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Effective fractionation of lignocellulose in herbaceous biomass and hardwood using a mild acetone organosolv process†

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Large-scale biorefineries converting lignocellulosic biomass into chemicals, fuels and energy require a cost-effective pretreatment process that can effectively fractionate the three main lignocellulose constituents from a wide variety of feedstocks. A mild organosolv process has been developed using acetone as solvent. Herbaceous biomass (wheat straw and corn stover), hardwood (beech, poplar and birch) and softwood (spruce and pine) were fractionated using near-identical process conditions: 140 °C, 120 min, 50% w/w aqueous acetone and sulfuric acid. For herbaceous biomass and hardwood, effective pretreatment and subsequent enzymatic cellulose hydrolysis into glucose was observed in combination with a high yield of monomeric hemicellulose sugars and lignin. In the case of softwood, poor delignification hampered enzymatic cellulose hydrolysis, despite efficient hemicellulose removal. To assess solvent stability, the impact of temperature, time and acid dose on the degree of acetone self-condensation was explored. The process conditions used for feedstock screening resulted in a 1.4% w/w conversion of acetone to mainly diacetone alcohol and mesityl oxide. For wheat straw, shortening the reaction time to 60 min resulted in reduced solvent self-condensation (1.0% w/w) and improved hemicellulose sugar yield (86%). In sum, effective fractionation was demonstrated for various herbaceous and hardwood feedstocks combined with limited acetone loss due to self-condensation.

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Introduction

The increasing demand for energy, fuels, chemicals and materials has led to concerns over greenhouse gas-induced climate change and future fossil petroleum shortages. To mitigate these threats, new technologies are being developed for balancing economic growth and its environmental impact by utilizing biomass components to replace petroleum derived energy carriers and chemicals. Lignocellulosic biomass, a renewable resource with high availability, has gained particular interest because it does not affect the world's food supply by direct land competition. A crucial factor for the realisation of a commercially viable biorefinery is to develop a cost-effective pretreatment of lignocellulose into bio-based intermediates from a variety of lignocellulosic feedstocks.[1]

Organosolv pretreatment can effectively fractionate biomass into its three main components: cellulose, hemicellulose and lignin. During the process, the hemicellulose is hydrolysed into monomeric sugars which may partially degrade to, for example, furfural. Temperature and/or acid-induced hydrolysis of the more labile ether linkages in lignin causes its partial depolymerisation and subsequent dissolution into the water-solvent mixture. The crystalline cellulose fraction is more resistant to hydrolysis and is recovered in the pulp. After fractionation, pulp and reaction liquid are separated and organic solvent removed from the liquor for recycling. The decrease in organic solvent concentration in the liquor results in precipitation and isolation of a high purity solid lignin.

The pulp enriched in cellulose can be used directly in fiber applications or enzymatically hydrolysed to glucose.[2,3] Monomeric sugars from the (hemi)cellulose fraction can be converted by fermentation into fuels such as bioethanol and building blocks for bio-based products such as itaconic, succinic, and lactic acid.[4–6] In addition, sugars can be converted into products such as furfural, hydroxy-methylfurfural and levulinic acid, which are well known as platform chemicals suitable for a wide variety of applications.[7] Alternatively, produced cellulose can be converted chemocatalytically into isosorbide, hexitols or even alkanes.[8–10] Finally, lignin is a

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potential renewable source for aromatic chemicals and performance products.[11–13]

A wide variety of organic solvents has been used for organosolv pretreatment, including alcohols, organic acids and ketones. Industrially feasible pretreatment processes should combine a good product yield and quality of all three lignocellulose constituents with cost-effective processing. In the past decades, ethanol and methanol have primarily been used because of their low cost and volatility, which facilitates solvent recovery.[14,15] However, the use of these solvents in combination with a typical temperature range of 160–220 °C and optionally an acid catalyst introduces process challenges such as reaction pressure and potential solvent loss due to di(m)ethylether formation. Furthermore, lignin condensation hinders effective depolymerisation into bio-based aromatics[16] and (m)ethylation of sugars and lignin,[17–19] introduces additional solvent loss and challenges in both downstream processing and product application.

Promising technologies are being developed for whole biomass utilisation, which generally aim for a high monomeric sugar yield next to the isolation of less-condensed lignin (using milder pretreatment conditions) and/or functional lignin derivatives. As an example, organic acids such as acetic and formic acid have been successfully used at atmospheric pressure.[20] Biphasic solvent systems using water and 2-methyltetrahydrofuran (2-MeTHF) have been shown to efficiently fractionate lignocellulose using oxalic acid as a catalyst.[21] Saccharification of mechanocatalytically treated beech wood using the same solvent system resulted in lignin depolymerisation with suppressed lignin recondensation.[22] Direct lignin depolymerisation into bio-based aromatics during pretreatment has been reported for various 'lignin-first' processes.[23,24,46] High hemicellulose retention in the pulp in some of these processes[23,24] enables effective utilization of this fraction.

