LIGNOFIBRE (LGF) PROCESS – A FLEXIBLE BIOREFINERY FOR LIGNOCELLULOSICS

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LignoFibre (LGF) organosolv process is a potential novel fractionation method for biomass, yielding cellulose, lignin and hemicelluloses. Depending on the solvent used, the LGF fractions have potential in numerous biomaterial and chemical applications. Unbleached acetic acid LGF cellulose fibres showed promising results as reinforcement fibres in composites, while bleached fibres could be used for dissolving pulps and cellulose derivatives as well as for nanocellulose manufacturing. Ethanol LGF pulps are suitable material for enzymatic hydrolysis and further fermentation of the monomeric sugars into bioethanol. Based on its material properties, sulphur-free LGF lignin has potential as an additive in bioplastics or in resin applications. The sugar-based compounds could serve as platform chemicals and studies on their recovery from the cooking liquor at high yield and purity are currently on-going.

Keywords: organosolv, acetic acid, ethanol, phosphinic acid, cellulose fibres, lignin, composite, nanocellulose, dissolving pulp, hydrolysability, bioethanol fermentation

INTRODUCTION

Global challenges, such as the increased demand of energy for the ever-growing population, global warming and the exhaustion of fossil energy sources, have led the society to search for solutions from bio-based materials and bioenergy. Novel process concepts and raw materials are actively sought in order to meet the demands of bioeconomy transformation. Biorefinery processes aim for the utilization of whole biomass producing energy, chemicals and polymers from non-food biomass. Organosolv processes, i.e. pulping of lignocellulosic biomass with organic solvents, have been known for several decades with a few processes, such as Acetosolv[1] using acetic acid and Alcell/Lignol[2,3] based on ethanol, having made progress to pilot scale and some even to industrial scale, such as the Formico biorefinery[4] based on Milox technology.[5] In recent years, the interest towards organosolv pulping processes has increased due to their potential as biorefinery concepts.

The LignoFibre (LGF) organosolv process developed at VTT is a potential new method for fractionation of biomass components.[6] The process is based on cooking with organic solvents, such as acetic acid or ethanol, at elevated temperatures (130-150 °C). The novelty of the process is the use of phosphinic acid (H_3PO_2), which is a reducing agent.[7] According to recent results,[8] the role of phosphinic acid in the acetic acid LGF process is suggested to be the catalysis of delignification by acidolysis *via* phosphinic acid esterification. In addition, phosphinic acid potentially protects lignin against typical condensation reactions taking place under acidic conditions.

LGF process is very flexible in terms of raw materials, being suitable for both hardwood and softwood, as well as for various annual plants, such as flax and grasses. The process yields high quality components, i.e. reactive and/or well hydrolysable cellulose fibres, sulphur-free lignin and sugar compounds, which are potentially exploitable as bio-based materials in various applications. The choice of solvent in the LGF process has an effect on the properties of the fractions obtained.[9,10] The properties and potential applications of these fractions are discussed in this paper.

EXPERIMENTAL

Cooking trials

The raw material used for the LGF organosolv cooking experiments performed with acetic acid was birch *(Betula pendula and pubescens)*. Prior to cooking, part of the chips were extracted with alkali by treating 1 kg of chips with 2.5 M NaOH at a temperature of 95 °C and liquid to wood ratio of 5:1 for one hour. After the removal of the alkali extracts, the chips were washed twice with water (1 and 2 hours, 5%) and pressed to solids content of around 52% with a FREX press (100 bar, 10 min). The un-extracted chips were also pressed in a similar way to solids content around 68%. The cooking trials were done with both extracted and un-extracted chips for 100 g batch size in an air bath digester fitted with 6 autoclaves (V = 1.0 L). Acetic acid (80%) was used as cooking solvent and 3.5% phosphinic acid on wood was added. The process temperature was 150 °C and the liquid to wood ratio 5:1. The temperature was raised to the final cooking temperature in 60 minutes. The treatment time at the cooking temperature was varied between 120 and 240 minutes. After cooking, the pulps were separated from the cooking liquor by filtration and washed by dilution washing, three times with 70% acetic acid and twice with water. The pulps were then left in water overnight and filtrated and screened the next day.

