Electronic Supplementary Information

Effective Fractionation of Lignocellulose in Herbaceous Biomass and Hardwood Using a Mild Acetone Organosolv Process

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Contents

Composition organosolv pulp and liquor

Compositional analysis was performed as described in the experimental section of the article.

Table S1 Composition of pulp.

(%dw)	Carbohydrates						Lignin[1]	Ash	Sum
	Arabinan	Xylan	Galactan	Glucan	Mannan	Rhamnan			
Paper section 1: Fractionation									
Wheat straw	[2]	1.6±0.0		60.0±0.4			7.0±0.1	23.4±0.3	92.0
Corn stover		3.8±0.0	0.2±0.0	64.5±0.3			6.5±0.1	19.0±0.1	94.0
Beech		5.1±0.2		72.3±0.6	0.8±0.0		10.8±0.5	0.9±0.2	90.0
Poplar		1.2±0.0		77.5±0.1	1.2±0.1		10.0±0.0	0.9±0.1	90.7
Birch		3.7±0.0		78.3±0.3	0.7±0.1		6.9±0.1	0.0±0.0	89.6
Spruce		0.8±0.0		57.8±0.1	1.6±0.0		31.6±0.4	0.2±0.0	92.0
Pine		0.9±0.0		59.3±0.7	1.8±0.1		29.3±0.0	0.0±0.0	91.4
Paper section 2: Acetone self-condensation									
1		4.1±0.1		58.6±0.2			7.8±0.2	20.5±0.1	90.9
2		1.6±0.0		60.0±0.4			7.0±0.1	23.4±0.3	92.0
3		3.1±0.0	0.3±0.0	63.1±0.2			6.7±0.1	21.5±0.1	94.7
4		1.8±0.0	0.3±0.0	61.5±0.0			7.1±0.2	23.4±0.1	94.0
5		1.7±0.0		62.3±1.2			6.6±0.0	24.0±0.3	94.6
6		2.9±0.0		59.2±0.3			7.0±0.1	23.2±0.1	92.3

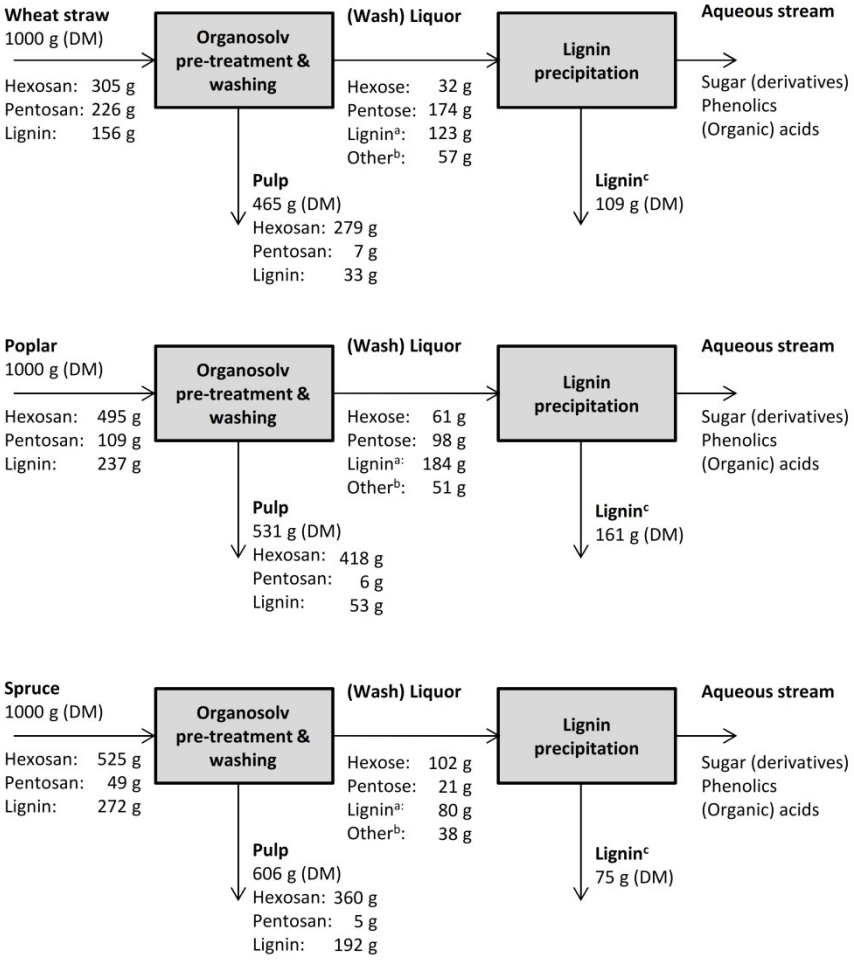
[1)] Sum of acid-insoluble and acid-soluble lignin, [2)] Empty cell: below detection limit.

