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Hydrotropic process for green biorefinery applications

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Preface

The thesis is based on the original publications that are listed below, and they are referred to in the text by the Roman numbers.

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II. Gabov, K., Gosselink, J. A. R., Smeds, I. A., and Fardim, P. (2014). "Characterization of lignin extracted from birch wood by a modified hydrotropic process," *Journal of Agricultural and Food Chemistry,* 62(44), 10759–10767.

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IV. Gabov, K., Oja, T., Deguchi, T., Fallarero, A., and Fardim, P. (2017). "Preparation, characterization and antimicrobial application of hybrid cellulose-lignin beads," *Cellulose,* 24 (2), 641–658.

Author's contribution

I. All experiments, interpretation of the results and writing the manuscript.
II. All experiments except the elemental analysis, NMR analyses, SEC and pyrolysis-GC-MS. Interpretation of the results and writing the manuscript.
III. All experiments except the pyrolysis-GC-MS, TMAH/Pyrolysis-GC-MS and elemental analysis. Interpretation of the results and writing the manuscript.
IV. All experiments except the SEM analysis, laser scanning confocal fluorescence microscopy and antibacterial studies. Interpretation of the results and writing the manuscript.

The work was mainly performed in the Laboratory of Fibre and Cellulose Technology. A part of the treatment experiments for publication no. I was carried out by the author in the Laboratory of Chemistry, Pulp and Energy, Luiz de Queiroz College of Agriculture, University of Saõ Paulo under co-supervision of Professor Francides Gomes da Silva Júnior. The funding for the work was received from the projects FEASEBIO and ProNatMat,

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List of abbreviations, acronyms and symbols

100C beads	Pure (100%) cellulose beads
4-O-Me-GluA	4-O-Methylglucuronic acid
60C40L beads	60% cellulose/40% lignin beads
75C25L beads	75% cellulose/25% lignin beads
90C10L beads	90% cellulose/10% lignin beads
AD	Air-dried
Ara	Arabinose
ASL	Acid-soluble lignin
ATR	Attenuated total reflection
CFU	Colony-forming unit
DMSO	Dimethyl sulfoxide
DP	Degree of polymerization
EDS	Energy-dispersive X-ray spectroscopy
FA	Ferulic acid
FE-SEM	Field-emission scanning electron microscope
FTIR	Fourier transform infrared spectroscopy
G	Guaiacylpropane lignin unit
Gal	Galactose
GalA	Galacturonic acid
Glu	Glucose
GluA	Glucuronic acid
H	Hydroxyphenylpropane lignin unit
HMDS	Hexamethyldisilazane
HMF	Hydroxymethylfurfural
LCC	Lignin-carbohydrate complex
Man	Mannose
MHC	Minimal hydrotrope concentration
MTBE	Methyl tert-butyl ether
ND	Never-dried
NSSC	Neutral sulfite semichemical pulping
O	Oxygen delignification
OD	Oven-dried
P	Hydrogen peroxide bleaching stage
*p*CA	*para*-Coumaric acid
PO	Pressurized hydrogen peroxide bleaching stage
PPU	Phenylpropane unit

Py-GC-MS	Pyrolysis-gas chromatography-mass spectrometry
Q	Chelation stage
S	Syringylpropane lignin unit
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
SXS	Sodium xylenesulfonate
TMAH	Tetramethylammonium hydroxide treatment
TMCS	Trimethylchlorosilane
TSB	Tryptic soy broth
UV	Ultraviolet
Xyl	Xylose

Abstract

Konstantin Gabov
Hydrotropic process for green biorefinery applications

Doctor of Science (Technology) Thesis, Åbo Akademi University, Faculty of Science and Engineering, Laboratory of Fibre and Cellulose Technology, Turku 2017

Keywords: hydrotropic process, sodium xylenesulfonate, birch wood chips, sugarcane bagasse, hydrotropic lignin, hydrotropic pulp, spent solution, NaOH/urea aqueous solution, lignin-cellulose beads, *Staphylococcus aureus, Escherichia coli*

Biorefinery is a concept of sustainable biomass processing into several useful products, such as fuels, materials, power and chemicals. The necessity of more extensive biomass utilization is governed nowadays by the intensively growing population and the increased pollution caused by the usage of oil-based products, which lead, among other things, to the global climate change. However, successful implementation of the biorefinery concept requires efficient fractionation technologies. Therefore, the aim of the present study was the investigation of a hydrotropic process as a method for biomass fractionation. The hydrotropic method is an environmentally friendly water-based process that possesses several attractive features, such as simple recovery of the hydrotropic solution and possibility to obtain, besides fibers, several by-products.

