Separation and Purification Technology 221 (2019) 226–235

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Pilot scale recovery of lignin from black liquor and advanced characterization of the final product

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ARTICLE INFO

Keywords:
Lignin recovery
Enzymatic treatment
Filtration
Characterization
Scale-up

ABSTRACT

Recently, the academic and industrial interest in lignin as a renewable resource for many valuable applications has been on the rise. However, the current biomass separation technologies are focused on obtaining high quality cellulose which can be further processed, *e.g.*, in the paper industry, resulting in a lignin of rather low quality. Moreover, lignin recovery from black liquor is often accompanied with filter clogging and a severe flux decline, limiting the cost-efficiency of its valorization. In this work, the pilot scale recovery of lignin from a black liquor derived from a mild soda pulping process of *Miscanthus x giganteus* chips is studied with the aim to develop a straightforward procedure that yields a high quality final product. A first pilot scale experiment demonstrated the pH to be crucial for optimal precipitation. Moreover, adding an enzyme mixture containing cellulases, hemicellulases and β-glucosidases, clearly enhanced the flocculation and filterability. Thorough characterization of the obtained lignin showed a native-like structure which can be related to the mild pulping conditions and revealed that the *p*-coumarates and ferulates were converted to the free acids as a result of the base catalyzed hydrolysis as well as the enzymatic cleavage of the ester linkages leading to the complete removal of the hydrophilic (poly)saccharides. Moreover, this resulted in a slightly more hydrophobic lignin material that was more amenable to flocculation. Building on lab scale experiments aimed at optimization of the process conditions, a second pilot scale experiment was performed resulting in improved precipitation and flocculation by means of acidification, an enzymatic treatment as well as the addition of a flocculant. This allowed for smooth filtration and resulted in a high purity of the isolated lignin.

1. Introduction

In light of the depletion of the fossil feedstocks and the ever-increasing environmental concerns about the use of these resources for the production of energy, materials and chemicals, it is important to investigate the exploitation of alternative resources, preferably of a renewable origin, that may replace the fossil ones. Lignocellulose is a type of non-edible biomass which may constitute a viable option for the sustainable production of fuels as well as many materials and chemicals. Lignocellulosic biomass is composed of three major biopolymers: cellulose, hemicellulose and lignin [1–7]. Cellulose is a polymer of glucose consisting of β-(1,4)-glucopyranoside chains. Hemicellulose is a branched polymer of pentose (xylose, arabinose) and hexose (mannose, glucose, galactose) sugars. Lignin is a complex aromatic polymer of phenylpropanoid units.

The smallest building blocks of lignin are aromatic C9 components, namely, coumaryl, coniferyl and sinapyl alcohols. These C9 building blocks are copolymerized forming a highly irregular three-dimensional cross-linked lignin polymer containing three substructures, *i.e.*, p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) (see Fig. 1). The exact structure and composition of the lignin depends on the type of plant (*i.e.*, tree, crop or grass) and the external conditions present during its

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https://doi.org/10.1016/j.seppur.2019.03.081
Received 26 October 2018; Received in revised form 25 March 2019; Accepted 26 March 2019
Available online 29 March 2019
1383-5866/

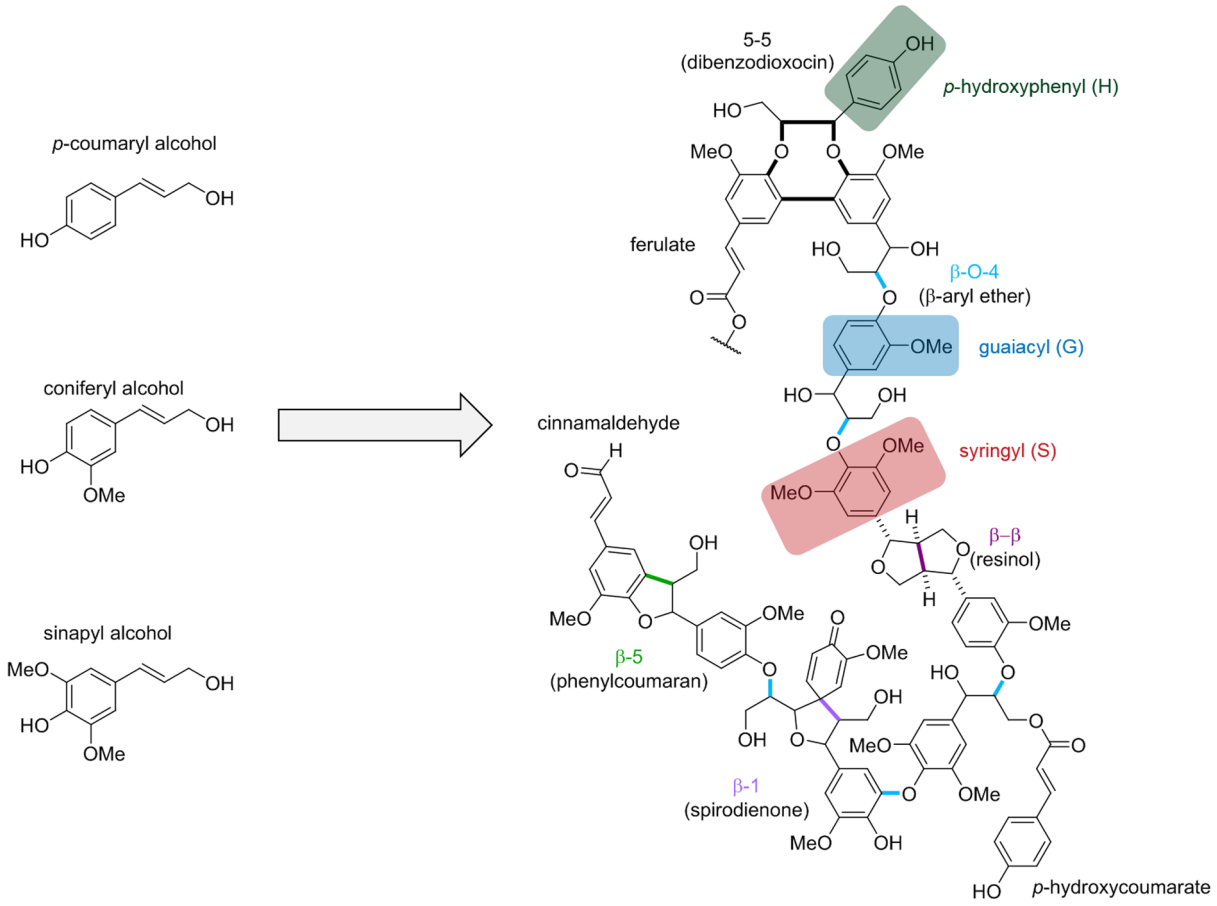


Fig. 1. A representation of a lignin structure formed from the three major monolignols, *i.e.*, p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol; Substructures: p-hydroxyphenyl (H) (green), guaiacyl (G) (blue) and syringyl (S) (red), dibenzodioxocin, ferulate, cinnamaldehyde, phenylcoumaran, resinol, spirodienone, p-hydroxycoumarate; Most common linkages: β-O-4 (blue), β-β (purple), β-5 (green), β-1 (violet), 5–5 (black). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

