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LIGNIN FRACTIONATION IN SEGMENTED CONTINUOUS FLOW

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Abstract

Lignins were fractionated in a segmented continuous flow mode using isocratic or gradient elution profiles of organic solvent systems at various flow rates. Fractionation was achieved at various temperatures against adjustable pressure regimes. Superior control of parameters such a temperature and pressure, in combination with the possibility of freely combinable solvent gradients allows facile fractionation and generation of industrially interesting fractions different in molecular weight properties and /or in physicochemical properties in a process that can be fully remotely controlled for automation and superior performance. Scale-up of the process is possible in linear and parallel mode, allowing for real continuous running. The developed process is compatible and combinable with upstream purification protocols and downstream lignin functionalisation.

Keywords

softwood kraft lignin,

wheat straw organosolv lignin

fractionation

segmented continuous flow

gel permeation chromatography

^{31}P NMR

1 Introduction

Almost one third of the mass of lignocellulosic biomass is comprised of polyphenolic oligomers and polymers, which are for the most part lignins.[1] Being already present in nature with an impressive array of different specifics,[2],[3] industrial processes aimed at isolating the cellulose and hemi-cellulose parts of various types of lignocellulosic biomass further modify the structure of the lignin component. Isolated lignins are thus often obtained as a rather low-quality by-products in biorefinery processes that were optimized with respect only to the cellulose components.[4],[5] Only recently, bigger global players and promising newcomers in the biorefinery business started to view the lignin-containing streams as an additional source for augmented revenues.[5] Consequently, different higher quality lignins are available nowadays for potential applications in sectors of material science, functional cosmetics and eventual biomedical devices:[6] *e.g.*, the LignoBoost lignin, that is obtained by a kraft process that improved the precipitation of lignin after standard kraft pulping,[7] and lignins obtained in industrialized organosolv pulping processes, *e.g.*, Alcell lignin[8] and CIMV Biolignin™.[9] Some characteristic structures of important types of lignins used in this study are shown in Figure 1.

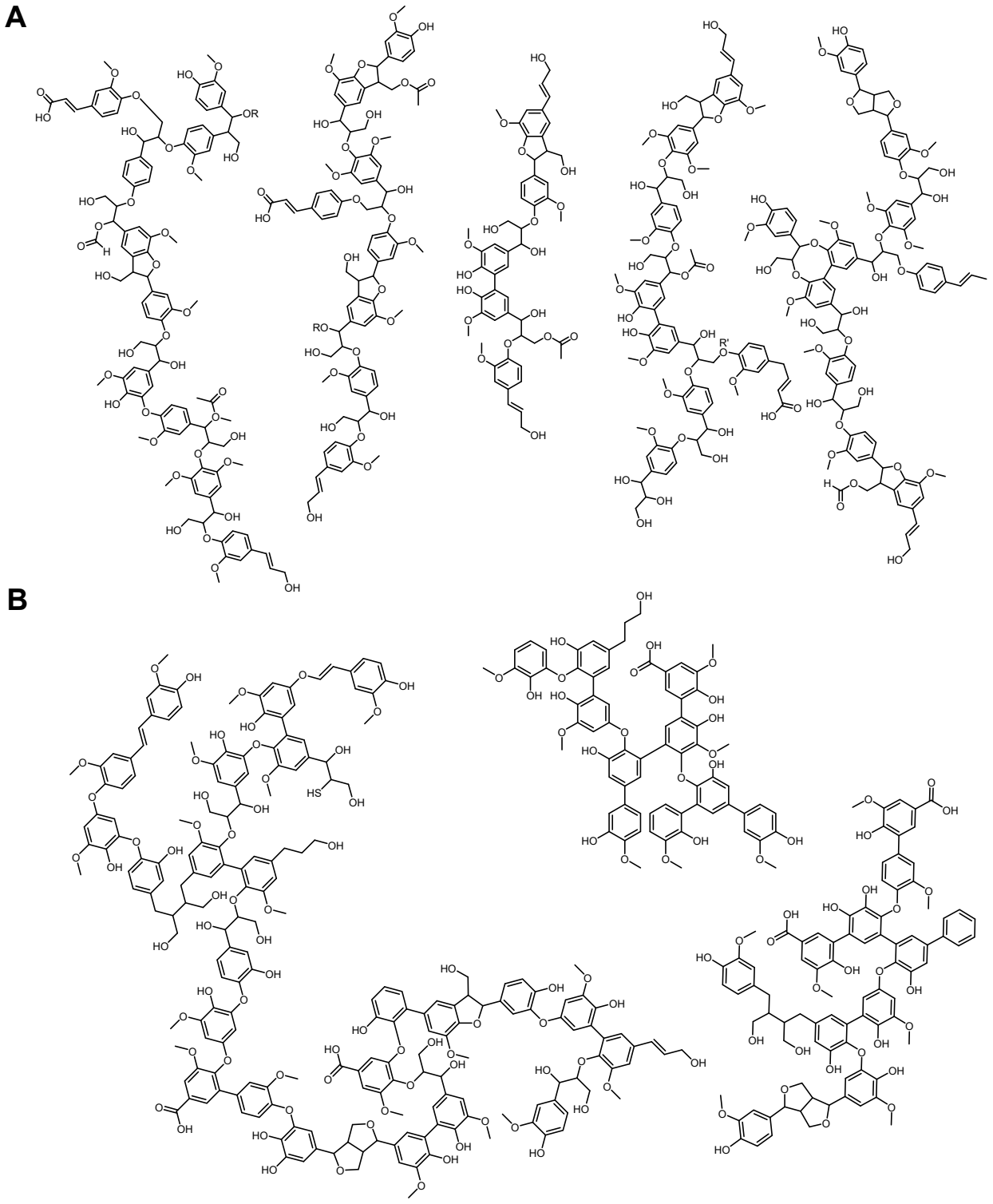

Figure Error! No text of specified style in document.**:** Lignin structures showing characteristic interunit bonding motifs and functional groups for different types of lignin: (A) [10] (A) complex structure of Lignoboost softwood kraft lignin (**KL**).[11] (B) Organosolv CIMV Biolignin™ (**WL**)

Any kind of higher value application, however, calls for a rather detailed structural understanding of the technically produced lignin of choice, and the determination of basic thermal characteristics.[12], Correlating structural features like, *e.g.*, the abundance of free phenolic residues with observed thermal behaviours have proven to be a powerful tool: thermal properties of an industrial lignin, and thereby its processibility in extruder-based applications could be tuned by modifying the concentration of free phenolic hydroxyl groups.[13–17]

While the functional groups without doubt play a significant role with respect to any kind of application, the polymer characteristics as such are important, especially the number average molecular weight (Mn) and the polydispersity (PD); most isolated lignins are characterized by a polydispersity that *a priori* prevents any use in higher value applications, independent of the Mn, which additionally differs significantly depending on the isolation process.[4] A conceptually simple way to arrive at lignins that exhibit, at least, an industrially acceptable polydispersity is fractionation of lignin. This idea of fractionating lignins has been investigated on various samples in the 1980s,[18] after initial attempts in the early 1950s;[19] recently, however, it re-gained momentum in connection with more specified investigations for industrial applications of lignins. Reported versions include sequential precipitation out of alkaline solutions,[20] fractional precipitation of re-dissolved kraft lignin in a gradually changed binary solvent system,[21] (sequential) extractions using different solvents[20,22–28] or just plain water[29] followed by adsorption as well as the fractionation by ultrafiltration of black liquor using ceramic membranes[30–32] as versatile options. Figure 2 shows flow diagrams of two common fractionation techniques: ultrafiltration and fractionated precipitation.

