Green Chemistry

Biphasic extraction of mechanocatalytically-depolymerized lignin from water-soluble wood and its catalytic downstream processing

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Supporting Information

Chemicals

Pellets from beechwood were comminuted with a blender. The sawdust was sieved. Powders with a particle size smaller than 250 µm were collected and used for the mechanocatalytic experiments. Sulfuric acid (95–97%, J. T. Baker), α-cellulose (76% glucans, 16% xylans, 6% humidity, 0.1% ash, an 1.9% others, Aldrich), Diethyl ether (99%, Aldrich), Tetrahydrofuran (Aldrich, 99.9%); 1,4-Dioxane (Aldrich, 99.8%); Ethane thiol (Aldrich, 97%); *N*,*O*-*bis*(trimethylsilyl)trifluoroacetamide (Aldrich, 99%); Boron trifluoride diethyl etherate (Aldrich, for synthesis); Pyridine (Aldrich 99.8%); Dichloromethane (aldrich, 99%), 2-Propanol (Aldrich, 99.8%), RANEY®Ni 2800 (aldrich), Dibutyl ether (Aldrich, ≥99%), 2-Methyltetrahydrofuran (Aldrich, ≥99%, Inhibitor-free) and Ethyl acetate (Aldrich, 99%) were used as received.

Wet impregnation with an H_2SO_4 solution in diethyl ether

Beechwood was suspended in a dilute diethyl ether solution of H_2SO_4 (150 mL); note: to avoid degradation of the substrates upon prolonged contact with the acidic solution, 0.065 mol L^{-1} H_2SO_4 solution was chosen for the acid impregnation. The suspension was shaken for 1 h (IKA shaker, KS 130 control, 350 rpm), and the organic solvent was removed under reduced pressure at 40 °C. A fine powder of loose particles was obtained. This procedure led to an acid loading of 0.8 ± 0.1 mmol H_2SO_4 per gram of substrate. The powder was immediately processed in a ball mill or stored in a closed vial and kept in a freezer (−10 °C) to prevent substrate decomposition that would normally occur to form a gray-black powder after several days of storage at room temperature.

Mechanocatalytic depolymerization

The mechanocatalytic depolymerization of lignocellulose was performed in a stainless steel vial (12 mL; 5 stainless steel balls of 4 g each) using a planetary ball mill (Fritsch, Pulverisette P7). The acid-impregnated substrate (1 g) was processed at 800 rpm. Full conversion of beechwood was achieved at a milling duration of 2 h. The mill was switched off every 0.5 h for 10 min to avoid overheating and thermal decomposition of the sample. The product was collected and kept in an air-tight vial at −10 °C prior to analysis or saccharification experiments.

Saccharification of 'water-soluble' beechwood in a biphasic system

1 g of depolymerized sample (H_2SO_4-impregnated samples milled for 2 h) was solubilized in H_2O/2-MeTHF (50:50 *vol*/*vol*) (10 mL). In a closed glass vial, the beechwood solution (10 mL) was heated at 145 °C for 1 h, whereupon separation of the solvents occured. The organic layer was separated from the water layer and was washed with three aliquots of water. Subsequently, 2-MeTHF was evaporated under reduced pressure at 50 °C. The aqueous solutions were set aside for HPLC analysis.

Extraction of lignin from beechwood (organosolv lignin)

Beechwood (16–17 g) was suspended in ethanol–water (50:50 *vol*/*vol)* (140 mL) in a 250 mL autoclave equipped with a mechanical stirrer. The suspension was processed at 180 °C for 3 h. In sequence, the mixture was left to cool down to room temperature. A reddish-brown solution was obtained after filtering off the lignocellulose fibers (pulp). Ethanol was partially evaporated at 60 °C using a rotoevaporator, leading to lignin precipitation. The solid was collected by filtration and was resuspended in hot water in order to remove hemicellulose sugars. Subsequently, the suspension was filtered and the solid was washed several times with hot water. Finally, the organosolv beechwood lignin was dried in an oven at 40 °C for 1 day.

Elemental analysis

CHNS/O elemental analyses were performed in triplicate for samples (2 mg) on a Vario Micro cube elemental analyzer. Average values of the triplicate measurements are reported.

Gel Permeation Chromatography (GPC)

To analyze the apparent molecular weight distributions, all samples (2–4 mg) were dissolved in THF (2 mL) and were filtered prior to injection. GPC analyses were performed at 60 °C on a Perkin–Elmer HPLC 200 apparatus equipped with four columns (2 × TSKgel Super HZ1000; TSKgel Super HZ2000; TSKgel Super HZ3000, 4.6 mm × ID 15.0 cm, Tosoh Bioscience), using inhibitor-free THF as the eluent (0.4 mL min^{-1}, S4 Aldrich). For detection, an ELSD detector was used. The ELSD response was normalized to 1. Apparent molecular weights are quoted relative to polystyrene standards (200 to 60,000 Da, Aldrich). GPC analyses were performed in triplicate for each sample, leading to very similar results. The chromatograms in Figure 2 show single experiments.

