Highly efficient semi-continuous extraction and in-line purification of high β-O-4 butanosolv lignin

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1. Methods

Flow-through setup

The flow-through system consist of a glass solvent reservoir (800 mL), pneumatic oscillatory pump (Williams; P250V225), pressure indicator (0-400 bar), GC oven (HP, 5890 series II), reactor (100 mL, cylindrical (length: 20 cm, diameter: 2.6 cm), SS 316L), Type K thermocouple with indicator, particulate filter (Swagelok, ¼ in. tube fitting, 2 micron pore size), adjustable back pressure regulator (Gommer B.V., BP-3 series, SS 316L) with pressure indicator (0-40 bar) and precision balance (Kern, PCB 2500-2). These units are connected by tubes and fittings (Swagelok, SS-316L, ½ and ¼ in.). Both openings of the reactor are closed with a glass filter (custom made, sintered, P2).

Continuous integrated mixer/seperator (CINC)

The CINC, a 3535-B Arrowhead Drive (2002) has a max rpm of 6000, max throughput of 2 L/min, 200 g filling mass and 0-100 °C temperature range and is connected to the extraction liquor output with a 2 mm silicon tube.

Figure S1: Schematic representation of the applied CINC setup (Schuur et al., 2008).

Synthesis of (2R,S,3R,4S,5R)-2-butoxytetrahydro-2H-pyran-3,4,5-triol (butyl xyloside): 10 g of D-xylose was heated at reflux conditions in a 90% n-BuOH/10% H₂O mixture containing 0.18 M H₂SO₄ for 2 hours. The reaction mixture was concentrated in vacuo to give the desired product as a viscous yellow oil which was characterized by NMR-spectroscopy without further purification.

2D Heteronuclear Single Quantum Coherence (HSQC) NMR spectroscopy: Structural analysis was performed by 2D HSQC NMR spectroscopy. 60 mg of butanosolv lignin was dissolved in 0.7 mL of d₆-acetone. A few drops of D₂O were added in order to ensure complete dissolution of the lignin. The NMR measurement was performed on a Bruker Ascend Neo 600 using the following parameters: (F2 = 11 to -1 ppm), (F1 = 160 to -10 ppm), nt = 4, ni = 512, d1 = 1.5, CNST [2] = 145 and the applied pulse sequence is hsqctgpsi2. Analysis was
performed with Mestrenova. The obtained values for the detectable linking motifs were divided by a factor of 1.3 as previous work showed that the 2D HSQC NMR measurement overestimated these values (Zijlstra et al., 2019b).

**31P-NMR Spectroscopy:** Hydroxyl content analysis was performed by 31P-NMR spectroscopy using a literature procedure (Constant et al., 2016). 50 mg of lignin was dissolved in a 1.6:1 pyridine/CDCl₃ mixture (1.5 mL). 200 μL of an endo-N-hydroxy-5-norbornene-2,3-dicarboximide stock solution (9.2 mg/mL) was added as internal standard and 50 μL of a Chromium acetylacetonate stock solution (5.6 mg/mL) was added as relaxation agent. Afterwards, 100 μL 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane was added as a derivatizing agent (Constant et al., 2016).

**Gel Permeation Chromatography (GPC):** The molecular weight of the isolated butanosolv lignin was determined by gel permeation chromatography (GPC) analysis. The analysis was carried out on a Hewlett Packard 1100 series THF-GPC. The lignin samples (10 mg) were dissolved in THF with toluene as a flow marker. Prior to analysis, the samples were filtered with a syringe filter (0.45 μm, PTFE). Analysis was performed with PSS WinGPC UniChrom.

**Biomass analysis:** Analysis of the carbohydrate and lignin content of the biomass sources and residual samples were performed following the NREL methodology (Sluiter et al. 2008). A 1,000 g dried raw biomass or residual biomass sample was added to a round bottom flask and 10 mL of a 72% aqueous H₂SO₄ was added. The resulting solution was incubated at 30 °C for 1 hour. After 1 hour, 280 mL of deionized water was added in order to obtain a 4% aqueous H₂SO₄ solution. The mixture was heated at reflux conditions for 1 hour and afterwards allowed to cool down to room temperature. Acid insoluble lignin was obtained by vacuum filtration and dried overnight in a vacuum oven at 60 °C. The amount of acid soluble lignin was determined by UV-VIS spectroscopy, using a background of a 4% sulfuric acid in deionized water. The samples were diluted in order to obtain an absorbance in the range of 0.7 – 1.0 at wavelength 240 nm.