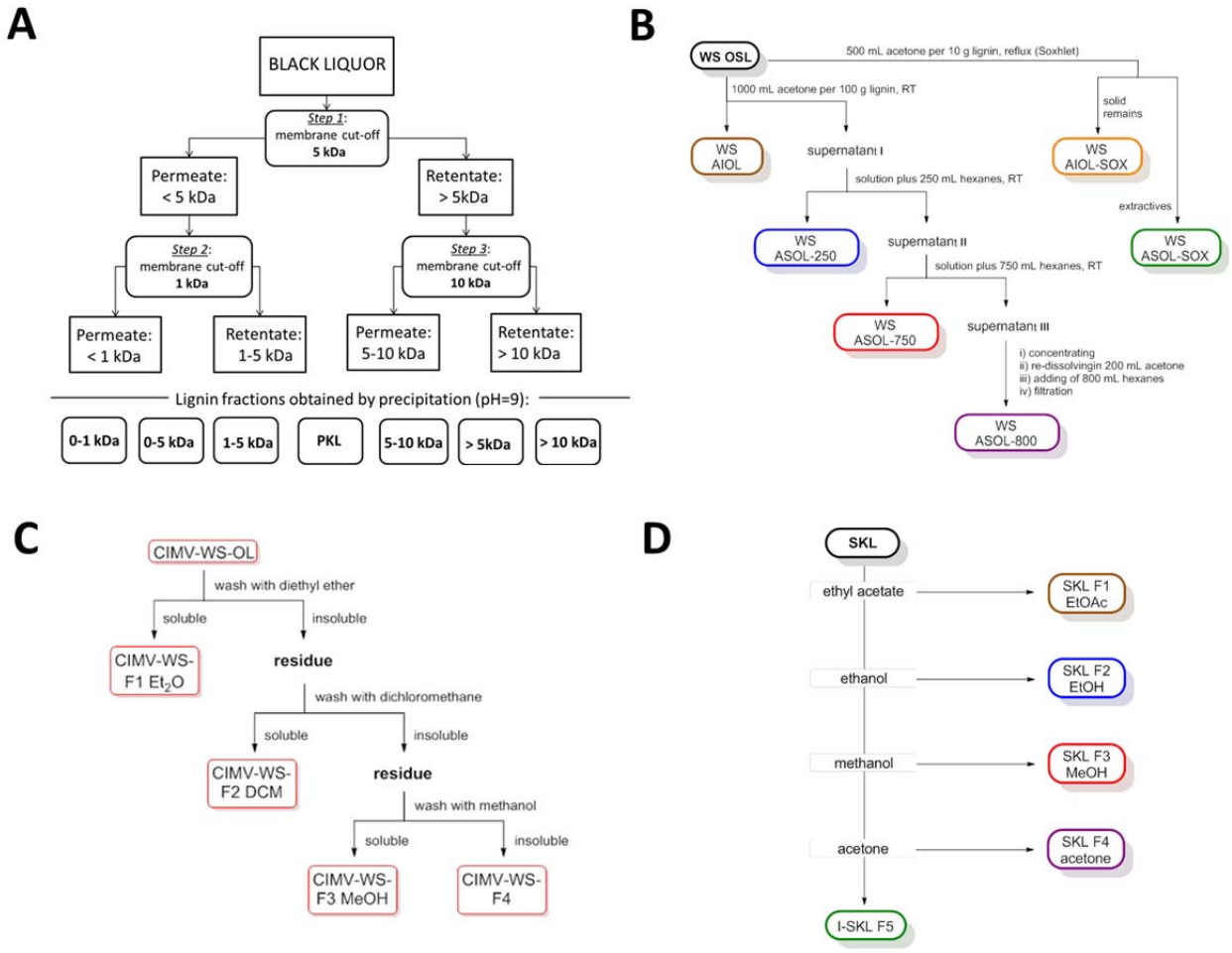

Figure 2: Examples for lignin fractionation processes: (A) ultrafiltration ;[31] (B) fractional precipitation;[33] (C) chromatography-based fractionation,[34] (D) fractionated solubilisation/filtration.[26]

The aforementioned techniques succeed in dividing a given batch of lignin into fractions that differ in molecular weight characteristics and that eventually additionally differ in terms of the distribution of functional group distributions. The processes discussed above do all suffer, to various extends, from the fact that a continuous run is difficult to realise. In terms of fractional precipitation (method A), the continuous production of solids that would need to filtered off is hard to realise. At maximum, a discontinued process is possible. In case of ultrafiltration (method B), membrane clogging is a known problem in continuous processing. In terms of chromatography-like / based fractionation of lignins

(method C), the presence of the remaining insoluble material, eventually in form of a mix with material forming the stationary phase in chromatography set-ups means the necessity of renewing the set-up when loading a new batch of material to be fractionated.

Since lignin fractionation is especially interesting for higher value added industrial applications, efforts towards more efficient processes have been reported, mainly in patent form: A column-based fractionation of lignins using organic solvents or mixtures of organic solvents with increasing polarity has been presented, in the course of which the lignin is mixed to various extends with inert materials inside the column. This method is not claimed to be scalable, nor remotely fully controlled for automation. Insoluble lignin cannot be directly isolated, since it is eventually mixed with an inert excipient material.

The present study reports an unprecedented lignin fractionation approach that is simply flexible and adaptable to the specific downstream needs and directly scalable. We started from the simple idea that the process of fractionated precipitation can be inverted, reversed and transferred into a segmented flow chemistry set-up, to become a continuous process of fractionated dissolution that can be fully automated and run continuously. More importantly, such a process is bimodal scalable by means of dimensions of the set-up as well as by means of overall run time; set-up parameters would ensure that a mixing of lignin with inert dummy materials is not necessary. This paper highlights that such a process is feasible using simple HPLC equipments, and that the fractions of various technical lignins realized in such a simple continuous process are comparable in quality and quantity to fractions obtained in traditional non-continuous batch-based fractional precipitation processes that were published before.