In the scope of the study, two raw materials, namely birch wood chips from Finland and sugarcane bagasse from Brazil, were treated with the hydrotropic method, and the obtained fractions were characterized employing different techniques. In addition, lignin hydrotropically extracted from birch wood was utilized for the preparation of lignin-cellulose particles.

The result of the fractionation of the birch wood chips showed that this raw material could be efficiently delignified with the process under the conditions employed. The modification of the solutions with formic acid, or hydrogen peroxide, or both improved considerably the delignification efficiency. Generally, the produced pulps were enriched in cellulose. Subsequent bleaching further increased the cellulose content. Therefore, the obtained cellulose fractions could potentially be used as dissolving grade pulps. Lignins extracted from the wood by the unmodified and modified (formic acid and hydrogen peroxide) hydrotropic solutions were isolated by

dilution of the spent solutions with water and filtration and had low contents of non-lignin compounds.

Sugarcane bagasse was fractionated with a hydrotropic process at different treatment temperatures and times. Generally, two fractions, namely cellulose and lignin, were obtained. The purity of both fractions and the yield of lignin were higher at more severe treatment conditions. The spent solutions from the treatments contained, besides lignin, also dissolved hemicelluloses, sugar monomers, furfural, acetic and formic acids. The content of the dissolved components varied depending on the treatment conditions.

Lignin extracted from birch wood with a conventional hydrotropic method was mixed with cellulose and shaped into beads employing 7% NaOH/12% urea aqueous solution as a solvent. The beads in the never-dried state were highly porous particles, and the lignin was evenly distributed in them. Antibacterial studies against the common pathogens *Staphylococcus aureus* and *Escherichia coli* revealed that the beads could inhibit the growth of *S. aureus*, and the extent of the inhibition correlated with the lignin content in the beads.

The results summarized in the thesis showed a great potential of the hydrotropic treatment for fractionation of hardwood and non-wood raw materials into valuable products, such as cellulose and lignin. Positive aspects of the hydrotropic treatment with the respect to the recovery of the named fractions are simple isolation of the extracted lignin from the solution and minimal losses of cellulose over the course of the treatment. In addition, both fractions can be produced with a high degree of purity, and they present excellent raw materials for further conversion. As was shown with sugarcane bagasse, besides the main streams, several other products (furfural and acetic acid) can be obtained as well. Overall, the results of the thesis can serve as a basis for further development of hydrotropic process-based biorefinery technology.

Sammanfattning

Konstantin Gabov
Hydrotropisk process för gröna bioraffinaderitillämpningar

Teknologiedoktorsavhandling, Åbo Akademi, Fakulteten för naturvetenskaper och teknik, Laboratoriet för fiber- och cellulosateknologi, Åbo 2017

Nyckelord: hydrotropisk process, sodium xylensulfonat, björkflis, sockerrörsblast, hydrotropisk lignin, hydrotropisk massa, restlut, NaOH/urea vattenlösning, lignin-cellulosa pärlor, *Staphylococcus aureus, Escherichia coli*

Bioraffinaderi är ett koncept som innefattar hållbar förädling av biomassa till flera nyttiga produkter såsom bränsle, material, energi och kemikalier. Behovet av en bredare användning av råvaror från biomassa har sin grund i jordens befolkningsökning och förorening med olja-baserade produkter, vilka bland annat bidrar till klimatförändringen. En framgångsrik implementering av bioraffinaderier behöver effektiva fraktioneringsmetoder och därför är syftet av den här forskningen att undersöka en hydrotropisk process som kan användas som metod för fraktionering av biomassa. Den undersökta hydrotropiska metoden är en miljövänlig vatten-baserad process med enkel återvinning av hydrotropen och en möjlighet att erhålla olika biprodukter.