The ethanol LGF organosolv cooking was performed at pilot scale for 20 kg (b.d.) batch of screened and dried *E. globulus* chips, using a forced circulation reactor of 250 L at a temperature of 130 °C with 3.5% H_3PO_2 and 15% water for 20 h. During cooking, the organosolv liquor was circulated through the chips continuously. After cooking, the pulp was washed with a hot ethanol:water (85:15) mixture, followed by washing with water. After LGF cooking, the alkaline extraction of the pulp was performed with 1M NaOH at 2.5% consistency overnight at room temperature.

Pulp analysis

The kappa numbers of the pulps were determined according to standard ISO 302 and intrinsic viscosities according to ISO 5351. The chemical composition of the pulps was determined as described in detail elsewhere.[9] Shortly, the samples were air dried and ground. To determine the carbohydrate and lignin composition, the samples were hydrolyzed with sulphuric acid and the resulting monosaccharides were determined by high-performance anion exchange chromatography (HPAEC) with pulse amperometric detection (PAD, Dionex ICS 3000 equipped with CarboPac PA1 column) according to NREL method.[11] The Klason lignin content, i.e. the insoluble residue from the hydrolysis, was determined gravimetrically. Acid soluble lignin in the hydrolysate was detected at 215 and 280 nm using the equation described by Goldsmid.[12]

Pulp bleaching

The acetic acid LGF pulp cooked from alkali-extracted chips (AE-LGF) was bleached with ODEDED sequence and the pulp cooked from un-extracted chips (U-LGF) with shorter ODED sequence due to its significantly lower kappa number after the oxygen delignification (16 for AE-LGF and 2.7 for U-LGF). Oxygen delignification (O) was carried out at 98 °C for 60 min at 12% consistency, and NaOH charge was 69 kg/t for AE-LGF and 82 kg/t for U-LGF (oven dry pulp). A high alkali charge was needed due to the low pH of the pulps. In the first chlorine dioxide stage (D0), the chlorine dioxide was charged according to the kappa number of the oxygen delignified pulp. The chlorine dioxide charges for AE-LGF and U-LGF pulps were 24.3 kg aCl/t and 9 kg aCl/t, respectively. The temperature in the chlorine dioxide stage was 60 °C, the reaction time 60 min and the consistency 9%. The temperature in the second D stage (D1), performed only for the AE-LGF pulp, was 70 °C, the reaction time 120 min, the consistency 9% and the chemical charge 17 kg aCl/t. In the third D stage (D2), the temperature was 70 °C, the reaction time 180 min, the consistency 9% and the chlorine dioxide charges 7 kg aCl/t for both pulps. In the first extraction (E1) stage, performed only for AE-LGF pulp, the temperature was 60 °C, the reaction time 60 min, the consistency 10% and the NaOH charge 15 kg/t. The second E stage, E2, was performed under similar conditions, with the exception of temperature, which was 70 °C, and NaOH charge, which was 8 kg/t.

Enzymatic hydrolysis and fermentation

The washed acetic acid and ethanol LGF pulp samples were suspended into 100 mM sodium citrate buffer at pH 5, 45 °C temperature and 1% consistency. Enzymatic hydrolysis was started by adding commercial Novozymes cellulase mixture (Celluclast 1.5 FP) at the dosage of 10 FPU/g dry weight and β-glucosidase (Novozym 188) at the dosage of 500 nkat/g dry weight. The suspensions were incubated at 45 °C with magnetic stirring for 72 hours, and the content of released sugars was followed. The 3,5-dinitrosalicylic acid (DNS) assay was used for the determination of reducing sugars.