2 Results and Discussion

2.1 SKL flow fractionation

The present fractionation effort focuses on the design and development of a refinement of technical lignins in a scalable and continuous fashion; this process can be best termed 'continuous segmented flow fractional dissolution of lignins'. In principle flow fractionation should be releasable under various/varying parameters, such as i) the pH of an aqueous solvent system; ii) salinity of aqueous salt solutions; iii) polarity of the solvents used, also by mixing solvents and / or solvent systems; iv) pressure in systems in combination with one of the options from i) to iii); v) temperatures in combination with one of the options from i) to iv); and vi) facultative use of molecular weight-sensitive filtering solid or flexible membranes. Besides, a monitoring in real time and inline is necessary to react immediately to any changes in the system, to guarantee optimum fraction qualities. In line monitoring is most easily realisable using a photo diode array (PDA) detector and additionally, not as substitute for the PDA, a refractive index (RI) detector. The flow fractionation system is also ideally remotely monitored and controlled using a computer equipped with suitable software packages. In this perspective, and with the objective of developing a fractionation methodology simple and feasible with the standard equipment available in laboratory a standard HPLC set-up was used: i) a pump suitable for pumping at various flow rates against various back-pressures pure solvents or freely selectable miscible and immiscible mixtures of two or more solvents; ii) a lignin-sample holder, *i.e.*, a simple glass column as used in segmented flow chemical processing under pressure; iii) an in-line detector or series of detectors, *i.e.*, a combination of a PDA and a RI detector, placed immediately downstream to the column suitable for the facile detection of organic material in the solvent; and iv) a means for collecting various volume fractions. Figure 3 shows a schematic representation of the general flow system.

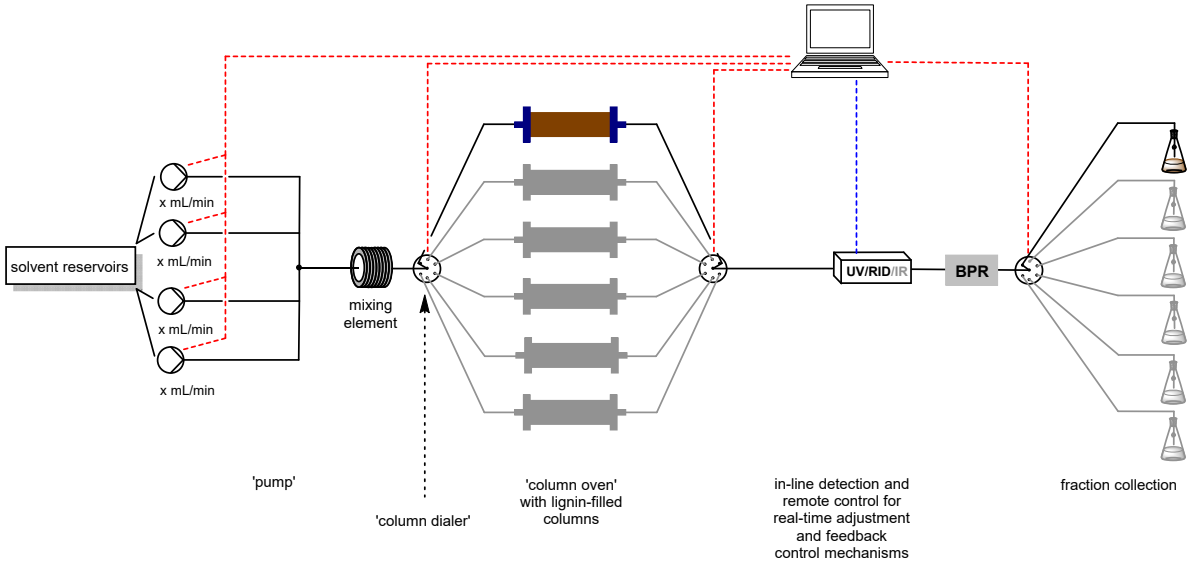

Figure 3: Schematic flow scheme with 'revolver'-like column change mechanism for scale-up.

A first trial was realised using 1 g of a well-studied **KL**,[11] transforming a basic fractionated solubilisation in acetone into a continuous flow fractionation using acetone. Based on the trace of the intensity of the band at $\lambda = 280$ nm recorded by the in-line PDA detector, the acetone-soluble kraft lignin (**ASKL**) fraction was collected; corresponding acetone-insoluble kraft lignin (**AIKL**) was recuperated from the column. Fractions were analysed by GPC and quantitative ^{31}P NMR, and results including isolated yields are given Table 1, entries 4 & 5; Figure 4B shows the GPC traces obtained in the new 'flow fractionation' in comparison to typical GPC traces for Soxhlet-type fractionations (Figure 4A).

Table 1: Numerical data for GPC and ^{31}P NMR analysis of softwood kraft lignin (**KL**) fractions.

entry	lignin	flow condi-tions [a]	yield [%]	Mn (PDI) [Da]	aliphatic OH [mmol/g]	aromatic OH [mmol/g]				acidic OH [mmol/g]
						cond.	G-type	H-type	total	
1	**PKL**	---	---	1900 (4.1)	1.96	1.96	1.96	0.27	4.19	0.38
2	**ASKL-SOX**	---	12	1450 (3.2)	1.59	2.07	2.64	0.32	5.03	0.58
3	**AIKL-SOX**	---	85	2000 (3.9)	1.72	1.68	1.77	0.24	3.69	0.38
4	**ASKL**	KL-A	21	1700 (2.8)	1.76	2.19	2.42	0.32	4.93	0.48
5	**AIKL**[b]	KL-A	78	6000 (3.7)	0.93	0.42	0.38	0.01	0.82	0.01
6	**MSKL**	KL-B	50	1400 (2.7)	1.52	1.71	2.55	0.19	4.44	0.31
7	**MIKL**[b]	KL-B	48	2200 (3.9)	0.86	0.47	0.7	0.11	1.33	0.16

a: Conditions: see Experimental Section.

b: Sample not fully soluble under standard analysis conditions.

The flow fractionation approach yields superior results in terms of separation of fractions compared to both Soxhlet-based fractionation or a fractional precipitation approach (Figure 5A and B). This can be explained by the favourable conditions that are created by the pressurised system and the effective use of incremental solvent volumes for the dissolution.

A slightly different situation is encountered when using methanol. Here, separation is more sluggish, probably due to the fact that the very polar methanol, under the pressurised conditions, is suitable to dissolve a wider heterogeneity of lignin oligomers with various characteristics, leading to a stronger overlap of molecular weights: the rather broadly overlapping fractions display striking differences in terms of distributions of aliphatic and aromatic OH-groups. ^{31}P NMR data show that methanol-soluble softwood kraft lignin (**MSKL**) exhibits much more condensed structure than corresponding **MIKL**.[35] Given the additionally the fact that the molecular weight of condensed aromatic structures were found to be systematically underestimated in GPC-based molecular weight determinations,[36] flow fractionation seems to effectively enhance thus a separation mainly based on physico-chemical characteristics such as H-bonding rather than pure molecular weight features.