formation (*i.e.* the climate and weather) [8,9]. Several different type of linkages, connecting the monomers via ether bonds and/or carbon-carbon bonds (*e.g.* β-O-4, 4-O-5, β-5, β-β, 5–5 (dibenzodioxocin) and β-1 structural motifs) can be found within the lignin polymer (see Fig. 1). The β-O-4 linkage is the most common one, often making up to about 50% of all linkages [10]. In the plant cell wall, the different biopolymers are involved in hydrogen bonding interactions and, additionally, hemicellulose and lignin are covalently cross-linked. All this adds to the structural complexity and to the challenge of extracting and separating the lignin from the carbohydrates. Indeed, isolated lignin often contains a small amount of carbohydrates, as a result of the lignin-carbohydrate complex (LCC) or due to newly formed lignin-hemicelullose linkages during pulping [11–14]. For example, milled wood lignin originating from the C_4 grass *Miscanthus x giganteus* was found to contain about 22.8% carbohydrates, mainly composed of xylose [8,15]. In general, benzyl ethers, benzyl esters and phenyl glycosides are the most common lignin-carbohydrate linkages, with the benzyl ester bonds being alkali-labile, and the phenyl-glycosidic bonds being rather alkali-stable. As for the benzyl ethers, the free-phenolic α-ether structures are reported to be unstable in a basic environment even at room temperature, while non-phenolic benzyl ether bonds are alkali-stable [16]. In the LCC in grasses, the alkali-labile ester type bonds strongly predominate. Moreover, in grass type LCCs the linkages are typically comprised of *p*-coumarate and ferulate structures which are bound to the polysaccharide via an ester and to the lignin via an ester or ether. Typically, more than half of the ferulates are linked with lignin through ether bonds (51.6–68.3%) and a lesser extent through ester bonds (31.7–48.4%), while the majority of *p*-coumarates is ester-bound to lignin (67.0–83.5%) [17,18].

Since the emergence of the lignocellulosic feedstock based biorefinery concept, research has focused on converting the cellulose and hemicellulose components into consumables such as fuels, chemicals, polymers and medicines [19,20]. In contrast, less attention was paid to lignin, leaving it currently underused. Traditionally, most large-scale industrial processes that use plant polysaccharides have burned lignin to generate the power needed to productively transform the biomass [21–23]. However, if viably isolated, lignin could be an interesting resource for materials applications or for the production of renewable aromatics. Alternative routes to produce aromatics from other renewable feedstocks have been investigated [24], but as lignin is the most abundant aromatic bio-component, the depolymerization of this polymer holds great appeal [20,25–27]. In addition to such bulk aromatic chemicals production, other (macromolecular) applications for lignin or its derivatives include use as antioxidant, UV stabilizer, surfactant, copolymer in polymers or filler in adhesives and resins [9,28–39]. As a result of the current academic and industrial interest in lignin valorization, the recovery of lignin from biomass or waste streams has also recently received increased attention.

Various different processes have been reported to separate cellulose, hemicellulose and lignin such as alkaline pulping, organosolv pulping, the sulfite process, enzyme or acid catalyzed hydrolysis, the Bergius-Rheinau process and steam explosion combined with a pulping or hydrolysis treatment [20,40–42]. It should also be noted that, in addition to its origin and the external conditions during the lignin formation, the method used to extract lignin from the biomass strongly affects the structure and composition of the obtained lignin. Hence, each of these procedures yields a significantly different type of final material.

Currently, alkaline pulping is most commonly used to separate the three major components from biomass, *i.e.*, more than 85% of the global lignin production occurs via such a procedure [22]. Alkaline pulping can be divided in two subcategories, namely, the Kraft process and the soda process. In the Kraft process, sodium hydroxide and sodium sulfide are used to solubilize the lignin and hemicellulose components while in the soda process only sodium hydroxide is used to avoid sulfur contamination of the final product. When, in addition to these chemicals, high temperatures and mechanical stirring are also employed in the process, the term chemi-thermomechanical pulping (CTMP) is used. In each of the alkaline type pulping processes, the liberated lignin ends up in the dark brown solution that is called the black liquor (BL).

Up to now, not many cost-efficient methods have been developed to recover lignin from black liquor, which is the main reason for the small quantities of lignin that are currently commercially available [43]. In literature, the state-of-the-art techniques typically comprise lignin precipitation by addition of, *e.g.*, carbon dioxide, sulfuric acid or chlorine dioxide to the black liquor followed by a membrane filtration using microfiltration, ultrafiltration or nanofiltration [28,44–60]. However, the filtration step often suffers from flux decline due to plugging of the filter cake and the filter medium [43,61,62]. In the LignoBoost™ process, which has been scaled up to industrial scale in 2013 at Domtar's Plymouth, North Carolina mill, this issue is resolved by re-dispersing and acidifying the filter cake, *i.e.*, cake re-slurrying, after which the slurry is filtered and washed by means of displacement washing [62].

Here, we report on the development and pilot scale application of a straightforward method for lignin isolation based on acidification, enzymatic treatment as well as the addition of a flocculant that enhances lignin flocculation and filterability. The pilot scale recovery of lignin from a black liquor obtained from a mild soda pulping process using *Miscanthus x giganteus* chips has been optimized. Lab scale experiments have been performed to provide more insight into the parameters important for lignin isolation and served to guide the accompanying pilot scale runs. Thorough characterization of the isolated lignin revealed structural features that could be related to the filterability of the black liquor. Moreover, the lignin was found to be a high quality, rather native-like material which is in strong contrast to the more condensed lignins that are for example obtained from the Kraft process. Therefore, it is expected that this material will be very susceptible for further processing under mild conditions and, hence, has a high potential for further valorization [63].

2. Materials and experimental procedures

2.1. Pilot scale experimentation

Pilot scale experiments were performed at the Bio Base Europe Pilot Plant using the setup shown in Scheme 1. The pulper (Scheme 1a) has a volume of 1800 L and a diameter of 166 cm containing a cutting disk with a diameter of 40 cm that can rotate at a maximum speed of 900 rpm. This reactor is heated using a heating jacket. The outlet line of the pulper is equipped with a typhoon inline mixer (Scheme 1b) that contains two mixing blades with a diameter of 14 cm. This mixer is able to rotate in the range of 293 and 2930 rpm. The rotor-stator gap of the high shear colloid mill (Fryma MZ 100) (Scheme 1c) is stepless adjustable resulting in a product fineness between 100 and 500 µm. The Vincent screw press CP4 (Scheme 1d) has a screen slot size of 350 µm and can be operated with a cone counter pressure between 0 and 6 bar and screw rotation speed between 0 and 90 rpm. The chamber filter press (Netsch) (Scheme 1e) has plate dimensions of 400 × 400 mm (5 L per plate), a maximum feed pressure of 15 bar and contains 6 20 µm polypropylene filter cloths with an air permeability at 200 Pa between 8 and 10 L min^{-1} dm^{-2}. The Uniq ultrafiltration unit (Scheme 1f) contains 6 spiral wound UFX pHt membranes with a molecular weight cut off of 2000 Da. The maximum pressure drop over this equipment is 1.1 bar and the maximum operating pressure is 6 bar. The black liquor treatment tank (Scheme 1g) and the second chamber filter press (Scheme 1h) are, respectively, identical to the pulper and the first chamber filter press.

2.1.1. Black liquor preparation through a mild soda pulping process

To prepare the black liquor, 100 kg *Miscanthus x giganteus* chips were fed to the pulper (see Scheme 1a) and mixed with hot water and a NaOH solution. When the chips formed a homogeneous slurry, a pulping cycle was started using the internal mixer as well as the typhoon inline mixer (see Scheme 1b). After the first mixing phase, high shear mixing started over the colloid mill (see Scheme 1c) at about 500 L h^{-1}. Initially, the gap distance of the colloid mill was set at about 200 µm. However, due to the feed pressure rising above 4 bar, it had to be increased periodically. The milled pulp was recirculated from the colloid mill to the pulper. After 2 h, the pulp was sent to the screw press at about 400–500 L h^{-1} (see Scheme 1d). There, the pulping liquor was separated from the fiber fraction. The latter contained about 40–50% moisture. However, the filtrate still contained fine particles. Therefore, the filtrate was sent over a filter press equipped with 6 chambers containing a 20 µm polypropylene filter cloth (see Scheme 1e). Initially, the filtrate flow was high, but about halfway through the batch, the pressure increased to 10 bar and the flow reduced drastically. Finally, compressed air was applied to remove excess moisture. A wet mud-like cake of about 15% dry mass could be discharged when opening the chambers. After this process, the filtrate, *i.e.*, black liquor, was clear and free from particles. This procedure was performed two times resulting in two black liquors denoted as BL1 and BL2.

