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VALORISATION OF HYDROLYSIS LIGNIN REST FROM BIOETHANOL PILOT PLANT: PROCESS DEVELOPMENT AND UPSCALING

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ABSTRACT

The aim of this work has been to find a scalable process to purify lignin and separate the cellulose-rich fraction from a poplar wood hydrolysis rest produced at a bioethanol pilot-plant. At initial laboratory optimization, a mild extraction of lignin was performed with 1 M NaOH at 90 °C to dissolve the lignin, that was further precipitated with sulfuric acid and this formed an easily filtrable powder. The main sub-product was a solid residue enriched in cellulose, which was successfully saccharified and tested for its fermentability.

Further upscaling of the method was done at Bio Based Europe Pilot Plant (BBEPP) in Belgium. In a first run 14.2 kg of purified poplar lignin was obtained and in a second run 128.6 kg was obtained. The wet fiber residue was processed by washing and enzymatic hydrolysis to obtain, 26.7 kg of concentrated sugar solution with 509.4 g/L of glucose.

1. Introduction

The aim of this work has been to further valorize hydrolysis lignin rest from poplar, obtained after a bioethanol production process (Proesa®) at BIOCHEMTEX (It). Poplar (*Populus spp.*) is a fast-growing tree existing in both native and cultivated forests in many continents in the world. The global poplar forest products output was reported at 687 M m^3 2015, of which 64% was attributed to indigenous forests and 36% to planted poplars, see FAO report 2016. The same report also states that the share of production of plywood and veneers are around 13 M m^3. From this production as high as 45% (Mantau et al., 2010) is lost as wood residue, giving clean wood shavings suitable for bioethanol production or for incorporation in particle boards. To make the production of 2nd generation bioethanol from agroforestry residues economically viable, it is highly important to co-valorize the main by-product, which is hydrolysis lignin (Kautto et al., 2014). This lignin, together with some residual cellulose fibers, can be dried and burnt for the recovery of energy, but this is not profitable enough to make it worthwhile, which is why in an integrated biorefinery scheme, new, more valuable industrial applications are looked for, see review by Collins et al. (2019). There are, however, many limitations to using lignin for technical applications, depending on its structure and impurities. A review of the problems and possible approaches to overcoming these limitations, presented by Vishtal and Kraslawski (2011), found that the main problems with crude hydrolysis lignin are that it has a high content of carbohydrates as cellulose, a high polydispersity of molecular weights and high water retention. These factors reduce its applicability in composites. On the other hand, this lignin is expected to have zero or low sulfur content.

A simple strategy to extract hydrolysis lignin from a residue after bioethanol fermentation has been used at the lab scale by Cotana et al. (2014), using biomass from *Arundo donax* L. (or giant reed). These authors used sodium hydroxide (NaOH) solution to dissolve the lignin and separate the solid cellulose rest, before precipitating the lignin with sulfuric acid and washing it with water. A similar approach, directly extracting lignin from ground poplar wood using NaOH, has been described by Sun et al. (2000). Dávila et al. (2017) extracted lignin from wine prunings using NaOH after an initial hydrothermal pre-treatment to degrade the hemicellulose. Using an NaOH solution to extract lignin, instead of using an organic solvent, reduces the risks of toxicity and inflammability and also reduces cost.

Within this work a similar extraction scheme has been adopted for the extraction of lignin, using NaOH followed by precipitation with sulfuric acid. To show the cascading use of the main sub-product, the

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Table 1
Starting material composition (dry weight %), obtained from BIOCHEMTEX bioethanol plant.

Starting material	Hemicellulose (Soluble in 8% NaOH) (%)[a]	α-Cellulose (%)[a]	Lignin (Klason) (%)[b]
Poplar hydrolysis lignin 1st Batch	16.3	10.1	69.3
Poplar hydrolysis lignin 2nd Batch	10.1	23.4	53

a: Methods Wise et al. (1946) followed by Han and Rowell (1996), b: TAPPI, T-222

solid, cellulose-rich residue was first hydrolyzed with a glycosidase cocktail and then used for fermentation. After initial laboratory optimizations, the processes have been upscaled at the pilot plant facilities available at the Bio Base Europe Pilot Plant (BBEPP) in Belgium to produce purified lignin and sugars, which were further fermented into 2,3-butanediol. This work was part of the work carried out in the EU-H2020-SPIRE project REHAP as a first step in the development of further transformation of bio-materials into high-value products for the construction sector.

2. Materials and methods

2.1. Raw material

Chemicals: Sodium hydroxide (Reagent grade) and Sulfuric acid (Synthesis grade) both from Scharlau. Poplar hydrolysis lignin: Samples, see Table 1, came from the Biochemtex bioethanol pilot plant in Italy, as a sub-product from their Proesa® process, which uses poplar veneer shavings as a raw material. The received material was dried in air to 6% moisture content, after which it was stored in an airtight plastic barrel. To get particles for extraction the cakes were first broken up with a hammer and then milled to a powder in an IKA hammer mill and passed through a 1 mm mesh sieve.

2.2. Lignin extraction at lab scale

The lignin extractions were then performed in 500 ml stirred glass reactors in differing extraction conditions, as seen in Table 2. The optimized lignin extraction method used 1 M NaOH at 90 °C for three hours to solubilize most of the lignin content in the poplar wood hydrolysis rest. The still warm slurry was filtered in a Büchner funnel to separate the solids from the black liquid which contained the lignin. To then precipitate the lignin, 2.5 M of sulfuric acid was added to acidify the solution to pH 1.5 and the solution was then heated to 70 °C and kept at that temperature for 1 hour. Throughout this procedure the stirring was maintained which provided a flocculated lignin product, which was easy to filter and wash with water. After drying in a ventilated oven at 80 °C a free-flowing light brown powder was obtained. The yields of dry lignin and solid rest were calculated as % of initial dry content of lignin cake used for the extraction. In repetitions of the extractions the repeatability of lignin and cellulose rich fractions were ± 1.5%.

