Supporting Information: Hydrotropic solutions enable homogeneous Fenton treatment of lignin

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Materials & Methods

Chemicals

Iron (II) sulfate heptahydrate (99.0%), hydrogen peroxide (30%), Kraft lignin (370959), 4-methyl-2-pentanone (MIBK, 99%), sodium hydroxide (98%), sodium xylene sulfonate (SXS, 90%), sodium sulfite (98-100%) and 2-methyltetrahydrofuran (2-MTHF, 99%) were purchased from Sigma-Aldrich. Sodium cumene sulfonate (SCS, 93%) was purchased from Julius Hoesch. The pH was adjusted with sulfuric acid (75%) from Carl Roth. Beech wood chips were supplied by Wilhelm Eder GmbH. The wood chips were dried at 50 °C prior to use. All chemicals were used without further purification and adjusted to the desired concentrations with deionized water.

Sample preparation

For the batch experiments, lignin samples were precipitated by the addition of 0.01 M H_2SO_4 with a dilution ratio of 1:10 v:v. Subsequently, the dispersion was centrifuged at 4083 g for 30 min. The precipitated solid was freeze-dried and resuspended in 2 mL of 1 M NaOH for SEC analysis.

For the SEC analysis of the continuous experiments, 2 mL of the aqueous lignin phase were diluted with 2 mL of deionized water or 3 wt.% sodium sulfite and 16 mL of 1 M sodium hydroxide solution. Sodium sulfite was initially used as quenching agent for the Fenton reaction. However, the addition of sodium hydroxide also stops the reaction and renders the sodium sulfite obsolete. Sodium sulfite does not change the SEC results significantly compared to water, see Figure S1 in the SI. One milliliter of the diluted sample was then transferred into a vial for analysis by SEC. Furthermore, for the analysis of organic acids a sample of the aqueous phase of the experiments with hydrotropic lignin was diluted 1:10 v:v with NaOH and analyzed according to the procedure described in the next section and in Di Marino et al.[1]

For the analysis of the organic phase by means of GC-MS, 1 mL of sample was transferred into a vial. The GC analysis of the organic phase was performed according to the procedure described in subsequent sections. The concentrations of the compounds listed in Table S1 were measured. The remaining sample volume after GC analysis was mixed with 1 mL of 1 M sodium hydroxide solution for SEC.

Further information on methods for UV-Vis, HPLC, LC-MS, GC-MS, MicroCT and NMR can be found in the next sections.

Batch setup

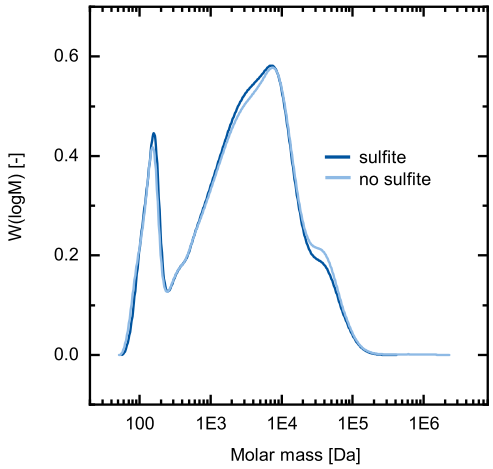


Figure S1: Comparison of SEC results for samples after 4.5 h of reaction time, which were either quenched or not quenched with sodium sulfite before 1 M NaOH was added.

Continuous setup

A Schott bottle with a side outlet near the bottom served as the reaction vessel. The reaction vessel was closed with a screw cap, which had three connections. The reaction mixture was circulated using a peristaltic pump (Ismatec, ISM831C) with 40 mL/min. The in situ extraction was performed with 150 mL MIBK. The aqueous lignin phase (5 g/L, 150 mL) was situated in the lower part of the reactor, the organic phase floated above. At the side outlet of the reaction vessel, the aqueous lignin phase was withdrawn by means of the peristaltic pump and recirculated via one of the three connections in the screw cap of the Schott bottle. During the descent through the organic phase, aromatic products were extracted from the aqueous phase. The pH value of the aqueous phase was adjusted to either 2 or 3. By means of a syringe pump (Harvard Pump 11 Elite) and a long cannula, 30 wt.% H_2O_2 was injected with a flow rate of either 1 mL/h or 2 mL/h into the aqueous lignin phase through a connection in the cap for three hours. The aqueous solution was agitated with a magnetic stirrer, which was kept below the point were organic and aqueous phase intermix. Samples were taken with a syringe through the third connection in the screw

cap: GC and SEC samples from the organic phase as well as SEC and LC samples from the aqueous phase. The first sample was taken before the pump for H_2O_2 flow was started. Additional experiments were performed with hydrotropic lignin extracted with 400 g/L SCS, to demonstrate the feasibility of the integrated two-step process consisting of pretreatment and valorization. After extraction, the solution was diluted to 100 g/L SCS, iron sulfate was added (1 mM), and the pH was adjusted to 2 with sulfuric acid.