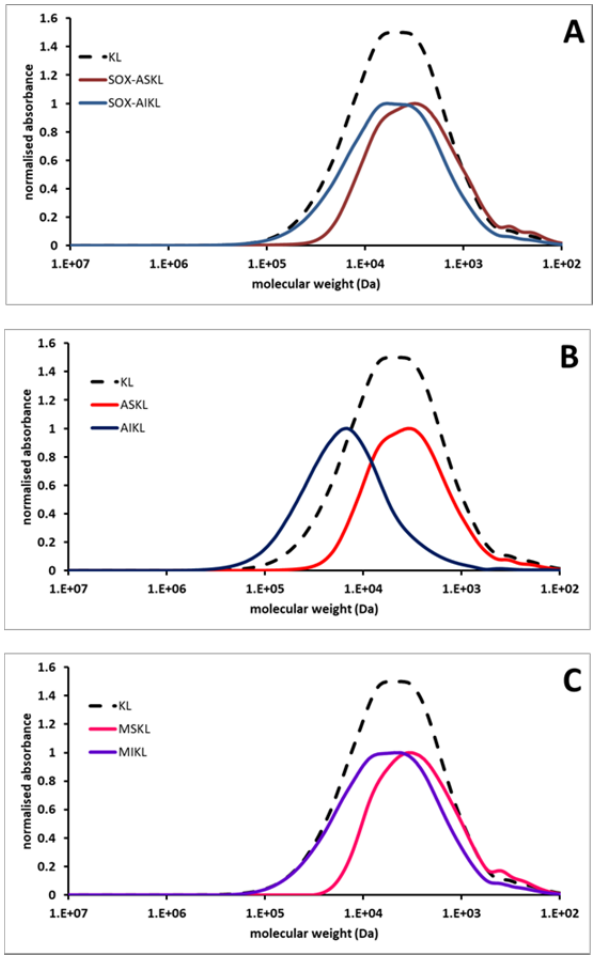

Figure 4: GPC profiles of various **KL** fractionation runs using single solvents: A) fractionation using acetone in a standard Soxhlet set-up; B) flow fractionation using acetone (Method KL-A, Table 1); C) flow fractionation using methanol (Method KL-B, Table 1). Conditions are described in the experimental section; numerical values are given in Table S1 in the Supplementary Information.

In order to improve fractionation efficiency, a step-gradient between hexane and acetone was tested using increasing polarities, following the idea of inverting and reversing a very successful fractionation protocol for **KL**.[21] Several fractions were obtained (Table S1, entries 1-5, Figure 5A). These fractions were acceptably separated with exception of fractions **20-80 SKL** and **0-100 SKL** (Table S1, entries 3 & 4, Figure 5A). These two fractions show a considerable overlap at the low molecular weight end, which can

be explained by the fact that the separation at room temperature using the step gradient in this region of polarity differences was insufficient. In order to correct this, fractionation with hexane-acetone mixtures was repeated using an elevated temperature of 70°C in order to increase solubility effectiveness (Table S1, entries 6-10, Figure 5B). Comparison of GPC elution profiles indicate a clear difference between the previously difficult fractions **20-80 SKL** and **0-100 SKL**. This is supported by the numerical analyses of the GPC data that indicate more distinct variations of the mean average molecular weights (Mns) of the obtained fractions with a rather constant polydispersity index (Table S1, supplementary information).

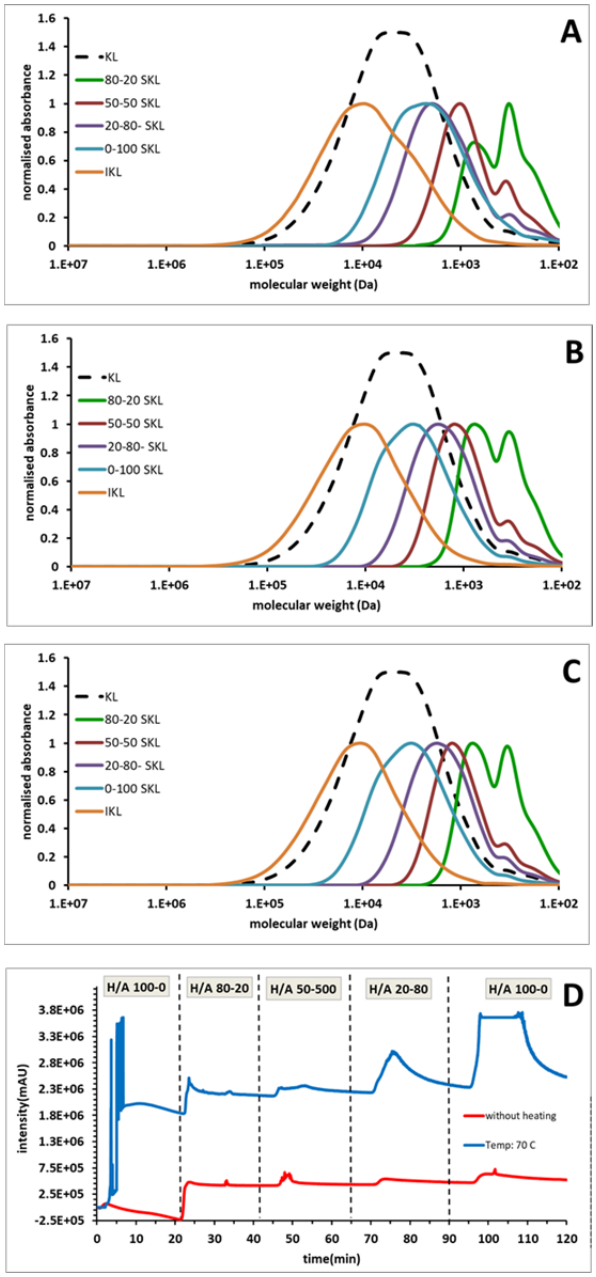

Figure 5: GPC profiles of various **KL** fractionation runs using hexane/acetone gradient systems: A) flow fractionation using hexane/acetone (Method KL-C); B) flow fractionation using hexane/acetone (Method KL-D); C) flow fractionation using hexane/acetone (Method KL-E); D) exemplary UV-profile of gradient runs used for KL-fractionation at ambient and elevated temperature. Conditions are detailed in the Experimental Sections: Numerical results are listed in Table S1 (Supplementary Information).

In a trial to further optimise this flow fractionation approach for technical lignins in terms of time efficiency, flow rate was doubled while maintaining other process parameters such as the optimum temperature of 70 °C constant. Results are presented in Table S1, entries 11-15 and Figure 5C. Elution profiles and numerical results indicate that the higher flow rate is not negatively affecting the separation capacity of the approach.