Två typer av råvaror användes i studierna, nämligen flis av nordisk björk och sockerrörsblast (bagass) från Brasilien. De här råvarorna behandlades med den hydrotropiska metoden och erhållna fraktioner analyserades med olika tekniker. Dessutom användes lignin, som var extraherad med den hydrotropiska metoden, för tillverkning av lignin-cellulosa partiklar (pärlor).

Experiment med björkflis visade att under de förhållanden som användes kunde den hydrotropiska metoden effektivt delignifiera den här råvaran. Tillsats av myrsyra, väteperoxid eller de båda i kombination till den hydrotropiska lösningen hjälpte att avsevärt förbättra delignifieringen. De producerade massorna hade en hög cellulosahalt, som ytterligare ökade efter blekning. Därför är dissolvingmassa ett möjligt användningsområde för de här massorna. Lignin som extraherats med den omodifierade och den modifierade (myrsyra och väteperoxid) processen isolerades genom tillsats av vatten till restlutarna och därpå följande filtrering. Ligninet hade låga halter av icke-lignin komponenter.

Sökerrörsblast (bagass) fraktionerades med den hydrotropiska metoden vid olika temperaturer och tider. Vanligen erhölls två fraktioner, nämligen cellulosa och lignin. Renheten av de båda fraktionerna och utbytet av lignin var högre vid de hårdare processförhållandena. Förutom utlöst lignin fanns det i restlutarna också hemicellulosor, monosackarider, furfural, ättik- och myrsyra. Halten av de utlösta komponenterna varierade med processförhållandena.

Lignin som extraherats från björkved med den konventionella hydrotropiska metoden blandades med cellulosa i en vattenlösning innehållande 7 % NaOH/12 % urea och den här lignin-cellulosa lösningen användes för att framställa partiklar som kallas pärlor. Icke-torkade pärlor hade en hög porositet och ligninet var jämnt utspritt i pärlorna. Antibakteriologiska prov mot vanliga patogener *Staphylococcus aureus* och *Escherichia coli* visade att lignin-cellulosa pärlorna förhindrade tillväxten av *S. aureus* och inhibitionen var högre när ligninhalten steg.

Resultaten av den har undersökningen visade att den hydrotropiska metoden har en stor potential för fraktionering av lövved och icke-ved råvaror till värdefulla produkter såsom cellulosa och lignin. De positiva sidorna av processen när det gäller återvinning av de nämnda fraktionerna är en enkel isolering av extraherat lignin från restluten och små förluster av cellulosa under processen. Dessutom kan de båda fraktionerna produceras med hög renhet och därför är de utmärkta råvaror för vidare förädlingar. Som det visades med sökerrörsblast kan förutom huvudprodukterna cellulosa och lignin också flera andra produkter erhållas såsom furfural och ättiksyra. På det hela taget, resultaten av denna avhandling kan stå som grund för ytterligare utvecklingar av ett bioraffinaderi baserat på hydrotropiska processer.

1 Introduction

1.1 Biorefinery

Biorefinery implies a sustainable process or a combination of processes of biomass conversion into a spectrum of valuable products which include fuels, power, chemicals and materials (Mussatto and Dragone 2016). The concept is analogous to the oil refinery, where crude oil is fractionated through a distillation process into several fractions, such as gasoline, diesel fuel, kerosene, lubricating oils and asphalts, which can be further upgraded/refined to other products (Cherubini 2010). Generally, as an example of biomass refinery one can think of pulp factories, as they process biomass and usually generate several products, such as fibers or cellulose fraction, bio-energy and tall oil, among others (Mikkola et al. 2016; Testova 2014).

The biorefinery concept is not new, and a large number of bio-based products were manufactured from wood and agricultural crops already at the beginning of the 20th century (Dodds and Gross 2007; Ragauskas et al. 2006). However, many of them had been replaced with oil-based counterparts by the 1960s (Ragauskas et al. 2006). Lately, biomass refinery has gained renewed interest driven by several factors and concerns. A main aspect is the environmental problem associated with the usage of petroleum-derived products. The fast growth of the world's population together with the rise in energy consumption per capita results in increased pollution of the planet by the generated waste, including solid and gaseous pollutants (Cherubini 2010; Mussatto and Dragone 2016). Extensive usage of oil and, in particular, the oil based transportation fuel leads to an increase in the greenhouse gas emissions, which, in turn, considerably influence the global climate change (Cherubini 2010; Mussatto and Dragone 2016). Utilization of biomass, which is a carbon-neutral resource, with simultaneous carbon capture and storage is seen as a way to reduce greenhouse gas emission and thus lessen the climate change (Mussatto and Dragone 2016).