Table S2 Composition combined organosolv liquor and wash liquor.

(mg/kg)	Arabi-nose	Xylose	Galac-tose	Glu-cose	Man-nose	Rham-nose	Furfu ral	Hydroxy-methyl furfural	Acetic acid	Formic acid	Levulinic acid
Paper section 1: Fractionation											
Wheat straw	1867	15132	788	2164	[1]	151	2051	536	2480	494	
Corn stover	1875	14056	981	2203		97	1881	324	2332		
Beech	1162	27291	1692	2550		929	1608	230	9785	1194	
Poplar	785	19405	1710	5896	4449	537	2008	284	7377	875	
Birch	580	25177	1021	2567		620	3425	151	9240	830	
Spruce	1215	3391	2426	9975	9364	187	1648	1385	3615	1055	357
Pine	1136	2986	1987	12810	7651	119	1794	1640	3835	1254	315
Paper section 2: Acetone self-condensation											
1	2283	15350	760	1753		161	1189	339	2636	434	274
2	1867	15132	788	2164		151	2051	536	2480	494	
3	2013	19375	821	2001		147	1403	127	2618		
4	1228	10347	506	1345		97	1097	81	1495		
5	1436	12699	609	1602		103	1320	96	1870		
6	1687	5580	362	1191	405	56	3246	656	2062	592	193

[1)] Empty cell: below detection limit.

Mass balance organosolv pretreatment



[a)] Lignin (derivatives) content determined by delignification of the feedstock. [b)] Sum HMF, furfural, levulinic, acetic and formic acid.
[c)] Lignin yield as determined by dilution of the combined liquor with water (4 °C, 4:1 w/w dilution ratio H_2O:liquor).

Fig. S1 Mass balance of organosolv pretreatment of three selected feedstocks.

Enzymatic hydrolysis organosolv pulps

Enzymatic hydrolysis was performed as described in the experimental section of the paper.

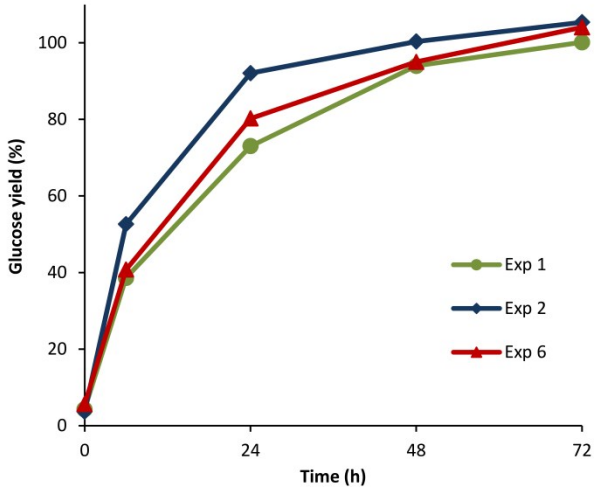


Fig. S2 Enzymatic hydrolysis of organosolv pulps from the acetone self-condensation section of the paper. Glucose yield based on glucan content of pulps.