The carbohydrate composition of the original material and the obtained residues (oven dried) was analyzed with HPAEC-PAD after acid hydrolysis (samples were diluted 20 - 50 times before injection). Therefore, 11 mg of each sample was mixed with 0.9 mL 72% (w/w) sulfuric acid and incubated for 1h at 30°C. After that 9.9 mL milliQ water was added, diluting the acid to 1 M. The incubation was continued for 3h at 100°C to fully hydrolyze the carbohydrates into monomers. All monomers were quantified by integrating the peak area of corresponding standards (arabinose, galactose, glucose, glucuronic acid, mannose, rhamnose, and xylose). Total sugar content was calculated as a sum of all neutral sugars and glucuronic acid.

High performance anion exchange chromatography (HPAEC) on a Dionex Ultimate 6000 system (Thermo Scientific, Sunnyvale, CA, USA) equipped with a CarboPac PA-1 column (2 mm x 250 mm ID) in combination with a CarboPac PA-1 guard column (2 mm x 50 mm ID) and PAD detection. System was controlled by the Chromeleon 7.2.9 software (Thermo Scientific, Sunnyvale, CA, USA). Elution of monosaccharides (0.25 mL min-1) was performed with a multi-step-gradient using the following eluents: A: 0.1M NaOH, B: 1M NaOAc in 0.1M NaOH, C: 0.2 M NaOH, and D: milliQ water. All analyzed monosaccharides elute in the first 20 min with 16% A, 84% D. Followed by 5 min with 45%A, 5%B, 50%D and 15 min with 60%A, 40% B. To regenerate the column it was flushed 12 min with 100% C by increasing flowrate in first 2 min to 0.35 mL min-1. Finally, the column is equilibrated for 12 min with 16%A, 84%D by decreasing the flowrate in the first 2 min to 0.25 mL min-1.
Table S1: Structural composition of the applied biomass (starting material) and the composition after organosolv extraction (residual). All values are given as a weight percentage (%w/w). * values are calculated based on the values for the glucose in the starting material and the residual biomass (assuming no loss of glucose) and the values for the lignin extraction efficiency.

|          | Walnut powder | Beech wood | Reed | Douglas Fir | Pine wood |
|----------|---------------|------------|------|-------------|-----------|
| Lignin   | 40            | 25         | 21.5 | 24.9        | 29.8      |
| Carbohydrate | 28.2        | 22.4       | 38.3 | 38.3        | 39.9      |
| Xylose   | 24.8          | 16.1       | 24.2 | 8.7         | 4.6       |
| Mannose  | 0             | 0          | 0    | 15.7        | 5.9       |
| Galactose| 1.4           | 2.7        | 1.4  | 6.2         | 3.9       |
| Arabinose| 2.0           | 3.6        | 12.4 | 7.7         | 25.5      |
| Glucose  | 25.2          | 47.9       | 39.5 | 43.0        | 31.6      |
| Total    | 94.4          | 99.3       | 94.5 | 106.2       | 101.3     |

Table S2: Composition of walnut powder as determined by different analysis methodologies. Values are based on 2D HSQC NMR analysis.

| Particle size fraction (mm) | Weight percentage |
|-----------------------------|-------------------|
| d > 0.425                   | 0                 |
| 0.300 < d < 0.425           | 49                |
| 0.212 < d < 0.300           | 41                |
| d < 0.212                   | 10                |

Table S3: Particle size distribution of walnut powder

Moisture content measurement: The moisture content of the biomass and the isolated lignin was determined with a PCE-MA 110 moisture meter. An ascension program to 120 °C was used during which the weight of the sample is tracked. At 120 °C the measurement is stopped if between 2 iterations the weight change is less than 0.2%. The obtained data was used to correct all yield calculations.

Sankey diagram: The Sankey diagram was made based on the following data and assumptions. The reported values between brackets were determined by the NREL analysis of the biomass. The other values are based on the determined mass of the isolated solid product (corrected for moisture content, butoxy-incorporation and carbohydrate impurities). It is assumed that no loss of product occurs in the separation steps. The reported value for lignin lost is the difference of the isolated lignin and the determined Klason Lignin content. The online tool SankeyMatic was used to generate the Sankey diagrams.
2. Comparison with other extraction procedures