Fermentation was carried out anaerobically in an Erlenmeyer flask (25 mL) in an incubator at 10% consistency. The same relative enzyme loading was used as in hydrolysis experiments (10 FPU/g Celluclast 1.5 L and 500 nkat/g Novozyme 188). After prehydrolysis of 6 h at 45 °C, the yeast (Red Star, Fermentis; a *Saccharomyces serevisiae* strain) was added with an OD_{600} of 3.5 (being ~1 g cell dry weight/L) to the flask to start the SSF and the temperature was lowered to 30 °C with slow shaking (100 rpm). The theoretical yield in fermentation was 0.51 g ethanol/g glucose.

Composite preparation and testing

For composite manufacturing, unbleached AE-LGF pulp was used. Before blend preparation, PLA pellets and fibres were dried in a vacuum oven for 6 h at 80 °C. Compounding of the components was performed at 170 °C for 10 min at a rotor speed of 50 rpm using a fully automated laboratory Brabender station. Specimens for the mechanical characterization were prepared by compression moulding using a Carver press. The compression moulding was carried out at 180 ºC with a pre-pressing step of 3 min at 50 atm and a pressing step of 2 min at 150 atm.

All the mechanical tests were performed at 50% relative humidity and 23 °C. The specimens had been conditioned under the same circumstances (50±5% relative humidity) for 24 h before testing. Young's modulus was determined according to ISO 527. An Instron 5 kN test machine operated at a crosshead speed of 30 mm/min was used for testing the specimens. The Charpy impact strength of the composites was tested according to EN ISO 179. A CEAST testing machine with a pendulum of 15J energy was used to measure the un-notched specimens (80 mm x10 mm x 4 mm). Ten specimens were tested for each material.

Triacetylation trials

To carry out the triacetylation synthesis, dried pulps were mixed with 20 mole equivalent (moleq) of acetic acid, 9 moleq of acetic anhydride and 0.08 moleq of concentrated sulfuric acid at 50 °C. After 15-120 min the cellulose began to dissolve in the reaction mixture. After additional 15 min, the reaction was completed. For destroying the excess of acetic anhydride, 80% acetic acid was added carefully. After the synthesis dispersion was cooled, cellulose acetate was precipitated by slow addition of water. The product was washed with water and dried at 105 °C.

The amount of acetyl groups was determined after saponification and titration. A value of 44% for the amount of acetyl groups corresponded to a degree of substitution of 3.0. Cellulose triacetate samples were also dissolved in a mixture of CH_2Cl_2/MeOH (90:10) to obtain solubility as a precondition for film casting.

Preparation and characterisation of nanocelluloses

Nanocellulose was manufactured from bleached U-LGF pulp. Nanocellulose grades prepared from once-dried bleached hardwood kraft were used as a reference.[13] The LGF pulp was ion-exchanged to the sodium form prior to fibrillation, as described previously.[14] The nanocelluloses were manufactured by an ultra-fine friction grinder (Masuko Supermasscolloider). The LGF pulp passed three times through the grinder.

The transmittance values of nanocelluloses were measured with a UV-Vis spectrometer (Perkin Elmer Lambda 900), as described elsewhere.[13] LGF nanocellulose was imaged using optical microscopy (OM)[13] and scanning electron microscopy (SEM). The sample was prepared for SEM analysis by critical point drying (CPD). The nanocellulose sample was diluted with milli-Q water to 0.1% consistency, homogenized with Ultra-Turrax and filtrated into membrane. The film was kept together with the membrane in 100% acetone overnight. Samples were taken to the critical point dryer (Baltec CPD 30). The dryer was cooled down to +8 °C and filled with CO_2. Mixing was applied, followed by a delay of a few minutes and exhaustion of the dryer, so that the samples remained under the liquid level. The filling and exhaustion of the dryer was repeated 8 times, until acetone was replaced with CO_2. At this point, the heating was started. When the temperature reaches the critical point, CO_2 will change from liquid phase into gas phase. The critical point in this case was 31 °C and 73.8 bar. Dried samples were placed on an aluminium stub with a double side carbon tape and coated with a thin layer of platinum. SEM images were carried out with Zeiss Merlin SEM by using InLens-detector and an accelerating voltage of 4 kV.