Pretreatment conditions:

- Exp. 1: 100 °C, 960 min, 200 mM H_2SO_4
- Exp. 2: 140 °C, 120 min, 60 mM H_2SO_4
- Exp. 6: 170 °C, 60 min, 35 mM H_2SO_4

NMR analysis of wheat straw acetone organosolv lignin

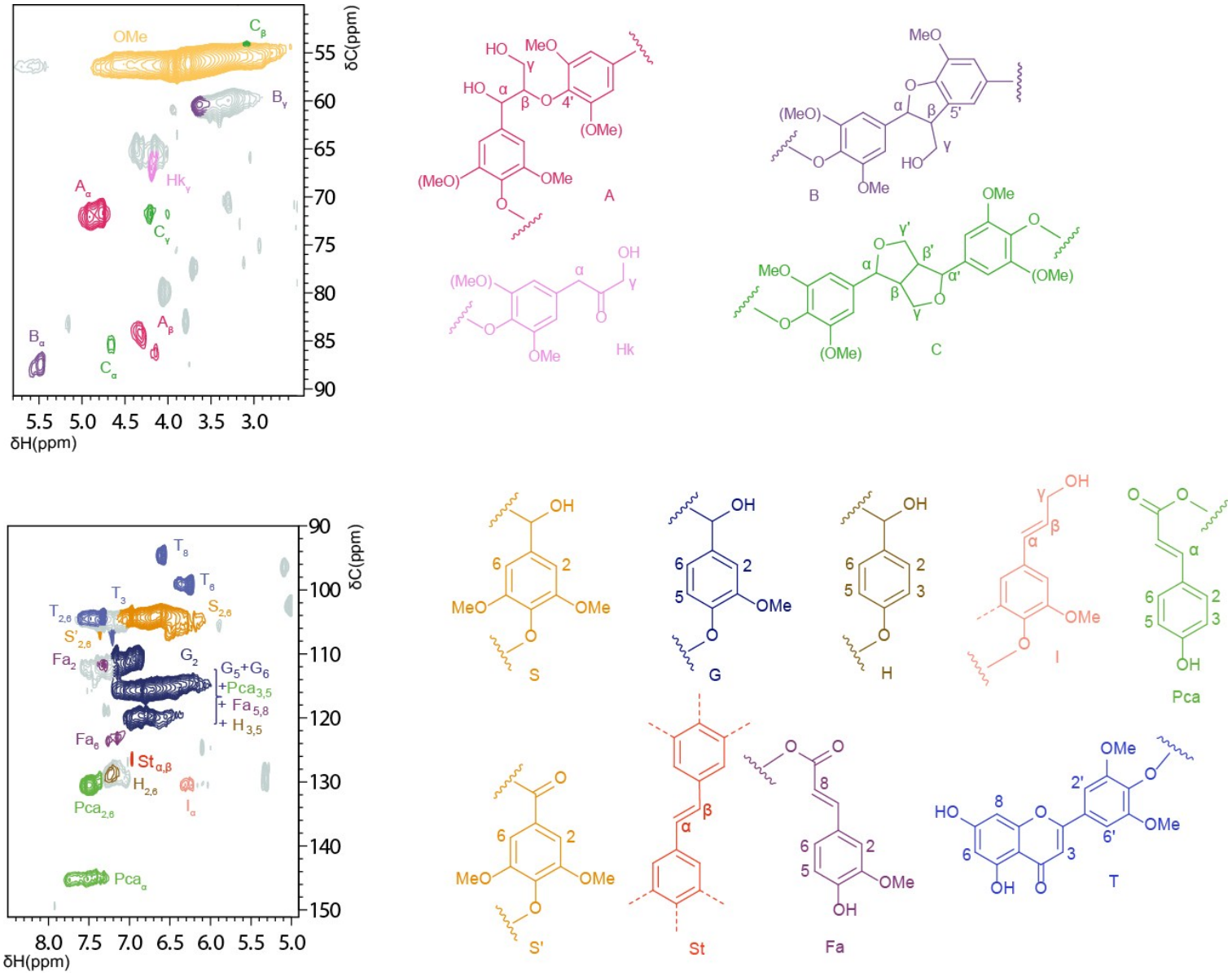


Fig. S3 2D HSQC NMR spectrum of wheat straw lignin obtained by mild acetone organosolv fractionation (140 °C, 60 min, 50% w/w aqueous acetone, 60 mM sulfuric acid, 10 L/kg DM). 2D NMR was performed as described in Constant et al. (2016). Courtesy of Utrecht University.