An interesting class of solvents for biomass fractionation are ketones.[25] However, studies involving the use of ketones as solvent for organosolv pretreatment are limited. Araque *et al.* used 50% w/w aqueous acetone as solvent to successfully pretreat Pinus radiata D Don at 195 °C.[26] Huijgen *et al.* and Jiménez *et al.* both reported a parameter study on the autocatalytic pretreatment of wheat straw using aqueous acetone at higher temperatures (*i.e.* 160–220 °C and 150–200 °C, respectively).[25,27] The clean fractionation process developed by NREL uses a ternary system with either aqueous ethanol or acetone and methyl isobutyl ketone (MIBK) for the sulfuric acid-catalysed fractionation of lignocellulosic feedstocks at lower temperatures (104–160 °C).[28–30]

In this study, we present a new acetone organosolv process that operates at a relatively low temperature.[31] Acetone is an excellent solvent for lignin dissolution as compared to ethanol.[25] Acetone has a high volatility and does not form an azeotrope with water, which contributes to a significant reduction in energy demand when ethanol is replaced by acetone.[32] Unfortunately, little is known about the stability of acetone during fractionation while solvent loss is a key parameter for a sustainable and cost-effective pretreatment process.[33] For every percent solvent loss, the additional costs for a process using 50% w/w acetone and a liquid–solid ratio of 5 L kg^{-1} feedstock is approximately 20$ per ton processed feedstock.

An advantage of a water miscible solvent such as acetone is its potential use in (gradient-based) pre-extraction of biomass. Removing non-lignocellulose components before fractionation increases the feedstock composition homogeneity and biorefinery product purity.[34] New developments focus on the lignocellulose enrichment of agricultural residues, industrial biodegradable waste and manure fibers. Increased biomass availability at lower prices and the valorisation of extractives towards fine chemicals/fertilizers can significantly improve the economy and sustainability of biorefineries.

Here, we limit our focus to mild acetone organosolv pretreatment of herbaceous biomass (wheat straw, corn stover), hardwood (birch, beech, poplar) and softwood (spruce, pine). First, fractionation data will be compared using near-identical process conditions. Secondly, solvent loss due to acetone self-condensation reactions will be explored for a range of process parameters. Overall, effective fractionation will be demonstrated for lignocellulose in herbaceous biomass and hardwoods.

Experimental

Materials and feedstocks

Technical acetone was obtained from VWR Chemicals; sodium azide 99.5%, sulfuric acid 98%, *o*-toluidine 99%, and thiourea 99% from Sigma-Aldrich; sodium acetate trihydrate and glacial acetic acid 100% from Merck. Ambient-dry wheat straw (The Netherlands), corn stover (USA) and birch (Finland), poplar (The Netherlands), spruce (Denmark) and pine (The Netherlands) chips were cut to a smaller particle size using a Retsch SM2000 cutter mill equipped with a 2 or 10 mm sieve (Table 2). Beech was commercially purchased in 0.75–2 mm size from Rettenmaier (Räuchergold HBK 750/2000). The moisture content was determined using a halogen moisture analyser (Mettler Toledo HR83, Columbus, OH). Acid neutralising capacity (ANC) of the milled feedstocks was determined by nitric acid titration to pH 2.0 at room temperature for 48 h.[35]

Fractionation

Lab-scale pretreatment experiments were performed in an autoclave reactor (2 L Kiloclave, Büchi Glas Uster AG, Switzerland) following a procedure published in earlier work.[36] A mixture of biomass, 50% w/w aqueous acetone (corrected for the biomass moisture content) and sulfuric acid as catalyst (Table 2) was heated to 140 °C and kept isothermal for 120 min, while stirring with an anchor stirrer at 100 rpm. After cooling below 25 °C, the slurry was measured for pH and filtered over a Whatman GF/D filter. The solids were first washed with 50% w/w aqueous acetone (2.5 L kg^{-1} initial dry biomass, 5 L kg^{-1} for wheat straw) followed by a wash with water

(2.5 L kg^{-1} initial dry biomass) to remove acetone from the pulp. A subsample was dried in a vacuum oven at 50 °C to determine the moisture content of the pulp as well as dry pulp yield. The remainder of the pulp was stored wet at −20 °C for enzymatic hydrolysis. The filtrate and first wash liquor were combined and samples were taken for analysis. The combined liquor was analysed for monomeric sugars using High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection and sugar degradation products/organic acids using High Performance Liquid Chromatography as previously published in Grisel *et al.* (2014).[17] The dissolved lignin was precipitated from the combined liquor by dilution with water (4 °C, 4 : 1 w/w dilution ratio H_2O : liquor) and collected by centrifugation at 3488*g* for 5 min. The lignin wet pellet was weighed and residual non-covalently bonded carbohydrates were removed by adding 2 parts of demineralised water and heating the mixture at 40 °C overnight. Subsequently, the liquid was decanted and the lignin dried at 50 °C in a vacuum oven as described in Smit *et al.* (2017).[37] Wheat straw pretreatment experiments for exploring acetone self-condensation, were performed as described above but with changes in reaction time, temperature and acid dose (Table 3).

Biochemical composition analysis

The summative composition of solids was analysed using procedures described in earlier work.[36] These procedures are modified versions of the NREL standard biomass analytical procedures.[38] In short, the content of lignin and carbohydrates was determined in duplicate as follows. The sample was milled with a cutting mill and hydrolysed in two steps: (1) 12 M (72% w/w) H_2SO_4 (30 °C, 1 h) and (2) 1.2 M H_2SO_4 (100 °C, 3 h). The solid residue was determined gravimetrically and its ash content was measured. The acid-insoluble lignin (AIL) content was based on the amount of ash-free residue, and acid-soluble lignin (ASL) was determined using UV-VIS absorption. Finally, the hydrolysate was analysed for monomeric sugars using High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection as previously published in Grisel *et al.* (2014).[17] For biochemical composition analysis of the feedstocks, the extractives were removed with two successive Soxhlet extractions using water and ethanol according to NREL/TP-510-42619 prior to hydrolysis.[38]