Lignin precipitation and analysis

The lignin in the spent liquor samples was precipitated by addition of excess water (5:1). The precipitate was separated from the liquid by centrifugation (7-27 minutes at 20 °C and 9000 rpm, corresponding to 10 300 g) and sequentially washed with water until a pH around 5 was reached. The samples were freeze-dried.

The content of acetyl groups in the LGF lignin was analysed by capillary electrophoresis (CE). 4-9 mg of sample was weighted into 1 mL of solvent (100 mM NaOH). The solution was first mixed in a Vortex mixer and then with ultrasound (30+30 min). The mixture was centrifuged at 14000 rpm for 5 minutes and the supernatant was taken for CE analysis. Reference treatment was performed with water. The CE analysis of detached acetyl groups was performed at pH 8.1 with the chemical and separation parameters as described elsewhere.[15] The phosphorous (P) analysis was performed after wet-combustion in hydrogen peroxide and nitric acid in a microwave oven. The P content was determined from the solution by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Analysis of spent liquor

The monosaccharide (arabinose, rhamnose, galactose, glucose, xylose and mannose) compositions of the spent cooking liquors were determined without acid hydrolysis and analysed by high-performance anion exchange chromatography (HPAEC) (Dionex ICS-3000) with pulse amperometric detection (PAD) (Dionex Corporation, Sunnyvale, CA, U.S.A.). The pre- and separation columns (Dionex CarboPac PA-1) were employed at 30 °C with a flow rate of 1 mL/min using the following eluents: milli-Q water, 100 mM

NaOH, 300 mM Na-acetate/100 mM NaOH, and 300 mM NaOH. The gradient of injecting different eluents was adapted from Tenkanen and Siika-aho.[16] 4 mL of sample was diluted to 10 mL with milli-Q water prior to analysis.

The furfural content was analyzed with a P/ACE MDQ capillary electrophoresis instrument with a photodiode array UV/Vis detector (PDA) (Beckman-Coulter, Inc., Fullerton, CA, USA). For the furfural detection and quantification, standards were prepared and analysed prior to actual sample analyses. Trizma base, sodium dodecyl sulphate (SDS), furfural and glacial acetic acid were purchased from Sigma (St. Louis, MO, USA). HCl and NaOH (Titrisol) were purchased from Merck (Darmstadt, Germany). Ethanol (99 wt%) was obtained from Altia (Altia, Rajamäki, Finland). A stock solution of 10 000 mg/L was prepared for furfural and the working standard solutions with a concentration range of 1-300 mg/L were made by appropriate dilutions of the stock solution with ion exchanged water. The stock solutions were stored in a refrigerator (+4 °C).

An optimized electrolyte solution for the furfural analyses consisted of 50 mM Trizma base and 100 mM sodium dodecyl sulphate (SDS) and 5% (v/v) ethanol. pH was adjusted to 8.3 with 1 M HCl prior to addition of alcohol.

In the analyses, direct detection method was used at a wavelength of 283 nm. The length of the capillary to the detection point (L_{det}) was 20 cm and the total length (L_{tot}) 30 cm, while the internal diameter was 50 µm and the outer diameter 375 µm. The temperature during analysis was 20 °C. A voltage of +15 kV was used and injection pressure and time were 0.5 psi (3.447 kPa) for 5 s. The capillary (Composite Metal Services, The Chase, Hallow, Worcester, UK) was conditioned by sequentially purging with 0.1 M NaOH (20 min), Milli-Q water (20 min), and buffer solution (20 min). The capillary was dipped in ion exchange water between rinse with buffer solution and sample injection to prevent the sample contamination with SDS. The LGF cooking liquor samples were diluted 1:5 (v/v) with 5% EtOH in H_2O, vortexed shortly and centrifuged at 14000 rpm for 5 minutes. A supernatant was further diluted with ion exchanged water prior to CE analyses. All the standards and samples were analyzed in duplicate.