2.3. Laboratory saccharification of solid fraction

After the filtration of the solubilized lignin, the solid, cellulose-rich fraction, was repeatedly washed with diluted HCl and water until the washing water approached neutrality. It was then dried. The resulting dry sample (264.5 g) was ground using a ZM 200 ultra-centrifugal mill (Retsch) to a particle size of 250 µm. In a 2-L hydrolysis reactor, a 15% (w/v) suspension of the ground sample was prepared in distilled water. A stir rate of 400 rpm was applied to the suspension and its temperature was adjusted to 45-50 °C. The pH-value was then adjusted to 5.0 with diluted HCl. Hydrolysis was started by adding the commercial enzyme preparation Cellic Ctec2 (Novozymes) to the suspension at 12.5 mg protein/g dry solid (1.25% w/w) and the process was carried out for 72 h. When the hydrolysis was finished, the remaining solid residue was separated from the glucose-containing supernatant by filtration and the resulting solution was then concentrated by freeze-drying ready for it to be tested for its fermentability.

2.4. Fermentation

Fermentations were carried out with the proprietary 2,3-butanediol-overproducing strain *Lactococcus lactis* 43103, according to (Roncal et al., 2016), in a 0.5-L fermenter containing 300 mL of YE-CSL medium (35 gL^{-1} yeast extract, 20 $g\,L^{-1}$ corn steep liquor), with an amount of lignocellulosic hydrolysate equivalent to 100 $g\,L^{-1}$ glucose as carbon and energy source. Lignocellulosic hydrolysate was used without autoclave sterilization in order to avoid the formation of potentially toxic by-products. Fermentation conditions were: temperature, 30 °C; stir rate, 750 rpm; dissolved oxygen concentration, 10%; air flow, 2 L $L^{-1}\,min^{-1}$; pH, 5.50. Fermentations were started with a 5% (v/v) inoculum grown in YEC medium (2% glucose, 10 $g\,L^{-1}$ yeast extract, 20 mM sodium citrate buffer, pH 7.0).

2.5. Pilot plant upscaling of lignin extraction

2.5.1. Alkaline extraction

Two batches of lignin cake were processed at pilot scale. In the first

Table 2
Laboratory extraction reactions on poplar hydrolysis lignin, L1-L11: batch 1 and in L12: batch 2.

Ref.	Loading (g)	NaOH (M)	Lignin solvation reaction	Lignin Precipitation	Solid rest Yield (%)	Lignin Yield (%)	Aromatic lignin absortivity (UV,280 nm) (g $L^{-1}\,cm^{-1}$)
L1	20	1	400 mL, 75 °C, 3 h	H_2SO_4 pH = 6	48.1	35.1	18.1
L2	20	1	400 mL, 75 °C, 3 h	H_2SO_4 pH = 1.5	37.6	34.1	21.8
L3	20	1	400 mL, 90 °C, 3 h	H_2SO_4 pH = 1.5	26.8	48.0	23.3
L4	20	1	400 mL, Autoclave 120 °C, 3 h	H_2SO_4 pH = 1.5	18.1	55.6	22.6
L5	20	1	400 mL, 90 °C, 5 h	H_2SO_4 pH = 1.5	30.2	47.9	21.9
L6	20	1	400 mL, 90 °C, 3 h	H_2SO_4 pH = 2.0	26.6	50.7	21.7
L7	40	2	400 mL, 90 °C, 3 h	H_2SO_4 pH = 1,5	29.6	49.2	21.8
L8	20	1	400 mL, 90 °C, 3 h	CO_2 pH = 7,7	26.8	35.3	19.7
L9	20	0.5	400 mL, 90 °C, 3 h	H_2SO_4 pH = 2.0	35.4	55.4	20.9
L10	40	1	400 mL, 90 °C, 3 h	H_2SO_4 pH = 1.5	29.0	53.0	20.7
L11	160	1	1600 mL, 90 °C, 3 h	H_2SO_4 pH = 1.5	28.8	54.5	21.3
L12	20	1	400 mL, 90 °C, 5 h	H_2SO_4 pH = 1.5	46.1	30.0	19.8