Another reason behind the interest in the biorefinery concept is the renewable nature of biomass and its availability. Furthermore, due to the wide range of raw materials and variation in the composition, the processing of biomass enables manufacture of a huge variety of products compared to the oil refinery (Cherubini 2010). Also, the possibility to obtain products with a unique structure and functionality that otherwise cannot be derived from

oil can be considered as an additional advantage of biomass refinery (Mussatto and Dragone 2016).

1.2 Biomass fractionation

One of the prerequisites for the successful implementation of the biorefinery concept is the development of the chemical fractionation methods that would enable techno-economically feasible recovery of the main constituents of the lignocellulosic material, namely cellulose, hemicelluloses and lignin, in a sufficiently pure form (Amore et al. 2016; Mikkola et al. 2016; Mussatto and Dragone 2016).

The fractionation into the constituents is generally performed by chemical treatments or a sequence of chemical processes. For example, the hemicellulose fraction can be extracted by a hydrothermal method that comprises treatment of lignocellulosic materials with water/steam at temperatures of 150–230 °C in an autocatalyzed mode or with the addition of an acid catalyst (Garrote et al. 1999). This process has been applied to various lignocellulosic materials, and a huge number of research papers devoted to the hydrothermal treatment can be found (Borrega et al. 2013; Song et al. 2008, 2013; Vallejos et al. 2015a). Extraction of lignin is accomplished by, for example, organosolv fractionation methods or other methods that are used in pulping technology, such as kraft and sulfite processes, and treatment with sodium hydroxide (soda pulping) (Amore et al. 2016; Mikkola et al. 2016). As a rule, delignification methods also extract and modify hemicelluloses. Several researchers have applied a combination of hydrothermal and organosolv processes to extract hemicelluloses and lignin from biomass, for example, from sugarcane bagasse and eucalyptus (Romaní et al. 2011; Vallejos et al. 2015b). During both hydrothermal treatment and delignification processes, cellulose generally undergoes several changes, such as reduction in the degree of polymerization (DP) and partial dissolution. However, it is usually affected to a lesser extent, because it is the most robust constituent of lignocellulosic biomass due to the high DP and the supramolecular structure (Garrote et al. 1999; Sjöström 1993a). Therefore, the residue remaining after the extraction of hemicelluloses and lignin constitutes a cellulose fraction with a certain degree of purity. This principle is used in the manufacture of dissolving grade pulps, which essentially consist of cellulose, by prehydrolysis treatment combined with a kraft/soda process (Andrade and Colodette 2014; Borrega et al. 2013; Sixta et al. 2006).

Research presented in this thesis was devoted to a special type of a fractionation method, a hydrotropic process. Generally, this treatment is an alternative to other delignification processes, some of which are listed above. Despite that the lignin extraction methods exist in a fully commercial scale and are very common, *e.g.* pulping processes, research on a hydrotropic method is still of great importance. This is related to the fact that the commercial kraft and less widespread sulfite processes have several drawbacks. Most important is that neither of the methods can be considered as environmentally friendly, because of the various types of pollution (Süss 2006). Besides this, these processes have complex systems for the recovery of the pulping chemicals and, in the case of the sulfite process, the recovery is performed only for certain types of bases, magnesium and sodium (Krotscheck and Sixta 2006).

The hydrotropic treatment is an environmentally friendly water-based process with a simple recovery of hydrotropic solution and a possibility to obtain several products (McKee 1954). These advantages over the commercial processes make this method an attractive candidate for the biorefinery applications. In this work, the hydrotropic method was applied to two raw materials, namely birch wood from Finland and sugarcane bagasse from Brazil as representatives of hardwood and non-wood types of biomass.

2 Background

2.1 Birch wood

Birch is an important industrial hardwood tree species. It is widely spread in the world, including the Nordic countries and Russia. Birch wood is traditionally used for pulping and papermaking, construction, manufacturing of plywood and furniture.