Molar mass distribution of lignins

Lignins obtained from wheat straw fractionation experiments (acetone self-condensation section of the paper) were analysed for molar mass using size exclusion chromatography (SEC) as described in Constant et al. (2016) method B. Mw = weight-average molar mass.

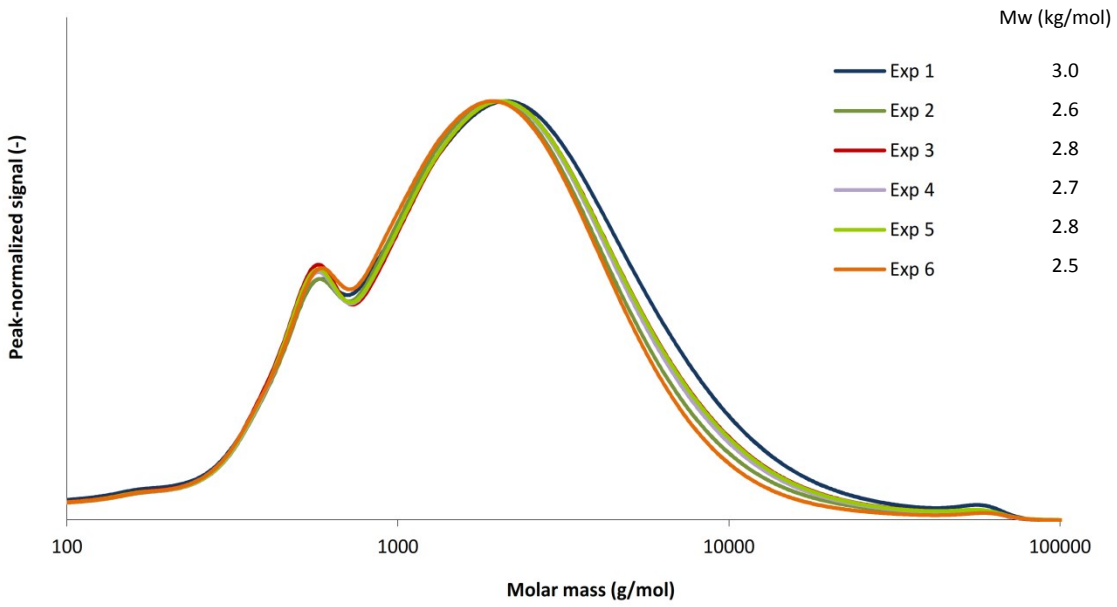


Fig. S4 SEC analysis of wheat straw lignins.