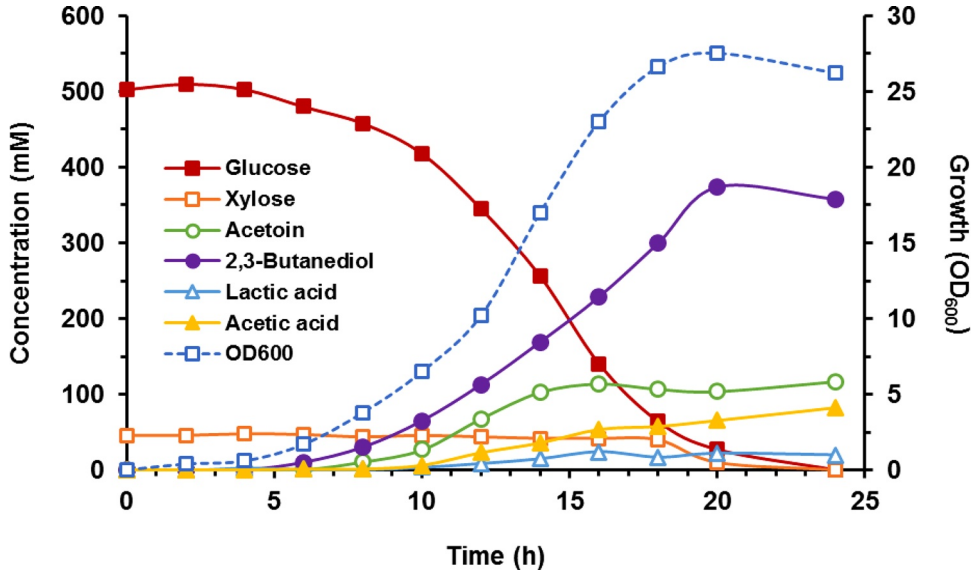

Fig. 1. Time-course of a batch fermentation of the solid rest hydrolysate by *L. lactis* 43103. Culture conditions were as described in the Materials and Methods section. 2,3-BDO: 2,3- Butanediol and OD600: Optical Density at 600 nm.

Fig. 2. Schenk filter press.

run, a 110 kg lignin cake was diluted in 620 kg RO water in an 800 L glass-lined reactor (Pfaudler DG-800), resulting in a slurry of 5.0 wt % DM. Next, 29 kg NaOH pellets were added and the mixture was stirred for 3 hours at 90 °C. After cooling to 40 °C, the solids were removed by using a Schenk filter press (Fig. 2), equipped with 25 chambers with a 5.5 L cake volume in each. Each chamber was sealed by a Mono sefar tetex 05-1020-SK-025 filter cloth with a surface area of 0.221 m^2. The filter press was loaded by pressurizing the reactor containing the lignin extract. Starting from 0.5 bar, the feed pressure was gradually increased in steps of 0.5 bar to 3.5 bar, after which the obtained filter cake was washed with 80 L RO water. Finally, the filter-cake was washed for a second time with 95 L RO water and stored at −20 °C. In the second batch, a 935 kg lignin cake was diluted in 3527 kg RO water in a 4500 L glass-lined reactor (Dietrich AE), after which 394 kg of a 50 wt% NaOH solution was added. After 3 hours of stirred incubation at 90 °C, the mixture was cooled to 40 °C, and subsequently fed to an Alfa Laval FOODEC 100 decanter (Fig. 3), operated at 5000 rpm, at a feed rate of 1000 L/h and a differential speed of 5 rpm. Under these conditions, the resulting torque on the decanter screw was found to be 0.1-0.25 kN m, well below the maximum value of 0.4 kN m.

2.5.2. Lignin precipitation and isolation

The obtained filtrates were loaded in an 800 L and 4500 L glass-lined reactor and acidified to pH 1.5 by adding 100 L 5 M H_2SO_4 and 8.18 L 96 % H_2SO_4 in the 800 L reactor, and 200 L 96 % H_2SO_4 in the 4500 L reactor for batch 1 and 2 respectively. Next, the mixtures were

Fig. 3. Alfa Laval FOODEC100 decanter.

Table 3
Fermentation parameters (for 2,3-butanediol production) for the solid rest hydrolysate and the pure glucose control.

Carbon and energy source	Concentration (gL^{-1})	Yield* (%)	Productivity ($gL^{-1}h^{-1}$)
Solid rest hydrolysate	33.7	70.2	1.68
Pure glucose (control)	33.4	70.1	2.78

* Yield is expressed as % of the maximum theoretical one, and is calculated considering the consumption of both glucose and xylose from the hydrolysate. The maximum theoretical yield is 0.50 g g − 1 for glucose and 0.30 g g − 1 for xylose.

stirred for 1 hour at 70 °C, after which they were cooled down to 40 °C. The precipitated lignin fractions were then separated using a 100 L Schenk or 450 L WFT filter press for batch 1 and 2 respectively. Both filter presses were equipped with sefar tetex 05-1020-SK-025 cloths. Upon complete filtration, the cakes of batch 1 and 2 were washed with 170 L and 1000 L acidified RO water, and subsequently pre-dried with compressed air for 60 minutes. Finally, the filter cakes were harvested and dried in a vacuum tray dryer at 60 °C for 28 hours.

2.6. Pilot plant upscaling of sugar recovery

The fibers from the second upscaling batch (using a second shipment of hydrolysis lignin raw material) were washed by resuspension in 5000 L of water, followed by separation on an Alfa Laval FOODEC 100 decanter, operated at 5000 rpm, at a feed rate of 1400 L/h and a differential speed of 5 rpm. The obtained 358 kg of wet fibers were then mixed with 588 kg water in a stirred tank and heated to 50 °C. Subsequently, the pH was adjusted to 5.2 by adding 720 g of 96 % H_2SO_4, followed by the addition of 19 kg Cellic®CTec2 enzyme. After 10 hours of incubation at 50 °C, the reaction mixture was heated to 70 °C to prevent post-contamination. The obtained sugar solution was

Table 5
Molecular weights and polydispersity of purified lignin samples determined by SEC, Laboratory samples L1, L3, L4 and L11 and two upscaled samples from BBEPP: L-scaled 1 and L-scaled 2

Sample	Mw	Mn	Mw/Mn
L1	11502	1630	7.0
L3	6005	1867	3.2
L4	7792	1806	4.3
L11	8593	1506	5.7
L-scaled 1	14247	1487	9.6
L-scaled 2	12613	1381	9.1

first clarified by passing over an Alfa Laval FOODEC 100 decanter, operated at 5000 rpm, at a feed rate of 1400 L/h and a differential speed of 5 rpm. Next, the light phase was passed over a centrifuge (Westfalia SA19) at 2000 L/h to remove most of the remaining particles. The obtained light phase was subsequently passed through a 10 kDA PES spiral wound ultrafiltration membrane (Alfa Laval UFX10 pHt). To minimize losses in the retentate, a 2:1 diafiltration was performed. Finally, the obtained filtrate was concentrated on a wiped film evaporator operated at 65 °C.