European birch wood (*Betula verrucosa* and *Betula pendula* species) contains 40–43% of cellulose, 20–25% of lignin, 28–35% of hemicelluloses, 2–3% of extractives and 0.3% of ash (Alen 2000a; Pinto et al. 2005; Sjöström 1993b; Testova et al. 2014).

Cellulose

Cellulose is a linear homopolysaccharide consisting of anhydrous β-D-glucopyranose units (Glu*p*) joined by C_1-C_4' glycosidic bonds (Figure 1). Each following unit is turned 180° relative to the preceding one, so the repeating unit of the polymer actually consists of two β-D-glucopyranose units, so-called cellobiose, with a length of 1.03 nm (Fengel and Wegener 1984a; Sjöström 1993c). The terminal units of cellulose macromolecules are divided into non-reducing and reducing end-groups. The former has an additional hydroxyl group at C_4, and the latter bears an OH group at C_1 position forming a cyclic hemiacetal that, in solutions, co-exists in the equilibrium with the acyclic structure bearing an aldehyde group (Sjöström 1993d).

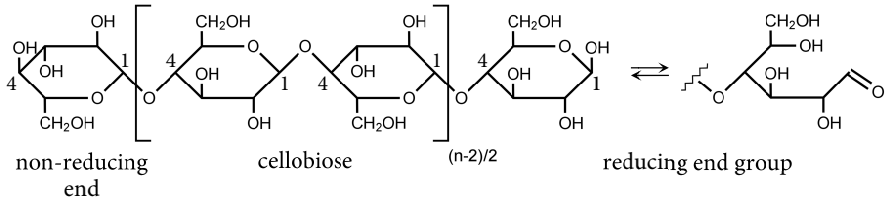

Figure 1. The structure of cellulose (Fengel and Wegener 1984a) and a reducing end unit of cellulose (Sjöström 1993d).

The hydroxyl groups of the cellulose macromolecules are engaged in intra- and intermolecular hydrogen bonding. The former render cellulose chains rigid, and they exist between O(6) of one unit and O'(2)H of the neighboring unit, and between O(3)H and O'(ring) of another unit (Sjöström 1993c). Intermolecular hydrogen bonds, O(3) to O''(6)H, result in the formation of supramolecular structures, cellulose bundles, that combine further to form

microfibrils. Microfibrils, in turn, constitute fibrils, which make up the cell wall (Sjöström 1993c). Cellulose in wood has a degree of polymerization of about 10,000 units, and it is 500–2,000 in technical wood pulps (Alen 2000a).

Hemicelluloses

Hemicelluloses constitute another group of polysaccharide-type structural components in wood. In contrast to cellulose, hemicelluloses are heteropolysaccharides, and they consist of different sugar units.

A main hemicellulose of hardwoods is 4-*O*-methylglucuronoxylan which amounts to 25–28% in European birch wood (Sjöström 1993b; Testova et al. 2014). The backbone of this polysaccharide consists of 100–200 anhydrous *β*-D-xylopyranose (Xyl*p*) units connected with each other by C_1-C_4' glycosidic linkages (Figure 2) (Fengel and Wegener 1984b). The anhydroxylose units are acetylated at C_2 or C_3 positions. On average, 10 xylose units bear 5–7 acetyl groups (Fengel and Wegener 1984b; Sjöström 1993c). The molar distribution between the unsubstituted, C_2-acetylated, C_3-acetylated and C_2+C_3-acetylated units is 44:24:22:10, respectively (Lindberg et al. 1973, birch xylan). 4-*O*-methyl-*α*-D-glucuronic acid (4-*O*-MeGluA) groups are linked to the backbone units by C_1-C_2' glycosidic bonds, and the ratio of Xyl to 4-*O*-MeGluA in hardwoods is about 10:1 (Fengel and Wegener 1984b; Sjöström 1993c). In the case of birch wood (*Betula pendula*), the ratio of 8.5:1 could be estimated from the methanolysis results presented elsewhere (Willför et al. 2005). Other authors reported a value of 14:1 for *B. pendula* (Pinto et al. 2005). Hardwood xylans also contain small quantities of rhamnose (Rha) and galacturonic acid (GalA) residues. They constitute a part of the backbone connecting the reducing xylose unit to the xylan chain in the following sequence: ···→4-*β*-D-Xyl*p*-1→3-*α*-L-Rha*p*-1→2-*α*-D-Gal*p*A-1→4-D-Xyl (Johansson and Samuelson 1977; Pinto et al. 2005).

