361 ± 927	3982 ± 955
?-Eicosene	1992	Extractive	320 ± 127	332 ± 15	1649 ± 124	2204 ± 241	1397 ± 137	1799 ± 548	545 ± 156	689 ± 156
Sinapaldehyde	1999	*p* / S	836 ± 121	824 ± 98	1816 ± 166	2619 ± 252	165 ± 25	66 ± 29	176 ± 47	165 ± 51
1-Methyl-9H-pyrido[3,4-b]indole	2005	Aromatic N	340 ± 97	394 ± 29	470 ± 66	257 ± 54	7027 ± 611	7226 ± 573	6864 ± 882	6124 ± 1804
Sinapyl alcohol	2008	*p* / S	2023 ± 292	1997 ± 632	8085 ± 1310	3985 ± 376	-139 ± 39	-170 ± 30	-117 ± 15	-109 ± 62
9H-Pyrido[3,4-b]indole	2019	Aromatic N	648 ± 213	842 ± 93	353 ± 18	333 ± 99	10036 ± 464	10081 ± 516	10402 ± 1996	8814 ± 1974
?-Heneicosene	2092	Extractive	262 ± 112	258 ± 6	1443 ± 132	1800 ± 214	1440 ± 125	1864 ± 572	580 ± 147	711 ± 158
?,?-Octadecadienoic acid	2145	Extractive	380 ± 155	582 ± 7	5484 ± 248	3263 ± 509	243 ± 47	109 ± 27	229 ± 71	240 ± 81
1-Docosene	2195	Extractive	336 ± 131	401 ± 10	1743 ± 134	2290 ± 287	1390 ± 131	1821 ± 591	510 ± 160	661 ± 167
Docosane	2198	Extractive	251 ± 98	442 ± 96	538 ± 27	536 ± 67	1502 ± 713	1681 ± 263	611 ± 347	1112 ± 575
1-Tricosene	2293	Extractive	271 ± 119	222 ± 9	1361 ± 94	1608 ± 135	1597 ± 131	2014 ± 643	534 ± 141	752 ± 221
Tricosane	2297	Extractive	668 ± 142	707 ± 26	1221 ± 15	790 ± 12	2311 ± 1211	2465 ± 442	1111 ± 603	1725 ± 987
?-Tetracosene	2393	Extractive	319 ± 133	245 ± 40	1358 ± 131	1767 ± 258	1477 ± 83	1986 ± 613	540 ± 145	637 ± 190
Tetracosane	2398	Extractive	795 ± 196	740 ± 37	986 ± 34	743 ± 65	2668 ± 1715	3005 ± 671	1199 ± 850	1759 ± 1429

?-Pentacosene	2493	Extractive	-180 ± 49	169 ± 11	1029 ± 104	1049 ± 185	1568 ± 103	2091 ± 515	427 ± 151	515 ± 164
Pentacosane	2498	Extractive	1103 ± 190	974 ± 166	-1509 ± 39	-1123 ± 78	3554 ± 1525	4244 ± 592	1536 ± 926	2151 ± 1340
?-Hexacosene	2594	Extractive	530 ± 194	408 ± 51	2604 ± 75	1814 ± 343	2308 ± 37	3084 ± 681	658 ± 169	774 ± 186
Hexacosane	2598	Extractive	1169 ± 165	1043 ± 357	1499 ± 49	996 ± 120	3963 ± 1650	5122 ± 706	1630 ± 950	2314 ± 1360
?-Heptacosene+Heptacosane	2697	Extractive	1668 ± 173	1398 ± 518	6923 ± 187	5283 ± 67	11142 ± 789	16343 ± 3418	2939 ± 975	3857 ± 979
Octacosene	2794	Extractive	776 ± 304	583 ± 166	2888 ± 172	3668 ± 492	3191 ± 128	4964 ± 1115	1236 ± 272	1640 ± 414
Octacosane	2797	Extractive	713 ± 93	871 ± 735	1287 ± 1	1000 ± 48	4608 ± 1307	6978 ± 1325	1872 ± 964	2899 ± 1275
Nonacosane	2900	Extractive	1151 ± 118	1093 ± 918	6166 ± 343	22774 ± 2091	11326 ± 908	16401 ± 2731	11669 ± 1814	13139 ± 3052
?-Nonacosene or Hexacosanol	2907	Extractive	-77 ± 7	18 ± 95	1700 ± 138	1443 ± 63	2519 ± 181	4786 ± 612	1161 ± 310	1468 ± 680
Hentriacontane	3100	Extractive	703 ± 80	455 ± 802	3447 ± 219	9137 ± 1136	7151 ± 633	10022 ± 1580	6871 ± 1315	8144 ± 1691
Octacosanol	3112	Extractive	1177 ± 398	319 ± 103	13212 ± 1217	15272 ± 1483	35228 ± 3478	57962 ± 4384	24777 ± 4662	30850 ± 7381
Stigmastan-3,5-diene	3158	Extractive	61 ± 32	49 ± 13	5778 ± 406	1211 ± 227	59 ± 37	77 ± 10	52 ± 30	71 ± 14
Sitosterol	3389	Extractive	65 ± 1	72 ± 18	10450 ± 574	7456 ± 818	536 ± 41	111 ± 39	67 ± 20	31 ± 89
14,16-Hentriacontanedione	3399	Extractive	64 ± 5	153 ± 95	126 ± 21	26006 ± 2455	188 ± 158	211 ± 106	168 ± 127	278 ± 108

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