2.7. High-Performance Liquid Chromatography (HPLC) analysis of sugars and fermentation metabolites

The concentrations of glucose, xylose, acetoin, 2,3-butanediol, lactate, acetate and ethanol in fermentation broth were measured by HPLC using an Aminex HPX-87H 300 × 7.8 mm (Bio Rad) column and a Microguard Cation H Refill Cartridge precolumn, with the following conditions: mobile phase, 0.01 N H_2SO_4; flow rate, 0.7 mL/min; column temperature, 55 °C. Peak quantification was performed with a refractive index detector.

2.8. Measurement of the biomass concentration

Cell growth was measured spectrophotometrically as the optical density at 600 nm (OD_{600}).

2.9. Fourier Transform Infrared Spectroscopy (FTIR) analysis

ATR-FTIR on vacuum-oven dried powder samples was done using a Nicolet iS5/ATR iD7 from Thermo Scientific. The transmittance spectra were transformed to adsorption and ATR-corrected.

2.10. ^{31}P-NMR measurement of lignin hydroxyl species

For ^{31}P NMR analyses, each lignin sample was accurately weighted (25 mg) and dissolved in N,N-dimethylformamide (150 µL) in 4 mL vial. After total dissolution, pyridine (100 µL), internal standard solution

Table 4
Mass balances from lignin purification upscaling. DM: Dry Mass and RO: Reverse Osmosis.

	Upscaling 1		Upscaling 2	
Process unit step	IN	OUT	IN	OUT
Soda cooking	110 kg lignin cake 33.05 % DM 620 kg RO water 29 kg NaOH pellets	750 kg slurry	935 kg lignin cake 38.2 % DM 3527 kg RO water 394 kg 50 wt% NaOH	4856 kg slurry
Fibre separation	750 kg slurry	687 kg black liquor 72 kg fibres	4856 kg slurry 55 kg rinse water	4540 kg black liquor 371 kg fibres
Lignin precipitation	687 kg black liquor 108 kg H2SO4 solution	714 kg filtrate 81 kg filter cake	4540 kg black liquor 200 kg H2SO4 (96 %) 2000 L rinse water	6230 kg filtrate 510 kg filter cake
Vacuum tray drier	81 kg wet filter cake 17.5 % DM	14.2 kg dry lignin 99.8 % DM	510 kg wet filter cake 24.3 % DM	128.6 kg dry lignin 99.7 % DM

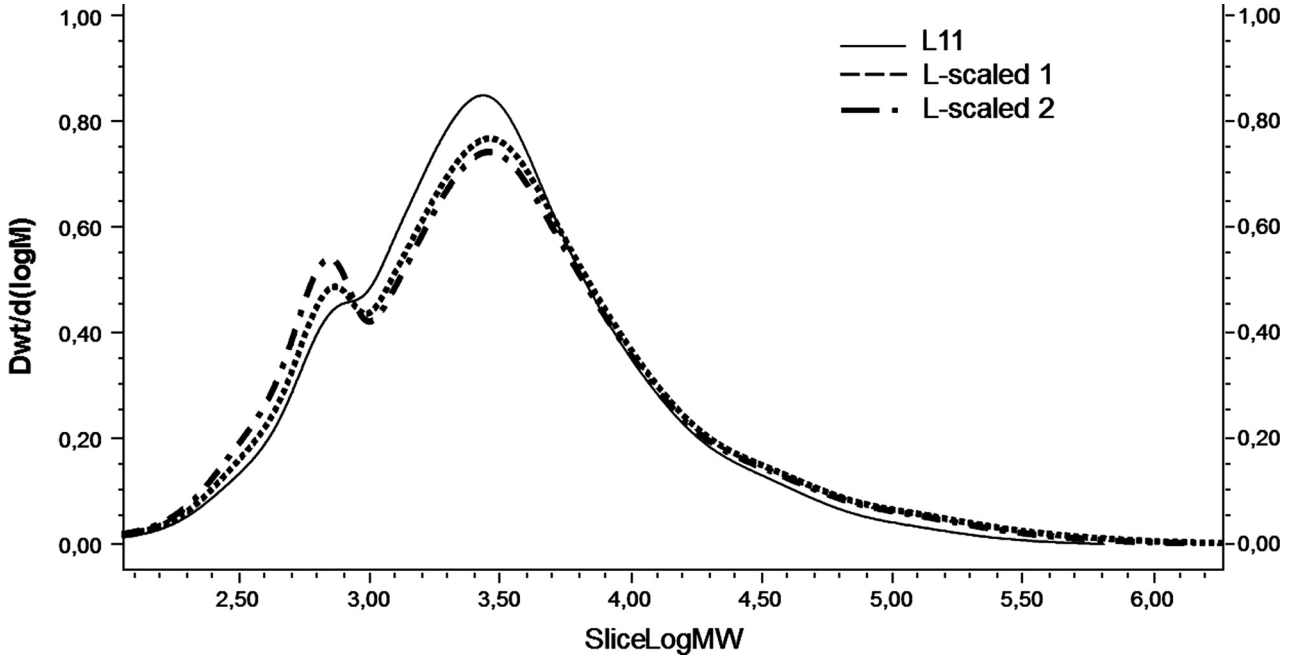

Fig. 4. Molar mass distributions against PSS standards for the laboratory sample L11 and two upscaled samples from BBEPP: L-scaled 1 and L-scaled 2.