Enzymatic hydrolysis

5 g (dry weight) of wet pretreated material was added to 50 mL liquid containing 0.05 M sodium citrate buffer pH 5.0 and 0.02% w/v sodium azide (corrected for moisture in sample and enzyme dose) according to NREL/TP-510-42629.[38] Enzymatic hydrolysis was performed for 72 h using an IKA ks4000 rotary shaker at 50 °C and 140 rpm. The commercial enzyme mixture Accellerase TRIO (DuPont Industrial Biosciences, Leiden, NL) was used for (hemi)cellulose hydrolysis. The enzyme activity of the enzyme batch was 33 FPU mL^{-1} and 2103 CMC U mL^{-1} (carboxymethyl cellulose assay), determined according to Ghose (1987).[39] After 0, 6, 24, 48 and 72 h samples were taken for colorimetric determination of the glucose concentration.[40] In short, 2 mL of reagent (9% v/v *o*-toluidine, 1.5% w/v thiourea in glacial acetic acid) was added to 20 μL of (diluted) sample and heated in a water bath at 90 °C for 8 min. After cooling in tap water for 4 min, the absorbance was measured at 635 nm. Glucose yield is expressed as percentage of the pulp glucan converted to glucose.

Analysis acetone self-condensation products

A Thermo Scientific DSQII Series Single Quadrupole GC/MS was used to analyse organosolv liquors. After split ratio injection, components were separated with a 30 m Phenomenex Zebron ZB-WAXplus fused silica capillary column (0.25 mm i.d. and 0.25 μm film thickness). The oven was programmed to start at 40 °C for 5 min, ramped to 245 °C at 10 °C per minute, and then held for 20 min. Data were collected with Thermo Xcalibur/QuanLab Forms software. The MS was operated in the full scan mode at a scan rate of 5 scans per second.

Results and discussion

Fractionation

Feedstock composition. Composition of the feedstocks is presented in Table 1 and shows the typical compositional differences between the types of biomass. Wheat straw and corn stover contain less lignin as compared to the woody feedstocks and have a relatively high content of non-lignocellulose components (extractives and ash). Typically, the major group of hemicelluloses found in herbaceous biomass is glucuronoarabinoxylan. Most hardwoods predominantly contain glucuronoxylan with varying amounts of glucomannan (beech < birch < poplar). Softwood hemicellulose composition is distinctly different, with galactoglucomannan as the principal component and to a lesser extent glucuronoarabinoxylan.[41] For glucan, no distinction can be made between glucan present as cellulose and glucan present as part of hemicellulose due to the experimental approach used for biochemical composition analysis.

Lignin content does not vary greatly for the woody feedstocks but the composition of lignin in softwood, hardwood and grasses is known to vary in the relative abundance of the *p*-coumaryl, coniferyl, and sinapyl alcohol monolignol subunits and the number of easy hydrolysable (*e.g.* β-O-4) and recalcitrant (*e.g.* 5–5) linkages.[16,42,43]

Acetone organosolv pretreatment. Organosolv fractionation of lignocellulose is designed to hydrolyse the hemicellulose fraction and partly depolymerise lignin so that both are solubilised into the liquor. Cellulose is recovered as a solid for fiber applications or (enzymatically) hydrolysed to glucose for conversion to fuels or chemical building blocks. Fractionation data have been grouped into C5 sugars (arabinose and xylose) and C6 sugars (galactose, glucose, mannose and rhamnose) for two reasons: (1) glucan is both present in the cellulose and hemicellulose, especially in softwood and (2) different monomeric C6 sugars (except rhamnose) can degrade to hydroxy-methylfurfural

Table 1 Feedstock composition

		Carbohydrates								
(%dw)	Extractives[a]	Glucan	Xylan	Mannan	Arabinan	Galactan	Rhamnan	Lignin[b]	Ash	Sum
Wheat straw[e]	7.8	29.8 ± 2.2	20.6 ± 1.2	[d]	2.0 ± 0.1	0.7 ± 0.0		15.6 ± 0.1	13.7 ± 0.3	90.2
Corn stover	9.1	33.9 ± 0.4	19.3 ± 0.1		2.1 ± 0.0	0.9 ± 0.0		16.9 ± 0.1	9.7 ± 0.1	91.9
Beech	2.2[c]	35.6 ± 0.5	18.8 ± 0.1		0.5 ± 0.0	0.9 ± 0.0	0.4 ± 0.0	24.8 ± 0.1	0.9 ± 0.0	84.1
Poplar[f]	4.9	44.6 ± 0.6	10.7 ± 0.1	4.2 ± 0.3	0.2 ± 0.0	0.7 ± 0.0		23.7 ± 0.7	0.6 ± 0.0	89.6
Birch[g]	3.8	37.3 ± 0.5	20.0 ± 0.3	1.4 ± 0.1	0.2 ± 0.0	0.6 ± 0.0	0.3 ± 0.0	22.3 ± 0.2	0.2 ± 0.0	85.9
Spruce[f]	4.8	40.5 ± 0.0	4.5 ± 0.0	10.2 ± 0.0	0.3 ± 0.0	1.8 ± 0.0		27.2 ± 0.2	0.3 ± 0.0	89.6
Pine[f]	4.2	37.9 ± 2.6	4.3 ± 0.4	10.4 ± 0.8	0.5 ± 0.0	1.6 ± 0.1		26.1 ± 0.2	0.3 ± 0.0	85.3

[a] H_2O and ethanol extractives combined, corrected for soluble ash. [b] Sum of acid-insoluble and acid-soluble lignin. [c] Only ethanol extractives, not corrected for soluble ash. [d] Empty cell: below detection limit. [e] Composition has been previously published in Smit and Huijgen (2017).[44] [f] Composition has been previously published in Galkin *et al.* (2016).[46] [g] Composition has been previously published in Ennaert *et al.* (2016).[9]

(HMF), and both the C5 sugars to furfural. Possible formation of humins from sugars and sugar derivatives[45] is not included in the C5 and C6 sugar product distributions.