Analytics

Size exclusion chromatography (SEC)

SEC was used to determine the average molecular weight of the different lignin fractions dissolved and of possible degradation compounds. Average molecular weight ($\overline{M_w}$) and polydispersity (D = $\overline{M_w}/\overline{M_n}$, where $\overline{M_n}$ is the number average molecular weight) were evaluated. Measurements were performed using an Agilent 1200 system equipped with a UV-Vis detector at a wavelength of λ= 280 nm. The eluent was prepared with water (HPLC grade, Carl Roth) with addition of 0.1 M sodium hydroxide (NaOH, 99 % , Sigma Aldrich) and 0.01 wt.% sodium azide (NaN_3, extra pure, Merck KGaA). The internal standard was a 12.5 g/L glucose monohydrate solution (biochemistry, Merck KGaA). One pre-column (8 x 50 mm) and three MCX gel columns (8 x 300 mm) were used at a flow rate of 1.0 mL/min at 40 ℃. The diameter of the gel particles was 5 µm, the nominal pore widths were 1000 $\mathring{A}$ for the three columns. Calibration was performed using narrowly distributed poly(styrene sulfonate) standards (Polymer Standards Service, Germany).

UV-Vis

Lignin concentrations were determined by means of a Thermo Scientific Genesys 10S UV-Vis-Spectrophotometer (Thermo Fisher Scientific Inc, USA). In order to determine the lignin concentration in the supernatant after the pretreatment, additional sample preparation was necessary. After the hydrotropic pretreatment, 5 mL of the supernatant were collected in a

Falcon tube. Then, the sample was diluted 1:10 v:V with 0.01 M sulfuric acid and stored for 12 h in order to precipitate the lignin. The solution was then centrifuged at an RCF of 4083 g for 10 min to separate the solids from the liquid phase. The supernatant was removed and only the solids remained inside the Falcon tube. The remaining solids were washed two times with 30 mL, respectively 20 mL, 0.01 M sulfuric acid and each time the supernatant was discarded after 10 min of centrifugation at 4083 g. Subsequently, the remaining solids were freeze dried to remove any residual liquid. After the freeze drying, lignin remained as a dark brown powder inside the Falcon tube. For UV-Vis measurements, the lignin was then dissolved in 1 mL of 1 M NaOH. A calibration curve was generated using Kraft lignin (Sigma Aldrich, Germany) solubilized at different concentrations in 1 M NaOH.

Enzymatic hydrolysis

For the enzymatic hydrolysis, cellulases from *Trichoderma reesei* (Aq. solution, 700 Units/g, Sigma Aldrich) were mixed with a buffer solution. The buffer enzyme mixture was composed of 85.95 vol.% 100 mM sodium acetate buffer solution at pH 4.8 and 14.05 vol.% of cellulases. Reaction tubes were placed in a thermo mixer (HLC-Heating Mixer MHR, Ditabis, Germany) at 45 ℃ and 1000 rpm. The hydrolysis was started by mixing 1 mL of enzyme solution with 0.1 g of biomass sample. Samples for glucose content determination were taken after 30 min, 4 h, 24 h and 48 h. The samples were rapidly cooled in ice to stop the reaction. A HPLC Agilent 1100 equipped with an organic acid resin column and precolumn with RI detector and 0.8 ml/min 5 mM sulfuric acid as eluent were used.

Gas chromatography

Gas chromatography mass spectrometry (GC-MS) (Agilent 6890 and N-Agilent 5975 MSD) equipped with a J&W 122-0132 DB-1MS capillary column (30 m, 0.25 mm n.d., film 0.25 mm) was performed in order to detect the monomers and oligomers produced during the electrochemical depolymerization and subsequent extraction. Helium was applied as carrier

gas with an initial flow of 0.8 mL/min. The initial temperature of the GC oven was 50 ℃ for 1 minute, thereafter the temperature was increased to 120 ℃ with a rate of 15 min^{-1} and finally 280 ℃ were reached with a rate of 25 min^{-1}. The two temperatures were kept constant for 6 and 2.5 minutes, respectively. All identified products by GC-MS measurement were quantified by external calibration curves. All identified products were purchased by Sigma Aldrich, purest available grade.

Table S1: List of chemicals which were measured in GC analysis.

Nr.	Compound	Chemical formula
1	α-methylstyrene	C_9H_{10}
2	guaiacol	$C_7H_8O_2$
3	p-cumenol	$C_9H_{12}O$
4	phenylacetic acid	$C_8H_8O_2$
5	4-hydroxybenzaldehyde	$C_7H_6O_2$
6	eugenol	$C_{10}H_{12}O_2$
7	vanillin	$C_8H_8O_3$
8	4-hydroxyacetophenone	$C_8H_8O_2$
9	acetovanillone	$C_9H_{10}O_3$
10	syringaldehyde	$C_9H_{10}O_4$
11	acetosyringone	$C_{10}H_{12}O_4$
12	bisphenol-A	$C_{15}H_{16}O_2$

Liquid chromatography coupled to electrospray ionization quadrupole time-of-flight mass spectrometry