Flow fractionation could be conveniently followed using an in-line PDA detector, allowing for collecting fractions following strictly the UV-vis signal recorded in real time. Once tailing of the signal died off comfortably in longer time limits (Figure 5D), fraction collecting vessels were exchanged. The graph nicely demonstrates the drastic changes in UV-intensity as soon as the novel solvent washes out a new fraction. Timing can be easily optimised and the process coupled to a distillation-based solvent recovery process, which would confer the presented approach with an unprecedented solvent efficiency.

2.2 WL flow fractionation

In light of the promising results obtained for the flow fractionation of **KL**, an emerging organosolv wheat straw lignin (**WL**) tested before in various other valorisation approaches was fractionated. The system for the continuous and scalable fractionation was left unaltered with respect to the **KL** settings. In order to test the general applicability of the inversion-inverting protocol and the impact of lignin type and quality, fractionation protocols similar to those used for **KL** have been run. Numerical results are reported in Table S2 (supplementary information). GPC elution profiles of various fractions and the conditions applied are compared in Figure 6.

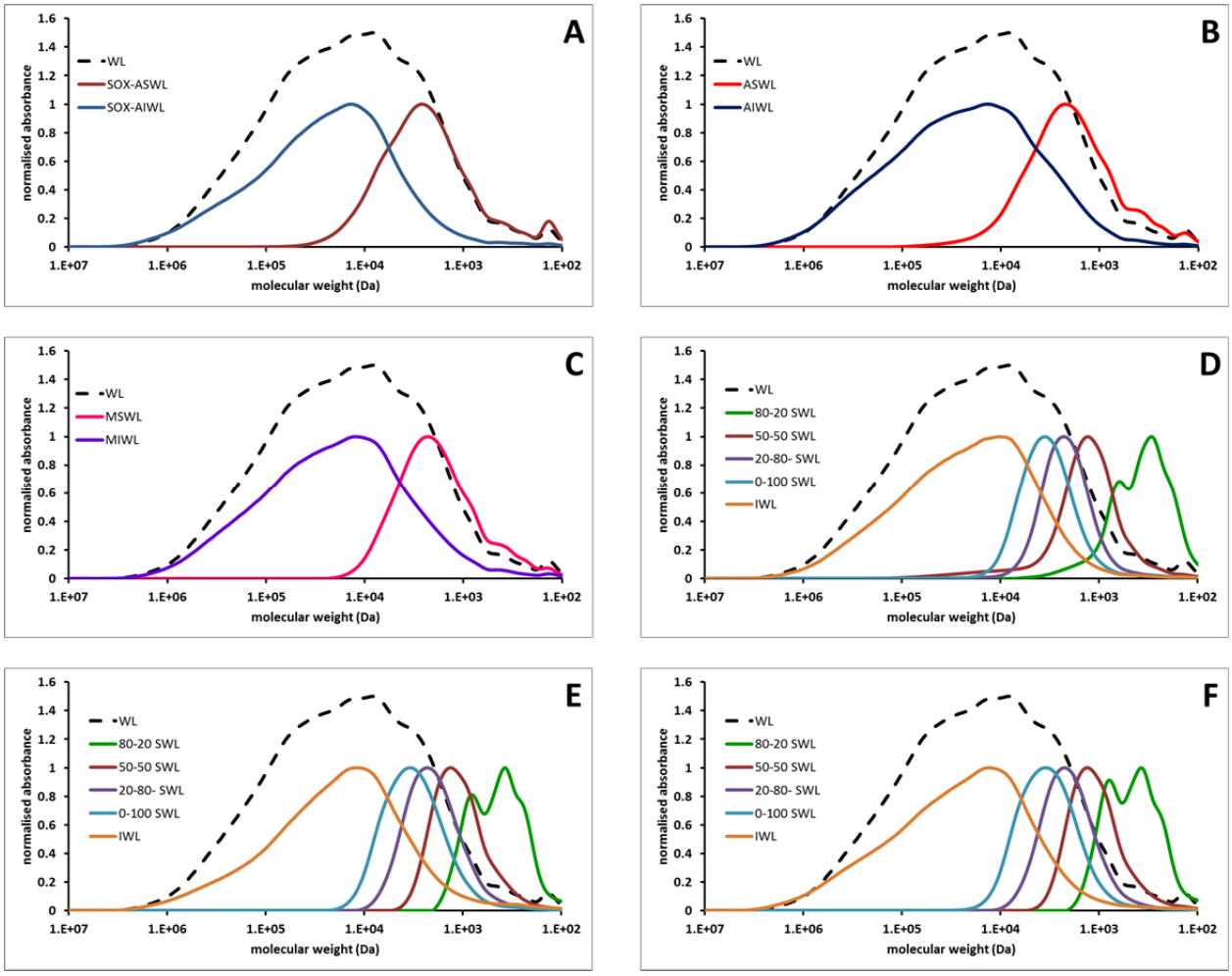

Figure 6: GPC profiles of various **WL** fractionation runs: A) standard Soxhlet-based fractionation using acetone; B) flow fractionation using acetone (Method WL-A); C) flow fractionation using methanol (Method WL-B); D) flow fractionation using hexane/acetone (Method WL-C); E) flow fractionation using hexane/acetone (Method WL-D); F) flow fractionation using hexane/acetone (Method WL-E).

Overall data analysis indicates that the flow fractionation system works equally well for organosolv lignins, irrespective of the fact that the solubility of organosolv lignins is very different with respect to the solubility of kraft lignins. Interestingly, the flow fractionation shows in this case that the methanol fractionation of lignin is delivering comparable results to an acetone fractionation in terms of molecular weight distribution. (Table S2, entries 2-5). Analyses by ^{31}P NMR, however, nicely underlines the

'hidden' effects, namely the enrichment of UV-invisible carbohydrate impurities in form of LCCs.in the **MSWL** fraction.[37] An increase in temperature does not change the effectiveness of the flow fractionation (Table 3, entries 6-15 supplementary). Overall fractionation performance can be maintained when increasing the flow rate.

Using the flow set-up, it was possible to achieve in a convenient scalable way a **WL**-fractionation using aqueous ethanol as green solvent system, adopting an approach for **SKL** by Jääskeläinen and coworkers.[38] Using the flow system, fractions obtained using pure water and aqueous solutions with 10 and 20% ethanol resulted very similar in their characteristics. (Table S2, entries 21-28). Using higher volume fractions of ethanol in water, clearly distinct, narrow fractions could be obtained; a selection is shown in Figure 7.

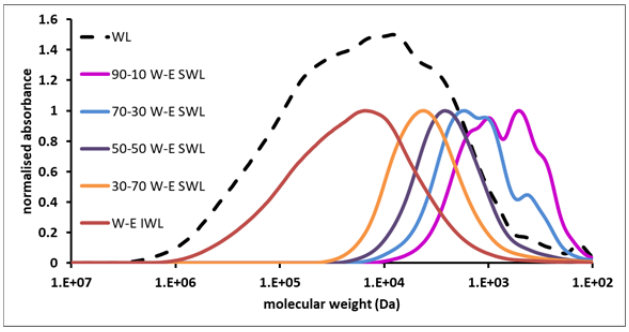

Figure 7: GPC profiles of a **KL** fractionation runs using a gradient system of aqueous ethanol (Method KL-F).