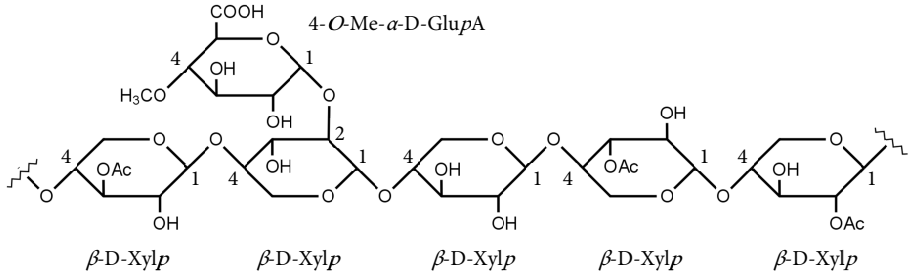

Figure 2. Structure of glucuronoxylan (Fengel and Wegener 1984b).

Glucomannan is less abundant in hardwoods compared to glucuronoxylan, and it constitutes 2–5% of wood weight. It is a linear polymer (Figure 3)

consisting of anhydrous *β*-D-glucopyranose and *β*-D-mannopyranose (Man*p*) units joined by C_1-C_4' glycosidic bonds (Sjöström 1993c). The ratio of Glu to Man is 1:1–2 (Sjöström 1993c), and the DP of the polymer is 60–70 units (Fengel and Wegener 1984b). It has been shown that glucomannan is also partially acetylated in hardwoods at the C_2 or C_3 positions of anhydro-*β*-D-mannopyranose units (Teleman et al. 2003).

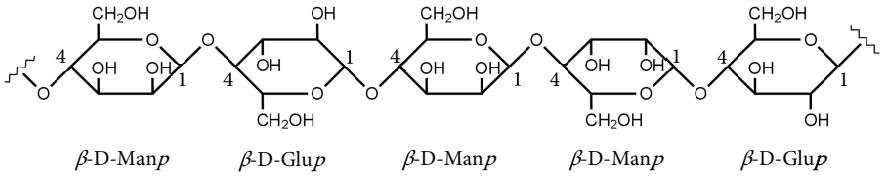

Figure 3. Structure of glucomannan (Sjöström 1993c).

Lignin

In general, lignin is an aromatic cross-linked polymer that consists of phenylpropane units (PPU), namely syringylpropane (S), guaiacylpropane (G) and phenylpropane (H) (Figure 4), connected to each other by aliphatic C-C' and ether C-*O*-C' bonds in a random manner.