RESULTS AND DISCUSSION

LGF process yields separate fractions of cellulose fibres, lignin and hemicelluloses. The yield and properties of the fractions are dependent on the solvent used as well as on the other process conditions. The results on the properties of the different fractions, as well as those related to some of their potential applications, are discussed below.

Cellulose fibres

When using acetic acid as the solvent, the majority of the hemicelluloses are hydrolysed during the LGF cooking stage, thus producing pulps with high cellulose (65-86% depending on the cooking conditions) and low hemicelluloses (8-16%) and lignin (4-6%) contents. After the LGF process, the fibre fraction can be utilized as such or further delignified using both ECF and TCF bleaching sequences.[9]

The unbleached LGF organosolv fibres have potential as re-inforcement fibres in composites. The composites containing 70% of polylactic acid (PLA) and 30% of LGF fibres were found to have stiffness properties (Figure 1a), as described by Young's modulus, and impact strength close to those containing birch kraft fibres (Figure 1b). The LGF cellulose fibres used in the composites were made from the alkali extracted chips, which were later found to have suffered from the alkali treatment. By optical microscopy evaluation, it was noted that the fibres were cut and their viscosity was also lower (640 mg/L) than that of the fibres prepared from the un-extracted chips (1020 mg/L). Further improvements in strength properties of the composites can thus be expected when LGF cellulose fibres with optimal fibre morphology and cellulose chain length are used.

The bleached LGF pulps have potential as dissolving pulps due to their high cellulose reactivity.[9] Preliminary results indicate that they can be used as raw material for cellulose triacetates, as shown by their almost complete triacetylation (DS = 3.0) and fair solubility (solution with slight turbidity). The pulp used for the triacetylation trials in this study was prepared from the alkali-extracted chips, which contrary to what was expected, contained more hemicelluloses (14%) than the LGF pulp made from un-extracted chips (8%). This result suggests that the xylan remaining in the chips after alkaline extraction was more resistant towards acid-induced hydrolysis in the subsequent LGF organosolv cooking stage. The solubility properties of triacetylated LGF pulps can thus be potentially improved by using LGF pulp with lower hemicelluloses content or by further removal of hemicelluloses from the pulp by cold caustic extraction (CCE).[9] Prospective applications of triacetylated LGF cellulose fibres include cellulose films and applications thereof. Another feasible application for acetic acid LGF

cellulose fibres is preparation of nano-scale cellulose fibrils, i.e. nanocellulose. The properties of LGF cellulose nanofibrils (CNF) were comparable to those of CNF grades prepared from dried hardwood kraft pulp (Table 1). Light transmittance of nanocellulose suspensions is influenced by many factors, with particle size being the main one and generally finer materials have higher transmittance than coarse materials.[13] After three passes in the refiner, the transmittance values of LGF and hardwood kraft (titled HW kraft coarse in Table 1) CNF were the same, indicating similar particle size.

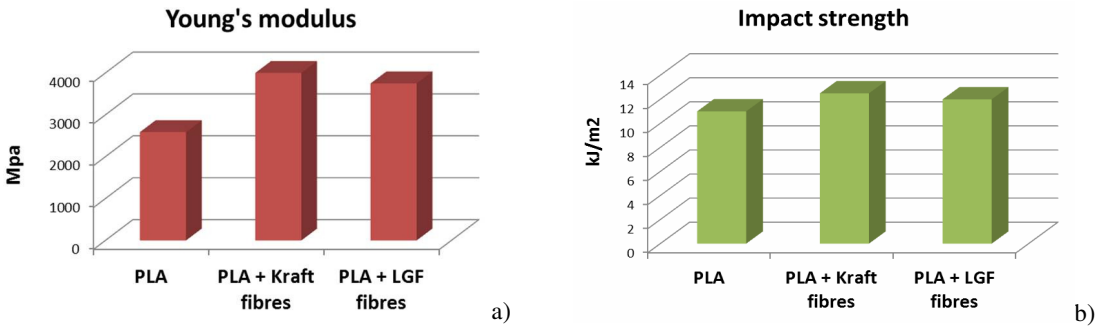

Figure 1: a) Young's modulus of PLA and PLA + pulp fibre composites; b) Impact strength of PLA and PLA + pulp fibre composites