2.3 WL flow purification

Previous studies suggest that the WS used in this study contains a series of impurities that can be expected based on the source and the isolation process. Such impurities were found to mainly comprise

carbohydrates, fatty acids, protein residues and silica. Based on various findings and discussions with the **WL** producer (Compagnie Industrielle de la Matière Végétale (CIMV), Levallois Perret, France),[33,39] it was tested whether these impurities can be 'washed' out using a series of conventional organic solvents. We adopted this approach for the flow fractionation in order to device a flow purification of wheat straw organosolv lignin. Results are given in Table 4, and GPC elution profiles are compared in Figure 8.

Table 2: Numerical data for GPC and ^{31}P NMR analysis of wheat straw organosolv lignin (**WL**) fractions obtained in flow using a sequence of various solvents.

entry	lignin	flow condi-tions [a]	yield [%]	Mn (PDI) [Da]	aliphatic OH [mmol/g]	aromatic OH [mmol/g]				acidic OH [mmol/g]
						cond.	G-type	H-type	total	
1	**PWL**	---	---	2100 (22)	1.37	0.80	0.57	0.29	1.66	0.45
2	**Et_2O-SWL**	WL-P	5.6	400 (1.5)	1.16	0.94	1.15	0.46	2.55	2.18
3	**DCM-SWL**	WL-P	8.2	800 (1.8)	1.24	1.38	1.06	0.36	2.80	0.46
4	**MeOH-SWL**	WL-P	20	1500 (2.0)	1.49	0.91	0.71	0.34	1.95	0.56
5	**IWL**[b]	WL-P	67	4400 (10)	0.39	0.32	0.17	0.12	0.61	0.14

a: Conditions: see Experimental Section.

b: Sample not fully soluble under standard analysis conditions.

a: Sample not fully soluble under standard analysis conditions.

b: Conditions used for flow fractionation of 1 g **WL**: Method WL-P: 0.75 mL/min isochratic flow, RT, 12 min Et_2O, 32 min DCM and 56 min MeOH, V_{total} = 76 mL.

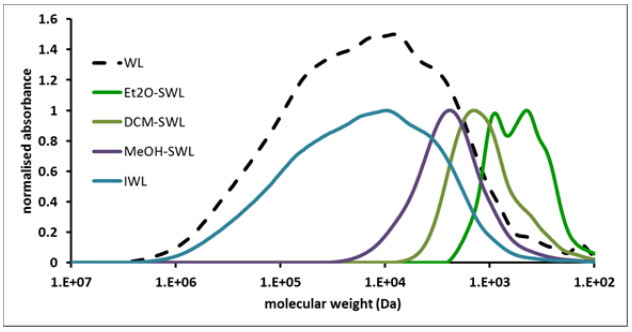

Figure 8: GPC profile of **WL** purification using various solvents in sequence (Method WL-P, Table 2).

Accumulated data suggest that **WL** can be effectively purified in a segmented continuous flow set-up as devised in this work. Very low molecular weight fractions rich in carboxylic acid groups can be washed out, methanol soluble fraction **MeOH-SWL**, potentially enriched in LCC structures can be separated. Although not shown in this work, such purified **IWL** could be directly subjected to additional fractionations using standard acetone-hexane mixtures, for example.

3 Conclusions

Using a conventional HPLC set-up and simple glass columns common in the field of segmented continuous flow chemistry, it is possible to arrive at a facile, flexible, scalable and fast lignin fractionation based on fractional dissolution strategies. Technical lignins of practical value in terms of available quantities and qualities can be efficiently and effectively fractionated using various solvents and solvent systems. Novel fractions are possible in this set-up that allows principally for a stepless change in solvents and the adjustment of temperatures and pressures in the system to improve fraction qualities.

Ongoing work is currently focussing on the use of aqueous solvent systems in the presented set-up and the targeted use of ionic liquids in miscible and biphasic systems for the generation of yet unprecedented lignin fractions.

4 Materials and methods

4.1 General

Softwood kraft lignin, **SKL**, was produced *via* the Lignoboost process[40] by Stora Enso, Kotka, Finland; wheat straw organosolv lignin was obtained from Compagnie Industrielle de la Matière Végétale (CIMV), Levallois Perret, France. Before use, lignins were kept at 40° C in an oven till constant weight. Solvents in appropriate grades were purchased from Sigma Aldrich and Carlo Erba and used as received if not stated otherwise.

4.2 Segmented flow fractionation of lignins

Typical fractionation hardware set-up in its simplest form comprises a Shimadzu HPLC instrument consisting of a controller unit (CBM-20A), a pumping unit (LC 20AT), a degasser unit (DGU-20A3), a column oven (CTO-20AC), a photo diode array detector (SPD-M20A), and a refractive index detector (RID-10A); the instrumental set-up is controlled using the Shimadzu LabSolution software package (Version 5.42 SP3).

A known quantity of dry solid lignin is placed in a 20x1 cm Omnifit glass column that is closed at one end with a fixed endpiece. After filling, the column is closed at the other end using an adjustable endpiece. Both endpieces are equipped with a 5 µm porous PTFE frit in order to prevent escape of solid lignin particles into the tubing. The column is placed in a column holder inside the column oven and connected to the solvent flow system via two switchvalves. The desired solvent gradient system is programmed, and the segmented continuous fraction is started as soon as the column oven reached the desired temperature. Fractions are collected according to absorbance signal changes monitored by means of the inline PDA- and RI-detectors. During the process, eventual noteworthy losses of solid mass inside the column are compensated by adjusting the adjustable endpiece in a way that the remaining solids stay

densely packed inside the column. Once detectors signalise that all soluble parts have been washed out for a given solvent (system), fractions are concentrated and solvent is recycled; solid remains in the column are collected as insoluble fraction. After drying at 40° C in an oven till constant weight, fraction yields are determined. Obtained fractions are analysed by means of GPC and quantitative ^{31}P NMR.

*Conditions used for flow fractionation of 1 g **KL**:* KL-A: acetone, 2 mL/min isochratic flow, RT, 45 min, V_{total}(acetone) = 90 mL; KL-B: methanol, 0.2 mL/min isochratic flow, RT, 390 min, V_{total}(methanol) = 78 mL KL-C: hexane/acetone step gradient (80/20, 50/50, 20/80, 0/100), 0.75 mL/min isocratic flow, RT, 150 min, V_{total}(hexane/acetone) = 113 mL; KL-D: hexane/acetone step gradient (80/20, 50/50, 20/80, 0/100), 0.75 mL/min isocratic flow, 70° C, 150 min, V_{total}(hexane/acetone) = 113 mL; KL-E: hexane/acetone step gradient (80/20, 50/50, 20/80, 0/100), 1.5 mL/min isocratic flow, 70° C, 75 min, V_{total}(hexane/acetone) = 113 mL.