The experiments were designed using an acid dose of 40 mM H_2SO_4 for the fractionation of woody feedstocks (Table 2). Small differences in the ANC of the woody feedstocks in combination with a liquid/solid ratio of 5 L kg^{-1} during fractionation affected the amount of free acid during fractionation. To correct for the acid neutralising capacity (ANC) of wheat straw, the acid dose for the fractionation of wheat straw was increased to 60 mM. Corn stover was added to the dataset using an acid dose matching the fractionation conditions of wheat straw. Besides acid dose, another factor influencing the pH is the degree of hemicellulose acetylation of the feedstocks. Fractionation deacetylates the hemicellulose fraction and acetic acid is released to the liquor. Acetic acid in the liquor is highest for hardwood *e.g.* 5.2, 3.6 and 4.8% (% w/w of the initial feedstock weight) for beech, poplar and birch respectively. Lower values were found for wheat straw (2.5%), corn stover (2.4%), spruce (1.7%) and pine (1.9%). Multiple factors can influence the pH and measurements at reaction temperature were not performed. When comparing different feedstocks, differences in acidity will affect fractionation results. Table 2 shows a small variation in pH of the slurry after fractionation.

Solid polymeric C6 sugar recovery (Table 2 and Fig. 2) is generally high. For softwood and to a lesser extent poplar, the observed lower polymeric C6 sugar recovery can primarily be attributed to hydrolysis of (galacto)glucomannan present in the hemicellulose fraction. The solubilised C6 fraction is mainly in the form of monomeric sugars with little degradation to hydroxy-methylfurfural (HMF) and levulinic acid (LA). Effective hemicellulose polymeric C5 sugar hydrolysis is demonstrated for all the feedstocks. Differences in hemicellulose solubilisation may partially be related to liquor acidity. Additional experiments monitoring the (oligomeric) xylose release over time revealed no significant differences in xylan hydrolysis rates between wheat straw, beech and pine (for details, see the ESI Fig. S5†). One of the key aspects of using acetone as solvent for the fractionation of lignocellulose is the hemicellulose derivatives composition.

Autocatalytic organosolv fractionation of wheat straw at high temperature (>205 °C) using 60% w/w ethanol or 50% w/w acetone (Fig. 1) results in rapid degradation of the solubilised xylose.[25] Wildschut *et al.* optimised the pretreatment of wheat straw using acid-catalysed ethanol organosolv.[36] At optimal fractionation conditions (190 °C, 60 min, 60% w/w aqueous ethanol and 30 mM sulfuric acid) 95.3% of the xylan was removed from the wheat straw with 29.5% converted to monomeric xylose and 34.4% to furfural. Further research

Table 2 Feedstock fractionation

	Fractionation conditions[a]				General fractionation data				
Feedstock	Mill size (mm)	L/S[b] (L kg^{-1} DM)	H_2SO_4 (mM)	Free H^+ [c] (mM)	pH liquor	Pulp yield (wt%)	C6 recovery (wt%)	C5 hydrolysis (wt%)	Delignification (wt%)
Wheat straw	10	10	60	67	2.1	46.5	91.4	96.8	79.1
Corn stover	10	10	56	67	1.9	48.0	89.3	91.5	81.5
Beech	2	5	40	50	2.2	47.6	94.3	87.3	79.4
Poplar	2	5	40	54	2.0	53.1	84.6	94.3	77.6
Birch	2	5	40	62	2.0	44.0	87.7	92.0	86.4
Spruce	2	5	40	68	1.9	60.6	68.5	89.5	29.7
Pine	2	5	40	68	1.9	60.7	74.2	88.6	31.6

[a] 140 °C, 120 min, 50% w/w aqueous acetone. [b] Liquid–solid ratio. [c] Acid dose corrected for the acid neutralising capacity (ANC) of the feedstock. Measured ANC's: wheat straw, 0.53; corn stover, 0.45; beech, 0.15; poplar, 0.13; birch, 0.10; spruce, 0.07; pine, 0.07 mmol H^+ per g dry feedstock.

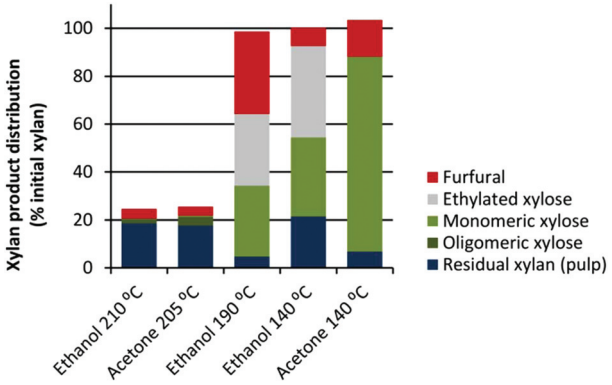

Fig. 1 The influence of process conditions on wheat straw xylan product distribution. Ethanol 210 °C and 190 °C data were previously published in ref. 36, ethanol 140 °C in ref. 31 and acetone 205 °C in ref. 25 (ethylated xylose was not analysed for ethanol 210 °C).