In order to allow comparison with literature data, a severity factor of this process has been calculated. The original severity factor, $\log(R_0)$, was developed by Chornet and Overend in 1987 [64] and is calculated according to Eq. (1). The variables t and T in this formula, respectively, represent the time in minutes and temperature in °C. Hence, this severity factor is very valuable for comparing data sets collected at different temperatures and times. A few years later, Chum et al. [65] and Abatzoglou et al. [66] developed the combined severity factor, $\log(R_0')$, in order obtain a better picture of the real severity of acidic processes (Eq. (2)). However, as soda pulping is an alkaline process, this combined severity factor has been slightly modified in order to account for the base concentration (see Eq. (3)).

$$R_0 = t.\ \exp((T - 100)/14.75) \quad (1)$$

$$\log(R_0') = \log(R_0) - pH \quad (2)$$

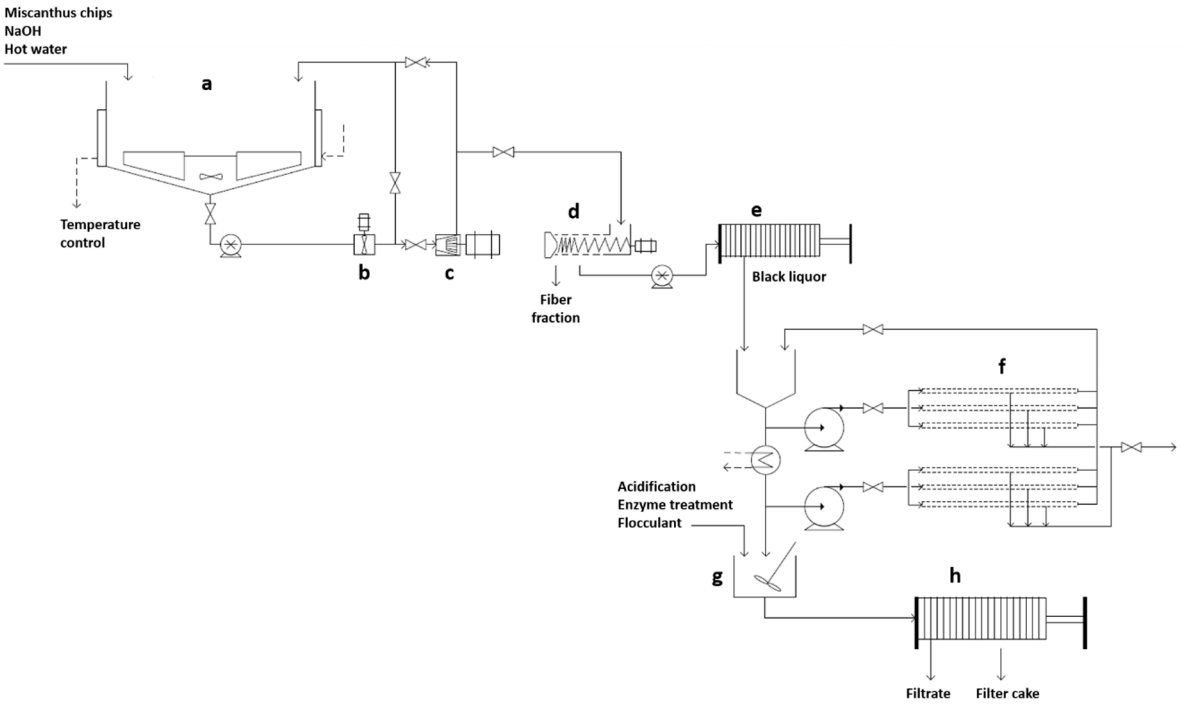
$$\log(R_0'') = \log(R_0) - pOH \quad (3)$$

2.1.2. Pilot scale lignin recovery

In this work, two pilot scale experiments were performed to recover lignin from the produced black liquor. The first experiment mainly aimed at identifying potential stumbling blocks while the conditions applied in the second pilot experiment have been chosen after performing some lab scale optimization experiments (as discussed in Section 3.2). Hence, the procedures used in both experiments are significantly different from each other.

In the first experiment, about 873 kg of the chamber filter press filtrate was fed to the ultrafilter which consisted of 6 spiral wound UFX pHt membranes with a 2 kDa cutoff (see Scheme 1f). Subsequently, the retentate was acidified to pH 5.2 using 5.9 kg of a 34% HCl solution (see Scheme 1g). The solution had a temperature of around 20 °C and was continuously circulated during acid dosing. Next, 4.65 kg of an Accellerase® Trio™ enzyme mixture containing cellulases, hemicellulases and β-glucosidases [67], was added and the slurry was heated to 45 °C. In order to allow time for the enzymes to act, the mixture was further circulated overnight. Afterwards, the warm suspension was filtered over 6 chambers with a 20 µm polypropylene filter cloth (see Scheme 1h). Finally, a wet filter cake was retained which was dried using compressed air.

In the second experiment, approximately 700 kg of the chamber filter press filtrate was immediately acidified to pH 4.8 using a 20%



Scheme 1. Process scheme of the pilot scale experiments; (a) pulper, (b) typhoon inline mixer, (c) high shear colloid mill, (d) screw press, (e) chamber filter press, (f) ultrafilter, (g) black liquor treatment tank, (h) chamber filter press.

sulfuric acid solution (see Scheme 1g). Subsequently, while stirring the mixture, the temperature was increased to 50 °C. When the desired temperature was reached, 1.7 kg of the Accellerase® Trio™ enzyme mixture was added and the mixture was incubated in the stirred tank reactor for 15 h at 50 °C. Afterwards, the warm slurry was fed to the filter press containing 6 chambers with a 20 µm polypropylene filter cloth (see Scheme 1h). This led to a first filter cake which was dried using compressed air. Subsequently, 16.4 kg of 8.5 w% of a epichlorohydrin-dimethylamine (EPIDMA) flocculant (FL 2949) solution was added to the remaining 448 kg of slurry. The slurry was stirred for 30 min after which it was fed again to the chamber filter press resulting in a second filter cake which was also dried using compressed air. The course of this second pilot scale lignin recovery experiment is also schematically shown in Scheme 2.

2.2. Lab scale experimentation

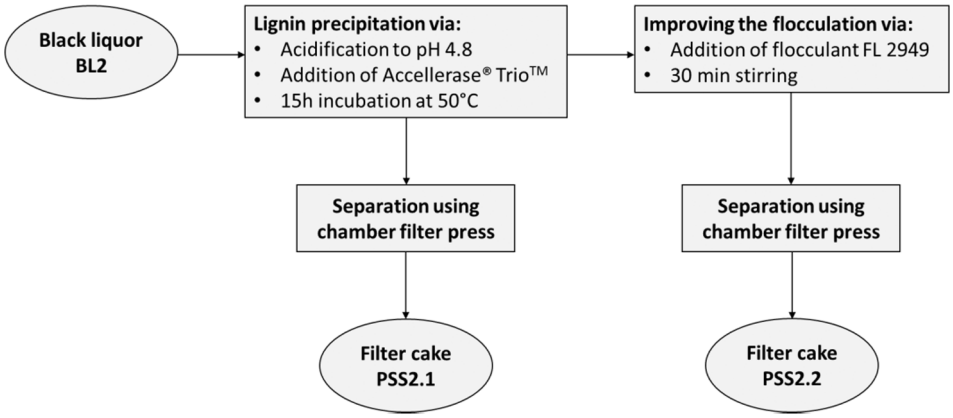
2.2.1. Lignin precipitation from the black liquor

At the lab scale, lignin was precipitated from black liquor BL1 using multiple methods, *i.e.*, by means of acidification, addition of the Accellerase® Trio™ enzyme mixture and addition of flocculants.