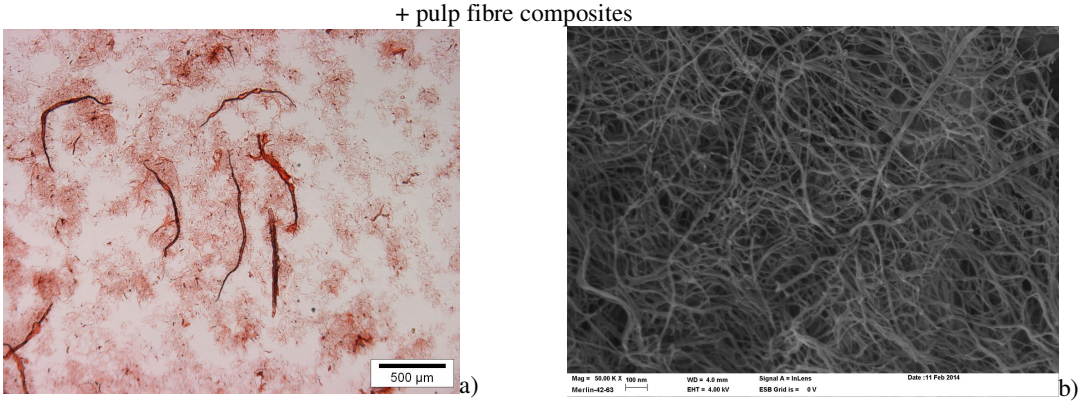

Figure 2: a) OM image of acetic acid LGF nanocellulose sample, scale bar 500 µm; b) SEM image of LGF nanocellulose sample, scale bar 100 nm

Table 1
Properties of LGF and HW kraft CNF grades[13]

	No. passes in refiner	Shear viscosity, mPa·s (10 rpm, 1.5%)	Transmittance, % (800 nm, 0.1%)
LGF coarse	3	25 200	34
HW kraft coarse	3	23 200	34
HW kraft fine	8	22 500	60

For cellulose nanofibrils, the viscosity generally increases with increased fibrillation degree due to decreased particle size. However, the size, the aspect ratio and the amount of fibrils are also known to affect viscosity, and the decrease in the aspect ratio due to cutting of fibres with increasing fibrillation degree may have the opposite, i.e. decreasing, effect on viscosity, as observed for sample HW kraft fine. Based on optical microscope images, LGF CNF was relatively homogeneous at macro/microscale (Figure 2a). SEM images showed that nano-scale dimensions for fibrils were obtained (Figure 2b).

When the LGF process is performed with ethanol as the solvent, more hemicelluloses (content around 18%) and lignin (25%) are retained with the cellulose fraction (53%). However, the ethanol based LGF process has been

shown to produce a fibre fraction well suitable for enzymatic hydrolysis and further fermentation of the monomeric sugars into bioethanol.[10] The enzymatic hydrolysis rate and degree, as well as the final bioethanol yield can be further improved by alkaline extraction, also providing additional valuable lignin and hemicelluloses fractions (Figure 3).