Solution-state NMR experiments

All spectra were acquired at 25 °C with a Bruker AV spectrometer (400 or 500 MHz 1H frequency) equipped with a BBFO probe head with z-gradient. Spectral widths of 20 ppm were used for the 1D ^{1}H spectrum. The relaxation delay for the 1D ^{1}H spectrum was 5.0 s following a 30-degree excitation pulse. For the 1D inverse-gated ^{13}C spectrum, the relaxation delay was set to 1.0 s following a 30-degree excitation pulse. ^{1}H-decoupling with the Waltz-16 sequence was applied during acquisition. The number of collected points was 64k for ^{1}H and for ^{13}C. The 1D ^{1}H spectra were processed using an exponential weighting function (lb 0.2 Hz) prior to Fourier transform. The 2D HSQC NMR (Bruker standard pulse sequence "hsqcetgpsi" with delay optimized for $^{1}J_{CH}$ of 145 Hz) were set up with spectral widths of 20 ppm and 180 ppm for ^{1}H- and ^{13}C-dimensions, respectively. The number of collected complex points was 2048 for ^{1}H-dimension with a recycle delay of 3.13 s (3.0 s relaxation delay and 0.13 s acquisition time). The number of transients for the HSQC spectra was between 12 and 24, and 512 time

increments were recorded in ^{13}C-dimension resulting in an overall experiment time of 6 to 12 h. For HSQC experiments, a squared cosine-bell apodization function was applied in both dimensions, followed by zero-filling to 1024 points in the ^{13}C-dimension prior to Fourier transform. The 1D ^{1}H NMR and 2D HSQC NMR spectra were processed using MestReNova 8.1.1 software. Noteworthy, HSQC spectrum data must be interpreted with caution, since the $^{1}J_{CH}$ dependence of polarization transfer in HSQC experiments is not suppressed in regular HSQC pulse sequences. As a result, the absolute intensity of cross peaks is not fully quantitative in the entire spectral range. Regular HSQC NMR experiments still offer extremely valuable (direct) semiquantitative information for characterization and comparison of lignins as well as whole plant cell compositions. Semiquantitative determination of volume integral ratios is possible for ^{1}H–^{13}C pairs in a similar chemical environment (*e.g.* Cα–Hα signals for the side-chain of lignin units or the C_2–H_2 and C_6–H_6 signals for lignin aromatic units), due to the fact that the $^{1}J_{CH}$ values for the specific entities are reasonably similar. Accordingly, for the different regions of the HSQC spectra, semiquantitative analysis was performed separately by integration of ^{1}H–^{13}C pairs of interest. The contents of G- and S-units, and S:G ratios, were quantified from single experiments.

Thioacidolysis of the lignin samples

The thioacidolysis reagent was prepared immediately before use; 2.5 mL of BF_3 etherate and 10 mL of ethane thiol were successively introduced into a 100 mL volumetric flask containing 20 mL of 1,4-dioxane and the final volume was adjusted to 100 mL with 1,4-dioxane. To the lignin samples (10 mg), 10 mL of thioacidolysis reagent was added in a tube fitted with a Teflon-lined screwcap under an atmosphere of argon. The thioacidolysis was allowed to proceed at 100 °C (in an oil bath) for 4 h with occasional shaking. The reaction tube was cooled in an ice bath and the reaction mixture, together with water to rinse the tube (3 × 5 mL), was poured over CH_2Cl_2 (analytical grade) to which the gas chromatography (GC) internal standard (hexadecane, 4 mg) had been added. The pH of the aqueous (upper) phase was adjusted to 3–4, as indicated by pH indicator paper, by the addition of aqueous 0.4 M $NaHC0_3$, and the two phases were together extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, and the solvent was then evaporated under reduced pressure at 40 °C. The oily residue was carefully redissolved in 0.5–1 mL of CH_2Cl_2 to recover a solution appropriately diluted for the following silylation and GC steps. 10 µL of this dried organic solution was trimethylsilylated (TMS) at room temperature with 50 µL of *N*,*O*-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA) and 5 µL of GC-grade pyridine in a 200 µL reaction vial fitted with a Teflon-lined screwcap, before GC analysis. GC×GC-FID analysis was performed in triplicate for each sample, leading to very similar results. The semiquantitative analysis reported in Table 2 refers to single experiments.