*Conditions used for flow fractionation of 1 g **WL***: WL-A: acetone, 0.2 mL/min isocratic flow, RT, 420 min, V_{total}(acetone) = 84 mL; WL-B: methanol, 0.2 mL/min isocratic flow, RT, 300 min, V_{total}(methanol) = 60 mL; WL-C: hexane/acetone step gradient (80/20, 50/50, 20/80, 0/100), 0.75 mL/min isocratic flow, RT, 230 min, V_{total}(hexane/acetone) = 173 mL; WL-D: hexane/acetone step gradient (80/20, 50/50, 20/80, 0/100), 0.75 mL/min isocratic flow, 70° C, 230 min, V_{total}(hexane/acetone) = 173 mL; WL-E: hexane/acetone step gradient (80/20, 50/50, 20/80, 0/100), 1.5 mL/min isocratic flow, 70° C, 65 min, V_{total}(hexane/acetone) = 98 mL. WL-F:water/ethanol step gradient (90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70), 0.75 mL/min isocratic flow, RT, 167 min, V_{total}(water/ethanol) = 125 mL. WL-P: 0.75 mL/min isochratic flow, RT, 12 min Et_2O, 32 min DCM and 56 min MeOH, V_{total} = 76 mL.

4.3 Gel permeation chromatographic analyses

For gel permeation chromatography (GPC), approx. 2-3 mg of lignin were dissolved in HPLC-grade dimethylsulphoxide (DMSO) (Chromasolv®, Sigma-Aldrich) containing 0.1% (m/v) lithium chloride (LiCl). A Shimadzu instrument was used consisting of a controller unit (CBM-20A), a pumping unit (LC 20AT), a degasser (DGU-20A3), a column oven (CTO-20AC), a diode array detector (SPD-M20A), and a refractive index detector (RID-10A)), and controlled by Shimadzu LabSolutions (Version 5.42 SP3). For separation, a PLgel 5 µm MiniMIX-C column (Agilent, 250 × 4.6 mm) was eluted at 70 ºC at 0.25 mL min^{-1} flow rate with DMSO containing 0.1% lithium chloride for 20 min Standard calibration is performed with polystyrene sulfonate standards in acid form (Sigma Aldrich, MW range 0.43 – 2.60 x 10^6 g mol^{-1}); lower calibration limits are verified by the use of monomeric and dimeric lignin models. Final analyses of each sample is performed using the intensities of the UV signal at λ = 280 nm employing a tailor-made MS Excel-based table calculation, in which the number average molecular weight (Mn) and the weight average molecular weight (Mw)) is calculated based on the measured absorption (in a.u.) at a given time (min) after corrections for baseline shift and drift as described before.[36] Analyses were run in duplicate.

4.4 Quantitative ^{31}P NMR analysis

A procedure similar to the one originally published and previously applied was used.[41,42] Approx. 30 mg of the lignin were accurately weighed in a volumetric flask and suspended in 400 µL of a solvent mixture of pyridine and deuterated chloroform ($CDCl_3$) (1.6:1 v/v) the above prepared solvent solution. One hundred microliters of the internal standard solution, *i.e.*, cholesterol at a concentration of 0.1 M in the aforementioned NMR solvent mixture, were added. 50 mg of Cr(III) acetyl acetonate were added as relaxation agent to this solution, followed by 100 µL of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (Cl-TMDP). After stirring for 120 min at ambient temperature, ^{31}P NMR spectra are

recorded on a Bruker 400 MHz or Bruker 700 MHz NMR spectrometer controlled by TopSpin software, using an inverse gated decoupling technique in the pulse sequence, with the probe temperature set to 20° C. The maximum standard deviation of the reported data is 0.02 mmol g^{-1}, while the maximum standard error is 0.01 mmol g^{-1}.[43,41] NMR data were processed with MestreNova (Version 8.1.1, Mestrelab Research).

List of Abbreviations

AIKL, acetone insoluble kraft lignin; **AIWL**, acetone insoluble wheat straw lignin; **ASKL**, acetone soluble kraft lignin; **ASWL**, acetone soluble wheat straw lignin; BPR, back pressure regulator; Cl-TMDP, 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane; DCM, dichloromethane; DMSO, dimethyl sulfoxide; GPC, gel permeation chromatography; HPLC, high pressure liquid chromatography; HW, hardwood; **IKL**, insoluble kraft lignin; IR, infrared; **IWL**, insoluble wheat straw lignin; **KL**, kraft lignin (softwood); **LS**, lignosulphonate; **MIKL**, methanol insoluble kraft lignin; **MIWL**, methanol insoluble wheat straw lignin; Mn, number average molecular weight; **MSKL**, methanol soluble kraft lignin; Mw, weight average molecular weight; MW, molecular weight; **MSWL**, methanol soluble wheat straw lignin; **MWL**, milled wood lignin; NMR, nuclear magnetic resonance; **OSL**, organosolv lignin; PDA, polydiode array; PD, polydispersity; PEEK, polyether ether ketone; **PKL**, parent kraft lignin; PTFE, poly(tetrafluoroethylene); PVF, polyvinyl fluoride; **PWL**, parent wheat straw lignin; RI, refractive index; **SKL**, soluble kraft lignin; **SWL**, soluble wheat straw lignin; THF, tetrahydrofurane; UV, ultraviolet.

Supporting Information

Supporting Information is available: Tables detailing numeric results obtained during the GPC and ^{31}P NMR analyses for the various fractions realised.

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References

[1] K. V. Sarkanen, C. H. Ludwig, *Lignins: Occurrence, Formation, Structure and Reactions*, Wiley-Interscience, **1971**.

[2] W. G. Glasser, S. Sarkanen, *Lignin: Properties and Materials*, American Chemical Society, Washington, DC, **1989**.

[3] Erich. Adler, *Ind. Eng. Chem.* **1957**, *49*, 1377–1383.

[4] D. S. Argyropoulos, *Materials, Chemicals, and Energy from Forest Biomass*, American Chemical Society, Washington, DC, **2007**.

[5] M. Aresta, A. Dibenedetto, F. Dumeignil, *Biorefineries, An Introduction*, De Gruyter, Berlin, Boston, **2015**.

[6] E. D. Bartzoka, C. Crestini, H. Lange, in *Handb. Polym. Pharm. Technol.* (Eds.: V.K. Thakur, nju K. Thakur), John Wiley & Sons, Inc., **2015**, pp. 127–203.

[7] P. Tomani, *Cellul. Chem. Technol.* **2010**, *44*, 53–58.

[8] J. H. Lora, C. F. Wu, E. K. Pye, J. J. Balatinecz, in *Lignin*, American Chemical Society, **1989**, pp. 312–323.