The amount of organic acids in the depolymerized lignin samples was determined by a method based on reversed-phase liquid chromatography coupled to electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-Q-ToF-MS)50 as described in Di Marino et al.[1] The 1260 infinity HPLC system (Agilent Technologies Inc.) was equipped with a Hi-Plex H, 300 mm x 7.7 mm column (Agilent Technologies Inc.) and coupled to UV detection (Agilent 1260 Infinity DAD) as well as ESI-Q-ToF-MS (Agilent 6530 Accurate Mass Q-ToF LC/MS). An injection volume of 10 mL, column temperature of 45 ℃, flow rate of 0.3 mL/min, UV wavelength of 220 nm, overall measurement time of 30 minutes and isocratic

elution with 20 vol% acetonitrile, 79.9 vol% ultrapure water and 0.1 vol% formic acid (2.65 M) were applied. ESI was used in negative ionization mode and with the following conditions: 180 capillary voltage 3000 V, nozzle voltage 1000 V, gas temperature 200 ℃, sheath gas flow 11 L/min, fragmentor 100 V, and skimmer 65 V. All samples were diluted 1:100 v:v with ultrapure water before analysis. Standards of malic acid, malonic acid, oxalic acid and succinic acid were prepared with concentrations of 0.125-4 mg/L in ultrapure water and measured with the method described above. Peak areas of organic acid standards obtained from the extracted ion chromatograms (EIC) and concentrations were correlated to gain linear standard curves for quantification. The software MassHunter (Agilent Technologies Inc.) was used for data collection and processing.

2D-NMR HSQC

Lignin contained in the liquid phase was precipitated by addition of sulfuric acid or water, 24 h, as described above, and freeze-dried. 2D Heteronuclear Single-Quantum Coherence (HSQC) NMR measurements were conducted to characterize the amount and the type of lignin chemical linkages. Measurements and analysis of the results were based on previous works.[2]

MicroCT

A Bruker Skyscan 1272 with 50 kV and 200 μA was used for the CT scans of the biomass.

SEM

In this work a Hitachi SEM S 3000 was used to investigate the surface changes of the wood structure.

Results & Discussion

Batch Fenton treatment

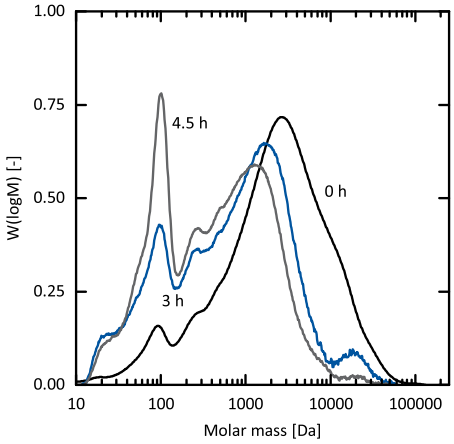
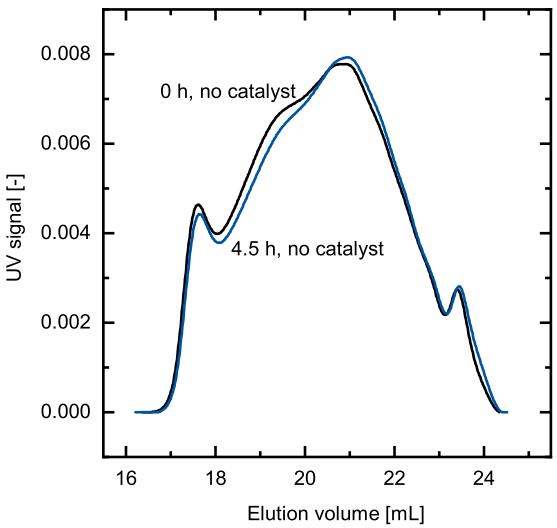
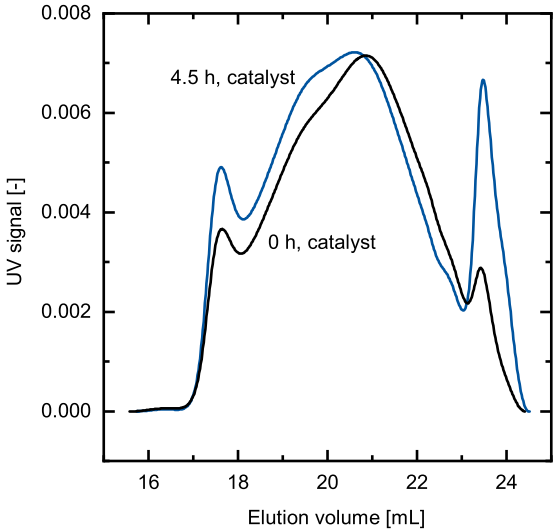


Figure S2: SEC results for the batch Fenton treatment of 5 g/L Kraft lignin in 400 g/L SCS, 1 mM $FeSO_4$, pH 3.



(a) SEC results of a batch experiment with 5 g/L lignin in 400 g/L SCS, pH 3 and the addition of 100 μL of 30 wt.% H_2O_2.