Fixed-bed organosolv experiments

Method: Fixed-bed organosolv experiments were performed using an Accelerated Solvent Extractor system (ASE™ 350, ThermoScientific™ Dionex™), an automated system for the extraction of solid and semi-solid samples at elevated temperature and pressure. A 100 mL stainless steel module was filled with 20 g dw of feedstock. The ASE 350 in standard operation mode placed the module in a 140 °C preheated oven and added premixed reaction liquid comprising of 50% w/w aqueous acetone and 40 mM H_2SO_4 until the pressure inside the module reached 100 bar. After preheating for 7 min, the module was kept isothermal for 10 min after which the liquor was purged from the module with nitrogen for 1 min. The cycle was repeated to obtain 6 liquid samples in total (approx. 50-55 g each). Thus, the feedstock was fractionated for 6 times 10 minutes (excluding the time to preheat the module). A sample of the liquor was post-hydrolysed in 1M H_2SO_4 for 120 min at 100 °C to hydrolyse oligomeric carbohydrates to monomeric sugars. The hydrolysate was analysed for monomeric sugars using HPAEC-PAD and the dissolved lignin was precipitated as described in the experimental section of the article.

Results: Figure S5 shows the fractionation data of wheat straw, beech and pine obtained from fixed-bed organosolv pretreatment experiments. Generally, wheat straw fractionation is lagging behind in the first 10 min as compared to the woody feedstocks, probably due to its higher acid neutralising capacity.

Straw, beech and pine pulp yield was 50.2, 44.2 and 59.1% respectively. Glucan conversion to (oligomeric) glucose is highest for pine and most likely originates from the galactoglucomannan present in the hemicellulose fraction of softwood. In addition, a higher glucose yield was observed for wheat straw compared to beech. Hemicellulose xylan hydrolysis to (oligomeric) xylose is similar for the three feedstocks after 30 min reaction time (or 3 runs).

Lignin precipitated from the liquor, presented as fraction of total lignin present in the feedstock, is similar for straw and beech. Pine shows a lower lignin yield as compared to straw and beech which is in line with the low delignification rates found for spruce and pine, as described in the paper.

The weight-average molar mass of the lignins (fig. S6) increased from 2.0 kg/mol in the first fraction to 3.6 kg/mol in the fourth fraction. Fractional isolation of lignin from lignocellulose can yield lignin fractions with different characteristics, including molar mass, given that lignin is a heterogeneous macromolecule which occurs in different compositions within various parts of the plant. Therefore, it is unclear to what extent molar mass distribution of lignins, as shown in fig. S6, is determined by process conditions, including possible condensation reactions between lignin and solvent, or its origin in the biomass.