In the acidification experiments, the pH of 20 mL black liquor was lowered by adding a 30 w% sulfuric acid solution. Once the desired pH was reached, the sample was centrifuged for 10 min at 3000 rpm. After decanting the supernatant, the precipitate was dried for 24 h at 90 °C. Finally, the mass of the precipitate was determined and the percentage of precipitate was calculated with respect to the amount of dissolved solids or dry matter present in the black liquor.

To investigate the effect of the Accellerase® Trio™ enzyme mixture on the lignin precipitation, 20 mL black liquor was first acidified to pH 4.75 using a 30 w% sulfuric acid solution. Subsequently, 0.18 mL of Accellerase® Trio™ enzyme mixture per gram of dry matter was added. Also, a reference sample in which no enzyme was added was included in the following steps of the procedure. All samples were placed in a shaker for 15 h at 40 °C. Note that the chosen pH and temperature are in the optimal operating range of the enzyme mixture [67].

Subsequently, 0.29 w% of flocculant (with respect to the total amount of black liquor) was added to some of the samples and the samples were homogenized. Several cationic flocculants, *i.e.*, Superfloc SD 2080, FL 2949, FL 4440, Chitoseen F and Tanfloc SH, were tested.



Scheme 2. Schematic representation of the second pilot scale lignin recovery experiment.

However, Superfloc SD 2080 resulted in a high viscosity making the sample not filterable, FL 2949 led to a very sticky precipitate making it impossible to transfer the entire precipitate to a filter, Chitoseen F proved to be difficult to dissolve in the acidified black liquor and Tanfloc SH led to a very low filtration rate. Hence, only FL 2949 was used further in this work.

A part of each sample was treated similarly to the end of the acidification experiments, *i.e.*, the mixture was centrifuged for 10 min at 3000 rpm, the supernatant was decanted, the precipitate was dried for 24 h at 90 °C and, after cooling for 10 min in a desiccator, the mass of the precipitate was determined and the percentage of precipitate was calculated with respect to the dry matter. The other part of each sample was used to estimate the filtration rate.

In order to estimate the filtration rate, a filter was placed in a funnel on which a fixed amount of sample was introduced. Thereafter, the amount of liquid that ran through the filter into a graduated cylinder per unit of time was measured. During the test, no additional sample was placed on the filter. Three types of filters were considered, *i.e.*, S&S types 589/2, 595 and 1505, containing, respectively, pores of 4–12 µm, 4–7 µm and 12–25 µm. As expected, it was observed that pore clogging was most severe when the filter with the smallest pores was used (*i.e.* S&S 595). In the pilot scale experiments (see Section 3.1.3), a filter with 20 µm pores was used, resulting in pore clogging after several hours on stream. However, to reduce the time in the lab scale experiments, it was decided to continue working with the filter with the smallest pores, *i.e.*, S&S 595. Note that a volume increase of about 30% occurred when the flocculant was added to the sample. Hence, in order to correctly interpret the filtration rate data, this was accounted for by dividing the filtration rate of these samples by a factor of 1.3.

2.3. Characterization techniques

2.3.1. Miscanthus x giganteus chips, black liquor and precipitate characterization

The amount of dry matter in the *Miscanthus x giganteus* chips was determined using the NREL/TP-510-42621 method, *i.e.*, drying at 105 °C [68]. Subsequently, the lignin, hemicellulose and cellulose content of the dry matter was characterized using the NREL/TP-510-42618 method [69].

The pH of the black liquors was determined with a digital Metrohm 827 lab pH meter. The density was determined by measuring the mass of the black liquor in a known volume. The amount of dissolved solids or dry matter was determined with the same method as used for the *Miscanthus x giganteus* chips, *i.e.*, the NREL/TP-510-42621 method [68]. After obtaining a dry sample, the inorganic or ash content was determined by combustion of that sample at 575 °C based on the NREL/TP-510-42622 method [70]. The amount of (Klason) lignin in the black liquor is obtained using the NREL/TP-510-42618 method, *i.e.*, by precipitation via acidification using a 72 w% sulphuric acid solution followed by gravimetric analysis [69].

The composition of the obtained precipitates, *i.e.*, ash and lignin content, was determined using the same techniques.

2.3.2. Gel permeation chromatography

Gel permeation chromatography (GPC) was performed on a Polymer Labs GPC 50 system, equipped with a series of three PLGel Mixed-E columns and a guard column and using THF spiked with 1 vol % acetic acid as mobile phase. Detection was done with an external Knauer UV detector at 280 nm and molecular weight determinations (M_n and M_w) were based on calibrations with polystyrene standards (M_n = 162, 570, 1060, 1400, 2240, 3690, 4760, 7130, 12,800 and 19,690). Prior to analysis the lignin was acetylated with pyridine/acetic anhydride (1:1) overnight and then dried under a stream of N_2 [71].

2.3.3. HSQC analysis

The 2D HSQC NMR spectrum was acquired on a Bruker Avance II 600 MHz spectrometer equipped with a 5 mm CPTCI ^{1}H–^{13}C/^{15}N/^{2}H cryogenic probe with z-gradients at 25 °C using the hsqcetgpsp.3 pulse program. Matrices of 2048 data points for the ^{1}H-dimension and 128 data points for the ^{13}C-dimension were collected with a relaxation delay of 1 s and spectral widths from 13 to −1 ppm and from 160 to 0 ppm for the ^{1}H and ^{13}C dimensions, respectively. The lignins were dissolved in DMSO-d_6 after overnight stirring (100 mg/600 µL) and chemical shifts were referenced to the solvent signal (2.50/39.5 ppm). The spectrum was processed using Bruker TopSpin 3.5 software and processed using squared cosinebell in both dimensions and LPfc linear prediction (32 coefficients) in F1. A semi-quantitative analysis of the HSQC spectrum was performed by integration of correlations peaks in the different regions of the spectrum. The relative quantity of side chains involved in the inter-unit and terminal substructures was expressed as a number per 100 aromatic units (S + G + H).

2.3.4. ^{31}P NMR analysis (hydroxyls and carboxylic acids)

The ^{31}P NMR measurement was performed according to a literature procedure [72]. The lignin sample was analyzed using the standard phosphitylation procedure using a dried (4 Å molecular sieves) solvent mixture composed of pyridine/deuterated chloroform (1.6/1.0 v/v). 40 mg of dried lignin was dissolved in the solvent mixture (400 µL) at room temperature overnight under continuous stirring. Stock solutions of the internal standard (cholesterol, 19 mg/mL) and relaxation reagent (chromium (III) acetylacetonate, 11.4 mg/mL) were prepared separately using the solvent mixture for dissolution. 200 µL and 100 µL were respectively added to the lignin mixture. Prior to analysis, 100 µL of derivatization reagent (2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane) was added and the mixture was transferred into a 5-mm-OD NMR tube. ^{31}P NMR spectrum was obtained on a Varian 400 MHz NMR spectrometer using a standard phosphorus pulse program with a relaxation delay of 5 s and 256 acquired scans. Chemical shifts were referenced from the sharp signal arising from the reaction product between residual water and 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane at 132.2 ppm.