after publication revealed that ethanol reacts with xylose to form ethyl-xylosides.[17,31,47] For the abovementioned experiment, 27.8% of the xylan was converted to ethyl-xylosides. New milder process conditions, as presented in this paper, were tested on the same wheat straw. At optimal conditions (140 °C, 120 min, 60% w/w aqueous ethanol and 60 mM sulfuric acid), 78.5% of the xylan was removed from the wheat straw with 32.9% converted to monomeric xylose and 7.7% to furfural. Although the lower temperature regime reduced sugar degradation to furfural, xylan conversion to ethyl-xylosides increased to 38.1%. This is likely due to the increase in acidity, where an acid dose of 30 mM H_2SO_4 results in 12 mM H^+ free acid during fractionation at 190 °C and an acid dose of 60 mM H_2SO_4 in 72 mM H^+ free acid at 140 °C. In addition, ethylation of lignin has been reported for auto- and acid-catalysed ethanol organosolv pretreatments.[48,49] Replacing ethanol by acetone as solvent eliminates unwanted ethylation of sugars and lignin. Without changing process severity (140 °C, 120 min, 50% w/w aqueous acetone and 60 mM sulfuric acid), the yield of monomeric xylose in Fig. 1 increases to 81.3%. Data presented in Fig. 2 show efficient polymeric C5 sugar conversion to monomeric sugars in the range of 58.6–79.0% for herbaceous biomass and hardwood combined with limited furfural formation (6.1–12.7%). For reasons unclear, relative monomeric C5 sugar yield is lower (36.6–38.7%) and furfural formation higher (21.6–24.9%) for spruce and pine. The sum of C5 sugar product distribution after fractionation ranges from 97.0% for poplar to 70.8% for spruce. Besides variation due to experimental/analytical error, an incomplete mass balance can be caused by: (1) presence of C5 oligomeric sugars solubilised in the liquor. However, post hydrolysis of the wheat straw liquor revealed no presence of oligomeric sugars in this case. (2) Decomposition of sugars and/or furfural to humins[45] or *via* alternative degradation pathways.[50] (3) Adsorption and/or condensation of furfural to other biomass components such as lignin. (4) Acetone and/or its degradation products react with sugars and/or furfural to components not detected.

Together with hemicellulose hydrolysis, lignin depolymerisation and subsequent solubilisation is an important mechanism for efficient fractionation of lignocellulose and valorisation of its three main components. The dominant linkage between monolignols present in lignin is the easily cleavable β-O-4 ether linkage, present in both softwood (±50%) and hardwood lignin (±60%).[12] Lignin present in herbaceous biomass contains all three types of lignin subunits *e.g.* *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S). Hardwood lignin consists of roughly equal proportions of guaiacyl and syringyl units. The extra methoxy group on syringyl forces the lignin to arrange in a more linear structure.[42] Softwood lignin primarily consists of guaiacyl units and forms a more branched structure with recalcitrant 5–5 or dibenzodioxocin linkages.[42] Herbaceous biomass and hardwood delignification

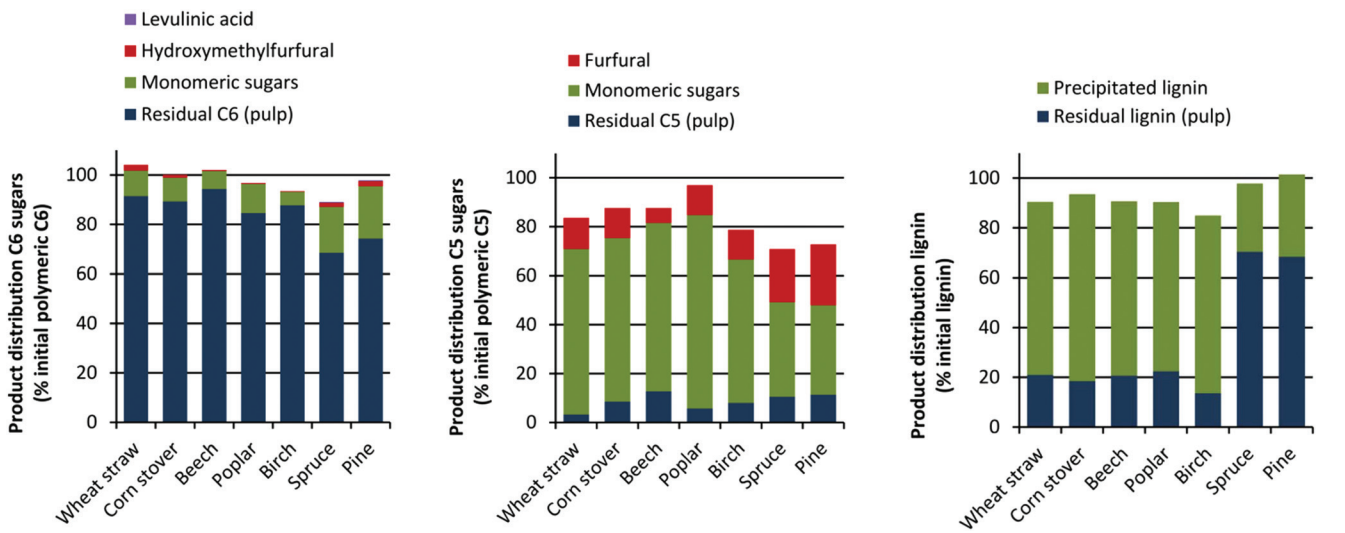

Fig. 2 Product distribution feedstock fractionation.

ranged from 77.6 to 86.4%. Despite of efficient hemicellulose hydrolysis, the delignification of spruce and pine is significantly lower, with only around 30% of the lignin solubilised. It seems that the process severity is too low for efficient softwood depolymerisation. Inability to fractionate softwood using mild organic solvent-based biorefinery concepts has also been reported by Grande *et al.*[51]

Shimada *et al.* showed a higher reactivity of guaiacyl carbocations (as compared to syringyl) towards lignin condensation.[52] Due to the relative abundance of guaiacyl units in softwood, a higher rate of lignin condensation could slow the softwood delignification rate.[53] However, fractionation of spruce at higher reaction severities using 150 °C, 50% w/w aqueous acetone and 40, 60 and 80 mM H_2SO_4 showed increased delignification of 61, 76 and 89% respectively.