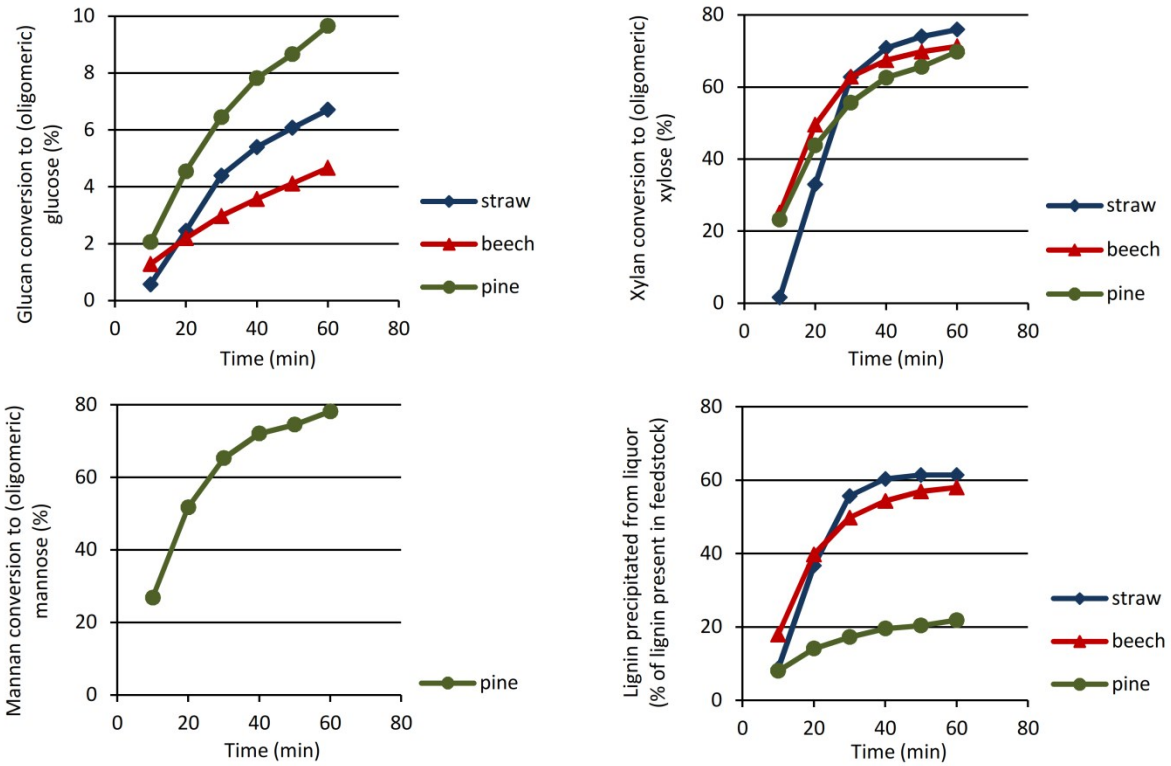


Fig. S5 Fixed-bed organosolv fractionation data of wheat straw, beech and pine.

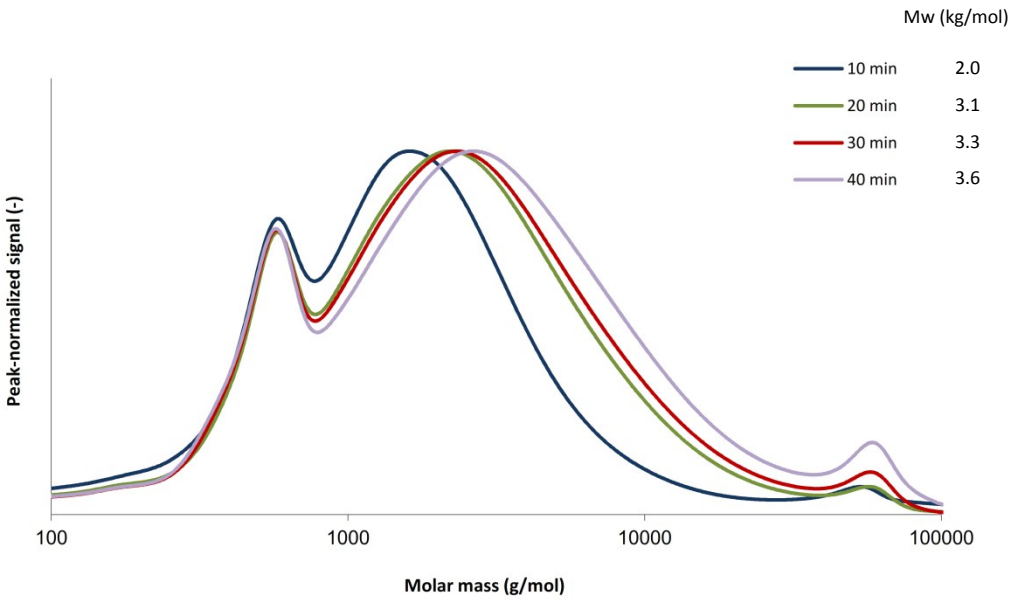


Fig. S6 Size exclusion chromatography (SEC) analysis of wheat straw lignins as precipitated from fixed-bed organosolv experiments at various reaction times. Mw = weight-average molar mass.

References

- S. Constant, H. L. Wienk, A. E. Frissen, P. de Peinder, R. Boelens, D. S. van Es, R. J. H. Grisel, B. M. Weckhuysen, W. J. J. Huijgen, R. J. A. Gosselink, P. C. A. Bruijnincx, Green Chem., 2016, 18, 2651-2665.