3. Results and discussion

3.1. First pilot scale lignin recovery experiment

To identify potential stumbling blocks in the recovery of lignin from pulp mill black liquor, a preliminary pilot scale experiment was performed. A black liquor was produced via mild soda pulping of *Miscanthus x giganteus* chips. The obtained black liquor was thoroughly characterized and then the lignin recovery by means of acidification, enzymatic treatment and filtration was studied. Finally, the obtained lignin was characterized using GPC and NMR analysis.

3.1.1. Mild soda pulping of Miscanthus x giganteus chips

Before starting the soda pulping process, the *Miscanthus x giganteus* chips were characterized, revealing that they contain 91% dry matter which in turn consists of 20.2% lignin, 27.0% hemicellulose and 49.2% cellulose.

The pilot scale soda pulping process was performed by high shear mixing 100 kg *Miscanthus x giganteus* chips with hot water and a NaOH solution. About 1000 kg of black liquor was obtained from this experiment. The modified severity factor of this process, $\log(R_0^{''})$ (see Eq. (3) in Section 2.1.1), was observed to be 0.12. Compared to other biomass fractionation processes such a severity factor can be considered to be very mild. In traditional Kraft pulping, wood fibers are treated with a 1 M NaOH and 0.25–0.7 M Na_2S aqueous solution at temperatures between 165 and 175 °C for 1–2 h. However, the conditions can vary extensively with the type of feedstock and the desired extent of delignification. As described by Rinaldi et al. [63], the delignification process can be categorized into 3 stages: the initial (10–15%), bulk (16–60%) and final (60–80%) delignification. During initial delignification, both the lignin dissolved in the liquor as well as the residual lignin in the pulp have significant quantities of β-ether linkages (around 50%). During this stage, the modified severity factor, $\log(R_0^{''})$, lies between 0.41 and 3.38 (see

Table 1
Pulping conditions and corresponding severity factors for the different stages in traditional Kraft pulping [63] and soda pulping of Miscanthus sinensis [73].

		T (°C)	t (min)	pH	$\log(R_0^{''})$
Traditional Kraft pulping [63]	Start	80	10	14.0	0.41
	Initial stage	150	80	14.0	3.38
	Bulk stage	150	160	14.0	3.68
	Final stage	170	250	14.0	4.46
Soda pulping of Miscanthus [73]	Very mild	80	30	13.4	0.29
	Intermediate	100	30	13.4	0.88
	Intermediate	80	180	13.7	1.37
	High	100	180	13.7	1.95

Table 1). In the later stages, it increases to 3.68 (bulk stage) and 4.46 (final stage) and the β-ether linkages are cleaved. Moreover, new recalcitrant, condensed and cross-linked C-C bonds are formed during the final stages resulting in a modified structure. Iglesias et al. [73] studied the effect of pulping time and temperature on the pulp yield and lignin solubilization in soda pulping of *Miscanthus sinesis*. Table 1, shows that using this type of feedstock which is typically more easy to degrade than hard wood, a modified severity factor of about 0.3 and below can be classified as very mild. Log($R_0^{''}$) values ranging from 0.88 to 1.37 can be classified as intermediate while a value of 1.95 can be considered as high. Hence, based on the very mild pulping conditions as demonstrated by the modified severity factor of 0.12 and a non-woody feedstock, a relatively high pulp yield and a lignin structure with significant quantities of β-ether linkages can be expected.

3.1.2. Black liquor characterization

The obtained soda pulping *Miscanthus x giganteus* black liquor (BL1) was characterized and compared with literature data of a similar soda pretreated *Miscanthus* [74], see Table 2. As a result of the soda pretreatment, the pH of the black liquor is in the alkaline range, *i.e.*, about 13. The density is about 1 g/mL indicating that the sample consists largely of water which was confirmed by evaporation of the solvent, yielding 41.1 ± 0.5 g/L of dry matter. Combustion of this dry matter revealed that about two-thirds is inorganic. Klason lignin analysis showed that 21.8 ± 0.3 w% of the dry matter consists of acid insoluble lignin. The acid soluble components are most likely carbohydrates and a small amount of acid soluble lignin. These results agree well with the results obtained by García et al. [74].

The entire batch of black liquor, *i.e.*, about 1000 kg, thus, contains about 8.96 kg of Klason lignin, while the 100 kg of *Miscanthus x giganteus* chips fed to the pulper comprised about 18.38 kg of Klason lignin. Hence, a pulp yield of 48.7% has been obtained which categorizes the described mild soda pulping process within the bulk delignification stage [63].

3.1.3. Lignin recovery

About 873 kg of the chamber filter press filtrate (see Scheme 1.e), *i.e.*, black liquor BL1 as characterized in Table 2, was fed to the ultrafilter with a 2 kDa cutoff (see Scheme 1f). The obtained filtrate was colored, yet lighter than the feed indicating some loss of lignin. Subsequently, the retentate was acidified to pH 5.2 (see Scheme 1g) yielding a light brown precipitate. However, no flocculation could be observed. Next, an Accellerase® Trio™ enzyme mixture was added and the slurry was recirculated overnight at a temperature of 45 °C. This enzyme cocktail has been optimized for the hydrolysis of both cellulose and hemicellulose [67]. Hence, this procedure is assumed to significantly reduce the carbohydrate content. Wallmo et al. described that the filtration resistance can be considerably lowered by reducing the hemicellulose content in the black liquor by means of a heat treatment and ultrafiltration [75]. Although the method is very different, the enzymatic treatment is expected to give rise to similar results. After the enzymatic treatment, a clearly visible flocculation of the precipitate occurred. Finally, the precipitate was filtered using the chamber filter press (see Scheme 1h). However, the feed pressure rapidly increased towards 10 bar, with the filtration rate was dropping to below 50 L h^{-1}, and the final filtrate still contained some turbidity. Hence, the final filtration was still not optimal. In the end, 16.4 kg of filter cake was obtained. In the remainder of the manuscript, this sample is denoted as PSS1 referring to 'sample from pilot scale experiment 1'.

Table 2
Black liquor characterization and comparison with literature data from García et al. [74] The w% are expressed relative to the total amount of dry matter present in the black liquor.

Black liquor	BL1	BL2	García et al. [74]
pH	12.92	12.52	12.64
Density (g/mL)	1.02 ± 0.02	1.02 ± 0.02	1.01
Dry matter (g/L)	41.1 ± 0.5	36.4 ± 0.5	42.4
Ash (w%)	67.2 ± 0.3	66.7 ± 0.3	67.9
Klason lignin (w%)	21.8 ± 0.6	18.5 ± 0.7	23.5

3.1.4. Characterization of the obtained lignin

By means of GPC, the weight-average (M_w) and number-average (M_n) molecular weights and the polydispersity (M_w/M_n) of the *Miscanthus* soda lignin PSS1 were found to be 6250 g/mol, 1530 g/mol and 4.08, respectively (see Fig. 2). These values are similar to what has been reported for *Miscanthus x giganteus* lignin which has been obtained using acid organosolv pulping processes, *i.e.*, acetic and formic acid fractionation [15]. However, compared to soda type lignins originating from different sources such as flax, straws, bagasse or hardwoods, which typically have a M_w in the range from 1500 to 5000 g/mol [76,77], the obtained molecular weight seems to be relatively high. Moreover, the molecular weight distribution of the obtained lignin is slightly broader than typically found for soda lignin, *i.e.*, ranging from 2.5 to 3.5 [76,77]. However, it should be noted that lignin from non-wood plants typically exhibits a higher polydispersity than wood lignin [76].