Hydrogenation of E-L, OS-L and MCP-L

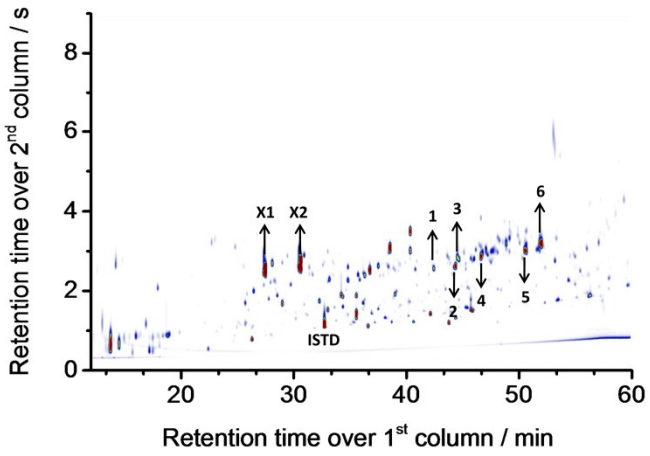
E-L, OS-L or MCP-L (0.5 g), Raney Ni (dry 0.15 g) and 2-PrOH (15 mL) were charged in an autoclave equipped with mechanical stirrer, under an atmosphere of argon. After purging the reaction vessel with H_2, the reactor was loaded with 70 bar H_2 (25 °C). The autoclave was heated to 200 °C. After 8 h at 200 °C, the autoclave was quenched in an ice-bath. The suspension was filtered using a pre-weighed Teflon filter. The filtrate (liquid product) was collected and the solvent was evaporated with a rotary evaporator at 40 °C. Raney Ni was digested with a 5 mol L^{-1} aqueous hydrochloric acid (HCl) solution, thus enabling determination of the amount of

unconverted solid lignin (solid residue). Reactions were performed in triplicate for each sample, leading to very similar results. The results reported in Figure 4 are average values.

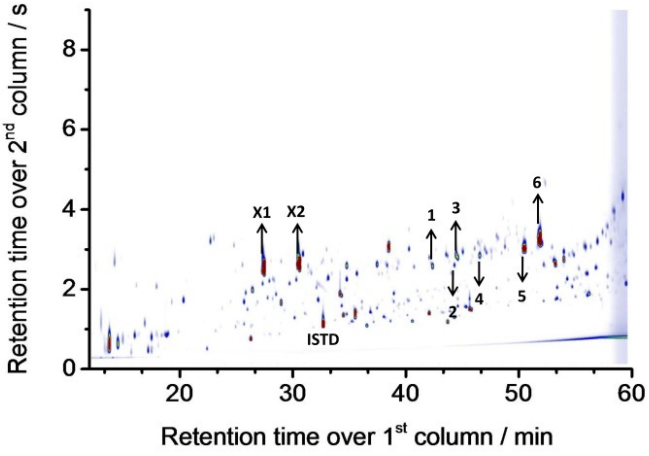
GC×GC-MS/FID Analysis

Lignin samples after hydrogenolysis and thiacidolysis were analyzed using 2D GC×GC-MS/FID spectrometry (first column: ZB-1HT 30 m, 0.25 mm ID, df 0.25 µm; second column: BPX50, 1 m, 0.15 mm ID, df 0.15 µm) in a GC-MS 2010 Plus (Shimadzu) apparatus equipped with a ZX1 thermal modulation system (Zoex). The injector temperature was 280 °C. The temperature program started with an isothermal step at 40 °C for 5 min. Next, the temperature was increased from 40 to 300 °C at a rate of 5.2 °C min^{-1}. The program finished with an isothermal step at 300 °C for 5 min. The modulation applied for the comprehensive GC×GC analysis was a hot jet pulse (400 ms) every 9000 ms. 2D chromatograms were processed with GC Image software (Zoex). The products were identified by a search of the MS spectrum with the MS libraries NIST 08, NIST 08s, and Wiley 9. In some cases, the structure was proposed by the analysis of the EI fragmentation pattern and by comparison of retention times with other samples. Semi-quantification of products was performed using GC×GC-FID images.

GC×GC-FID analysis of E-L after thioacidolysis assay (sample was silylated before injection)



GC×GC-FID analysis of OS-L after thioacidolysis assay (sample was silylated before injection)



GC×GC-FID analysis of MCP-L after thioacidolysis assay (sample was silylated before injection)

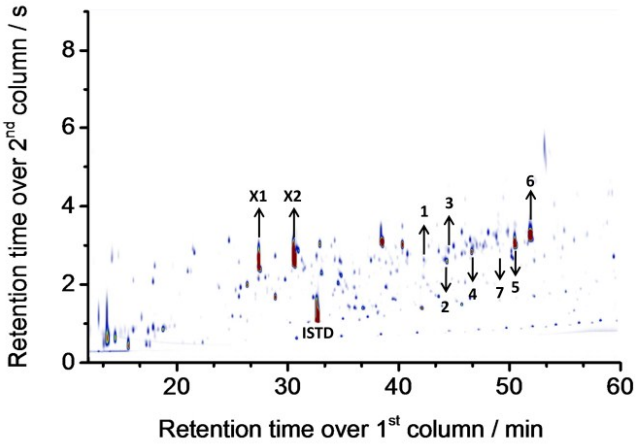


Table 1. Yields of thioacidolysis products (µmol/g lignin)

Substrate	E-L (µmol/g lignin)	MCP-L (µmol/g lignin)	OS-L (µmol/g lignin)
1	25.7	3.4	30.6
2	64.9	15.0	9.3
3	41.1	5.5	64.7
4	65.2	16.8	20.1
5	75.0	113.2	221.4
6	145.0	223.1	662.1
7	-	1.47	-