Table 6
Amounts of different hydroxyl group species (mmol/g) in lignin samples by ^{31}P-NMR analysis

Sample	Aliphatic OH	Carboxylic acid	Condensed + Syringyl	Guaiacyl + Catecols	p-OH-phenyl	Phenolic OH	Total OH
L1	3.00	0.24	1.42	0.72	0.08	2.22	5.46
L3	0.72	0.38	2.33	0.87	0.17	3.38	4.48
L4	1.55	0.44	2.29	1.02	0.16	3.46	5.46
L11	2.50	0.48	1.77	0.87	0.15	2.79	5.77
L-scaled 1	1.53	0.74	1.01	0.56	0.17	1.73	4.00
L-scaled 2	2.09	0.56	1.33	0.49	0.21	2.03	4.68

Table 7
Impurities composition of purified lignin fractions (NREL, 2012)

Sample	Glucose (%)	Xylose (%)	Ashes (%)
L11	0.21	0.38	0.10
L-scaled 1	0.66	0.26	6.39
L-scaled 2	0.55	0.28	13.4

(ISTD) (200 µL) *endo*-N-Hydroxy-5-norbornene-2,3-dicarboximide (e-HNDI, 0.005 mmol) in pyridine/$CDCl_3$ (1.6/1, v/v) and $Cr(acac)_3$ solution (50 µL) (11.4 mg/1 mL) in pyridine/$CDCl_3$ (1.6/1, v/v) were added. Then, phosphitylation reagent (150 µL) 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphopholane [P.R.(II)] was added dropwise. Finally, $CDCl_3$ (300 µL) was added to the solution and a clear brown to black solution was achieved.

Freshly-prepared samples were measured with ^{31}P NMR immediately after preparation at room temperature. A Bruker 500 MHz NMR spectrometer was used for the measurement. Chemical shifts are reported relative to the sharp signal (132.2 ppm) originating from the reaction between water and P.R. II. The following NMR parameters were used: scans = 1024, pulse delay = 5 s, 90° pulse and line broadening = 2 and default baseline correction. The method is based on Granata and Argyropoulos (1995).

2.11. Molar mass determination by Size Exclusion Chromatography (SEC)

The molar mass measurements were performed with size exclusion chromatography (SEC) using alkaline eluent (0.1 M NaOH). Samples were dissolved in 0.1 M NaOH and diluted for the measurement concentration ca 1 g/L. The samples were filtered (0.45 µm) before the measurement. The measurements were performed in 0.1 M NaOH eluent (pH 13, 0.5 ml/min, T = 25 °C) using PSS MCX 1000 & 100000 Å columns with a pre-column. The elution curves were detected using Waters 2998 Photodiode Array detector at 280 nm. The molar mass distributions (MMD) were calculated against polystyrene sulphonate (10 x PSS, 1600-267200 g/mol) standards, using Waters Empower 3 software.

- Mn = number average molar mass = $\sum M \cdot n / \sum n$
- Mw = weight average molar mass = $\sum M \cdot w / \sum w$
- PD = polydispersity (Mw/Mn)

2.12. UV spectroscopy to estimate aromatic lignin content

Dry lignin samples were weighed close to 10 mg and dissolved in 10.00 ml solvent mixture: dioxane/0.2 M NaOH (1:1). Then 2 ml was diluted to 25.00 ml in 0.2 M NaOH and the absorbance measured at λ = 280 nm in a quart's cuvette. The aromatic content was estimated using the extinction coefficient α = 20 L/(g*cm).

3. Results and Discussion

3.1. Laboratory lignin extraction and separation of cellulose enriched solid

The simple and mild lignin extraction method was optimized by varying the parameters: NaOH concentration, extraction temperature and reaction time, see Table 2. It was found that when extracting for