The absence of cross-peaks characteristic for cellulose or hemicellulose in the 2D HSQC NMR analysis indicates that the lignin recovered from the first pilot scale experiment, *i.e.*, PSS1, is very clean, *i.e.*, free from significant carbohydrate or extractive components. As expected for *Miscanthus x giganteus*, it is composed of a mixture of syringyl (S), guaiacyl (G) and *p*-hydroxyphenyl (H) units, and, surprisingly, the lignin structure is rather native-like (see Fig. 3). In particular, large amounts of sensitive β-aryl ether (β-O-4, A) units are clearly present (44 linkages per 100 aromatic units), together with other sensitive structural units such as phenylcoumarans (β-5, B) and cinnamyl alcohol (X) end groups. The composition and interunit abundances are consistent with values reported for native grass lignins

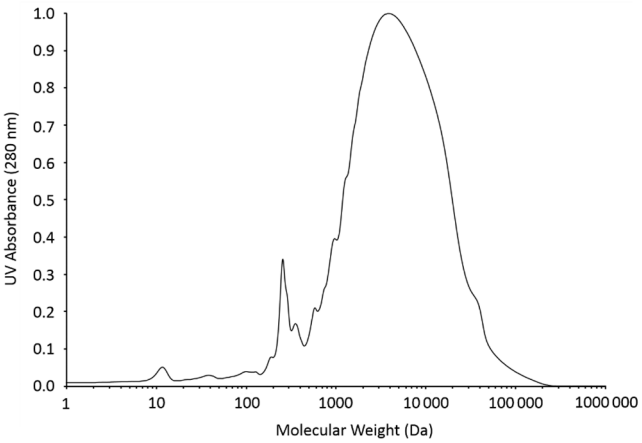


Fig. 2. GPC analysis of the lignin PSS1 isolated after pilot scale soda pulping of *Miscanthus x giganteus*.

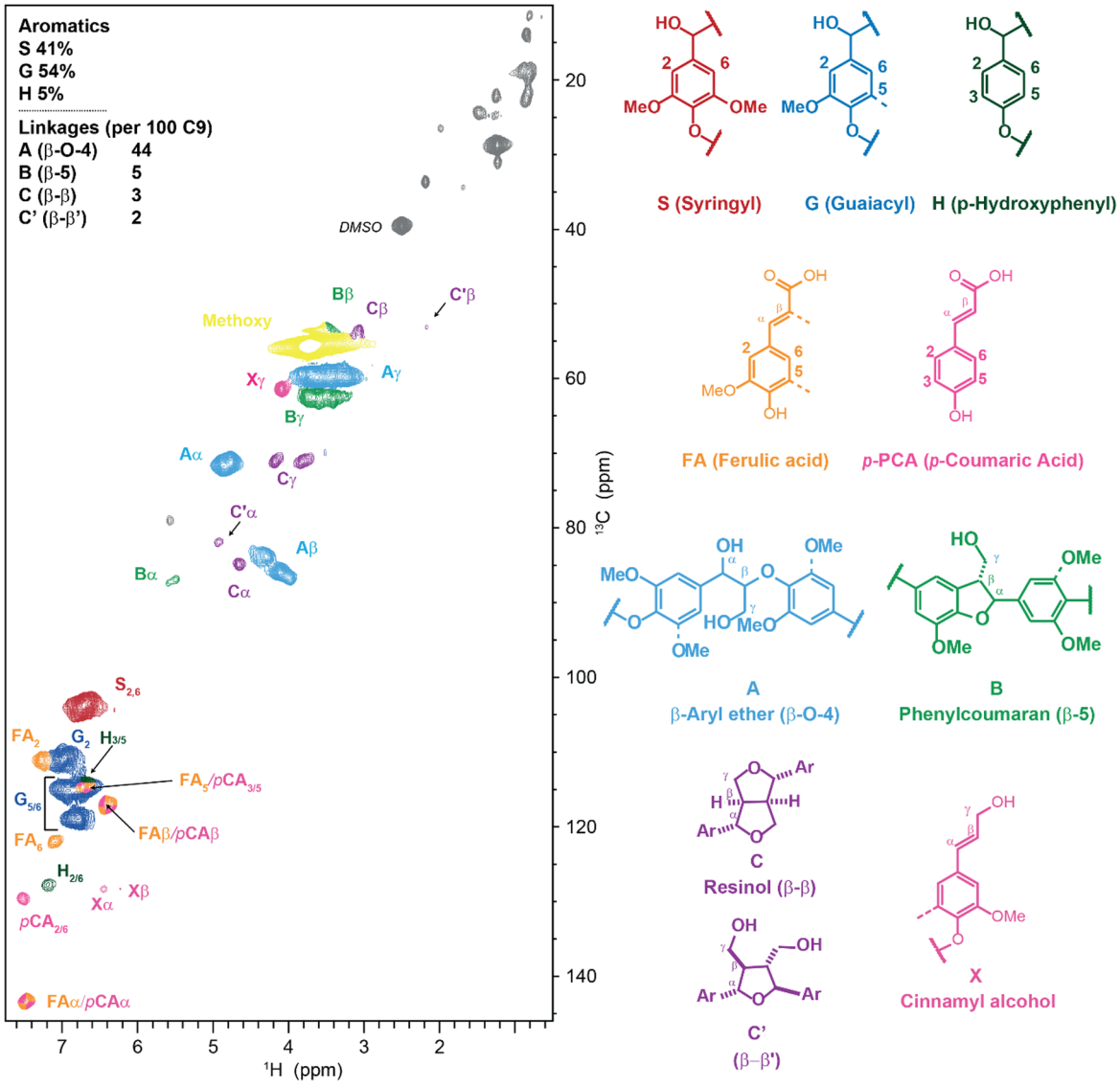


Fig. 3. HSQC analysis of the lignin PSS1 isolated after pilot scale soda pulping of *Miscanthus x giganteus*.

[63,78], but are in striking contrast to those reported for P1000 soda lignin which contains about 13 times less β-O-4 linkages [72]. This can be related to the mild nature of this particular soda pulping procedure. Another interesting feature of the lignin is that the *p*-coumarates (PCA) and ferulates (FA) [17,18,78], appear to be converted to the corresponding free acids based on the ^{13}C chemical shift of the β-carbon. This is consistent with base-catalyzed hydrolysis [79,80] and the enzymatic cleavage [81] of ester linkages during the pulping process and the enzymatic treatment, respectively, and is supported by the ^{31}P NMR analysis detailed further on. Considering that a fraction of the *p*-coumarates and ferulates are bonded to the polysaccharides as well as to the lignin via an ester linkage [17,18], some of these acids are likely present as monomers, which is consistent with the observation of low molecular weight components during GPC analysis (see Fig. 2).

The ^{31}P NMR analysis of lignin sample PSS1 is shown in Fig. 4 and indicates that the lignin is rich in aliphatic hydroxyls (2.95 mmol/g) and relatively low in phenolics (total 0.59 mmol/g), consistent with the native-like structure observed by 2D HSQC NMR. Of the phenols present, guaiacyl type units are the most abundant (0.41 mmol/g), with smaller amounts of 5-substituted (*e.g.* S units, 0.09 mmol/g) and H (0.09 mmol/g) units. This data is again consistent with a non-degraded lignin structure. For instance, similar phenolic contents were found in lignins obtained from mild pretreatments such as milled wood or enzymatic pulping (0.70–1.2 mmol/g) [82,83]. A notable feature of this lignin is the high carboxylic acid content (0.60 mmol/g), supporting the HSQC NMR analysis, which suggested that hydrolysis of coumarate and ferulate groups (generating free carboxylic acids) had occurred during the chemi-thermomechanical pulping and subsequent enzymatic treatment.

3.2. Lab scale optimization

The first pilot scale lignin recovery experiment revealed the pH as a crucial parameter for an optimal precipitation. Moreover, adding an Accellerase® Trio™ enzyme mixture clearly has a positive effect on the flocculation and the filterability. However, the final filtration was still not optimal, *i.e.*, the feed pressure rapidly increased, filtration rate was rather low and the final filtrate was still somewhat turbid. Therefore, the lignin recovery from the black liquor was further investigated at the lab scale. The effect of acidification of black liquor BL1 and addition of Accellerase® Trio™ enzyme mixture and flocculant FL 2949 to that black liquor on the lignin precipitation, filtration rate and the purity of the final product was investigated.