The incomplete lignin product distribution for herbaceous biomass and hardwood might be due to the presence of 10% w/w aqueous acetone-soluble lignin (derivatives) that do not precipitate upon liquor dilution with water. On the other hand, co-precipitation of feedstock solvent-extractives (long chain fatty acids, terpenes and waxes) and condensation reactions between lignin and, for example, proteins can lower the lignin purity slightly and have some influence on the lignin mass balance. The effective lignin yield in an industrial-scale process will be determined by downstream processing design for lignin isolation from the liquor and solvent recovery. A separate processing step removing residual lignin (derivatives) and phenolics from the aqueous stream might be needed in order to remove inhibitors for sugar fermentation or chemocatalytic conversion of the hemicellulose sugars.

Pulp enzymatic hydrolysis. Enzymatic hydrolysis of the pretreated feedstock was performed to assess pulp cellulose saccharification. Glucose yield was determined using an enzyme dose of 10 FPU g^{-1} dry pulp and a 10% w/v consistency. Full enzymatic cellulose conversion to glucose was achieved with wheat straw and corn stover pulp at the applied conditions (Fig. 3). The *o*-toluidine colorimetric assay slightly overestimates sugar concentrations as compared to HPAEC sugar analysis causing the glucose yield to exceed 100% when all pulp glucan is converted to glucose.[44] Furthermore, no correction was made for the increase in hydrolysis liquid volume as a result of relatively high sugar concentration buildup in a 10% consistency enzymatic hydrolysis. Glucose yields from poplar, beech and birch pulp are 72, 76 and 89% respectively (Fig. 3). Pulp cellulose digestibility of the spruce and pine pulp is limited. The high residual lignin content possibly impairs accessibility of the enzymes to the cellulose and/or deactivates the enzymes by irreversible binding to the lignin.[54,55]

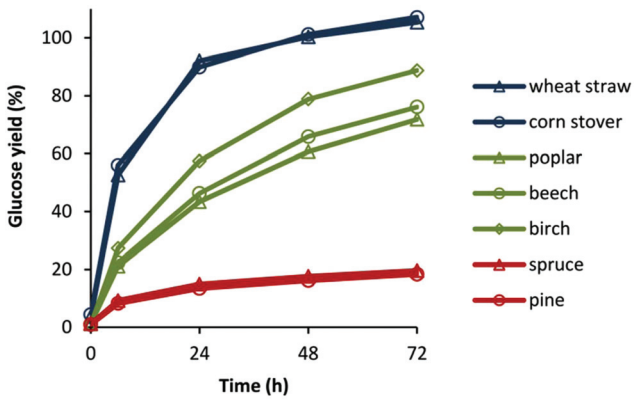

Fig. 3 Enzymatic cellulose digestibility of organosolv pretreated pulps (average of duplicates, relative standard deviation <5%).

Acetone self-condensation

Limited loss of solvent is a prerequisite for an economically viable organosolv biorefinery. Solvent losses due to incomplete recycling, solvent–solvent and solvent-product reactions can have a major impact on process economics. A potential solvent reaction is acetone self-condensation. This study focusses on analysis of the self-condensation products in the liquor. The interaction between acetone (self-condensation products) with carbohydrates, (hydroxymethyl) furfural and lignin is out of scope for this study.

Fractionation. Process conditions for the fractionation of wheat straw were designed to assess the influence of temperature, time and acid dose on acetone self-condensation kinetics while maintaining a similar level of fractionation.

The general fractionation data and product distribution in Table 3 and Fig. 4 show limited variation in polymeric C6

Table 3 Wheat straw fractionation

	Fractionation conditions[a]				General fractionation data				
Exp:	T (°C)	Time (min)	H_2SO_4 (mM)	Free H^{+} [b] (mM)	pH liquor	Pulp yield (wt%)	C6 recovery (wt%)	C5 hydrolysis (wt%)	Delignification (wt%)
1	100	960	200	347	1.3	48.8	93.6	91.2	75.7
2	140	120	60	67	2.1	46.5	91.4	96.8	79.1
3	140	60	60	67	1.7	47.3	98.3	93.4	79.7
4	140	30	100	147	1.3	46.2	93.5	96.3	79.1
5	140	15	140	227	1.2	49.5	101.2	96.2	79.2
6	170	60	35	17	2.8	47.7	92.6	94.0	78.6

[a] Using a liquid/solid ratio of 10 L 50% w/w aqueous acetone per kg dw straw. [b] Acid dose corrected for the acid neutralising capacity of the feedstock.