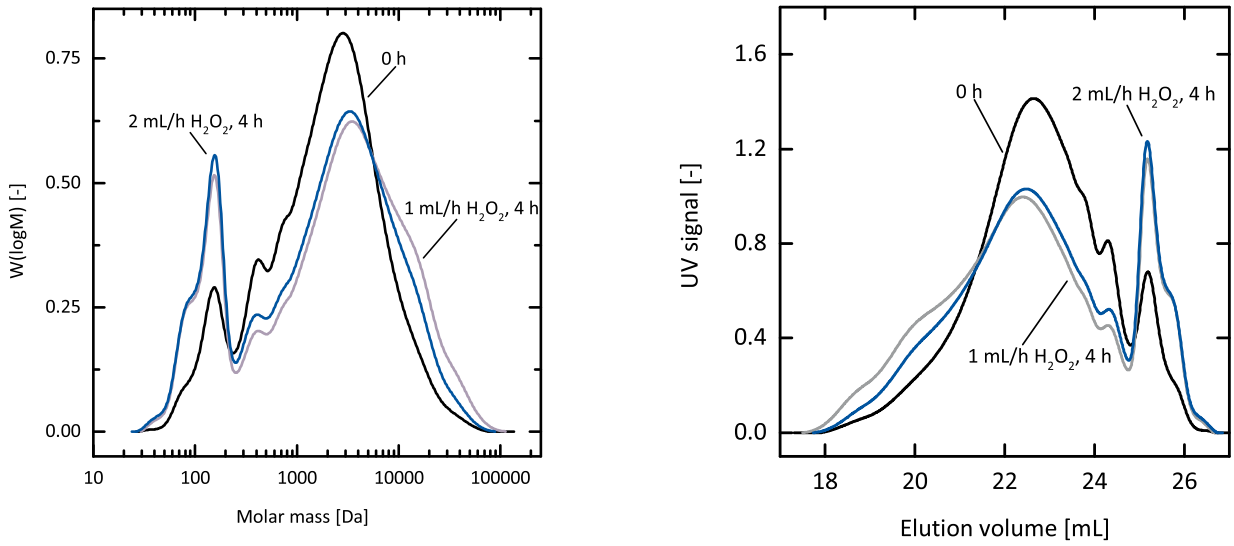
(b) SEC results of a batch experiment with 5 g/L lignin in 400 g/L SCS, 1 mM $FeSO_4$, pH 3 and the addition of 100 μL of 30 wt.% H_2O_2 after 0h, 1.5 h and 3h.

Figure S3: SEC results for the batch Fenton treatment of 5 g/L Kraft lignin with and without the iron catalyst.

Table S2: Total GC yield of aromatics of the batch depolymerization of Kraft lignin.

Experiment Nr.	Total GC yield
-	%
1	0.70
2	0.29
3	2.97
4	0.12

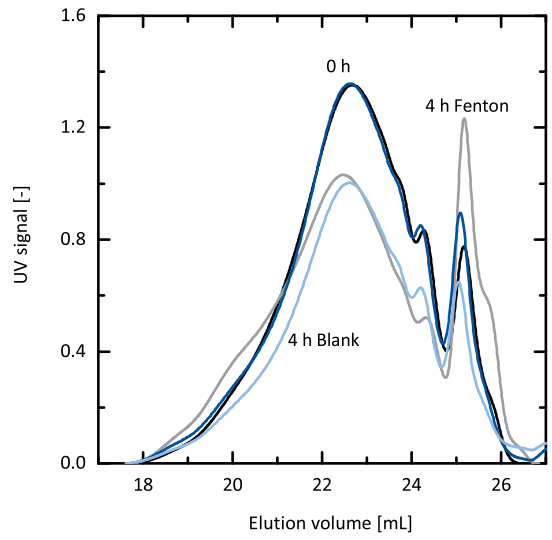
Continuous Fenton treatment



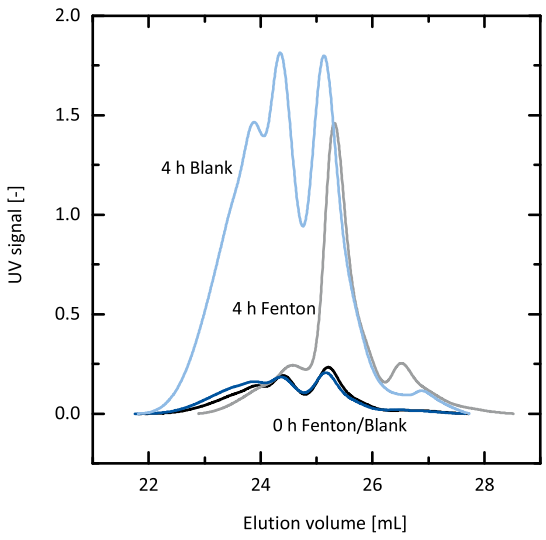
(a) Molar mass distribution

(b) Elugram

Figure S4: SEC results continuous Fenton treatment of 5 g/l Kraft lignin in 400 g/l SCS, pH 2, 1 mM $FeSO_4$ and different hydrogen peroxide flow rates.