3.2.1. Precipitation by acidification

In order to identify the optimal pH to precipitate lignin from the black liquor, the effect of acidification was studied. The pH of the

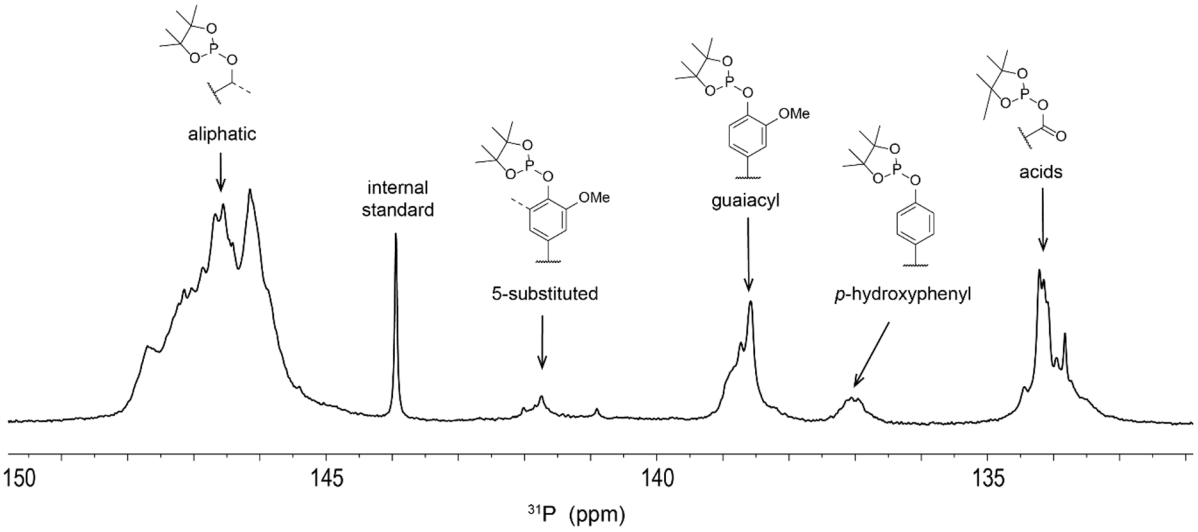


Fig. 4. ^{31}P NMR analysis of the lignin PSS1 isolated after pilot scale soda pulping of *Miscanthus x giganteus*.

samples was lowered by adding a 30 w% sulfuric acid solution and the amount of precipitate was measured gravimetrically. Fig. 5 shows the percentage of precipitate with respect to the amount of dissolved solids or dry matter present in the black liquor. At basic conditions, little to no precipitation is formed, *i.e.*, below 3%. In this environment, the functional groups of lignin are most likely ionized making the molecule readily soluble in water. However, starting from a pH of 6 the percentage of precipitate rapidly increases up to 45%. At this pH, the functional groups are protonated resulting in a lower hydrophilicity as well as a lower electrostatic repulsion between the different lignin molecules leading to a lower solubility [84]. This result is constituent with literature where for a similar type of lignin an increase up to 41% between pH 6 and 4 has been observed. After which it further increases to 45% and even 53% at pH 2.5 and 0.7, respectively [74]. However, in this case, the percentage of precipitate seems to be more stable as a function of pH. Moreover, it should be noted that as only 21 w% of the dry matter consists of lignin, also some other components such as inorganic ash or carbohydrates, have been precipitated.

3.2.2. Effect of enzymes and flocculants on the precipitate purity and filtration rate

Next, some lab scale experiments were performed to elucidate the effect of enzymes on the precipitate filtration rate and purity. Additionally, flocculants have been considered to enhance the precipitate formation. Lignin was precipitated from black liquor using three methods, *i.e.*, (i) acidification to pH 4.75 resulting in sample LSS1 (referring to 'lab scale sample 1'), (ii) acidification to the same pH followed by a treatment for 15 h at 40 °C with the Accellerase® Trio™ enzyme mixture resulting in sample LSS2 and (iii) these two steps followed by the addition of flocculant FL 2949 resulting in sample LSS3. The pH of 4.75 was opted for because it results in a good precipitate formation from black liquor, see Section 3.2.1, and it is in the optimal operating range of the enzyme mixture [67]. In order to mimic the filtration issues at the pilot scale, the filtration rate of the samples was measured using a filter that exhibits rapid pore clogging, *i.e.*, filter S&S 595. Subsequently, the precipitates formed using the three procedures were thoroughly characterized, see Table 3.

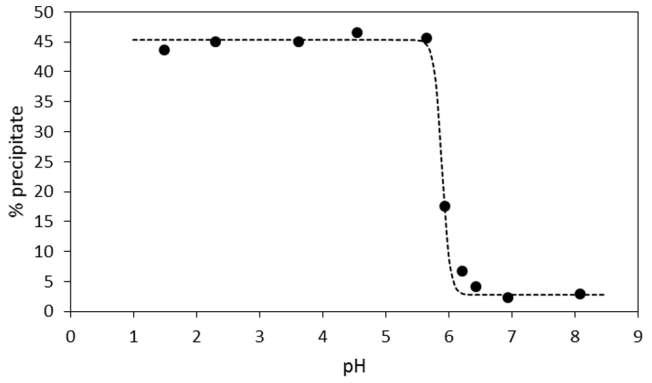


Fig. 5. Percentage of precipitate with respect to the total dry matter content formed as a function of pH; dashed line is intended as visual aid only.

As mentioned in the previous section, about 45 w% of the dry matter present in the black liquor precipitates when the pH is lowered to 4.75. About 44 w% of the precipitate at acidic conditions consists of Klason Lignin. This corresponds to 20 w% with respect to the total amount of dry matter present in black liquor BL1 (see Table 2) and a yield of 91.3% with respect to the total amount of lignin present in that black liquor. Additionally, sample LSS1 exhibited a low filtration rate of about 0.76 mL/min. When the black liquor was treated with the Accellerase® Trio™ enzyme mixture, *i.e.*, sample LSS2, about 12 w% less precipitate was formed. However, most notably, the amount of Klason lignin that precipitated was the same. Hence, the purity of the sample increased, *i.e.*, about 61% versus 44%. This is largely due to a low acid soluble content in the precipitate caused by the ability of the enzymes to cleave ester and glycosidic bonds. As a result, the carbohydrates present in the black liquor are reduced in size, and have more functional groups, making them more soluble in water. Moreover, the enzymes are able to remove the residual carbohydrates from the lignin backbone [81] yielding slightly more hydrophobic lignin particles that retain less water. As a result, a higher filtration rate, *i.e.*, 1.63 mL/min, was observed. Sample LSS3 which was precipitated using flocculant FL 2949 contains more organic matter than the one that was solely treated with enzymes. This is most likely due to the presence of the flocculant in the sample. Additionally, the flocculant might also result in the precipitation of more acid soluble components resulting in a decreased purity, *i.e.*, 52.5% versus 60.7%. On the other hand, the filtration test showed that the filtration rate drastically increased, *i.e.*, up to 3.69 mL/min, when the flocculant was used.