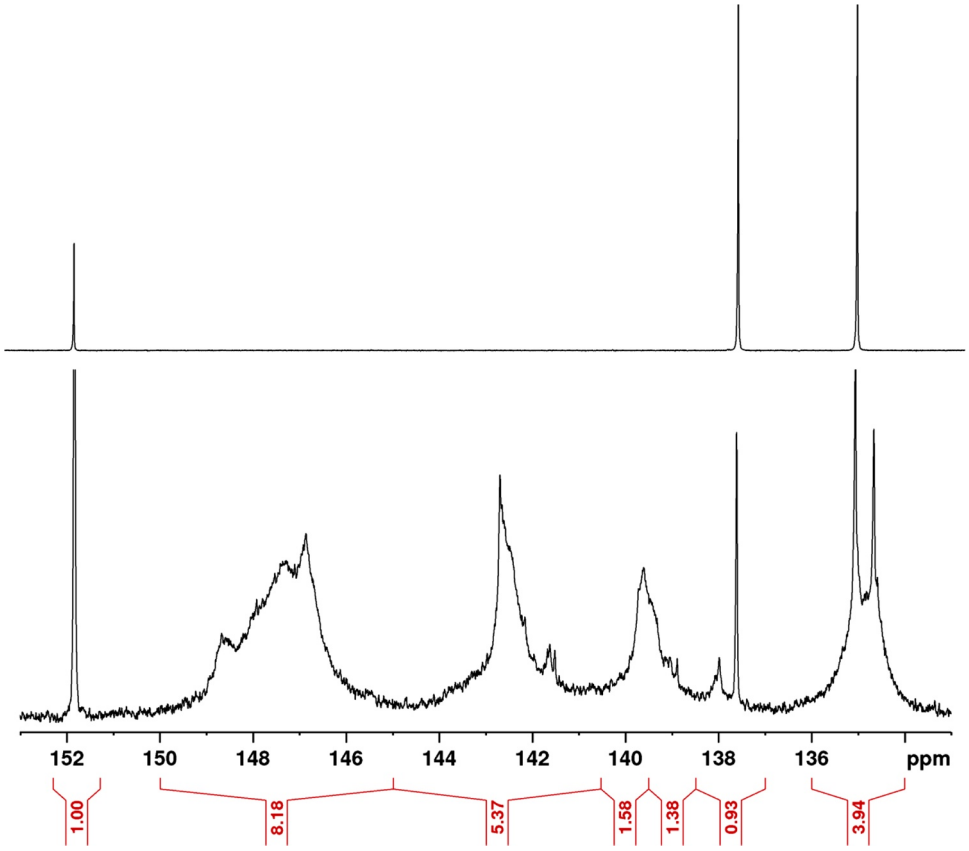

Fig. 5. 31 P NMR spectrum of p-hydroxybenzoic acid (upper) and L-scaled 1.

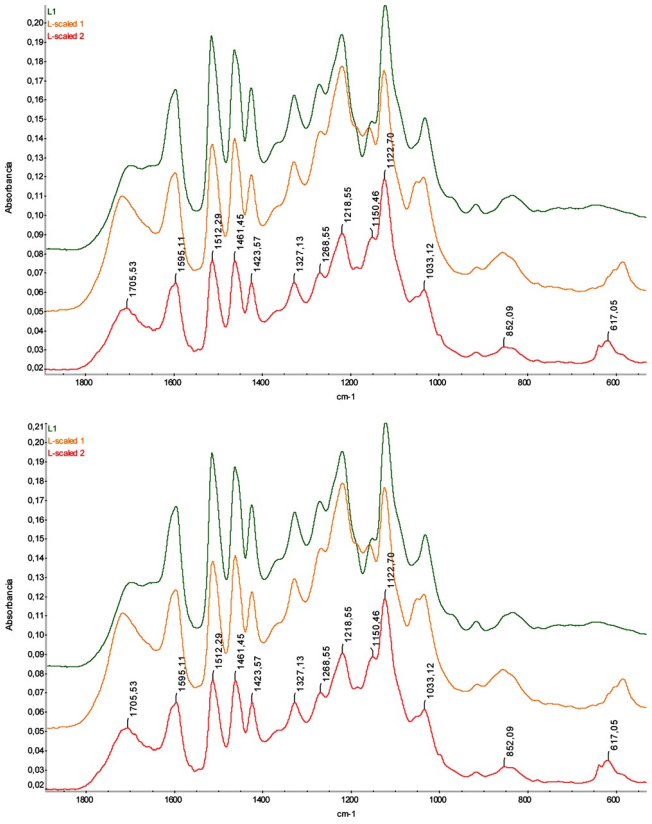

Fig. 6. ATR-FTIR adsorption spectra of lignin fractions, L11 (green), L-scaled 1 (yellow), L-scaled 2 (red).

3 h, the lignin yield increased with the temperature up to 120 °C, but the aromatic lignin absorptivity (an indication for lignin purity) was slightly higher at 90 °C. Typical absorptivity value for lignin is around 20 L/g cm. Absorptivity measured at a lower value often reflects the presence of impurities in the sample, while higher values can be interpreted as an indication of pure lignin. Also, taking in account that the purpose of this exercise was to scale up the process in a pilot plant, where 120 °C would need pressurized vessels, the further reactions were performed at 90 °C. Increasing the reaction time to 5 h or the NaOH concentration to 2 M did not improve the lignin yield nor phenolic content either.

For the acid precipitation of lignin, sulfuric acid was used because it is more compatible with the stainless steel equipment used in pilot and industrial plants than the corrosive hydrochloric acid. The best results, of filterability of precipitated lignin, were obtained when reducing the pH to 1.5 with 2.5 M sulfuric acid. Attempts at using a higher pH all produced suspensions that were very difficult to filter. It was also found that by heating up the acidified lignin suspension to 70 °C for 1 h, the lignin precipitated out in a form that was easy to filter and wash with water. This behavior was explained by Fatehi et al. (2016) who also found that acidifying to pH1.5 with sulfuric acid increased lignin precipitation of spent and prehydrolysis liquors. The suggested reason was that residual hemicelluloses got hydrolyzed at that pH. Furthermore, the increased temperature also helps to precipitate the lignin. A study from Scultze et al. (2016) showed how lignin suspensions agglomerates into round droplets at increased temperatures, a phenomenon they called “droplet formation temperature”. This temperature depended on the type of lignin and they showed examples approximately between 70-80 °C in water.

The two hydrolysis lignin residues from BIOCHEMTEX were extracted using the optimized method. The obtained dry weight yield of purified lignin was 54.5% when starting with 69.3% of the initial Klason lignin content (batch 1) and a 30% yield was obtained when the

Klason lignin starting material was 53% (batch 2).