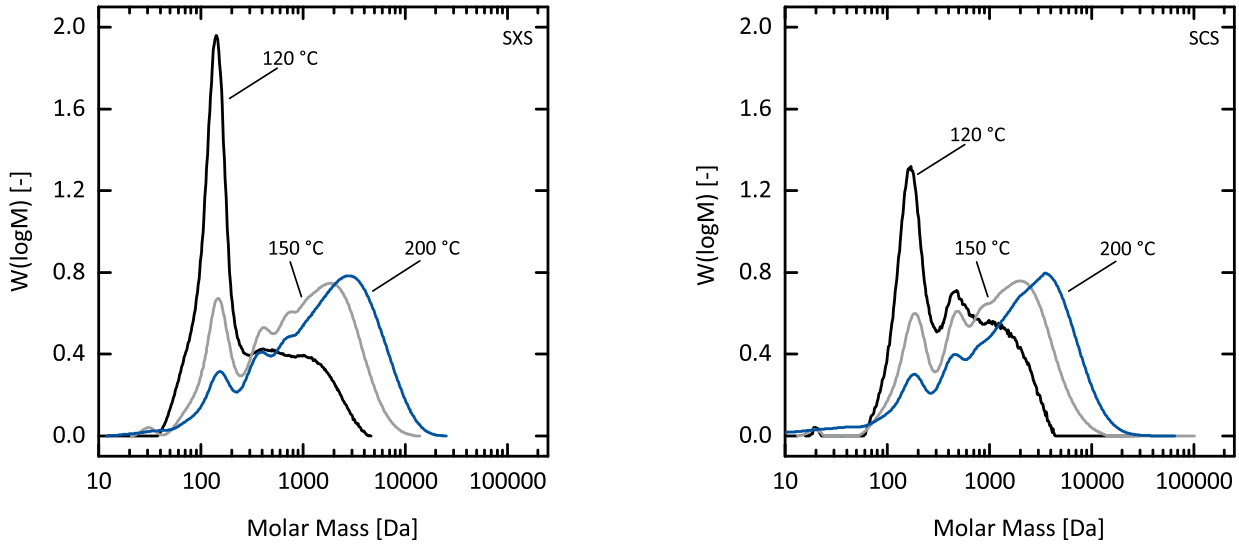


(a) Comparison of SEC elugrams of the aqueous phase of blank experiment and of the Fenton experiment.



(b) Comparison of SEC elugrams of the organic phase of blank experiment and of the Fenton experiment.

Figure S5: Comparison of SEC elugrams of the blank experiment and of the Fenton experiment.



(a) Molar mass distribution of extracted lignin after wood pretreatment in SXS.

(b) Molar mass distribution of extracted lignin after wood pretreatment in SCS.

Figure S6: Molar mass distribution of lignin extracted in a pretreatment with SXS, respectively SCS, at 120, 150 and 200 ℃ for 6 h.

Table S3: Average molecular weight M_w and polydispersity D of hydrotropic and Kraft lignin.

SCS

Temperature [℃]	120	150	200
M_w [g/mol]	774	1597	2903
D	3.00	3.45	3.71

SXS

Temperature [℃]	120	150	200
M_w [g/mol]	459	1356	2449
D	2.66	3.59	3.88

Kraft

Temperature [℃]	RT	
M_w [g/mol]	3702	
D	5.65	

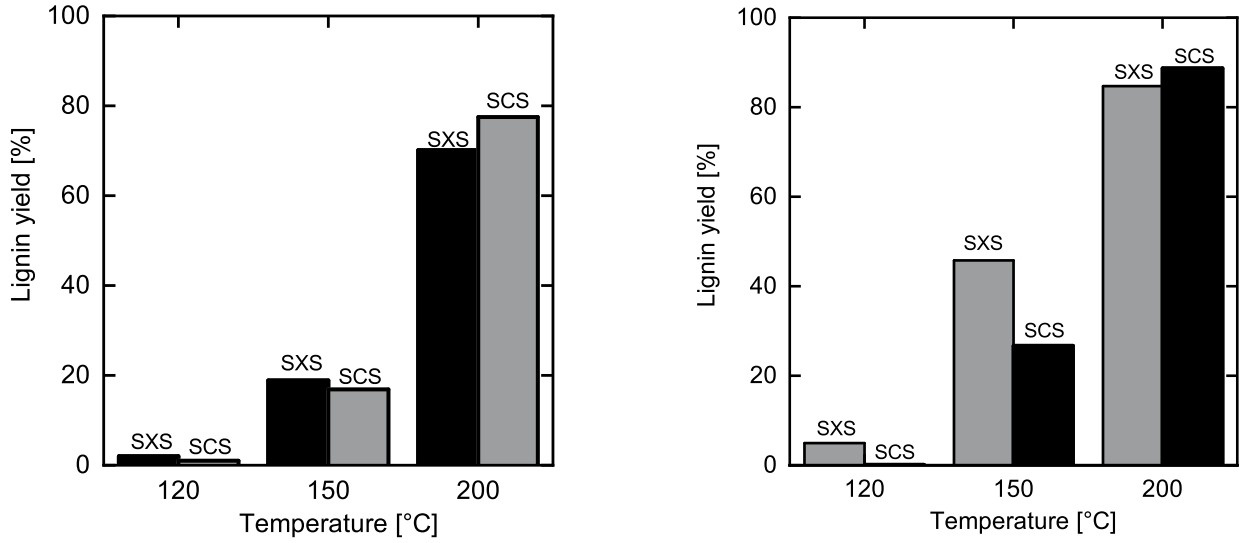


Figure S7: Quantification (UV-Vis left, gravimetric right) of extracted lignin relative to absolute lignin content in raw beech wood with SCS/SXS pretreatment at 120, 150 and 200 ℃ for 6 h.