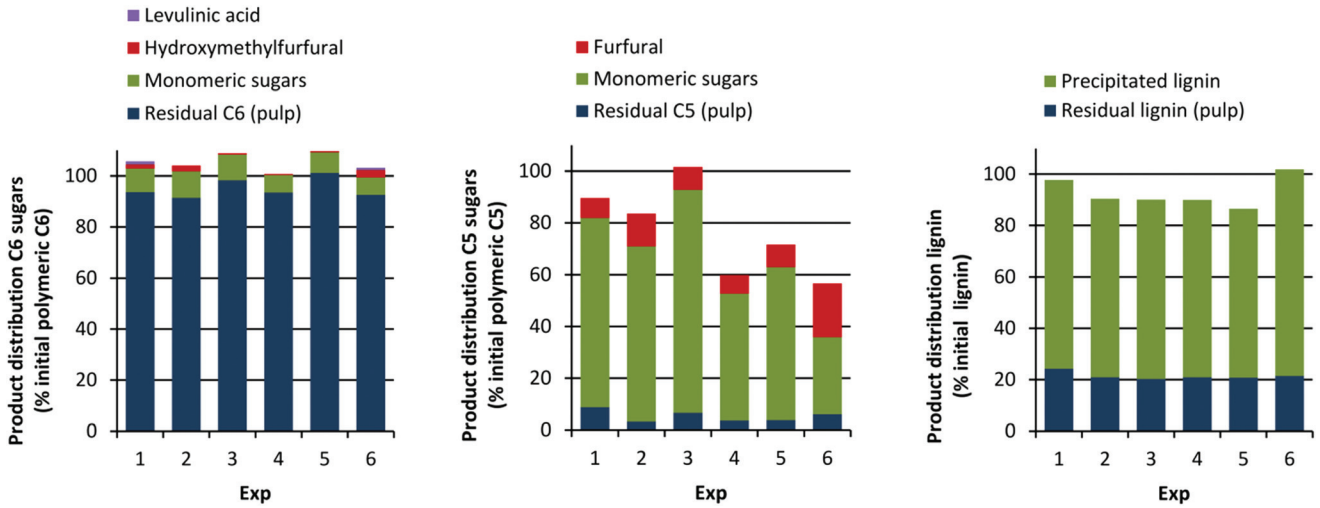

Fig. 4 Product distribution wheat straw fractionation.

sugar recovery, polymeric C5 sugar hydrolysis and delignification. The impact of process parameters is most clearly demonstrated by the hemicellulose C5 product distribution. Efficient fractionation including a high yield of monomeric C5 sugars is obtained in experiment 1 using a low temperature, a long residence time and high acid dose. At the previously described optimum at 140 °C, overall wheat straw valorisation improves when the reaction time is shortened from 120 to 60 min (exp. 2 and 3). In this case, 86.2% of polymeric C5 is converted to monomeric sugars.

Decreasing reaction time and increasing the acid dose at 140 °C (exp. 4 and 5) lowers monomeric C5 sugar yield, but no increase in furfural formation is observed. The experiment at 170 °C (exp. 6) resulted in efficient wheat straw fractionation but with a low C5 monomeric sugar yield, increased sugar degradation to furfural and suspected pseudolignin formation.[56,57]

Acetone self-condensation. Catalytic self-condensation of acetone is a complex reaction and numerous products are possible *via* competitive self-condensation and cross-condensation between the same or different ketones that are formed in the reaction. The aldol condensation of acetone initially produces diacetone alcohol (DAA) which dehydrates to form mesityloxide (MO).[58] At gas-phase reaction conditions below 550 K, formation of DAA and MO is the main process.[59] Further condensation of MO with acetone produces phorone, mesitylene, isophorone, 3,5-dimethylphenol and 2,3,5-trimethylphenol.[60]

Fractionation experiments with wheat straw were supplemented with blank runs (Table 4) where no wheat straw was added and the acid dose adjusted to compensate for the absence of acid neutralising capacity of the straw. The filtered liquor and pulp wash liquor were combined and analysed for the abovementioned components. Acetone self-condensation

Table 4 Acetone self-condensation product analysis

	Fractionation conditions				Acetone self-condensation products (mg kg^{-1} organosolv liquor)						
Exp	T (°C)	Time (min)	H_2SO_4 (mM)	Straw[a]	Diacetone alcohol (DAA)	Mesityl oxide (MO)	Mesitylene	Isophorone	3,5-Dimethyl phenol	2,3,5-Trimethyl phenol	MO/DAA ratio
1	100	960	200	+	4215	5115	<5	<10	<10	<10	1.2
				−	5643	6618	23	<10	<10	<10	1.2
2	140	120	60	+	881	2155	<5	<10	<10	<10	2.4
				−	1944	4763	13	<10	<10	<10	2.5
3	140	60	60	+	702	1762	<5	ND[b]	ND	ND	2.5
				−	1347	3162	<5	ND	ND	ND	2.3
4	140	30	100	+	629	1426	<5	ND	ND	ND	2.3
				−	1693	3720	13	ND	ND	ND	2.2
5	140	15	140	+	797	1960	<5	ND	ND	ND	2.5
				−	1765	4017	16	ND	ND	ND	2.3
6	170	60	35	+	215	399	<5	<10	<10	<10	1.9
				−	1343	4783	30	10	<10	<10	3.6

[a] + straw present, − straw absent during fractionation. [b] ND = not determined.

products are limited to mainly DAA and MO (Table 4) at the applied process conditions. Solvent loss in the blank runs due to acetone self-condensation reactions (Fig. 5) is highest for experiment 1 where a relatively low temperature is combined with a long reaction time and a high acid dose. For the fractionation optimum at 140 °C a significant reduction in DAA and MO concentration in the liquor is observed when the reaction time is halved to 60 min. A further reduction of the reaction time at 140 °C while increasing the acid dose (exp. 4 and 5) does not reduce acetone self-condensation. Wheat straw addition to the reaction mixture decreases the amount of condensation products found in the liquor. The difference is only 8.4% in exp. 1 but increases to 47.6% in exp. 2 and 87.9% in exp. 6, suggesting a strong relation with temperature. However, it is unclear at this point whether wheat straw components reduce acetone self-condensation kinetics, condensation products react with or adsorb to wheat straw components or other mechanisms play a role. The MO : DAA ratio (Table 4) increases from 1.2 for exp. 1 to an average of 2.4 for exp. 2–5 and 3.6 for exp. 6.