3.3. Optimized pilot scale lignin recovery

A second pilot scale experiment was performed to validate the insights obtained from the lab scale experiments. For this purpose a second batch of black liquor (BL2) was produced by the mild soda pulping of *Miscanthus x giganteus* chips. This pulping procedure was

Table 3
Lab scale filtration rate and characterization results of the precipitate formed at different conditions, *i.e.*, (i) LSS1 at pH 4.75, (ii) LSS2 treated with the Accellerase® Trio™ enzyme mixture (AT) at pH 4.75 and 40 °C and (iii) LSS3 treated with the Accellerase® Trio™ enzyme mixture (AT) at pH 4.75 and 40 °C followed by the addition of flocculant FL 2949 (FL). The filtration rate is defined as the volume of black liquor per minute passing through filter type S&S 595. In the case of the sample which was precipitated using flocculant FL 2949 the measured value was divided by a factor of 1.3 in order to compensate for the increased volume. The w% precipitate is expressed relative to the total amount of dry matter present in black liquor BL1 (see Table 2). The other w% are expressed relative to the total mass of precipitate formed.

Precipitation conditions	LSS1; pH 4.75	LSS2; AT at pH 4.75	LSS3; AT at pH 4.75 and FL
Filtration rate (mL/min)	0.76 ± 0.09	1.63 ± 0.08	3.69 ± 0.44
Precipitate (w%)	45.1 ± 1.0	33.3 ± 1.0	35.0 ± 1.0
Ash (w%)	33.0 ± 1.0	27.9 ± 1.2	20.9 ± 1.0
Klason lignin (w%)	44.1 ± 0.4	60.7 ± 0.3	52.5 ± 0.6

identical to the one previously used. The obtained black liquor was thoroughly characterized. Subsequently, the recovery of the lignin was performed, guided by the lab scale experiments, which indicated that using the Accellerase® Trio™ enzyme mixture significantly enhanced the filtration rate as well as the purity, while the use of this enzyme mixture in combination with flocculant FL 2949 led to an even further increase in filtration rate but at the expense of the purity. Therefore, in first instance, it was opted to scale up the former procedure. However, again some issues arose during the filtration and it was decided to add some flocculant. Finally, the precipitates formed before and after flocculant addition were characterized.

3.3.1. Black liquor characterization

The second batch of black liquor, *i.e.*, BL2, was characterized using the same procedures as before, see Table 2. BL2 contained a slightly lower amount of dry matter than BL1 and the black liquor of García et al. [74], *i.e.*, 36 g/L compared to 41 g/L and 42 g/L. The ash content was identical in BL1 and BL2, *i.e.*, about 67 w%. But within the organic matter content, BL2 consisted of slightly less lignin than BL1. However, overall it can be concluded that the properties of black liquor BL2 were very similar to these of BL1 as well as the results obtained by García et al. [74].

3.3.2. Lignin recovery

Scheme 2 shows a schematic overview of the different steps of the second pilot scale lignin recovery experiment. This experiment started by acidifying approximately 700 kg black liquor BL2 to pH 4.8, adding 1.7 kg of the Accellerase® Trio™ enzyme mixture and incubating the mixture in the stirred tank reactor for 15 h at 50 °C (see Scheme 1g). Hence, compared to the first pilot scale lignin recovery experiment, this corresponds to a slightly lower pH, a lower amount of the enzyme mixture and a higher incubation temperature. Subsequently, the slurry was fed to the chamber filter press (see Scheme 1h). However, after 4 h the filter was clogged and the feed pressure increased dramatically. Therefore, the procedure was stopped early but a first filter cake (denoted as PSS2.1 which refers to 'pilot scale experiment 2 sample 1') could be recovered from the chamber filter press. In order to improve the filterability, 16.4 kg of 8.5 w% flocculant FL 2949 solution was added to the remaining 448 kg of slurry. The slurry was stirred for 30 min after which it was fed again to the chamber filter press. This time there was hardly any pressure build up and the entire slurry could be filtrated without any issues and a second filter cake (denoted as PSS2.2, *i.e.*, 'pilot scale experiment 2 sample 2') was recovered.

3.3.3. Characterization of the obtained filter cakes

After removing the excess water from the obtained filter cakes, the amount of precipitated dry matter and the composition of both filter cakes was determined (see Table 4). First of all, the flocculation enhancing properties of FL 2949 are reflected in the strongly enhanced recovery of dry matter from the black liquor, *i.e.*, 22.6 w% after addition of this component versus 13.4 w% without. One could expect that this enhanced recovery of dry matter would have a detrimental effect on the lignin purity of the sample, something that was indeed observed in the laboratory scale experiments. This turned out not to be the case at the pilot scale, however. The filter cake obtained using both the enzyme mixture and flocculant FL 2949 exhibited a purity of about 83 w% while the one obtained using only the enzyme mixture exhibited a purity of about 77 w%. This means that full lignin recovery was reached when the black liquor was treated with both methods while only 55.8 w% lignin yield was obtained when using only the enzyme treatment. Moreover, it can be noted that both purities obtained at the pilot scale are very high compared to those obtained in the lab scale experiments. This is mainly due to the low amount of ash or inorganic components that was retained in the filter cakes. Additionally, filter cake PSS2.2 contains a relatively low amount of acid soluble components. This shows that extrapolation of lab scale conditions towards the pilot scale is not straightforward. It is important to mention that the stirring system in the tank at pilot scale is more powerful than using the shaker for the vials at lab scale. Most likely, this has an effect on the particle size of the precipitate and on the catalytic efficiency of the enzymes. However, based on the insights obtained at the lab scale, a successful pilot scale experiment could be performed resulting in an easily filterable slurry and lignin filter cake with a high purity.

Table 4
Characterization the two filter cakes obtained in the second pilot scale lignin recovery experiment, *i.e.*, PSS2.1 obtained after treatment with the Accellerase® Trio™ enzyme mixture (AT) at pH 4.8 and 50 °C and PSS2.1 obtained after treatment with the Accellerase® Trio™ enzyme mixture (AT) at pH 4.8 and 50 °C followed by the addition of flocculant FL 2949 (FL). The w% precipitate is expressed relative to the total amount of dry matter present in black liquor BL2 (see Table 2). The other w% are expressed relative to the total mass of dried filter cake.

Precipitation conditions	PSS2.1; AT at pH 4.8	PSS2.2; AT at pH 4.8 and FL
Precipitate (w%)	13.4 ± 0.4	22.6 ± 0.6
Ash (w%)	2.5 ± 0.1	4.3 ± 0.1
Klason lignin (w%)	77.2 ± 0.6	83.4 ± 0.7

4. Conclusions

A pilot scale procedure for the mild soda pulping of *Miscanthus x giganteus* chips and, the subsequent lignin recovery from the black liquor, has been developed. In a first pilot scale experiment, the lignin was recovered by means of acidification, an enzymatic treatment and subsequent filtration. The enzymatic treatment combined with the base catalyzed hydrolysis during the pulping process, resulted in the conversion of the *p*-coumarates and ferulates to free acids. Moreover, the enzymatic treatment also significantly improved the flocculation of the precipitate. However, during the filtration the filter pores clogged, leading to a rapid increase of the pressure, a low filtration rate and a filtrate which still contained some turbidity. Lab scale experiments indicated that the enzymes significantly enhance the filtration rate and increase the purity of the precipitated lignin. Combining the enzymatic treatment with a flocculant further increases the filterability but at the expense of the purity. However, this was not fully confirmed at the pilot scale. There, combining both treatments was found to be necessary to obtain an optimal filterability. Moreover, the lignin purity was found to be higher when a flocculant was used. However, it can be concluded that a straightforward pilot scale procedure was developed for the successful recovery of lignin from a black liquor prepared by a mild soda treated *Miscanthus x giganteus*, yielding a high quality native-like lignin which could have a bright future in many high-value applications.

Acknowledgements

The pilot scale experiments at Bio Base Europe Pilot Plant were performed in the framework of the EU Interreg Flanders-The Netherlands project Bio-HArT. J.L. is a postdoctoral fellow of the Research Foundation - Flanders (12Z2218N). C.L.S. and P.C.A.B. acknowledge the Catchbio program for financial support.

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