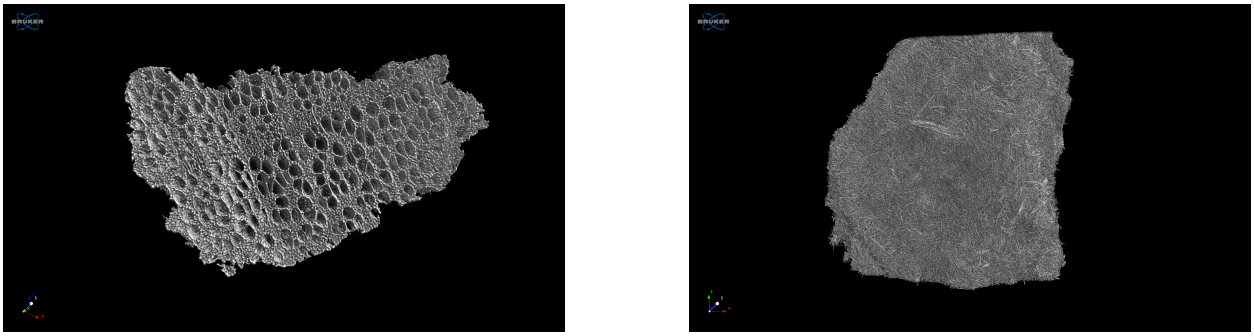


Figure S8: MicroCT images of raw beech wood (left) and beech wood after treatment in SCS at 200 ℃ for 6 h (right)

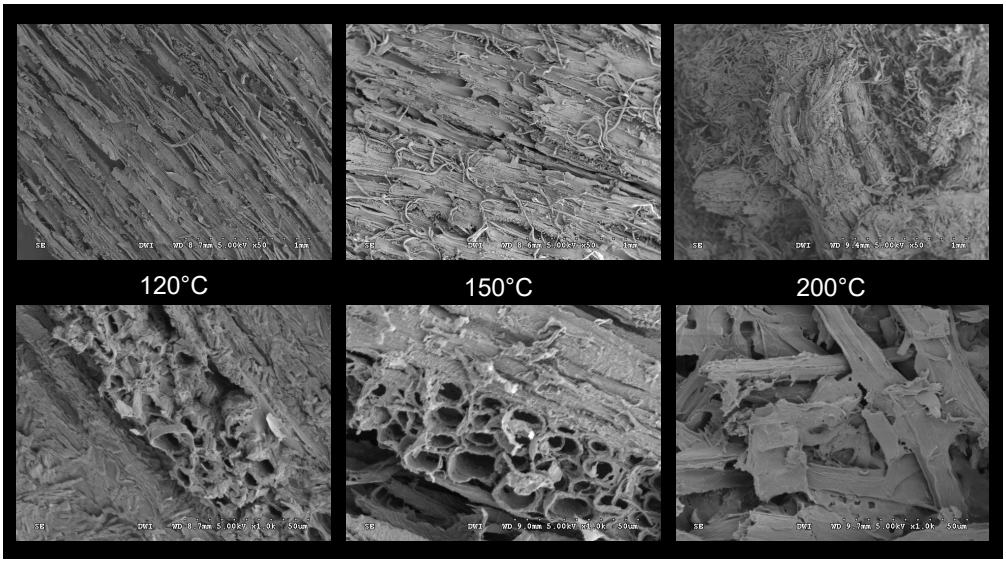
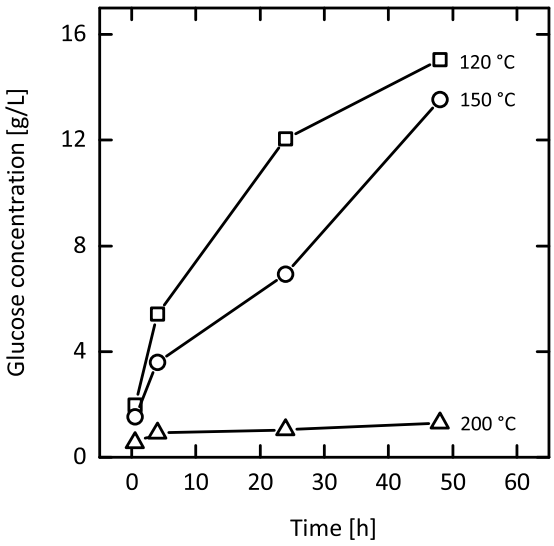
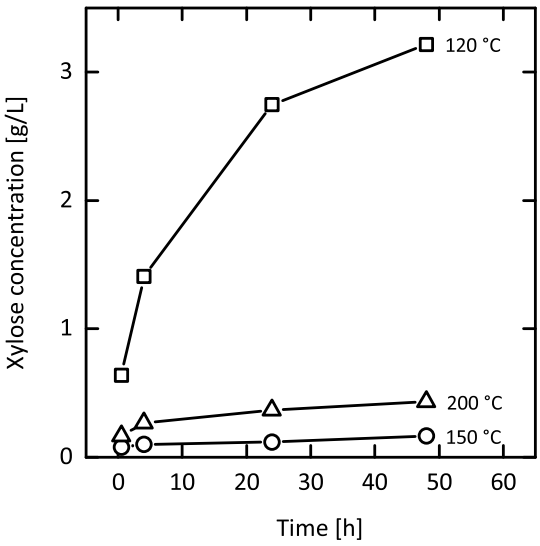