3.2. Laboratory saccharification of solid rest and fermentation of 2,3-butanediol

Hydrolysis of the solid rest resulted in the production of 23.3 g/L of glucose after 72 h in the hydrolysis medium, with around 75% of this being released after 24 h, and 94% after 48 h. In addition, a limited amount of xylose (1.2 g/L) was also released, representing less than 5% of the total sugars released. The final glucose and total sugars (glucose + xylose) yields, related to the starting lignin cake mass, were 15.5% and 16.3%, respectively.

The final sugar solution, following hydrolysate filtration and concentration by freeze-drying, contained 249 g/L of glucose and 15.2 g/L of xylose, and was the hydrolysate used as carbon and the energy source for the fermentability tests.

Batch fermentations were performed with a proprietary strain of overproducing 2,3-butanediol, to confirm the fermentability of the saccharified solid rest (as described in materials and methods). A starting hydrolysate concentration equivalent to 10% (w/v) glucose was used under standard fermentation conditions. The time-course of a representative fermentation is shown in Fig. 1.

The hydrolysate allowed for an excellent growth of the bacterial strain, with both growth and 2,3-butanediol production running in parallel and showing most fermentation parameters comparable to those obtained with pure glucose controls (Table 3).

Fermentation was finished by around 20 h, when glucose and xylose were completely used up, and growth and metabolites reached their maximum values. 2,3-Butanediol was the main fermentation product (33.7 g/L), with a significative accumulation of its precursor acetoin (10.3 g/L). As the bacterial strain used can be utilized, in addition to the glucose and xylose as carbon and an energy source, and it also produces 2,3-butanediol, product yields should be calculated considering the consumption of both sugars. Accordingly, the yield of 2,3-butanediol reached 70.2%, which was increased to 92.1% when considering both products of the C4 metabolic pathway (2,3-butanediol and acetoin).

Notably, lactic acid production was scarce (only around 2 g/L), which means that almost all metabolic flux was directed to the production of C4 compounds. Acetic acid production was higher (close to 5 g/L), but it occurred mainly at the final stage of fermentation, when xylose was utilized (xylose is metabolized by this strain through the phosphoketolase pathway, producing one mole of acetic acid per mole of xylose).

The only fermentation parameter negatively affected when using the solid rest hydrolysate was productivity, which decreased by 40% compared to the use of pure glucose. This decrease was the result of the fermentation delay observed (by around 6-8 h) with respect to the control with pure glucose, which is likely to have been a consequence of the presence of putative inhibitors that, although not toxic enough to prevent growth, do probably extend its lag phase.

In conclusion, solid rest hydrolysate fermentation resulted in a metabolite profile, titers and yields very similar to those obtained with pure glucose as carbon and energy source, which supports the suitability of the lignin cake hydrolysate as a fermentation feedstock and allows an enhanced valorisation of this lignocellulosic residue.

3.2.1. Pilot plant upscaling at BBEPP

In upscaling 1, a 110 kg lignin cake from BIOCHEMTEX was processed to purify the lignin based on the purification protocol developed by TECNALIA. This process involves five main steps: soda extraction, filtration, lignin precipitation, filtration and drying, as described in section 2.5. From this first upscaling run 14.22 kg of dried purified lignin was obtained, corresponding to a total yield of 39 % (Table 4). A second run, starting with a 935 kg lignin cake, was performed under similar conditions, except for Filtration N°1, in which the Schenk filter press (Fig. 2) was replaced by a decanter step (Fig. 3), due to its ease of operation and its ability to handle large volumes. The latter yielded 128.6 kg of dry lignin, corresponding to a yield of 36% (Table 4). These lignin products are being evaluated by project partners for high-value applications, such as concrete plasticizers. To improve the biorefinery economics, it is though important to find an industrial application for this purified lignin co-product, that could be paid a higher price for. In the case of making bioethanol with an organosolv pre-treatment, it was estimated that the lignin price should be 1000 USD/ metric ton to produce the bioethanol with the same price as the ethanol market price 2013 (Kautto et al., 2014).

3.2.2. Sugar recovery from cellulose rich solid fraction

The wet fibres produced in run 2 of the lignin purification upscaling were further processed to isolate carbohydrates from this fraction. To that end, the 371 kg of wet fibres were washed with 5000 L of RO water, and hydrolysed using Cellic®CTec2 enzyme. Starting from 68 kg DM fibre residue (358 kg washed fibres, 19 wt% DM), a 966 kg carbohydrate solution containing 25.2 g/L glucose was obtained. Considering the glucose concentration present in the enzyme solution (2%), a conversion yield of 30% was achieved. After purification and concentration, 26.7 kg of product solution was obtained with 509.4 g/L glucose, 22.9 g/L xylose, 3.8 g/L acetic acid, a density of 1.26 kg/L and a dry-matter content of 64.37 wt%.

3.3. Characterization of purified lignin

The effect of purification conditions on the molar mass distribution of lignin was evaluated by SEC measurements (Table 5). There was only a minor difference between samples obtained at temperatures 90 °C and 120 °C (samples L3 and L4), while sample L1 purified at 75 °C had significantly higher weight average molar mass (Mw). The differences in the number average values (Mn) are much smaller and even in the opposite direction. This is probably due to the sensitivity of Mw towards large molecular lignin-carbohydrate complexes (LCC) that may be more abundant in the extract at lowest temperatures. Also, the higher precipitation pH (6 vs 1.5 in samples L3 and L4) may favour the presence of lignin-carbohydrate complexes.