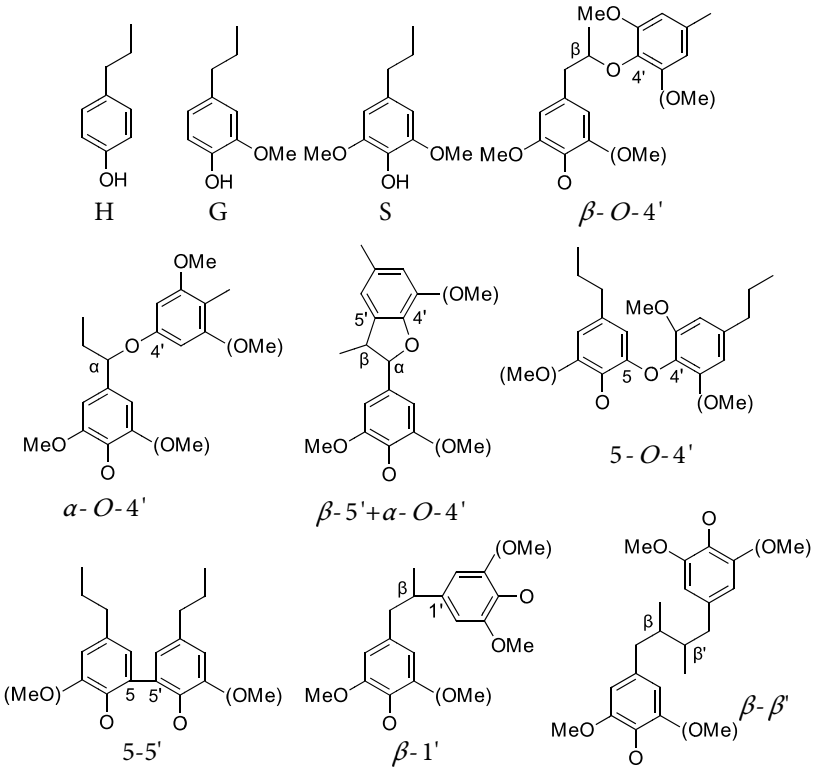

Figure 4. Lignin units and interunit linkages (Sjöström 1993e).

Birch wood lignin can be regarded as syringyl-guaiacyl one. The S/G ratio for *Betula pendula* lignin is in the range of 2–2.4 (Pinto et al. 2005; Rauhala et al. 2011; Rencoret et al. 2012). The main linkages between the lignin units (Figure 4) and their amount in birch wood are shown in Table 1. As can be seen from the table, *β-O*-4' is the main linkage in birch wood lignin. Generally, it is the most predominant type of bond in both softwood and hardwood lignins (Sjöström 1993e). Other values for the abundance of *β-O*-4', *β*-5' and *β-β*' linkages in birch wood (*Betula pendula*) lignin found in literature are 40–55, 2–5 and 7–10 per 100 PPU, respectively (Balakshin et al. 2011; Lundquist 1991, 1992; Pinto et al. 2005; Rauhala et al. 2011).

Table 1. Linkages in birch wood (*Betula verrucosa*) lignin (Adler 1977; Sjöström 1993e).

Linkage	Dimer structure	per 100 PPU
β-O-4'	Arylglycerol-β-aryl ether	60[a]
α-O-4'	Noncyclic benzyl aryl ether	6–8
β-5'	Phenylcoumaran	6
5-5'	Biphenyl	4.5[b]
4-*O*-5'	Diphenyl ether	6.5[c]
β-1'	1,2-Diaryl propane	7
β-β'	Linked through side chains	3

[a]22–28 of G type and 34–39 of S type.
[b]G type.
[c]1 of G type and 5.5 of S type.

Lignin bears various functional groups. Among them are methoxyl, phenolic and primary and secondary aliphatic hydroxyls. The quantity of these groups in birch wood (*Betula pendula*) lignin is 1.5, 0.2, 0.5 and 0.6 per PPU, respectively. If expressed in mmol/g, the content of aliphatic and phenolic OH groups is 5.4 and 1.2, respectively (Rauhala et al. 2011). In addition, carboxyl groups are present in the lignin in amount of 0.18 mmol/g (Rauhala et al. 2011).

In wood, lignin is chemically connected to hemicelluloses through ester, ether and glycosidic bonds forming a so-called lignin-carbohydrate complex (LCC). Examples of the possible combinations can be an ester bond between a lignin unit and 4-*O*-Me-glucuronic acid of xylan and ether linkages with arabinose and mannose units of arabinoglucuronoxylan and glucomannan (Sjöström 1993e).

2.2 Sugarcane bagasse

Sugarcane is a major crop used for the manufacture of table sugar or sucrose followed by sugar beet. Sugarcane is cultivated in tropical and subtropical countries, and in 2013, its production accounted for 1.9 billion tons with Brazil being the top sugarcane-producing country (O'Hara 2016). Other major producers include, in descending order, India, China, Thailand and Pakistan (O'Hara 2016).

When sugarcane is harvested, the leaves and the top parts of the plant are removed from the stalks and are left in the field to decompose, or they are collected to be burnt (O'Hara 2016). Only the stalks are delivered to the factory for the sugar manufacturing process (O'Hara 2016). The sugarcane stalks contain 65–75% of water, 10–18% of fiber material, 10–15% of sucrose and a small percentage of other soluble material (Mann 2016). In the factory, they are shredded, and the sugar juice is extracted from the crushed material in a set of mills and/or diffusers with the assistance of water (O'Hara 2016). The fibrous material remaining after the juice extraction is called bagasse. Generally, sugar mills generate about 260–280 kg of wet bagasse (dry content of 50%) from 1 ton of sugarcane (Clauser et al. 2016; Seabra et al. 2010), and most of it is burnt in boilers to produce steam and generate electricity to cover the energy demand needed for the factory operation (O'Hara 2016). However, bagasse obtained in the course of sugar manufacturing contains more energy than is needed by the factory and, therefore, upon optimization of the incineration technology, a high amount of this agro-industrial waste will be available for the conversion into other high-added value products. The manufacture of high-added value products from the bagasse, in turn, will help the sugar factories to diversify their economy and to make the profitability less dependent on the sugar price on the global market (O'Hara 2016).