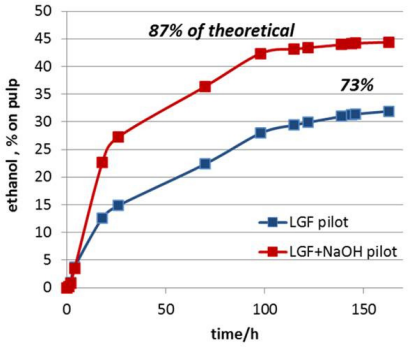

Figure 3: Bioethanol yield of ethanol LGF pilot pulps

According to literature, cellulose fibres obtained by acetic acid treatments have a decreased susceptibility to enzymatic hydrolysis, probably due to acetylation of cellulose during treatment.[17] Binding of cellulase enzyme to cellulose takes place *via* hydroxyl groups, and substitution of hydroxyl groups with acetyl groups during acetic acid pulping inhibits their interaction. Despite this, 80% sugar release on pulp carbohydrates was achieved from the acetic acid LGF pulps by enzymatic hydrolysis. However, the sugar release was lower compared to the ethanol LGF fibres.

Lignin

Regardless of the cooking solvent used, the LGF process yields sulphur-free lignin with substantial amount of reactive phenolic functionalities for biomaterial applications, such as bioplastics or thermoset resins. The glass transition temperature of ethanol LGF lignin is lower, around 130 °C, compared to a number of technical lignins (kraft, soda, steam explosion), being in that sense the most thermoplastic of these.[18] However, chemical modification is still needed to obtain mouldable thermoplastic lignin, e.g. for thermal processing of plastic composite materials.

Lignin of high purity (>93%) is isolated from the LGF spent cooking liquor by simple water precipitation with nearly 80% yield.[8,18] Structurally, acetic acid and ethanol LGF lignins are clearly different from native lignin as the main part of the β-aryl ether bonds are cleaved during the process. However, no evidence of lignin condensation reactions has been found in the structural analysis of acetic acid LGF lignin. With acetic acid solvent, the lignin is also acetylated to some extent (acetyl content 4.3 wt%) and contains approximately 0.5 wt% of phosphorus.[8] The average molar mass (Mw) of LGF lignin is around 3000-3500 g/mol and the molar mass distribution is narrow (PD = 1.6). The low molar mass level of LGF could be beneficial for example in polyurethane (PU) resin applications to prevent extensive increase of viscosity, or to improve the lignin solubility.[18]

Hemicelluloses

In acetic acid LGF process, the polymeric hemicelluloses can be recovered prior to cooking by an alkaline extraction of the chips. Under the conditions used in this work, 23% of the original xylan in the wood could be recovered in this manner.[19] Alternatively, the hydrolysed hemicelluloses can be found from the spent cooking liquor. For example, after cooking for 120 minutes, 25% of the xylan originally present in the wood was found in the pulp, while 37% was detected as xylose in the cooking liquor and 16% as furfural. The un-accounted material, comprising around 20% of the original xylan, most probably consists of further reaction and polymerization products of xylose and furfural, often referred to as humins[20] or pseudo-lignin.[21,22,23]

In ethanol LGF process, the hemicelluloses are either utilized together with the cellulose in fermentation or separated by alkaline extraction together with some lignin.

The sugar-based compounds obtained with both of these solvents have potential as platform

chemicals. Studies on how to recover the hydrolysed hemicelluloses from the spent cooking liquor with high yield and purity are currently on-going.

CONCLUSION

The Lignofibre (LGF) organosolv process is a novel method for fractionation of lignocellulosics for production of high-quality biomass components from a diversity of raw materials. LGF cellulose fibres have many potential applications, such as reinforcement fibres in composites, as raw materials for cellulose derivatives and applications thereof and for production of nano-scale cellulose fibres or bioethanol. High purity sulphur-free LGF lignin can be isolated by simple water precipitation with nearly 80% yield and has potential as component in bioplastics and in resin applications. The hydrolysed hemicelluloses can be found in the spent cooking liquor, mainly as monosaccharides and their further reaction products, such as furfural. The sugar-based compounds have potential as platform chemicals, and their recovery from the cooking liquor is currently studied. LGF process also offers possibilities for producing biomaterials for applications beyond the ones studied in this paper. The flexibility of the process enables it to be tailored to meet the needs for each application.

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