The effect of upscaling on molar mass distributions was studied by analyzing samples L11, L-scaled 1 and L-scaled 2 (Table 5). They were all obtained at 90 °C with precipitation at pH 1.5, either at large lab scale or at pilot scale. The large-scale lab sample L11 was comparable to the corresponding small-scale sample L3, but for the two pilot-scaled lignins the Mw was significantly higher, accompanied by notably low Mn values and thus high polydispersity. This could be a result of the upscaling in itself, but it could also be an effect of variable quality of different raw material batches, that were shipped at different occasions for each extraction trial. None of the samples contained relevant amounts of carbohydrate impurities as seen in Table 7, suggesting that lignin-carbohydrate complexes do not explain the high weight average molar mass.

The shape of the molar mass curves in Fig. 4 illustrates the phenomena discussed above. In addition, it indicates the presence of a peak in the small molecular region, especially abundant in the BBEPP samples. Its origin is most probably p-hydroxybenzoic acid. Model compound experimentation showed that it has the same elution time in the applied SEC system. The presence of p-hydroxybenzoic acid was further confirmed by ^{31}P NMR.

The frequency of various types of hydroxyl groups of the lignins was determined by ^{31}P NMR after phosphitylation (Table 6).

As seen in Table 6, there was no systematic variation in the values that could be explained by the differences in the extraction conditions. The main phenolic hydroxyl species in all samples were syringyl (+condensed guaiacyl) and guaiacyl/catechol type structures, as expected for a hardwood lignin. The total content of phenolic hydroxyl content is rather low in comparison to other technical lignins. Together with the high molar mass it may be an indication of native-like

structure.

The aliphatic OHs are sensitive to the presence of carbohydrate impurities, and thus need to be interpreted with caution. The visual appearance of the ^{31}P NMR spectra indicate that the samples contain a small molecular contaminant inducing sharp signals, together with the typical broad signals of the polymeric lignin (Fig. 5). The chemical shifts of the two sharp signals are in the carboxylic acid and p-hydroxyphenyl ranges. This suggests that the contaminant could be p-hydroxybenzoic acid that is known to be present in poplar lignin, esterified to the gamma carbon. This ester linkage could be hydrolyzed during the pretreatment. Release of p-hydroxybenzoic acid from poplar has earlier been reported to take place during alkaline aerobic oxidation of poplar lignin (Schutyser et al., 2018). The comparison between one of the lignin samples (L-scaled 1) and reference sample p-hydroxybenzoic acid confirms the identity of the sharp signals. The finding may have practical relevance, as p-hydroxybenzoic acid is a potential renewable precursor to replace fossil-based phenol, e.g. for the synthesis of paracetamol (Ralph et al., 2019).

To see structural differences between upscaled and laboratory purified lignin samples ATR-FTIR spectra are compared in Fig. 6. In principle the same purification procedure was used but with different lots of poplar hydrolysis rests after bioethanol production. The bands in three spectra are similar to those reported by Sun et al. (2000) when analysing alkaline lignin from poplar wood. No intense poly-saccharide bands were identified, suggesting rather pure lignins, even though the small peak in all spectra near 1150 cm^{-1} could be from residual sugars. The two upscaled lignins have a band at 1705 that could be from carboxylic groups, as in free acid form or in ester groups from *p*-hydroxybenzoic acid known to be present in poplar wood, Sun et al. (2000). This peak could also be from carbonyls in uronic acid groups in residual hemicellulose. It is, though, clear that in the spectra for laboratory-purified lignin (L11) this peak has been reduced and displaced to 1696 cm^{-1}, more characteristic for unconjugated ketone. This suggests that the upscaling reactions have not been as effective as the reaction in the laboratory. The typical assignations for the poplar lignin are seen in all spectra as: O-H stretching vibration around 3399 cm^{-1}, C-H vibration stretch in CH_2 and CH_3 groups at 2937 and 2842 cm^{-1}, aromatic skeleton vibrations at 1595, 1512 and 1423 cm^{-1}, C-H deformations and aromatic ring vibrations at 1461 cm^{-1}. As being a hardwood lignin with high a syringyl content the intensity of the bands is strong at 1327 and 1122 cm^{-1}. A relatively weaker intensity of the bands is found at 1268, 1150 and 1033 cm^{-1}, assigned with guaiacyl units in the lignin molecules.

4. Conclusions

A simple method was used for lignin purification from a poplar hydrolysis rest and it was successfully scaled, producing 128.6 kg of dry lignin (Yield 36%). The lignins were pure in respect to carbohydrate impurities, but care must be taken to prevent accumulation of inorganics during the purification process. The lignin has high molar mass and characteristics of native lignin structure. This kind of lignin may be found to be useful in material applications, such as composites.

The solid cellulose-rich residue was enzymatically hydrolyzed, giving a 30% conversion yield and after purification and concentration it produced a 26.7 kg solution, containing 509.4 g/L of glucose. The fermentability of the glucose-rich hydrolysate was confirmed at laboratory-scale in 2,3-butanediol batch fermentations using *L. lactis* 43103 as test microorganisms. The results support the suitability of the solid rest hydrolysate as a fermentation feedstock, allowing an enhanced valorisation of this lignocellulosic residue.

CRediT authorship contribution statement

Ingemar Svensson: Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Tomás Roncal:** Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Karel De Winter:** Investigation, Data curation, Writing - original draft, Writing - review & editing. **Anoek Van Canneyt:** Formal analysis, Investigation, Data curation, Writing - review & editing. **Tarja Tamminen:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing - review & editing, Supervision, Project administration. **Atte Mikkelson:** Formal analysis, Investigation, Data curation, Writing - review & editing. **Aitor Barrio:** Conceptualization, Methodology, Validation, Resources, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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