Figure S9: SEM images of beech wood after treatment in SCS at 120, 150 and 200 ℃ for 6 h

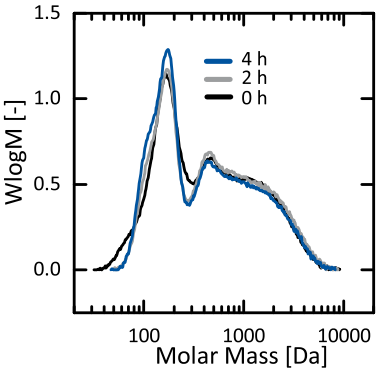


(a) HPLC results for the glucose concentration during the enzymatic hydrolysis of hydrotropic (SXS) beech wood.

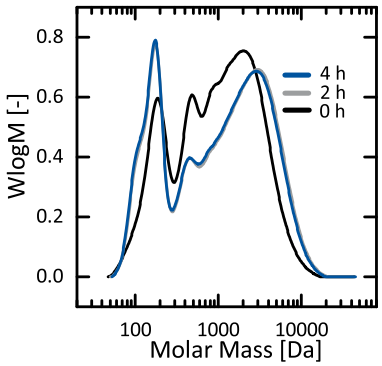


(b) HPLC results for the xylose concentration during the enzymatic hydrolysis of hydrotropic (SXS) beech wood.

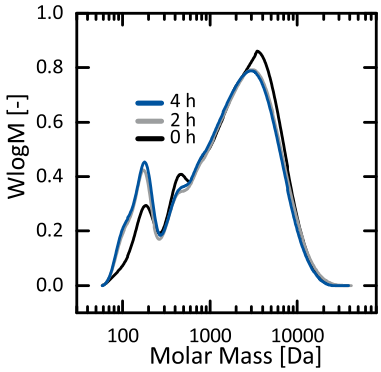
Figure S10: HPLC results for the sugar concentration during the enzymatic hydrolysis of hydrotropic (SXS) beech wood.



(a) SEC results for Fenton treatment of hydrotropic lignin extracted at 120 ℃.



(b) SEC results for Fenton treatment of hydrotropic lignin extracted at 150 ℃.



(c) SEC results for Fenton treatment of hydrotropic lignin extracted at 200 ℃.

Figure S11: SEC results of Fenton treatment of hydrotropic lignin, which was extracted at 120, 150 and 200 C and 400 g/L SCS. The hydrotropic salt concentration was then diluted to 100 g/L, the pH value was adjusted to 2 and the hydrogen peroxide flow rate was adjusted to 1 mL/h.