| Group               | Conditions                                         | Biomass source                      | Solvent efficiency (mg lignin extracted /gram solvent) |
|---------------------|----------------------------------------------------|-------------------------------------|-------------------------------------------------------|
| This work           | 9:1 nBuOH/H₂O 0.18 M H₂SO₄, 120 °C                | Walnut                              | 50                                                    |
| Bauer et al.        | 19:1 EtOH/H₂O 0.20 M HCl, 100 °C                  | Miscantus Giganteus                 | 10                                                    |
| Lancefield et al.   | 19:1 nBuOH/H₂O 0.20 M HCl, 120 °C                 | Walnut                              | 32                                                    |
| Luterbacher et al.  | 9:1 1,4-dioxane/formaldehyde 0.10 M HCl, 100 °C   | Beech and transgenic Poplar         | 16                                                    |
| Huijgen et al.      | 1:1 Acetone/H₂O 0.015 M H₂SO₄, 190 °C             | Beech, Poplar, Birch               | 19                                                    |
| Huijgen et al.      | 6:4 EtOH/H₂O 0.015 M H₂SO₄, 190 °C                | Wheat Straw                         | 13                                                    |
| Grande et al.       | 1:1 MeTHF/H₂O 0.05 M FDCA, 160 °C                 | Beech                               | 18                                                    |
| Sun et al.          | 7:3 2-PrOH/H₂O 220 °C                             | Eucalyptus                          | 20                                                    |
| Hensen et al.       | MeOH Varying acids, 160 °C                        | Oak sawdust                         | 9                                                     |
| Ragauskas et al.    | 85:15 1,4-BDO/H₂O 0.015 M H₂SO₄, 170 °C           | Eucalyptus                          | 33                                                    |
| Bacher et al        | 12:44:44 SO₂/Alcohol/Water 155 °C                 | Sugarcane straw                    | 47                                                    |

Table S4: Solvent efficiency of a wide range of lignin extractions, all showing a lower solvent efficiency than our reported extraction procedure in this work. (Bauer et al., 2012; Wildschut et al., 2013; Shuai et al., 2016; Lancefield et al., 2017; Smit and Huijgen, 2017; Wang et al., 2017; Ouyang et al., 2018; Weidener et al., 2018; Dong et al., 2019)
3. Extraction and work-up flow schemes

Figure S2: Schematic representation of the influence of the reactor loading on the biomass level. The small cylindrical tube used as reactor causes an increased contact time of the solvent with biomass, resulting in an increased lignin extraction.
Figure S3: Schematic representation of the flow-schemes of flow-through extraction coupled with the traditional precipitation work-up procedure.
Figure S4: Schematic representation of the flow-schemes of flow-through extraction coupled with biphasic extraction and precipitation.

Figure S5: Isolated lignin obtained with the traditional work-up procedure (top) and the biphasic extraction + precipitation (bottom).
4. Traditional work-up issues

Figure S6: Precipitation of the concentrated butanosolv extraction liquor in water in the absence of sodium sulfate (left) and precipitation with sodium sulfate added to the water (right) which clearly enhances lignin flocculation.

Figure S7: Solubility test of butyl-xyloside in water, clearly showing the mediocre solubility of modified xylose. During the lignin precipitation step part of the butyl-xyloside will also precipitate and be isolated as a solid product.
5. Butyl-xyloside determination

**Figure S8:** 2D-HSQC NMR (d$_6$-acetone) overlap of D-Xylose (red) and butyl-xyloside (green).

**Figure S9:** 2D-HSQC NMR overlap (d$_6$-acetone) of butyl-xyloside (green) and butanosolv lignin (red).
Figure S10: 2D HSQC NMR spectrum (d$_6$-acetone) of the product obtained after basic treatment of butyl-xyloside, showing partial reversion to D-xylose.

| Sample                        | Aromatic -OH content | Aliphatic -OH content | Ratio Aromatic:Aliphatic |
|-------------------------------|----------------------|-----------------------|--------------------------|
| Butanosolv lignin            | 2.65                 | 9.13                  | 1:3.45                   |
| After caustic soda treatment | 2.89                 | 8.93                  | 1:3.09                   |

Table S5: Aromatic -OH and Aliphatic -OH content of butanosolv lignin and prior and after caustic soda treatment (5-substituted -OH was set at 1.00) as determined by $^{31}$P-NMR analysis.
6. Molecular weight of lignin obtained by biphasic separation

![Figure S11: Molecular weight distribution of the organic phase lignin (OPL), CINC residual lignin (CRL) and water soluble lignin (WSL) fraction after biphasic treatment determined by GPC.](image)

| Lignin type                    | Weight average molecular weight (Da) |
|--------------------------------|--------------------------------------|
| Organic phase lignin          | 3900                                  |
| CINC residual lignin          | 6900                                  |
| Water Soluble lignin          | 1900                                  |

*Table S6: Weight average molecular weight of the different lignin isolated with biphasic extraction.*

7. Butanosolv pine wood extraction

![Figure S12: 2D HSQC NMR spectrum (d<sub>6</sub> acetone) of the product obtained after butanosolv extraction of pine wood.](image)
8. Literature

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