Electronic Supplementary Information

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

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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 | Ash | Sum |
|-------|---------------|--------|-----|-----|
| Paper section 1: Fractionation | | | | |
| Wheat straw | 1.6±0.0 | 60.0±0.4 | 7.0±0.1 | 23.4±0.3 | 92.0 |
| Corn stover | 3.8±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 | 90.0 |
| Poplar | 1.2±0.0 | 77.5±0.1 | 1.2±0.1 | 10.0±0.0 | 90.7 |
| Birch | 3.7±0.0 | 78.3±0.3 | 0.7±0.1 | 6.9±0.1 | 89.6 |
| Spruce | 0.8±0.0 | 57.8±0.1 | 1.6±0.0 | 31.6±0.4 | 92.0 |
| Pine | 0.9±0.0 | 59.3±0.7 | 1.8±0.1 | 29.3±0.0 | 91.4 |

Table S2 Composition combined organosolv liquor and wash liquor.

| (mg/kg) | Arabinose | Xylose | Galactose | Glucose | Mannose | Rhamnose | Furfural | Hydroxymethylfurfural | Acetic acid | Formic acid | Levulinic acid |
|---------|-----------|--------|-----------|---------|---------|----------|----------|-----------------------|------------|-------------|---------------|
| Paper section 1: Fractionation | | | | | | | | | | | |
| Wheat straw | 1867 | 15132 | 788 | 2164 | 151 | 2051 | 536 | 2480 | 494 |
| Corn stover | 1875 | 14056 | 981 | 2203 | 97 | 1881 | 324 | 2332 | 1194 |
| Beech | 1162 | 27291 | 1692 | 2550 | 929 | 1608 | 230 | 9785 | 1194 |
| Poplar | 758 | 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 | 1897 | 12810 | 7651 | 119 | 1794 | 1640 | 3835 | 1254 | 315 |

Paper section 2: Acetone self-condensation

| 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| 2283 | 15350 | 760 | 1753 | 161 | 1189 | 339 | 2636 | 434 | 274 |
| 1867 | 15132 | 788 | 2164 | 151 | 2051 | 536 | 2480 | 494 |
| 2013 | 19375 | 821 | 2001 | 147 | 1403 | 127 | 2618 | 1194 |
| 1228 | 10347 | 506 | 1345 | 97 | 1097 | 81 | 1495 | 830 |
| 1436 | 12699 | 609 | 1602 | 103 | 1320 | 96 | 1870 | 1055 | 357 |
| 1687 | 5580 | 362 | 1191 | 405 | 56 | 3246 | 656 | 2062 | 592 | 193 |

1i Sum of acid-insoluble and acid-soluble lignin, 2i Empty cell: below detection limit.
Mass balance organosolv pretreatment

- Wheat straw
  - 1000 g (DM)
  - Hexosan: 305 g
  - Pentosan: 226 g
  - Lignin: 156 g
  - Organosolv pre-treatment & washing
    - Pulp 465 g (DM)
      - Hexosan: 279 g
      - Pentosan: 7 g
      - Lignin: 33 g
    - (Wash) Liquor
  - Lignin precipitation
    - Lignin\(^a\): 109 g (DM)
      - Hexose: 32 g
      - Pentose: 174 g
      - Lignin\(^b\): 123 g
      - Other\(^c\): 57 g
    - (Sugar derivatives) Phenolics (Organic) acids

- Poplar
  - 1000 g (DM)
  - Hexosan: 495 g
  - Pentosan: 109 g
  - Lignin: 237 g
  - Organosolv pre-treatment & washing
    - Pulp 531 g (DM)
      - Hexosan: 418 g
      - Pentosan: 6 g
      - Lignin: 53 g
    - (Wash) Liquor
  - Lignin precipitation
    - Lignin\(^d\): 161 g (DM)
      - Hexose: 61 g
      - Pentose: 98 g
      - Lignin\(^e\): 184 g
      - Other\(^f\): 51 g
    - (Sugar derivatives) Phenolics (Organic) acids

- Spruce
  - 1000 g (DM)
  - Hexosan: 525 g
  - Pentosan: 49 g
  - Lignin: 272 g
  - Organosolv pre-treatment & washing
    - Pulp 606 g (DM)
      - Hexosan: 360 g
      - Pentosan: 5 g
      - Lignin: 192 g
    - (Wash) Liquor
  - Lignin precipitation
    - Lignin\(^g\): 75 g (DM)
      - Hexose: 102 g
      - Pentose: 21 g
      - Lignin\(^h\): 80 g
      - Other\(^i\): 38 g
    - (Sugar derivatives) Phenolics (Organic) acids

\(^a\) 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\(_2\)O: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$_2$SO$_4$
- Exp. 2: 140 °C, 120 min, 60 mM H$_2$SO$_4$
- Exp. 6: 170 °C, 60 min, 35 mM H$_2$SO$_4$
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$_2$SO$_4$ 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$_2$SO$_4$ 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.

| Mw (kg/mol) | Time (min) |
|-------------|------------|
| 2.0         | 10 min     |
| 3.1         | 20 min     |
| 3.3         | 30 min     |
| 3.6         | 40 min     |

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.
- 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. Bruijinincx, Green Chem., 2016, 18, 2651-2665.