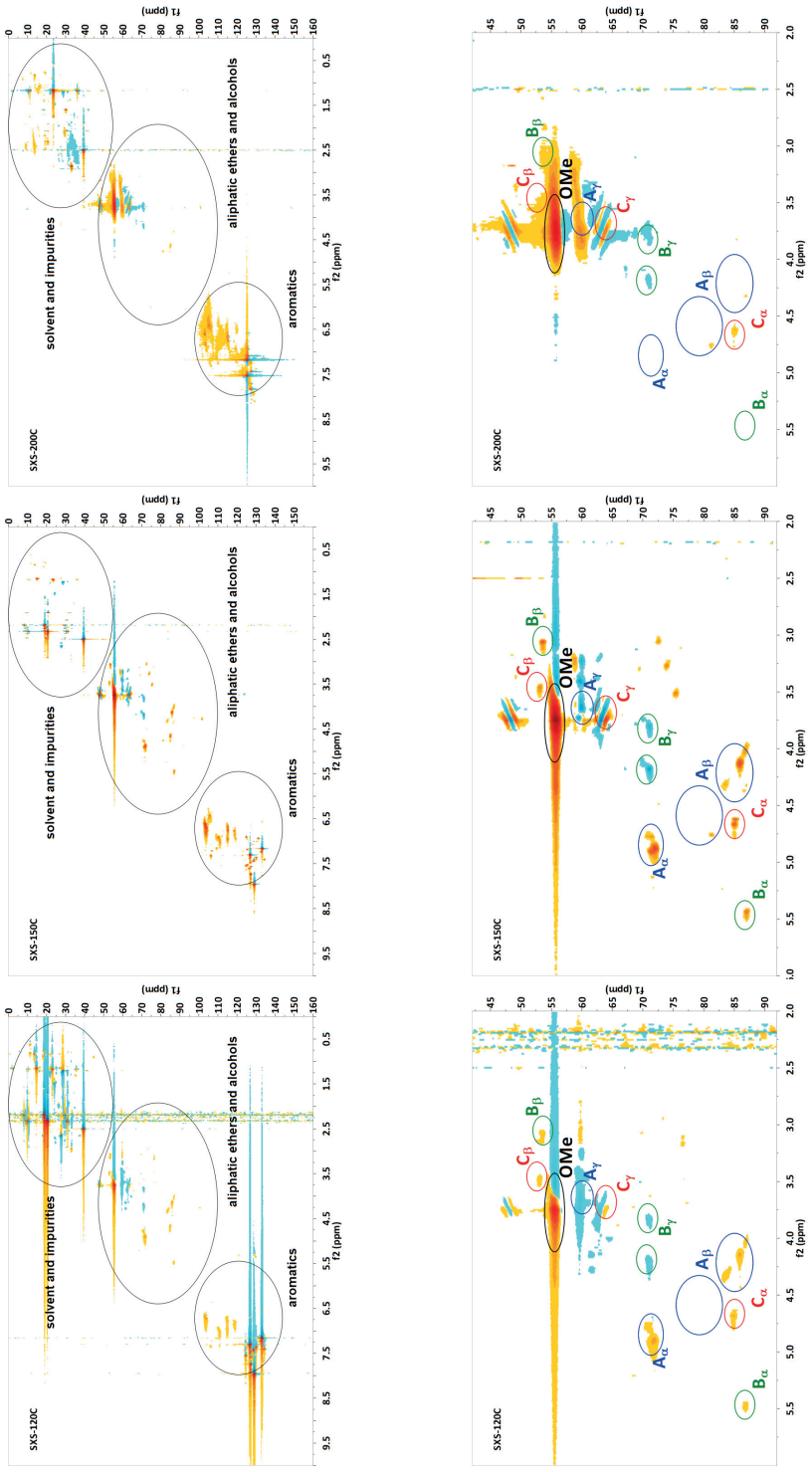


Figure S12: 2D-NMR of hydrotropic lignin (SXS) for pretreatment temperatures of 120, 150 and 200 ℃.

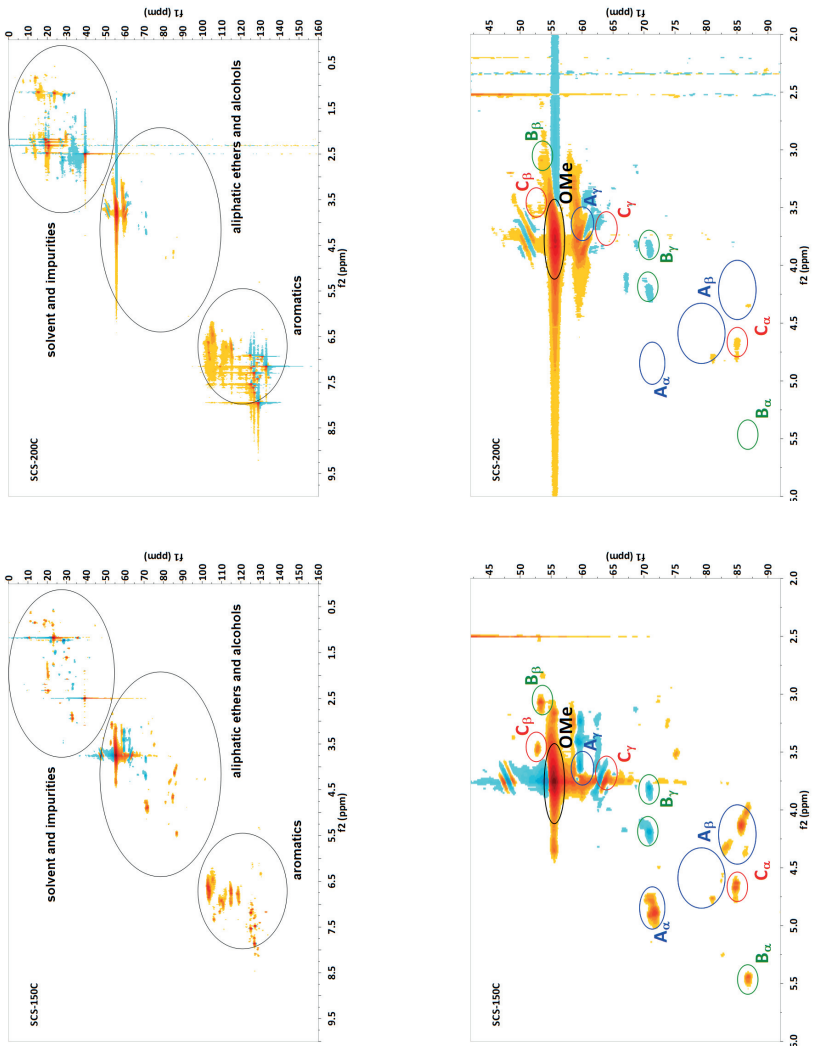


Figure S13: 2D-NMR of hydrotropic lignin (SCS) for pretreatment temperatures of 150 and 200 °C.

References

(1) Di Marino, D.; Jestel, T.; Marks, C.; Viell, J.; Blindert, M.; Kriescher, S. M. A.; Spiess, A. C.; Wessling, M. Carboxylic Acids Production via Electrochemical Depolymerization of Lignin. *ChemElectroChem* **2019**, *6*, 1434–1442.

(2) Dabral, S.; Mottweiler, J.; Rinesch, T.; Bolm, C. Base-catalysed cleavage of lignin β-O-4 model compounds in dimethyl carbonate. *Green Chemistry* **2015**, *17*, 4908–4912.