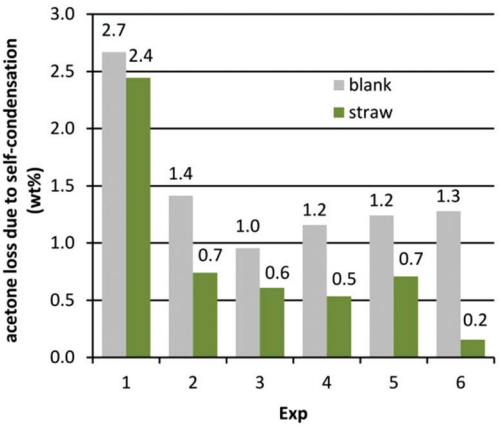

Fig. 5 Solvent loss due to acetone self-condensation.

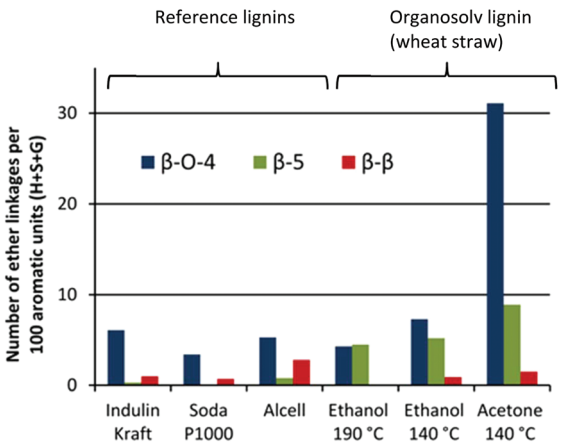

Fig. 6 Number of ether linkages in lignins determined by 2D HSQC NMR.[16] Data reference lignins were published in ref. 16.

Upon addition of wheat straw the DAA and MO concentration decreases but the ratio remains unchanged (except for exp. 6). Experiments performed on a different batch of wheat straw using conditions as exp. 3 revealed a minor increase (0.05%) in solvent loss when 60% w/w acetone is used for fractionation.

Although the observed solvent loss due to acetone self-condensation is limited, direct condensation of acetone to carbohydrates, sugar derivatives and lignin could contribute to additional solvent losses. However, the wheat straw carbohydrate product distribution in exp. 3 does not indicate substantial acetone condensation to carbohydrates and their derivatives. A follow-up study is required on solvent chemistry and the impact on downstream processing design, product (carbohydrate and lignin) properties and the economic viability of the process.

Lignin characterisation

As a first analysis of the properties of mild acetone organosolv lignins, lignin was isolated from wheat straw using optimum conditions (*i.e.*, 60 min, see Fig. 4, exp. 3) and characterised. The lignin contained 1.5% w/w residual carbohydrates, primarily arabinose and xylose, as compared to 0.5% w/w for wheat straw lignin resulting from classical ethanol organosolv.[16] The molar mass distribution was determined by size exclusion chromatography (SEC) as described in Constant *et al.* (method B).[16] The weight-average molar mass (M_w) of the lignin was 2.9 kg mol^{-1} as compared to 2.0 kg mol^{-1} for ethanol 190 °C lignin[16] and 2.3 kg mol^{-1} for ethanol 140 °C lignin. It seems unlikely that this higher M_w is due to lignin condensation reactions, since SEC analyses of the lignins resulting from the experiments given in Table 3 showed no increase in lignin molecular weight upon a longer reaction time (exp. 2 and 3) or higher process temperature (exp. 6) (see the ESI Fig. S4†). 2D HSQC NMR analysis of the mild acetone organosolv lignin from wheat straw showed remarkable characteristics as compared to classical high-temperature ethanol organosolv lignins. A high number of ether linkages per 100 aromatic units was determined, in particular β-O-4 linkages, which would suggest a less-condensed lignin as compared to ethanol organosolv or commercial reference lignins (Fig. 6). A high abundance of β-O-4 ether linkages is crucial for many chemo-catalytic depolymerisation routes.[16,61,62] Remarkably, the peak generally attributed to the γ-proton of the β-O-4 linkage was not observed in the NMR spectrum (Fig. S3† *cf.* Fig. 2d in ref. 16). In addition, the peaks belonging to the β-proton of the β-O-4 linkage changed. Apparently, chemical changes to the β-O-4 linkage seem to have occurred. Future work should characterise mild acetone organosolv lignins originating from various feedstocks in much more detail in order to elucidate the pretreatment chemistry, including possible lignin-solvent condensation reactions, and to assess potential lignin applications.

Conclusions

A novel mild acetone organosolv process was developed that effectively fractionates herbaceous biomass and hardwood into

its main components: cellulose, hemicellulose derivatives and lignin. Key features of this process are the good enzymatic digestibility of pulp cellulose to monomeric sugars, the high yield in monomeric hemicellulose sugars and the isolation of potentially less-condensed lignin. Process conditions applied were too mild for efficient softwood delignification hampering enzymatic cellulose hydrolysis. Solvent loss due to acetone self-condensation was found to be limited. The effect of condensation products on biorefinery downstream processing and product properties is subject to further study. Optimisation of process conditions for wheat straw fractionation resulted in near-complete conversion of the hemicellulose with 86% yield of monomeric C5 sugars and reduced solvent loss while maintaining an excellent cellulose recovery (98%) and delignification (80%).

Conflicts of interest

There are no conflicts to declare.

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