[9] M. Delmas, M. Benjelloun, *Process for the Separation of Lignins and Sugars from an Extraction Liquor*, **2011**, WO2011154293 (A1).

[10] C. Crestini, F. Melone, M. Sette, R. Saladino, *Biomacromolecules* **2011**, *12*, 3928–3935.

[11] C. Crestini, H. Lange, M. Sette, D. S. Argyropoulos, *Green Chem.* **2017**, *19*, 4104–4121.

[12] C. Heitner, D. Dimmel, J. Schmidt, *Lignin and Lignans: Advances in Chemistry*, CRC Press, **2010**.

[13] L. C.-F. Wu, W. G. Glasser, *J. Appl. Polym. Sci.* **1984**, *29*, 1111–1123.

[14] Y. Li, S. Sarkanen, *Macromolecules* **2002**, *35*, 9707–9715.

[15] Y. Li, S. Sarkanen, *Macromolecules* **2005**, *38*, 2296–2306.

[16] H. Sadeghifar, C. Cui, D. S. Argyropoulos, *Ind. Eng. Chem. Res.* **2012**, *51*, 16713–16720.

[17] C. Cui, H. Sadeghifar, S. Sen, D. S. Argyropoulos, *BioResources* **2013**, *8*, DOI 10.15376/biores.8.1.864-886.

[18] K. Lundquist, T. K. Kirk, *Tappi J* **1980**, *63:1*.

[19] C. Schuerch, *J. Am. Chem. Soc.* **1952**, *74*, 5061–5067.

[20] P. Mousavioun, W. O. S. Doherty, *Ind. Crops Prod.* **2010**, *31*, 52–58.

[21] C. Cui, R. Sun, D. S. Argyropoulos, *ACS Sustain. Chem. Eng.* **2014**, *2*, 959–968.

[22] R. W. Thring, M. N. Vanderlaan, S. L. Griffin, *J. Wood Chem. Technol.* **1996**, *16*, 139–154.

[23] N.-Y. Teng, I. Dallmeyer, J. F. Kadla, *Ind. Eng. Chem. Res.* **2013**, *52*, 6311–6317.

[24] R. J. A. Gosselink, D. J. E. G. van, J. E. de, E. L. Scott, J. P. M. Sanders, J. Li, G. Gellerstedt, *Holzforschung* **2010**, *64*, 193–200.

[25] F. Zikeli, T. Ters, K. Fackler, E. Srebotnik, J. Li, *Ind. Crops Prod.* **2014**, *61*, 249–257.

[26] A. Duval, F. Vilaplana, C. Crestini, M. Lawoko, *Holzforschung* **2015**, *70*, 11–20.

[27] G. Griffini, V. Passoni, R. Suriano, M. Levi, S. Turri, *ACS Sustain. Chem. Eng.* **2015**, *3*, 1145–1154.

[28] V. Passoni, C. Scarica, M. Levi, S. Turri, G. Griffini, *ACS Sustain. Chem. Eng.* **2016**, *4*, 2232–2242.

[29] P. J. Hernes, A. C. Robinson, A. K. Aufdenkampe, *Geophys. Res. Lett.* **2007**, *34*, L17401.

[30] A. Toledano, A. García, I. Mondragon, J. Labidi, *Sep. Purif. Technol.* **2010**, *71*, 38–43.

[31] O. Sevastyanova, M. Helander, S. Chowdhury, H. Lange, H. Wedin, L. Zhang, M. Ek, J. F. Kadla, C. Crestini, M. E. Lindström, *J. Appl. Polym. Sci.* **2014**, *131*, n/a-n/a.

[32] A. Duval, S. Molina-Boisseau, C. Chirat, *Holzforschung* **2014**, *69*, 127–134.

[33] H. Lange, P. Schiffels, M. Sette, O. Sevastyanova, C. Crestini, *ACS Sustain. Chem. Eng.* **2016**, *4*, 5136–5151.

[34] R. J. A. Gosselink, D. P. Van, E. Van, *Fractionation of Technical Lignin*, **2015**, WO2015178771 (A1).

[35] C. Crestini, H. Lange, M. Sette, D. S. Argyropoulos, *Green Chem* **2017**, *19*, 4104–4121.

[36] H. Lange, F. Rulli, C. Crestini, *ACS Sustain. Chem. Eng.* **2016**, *4*, 5167–5180.

[37] R. Ebrahimi Majdar, A. Ghasemian, H. Resalati, A. Saraeian, C. Crestini, H. Lange, *Polymers* **2019**, *11*, 225.

[38] A.-S. Jääskeläinen, T. Liitiä, A. Mikkelson, T. Tamminen, *Ind. Crops Prod.* **2017**, *103*, 51–58.

[39] L. Mbotchak, C. Le Morvan, K. L. Duong, B. Rousseau, M. Tessier, A. Fradet, *J. Agric. Food Chem.* **2015**, *63*, 5178–5188.

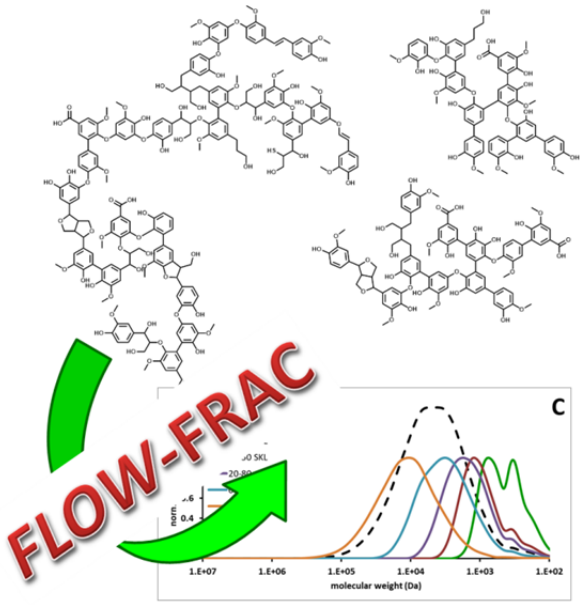
[40] F. Öhman, H. Theliander, P. Tomani, P. Axegard, *Method for Separating Lignin from Black Liquor*, **2013**, US8486224 B2.

[41] A. Granata, D. S. Argyropoulos, *J. Agric. Food Chem.* **1995**, *43*, 1538–1544.

[42] X. Meng, C. Crestini, H. Ben, N. Hao, Y. Pu, A. J. Ragauskas, D. S. Argyropoulos, *Nat. Protoc.* **2019**, *14*, 2627–2647.

[43] D. S. Argyropoulos, *Res. Chem. Intermed.* **1995**, *21*, 373–395.

TOC-graphic and synopsis



Commercially available softwood kraft lignin and wheat straw organosolv lignin were effectively fractionated in a dynamic segmented flow process, paving the way to facile larger scale production of lignin fractions using fractionated dissolution protocols.