Sugarcane bagasse consists of 40–45% of cellulose, 20–25% of lignin, 25–30% of hemicelluloses and minor amounts of other compounds, such as inorganic materials and extractives (Andrade and Colodette 2014; Canilha et al. 2012; Clauser et al. 2016), and it is a potential low-value high-volume biomass resource for biorefinery technology. Comparing the values for the chemical composition of sugarcane bagasse with those for birch wood (section 2.1), one can observe that these two raw materials are similar to each other with respect to the content of the structural components. However, the structure of the constituents, in particular, hemicelluloses and lignin is

different. Therefore, a brief description of the composition of the bagasse hemicelluloses and lignin should also be presented.

Hemicelluloses

A main hemicellulose in sugarcane bagasse is arabinoglucuronoxylan (Morais de Carvalho et al. 2017). The description below is based on the work by Morais de Carvalho et al. (2017), who isolated this hemicellulose using dimethyl sulfoxide (DMSO) from the peracetic acid-delignified sugarcane bagasse and characterized it with the help of different methods. Generally, its structure is similar to that of birch wood xylan, except that the OH groups of the anhydroxylose units in the backbone of the hemicellulose are also substituted with *α*-L-arabinofuranose connected by C_1-C_3' glycosidic bonds. The content of the arabinose and 4-*O*-Me-glucuronic acid units is 5 and 1, respectively, per 100 xylose units. The content of acetyl groups is 8.7%. The *O*-acetyl groups are attached either to C_2 or C_3 atoms of the xylose rings or to both atoms simultaneously. The ratio of such units, and also those not bearing any acetyl groups, is 11:16:3:70, respectively.

Besides arabinoglucuronoxylan, sugarcane bagasse also contains other hemicelluloses. The results of several studies have shown that upon hydrolysis or methanolysis, sugarcane bagasse also produces other non-cellulosic carbohydrate units, such as mannose and galactose (Alves et al. 2010; de Carvalho et al. 2015; Szczerbowski et al. 2014). The content of each is usually below 1% based on bagasse (Alves et al. 2010; de Carvalho et al. 2015; Szczerbowski et al. 2014).

Lignin

The S/G ratio of sugarcane bagasse varies in literature due to probably different procedures used for the determination as well as due to the variation among the raw materials. The reported values are 1.1 (de Carvalho et al. 2015), 1.3–1.6 (del Río et al. 2015) and 0.8–1.3 (Lopes et al. 2011). It has also been shown that the number of H units is small, 2–3 in 100 PPU (del Río et al. 2015).

Besides the classical lignin units, sugarcane bagasse contains residues of *p*-coumaric and ferulic acids and tricin (Figure 5). These were found in amounts of, respectively, 68, 26 and 2 mol% based on the total sum of S, G and H units (del Río et al. 2015). Tricin is a flavon, and it is incorporated into the lignin structure being connected to another lignin unit through a 4-*O*-*β*' linkage. *p*-Coumaric acid acylates C_γ-OH of the lignin units, predominantly S units, whereas ferulic acid is mainly attached to the hemicelluloses (del Río et al. 2015).

The main lignin linkage is *β-O*-4', which accounts for 83% of all linkages (del Río et al. 2015). It is represented by the *β-O*-4' structure and *β-O*-4' with acylated C_γ-OH in Figure 5. In addition, 3% of all *β*-aryl ether linkages is found in the structure of C_α-oxidized *β-O*-4'. Other linkages and structures found in sugarcane bagasse lignin are: 6% phenylcoumaran (Figure 4, *β*-5'+*α-O*-4'), 2% resinol (Figure 5), 4% tetrahydrofuran, 2% *α,β*-diaryl ether and 3% spirodienone (del Río et al. 2015).

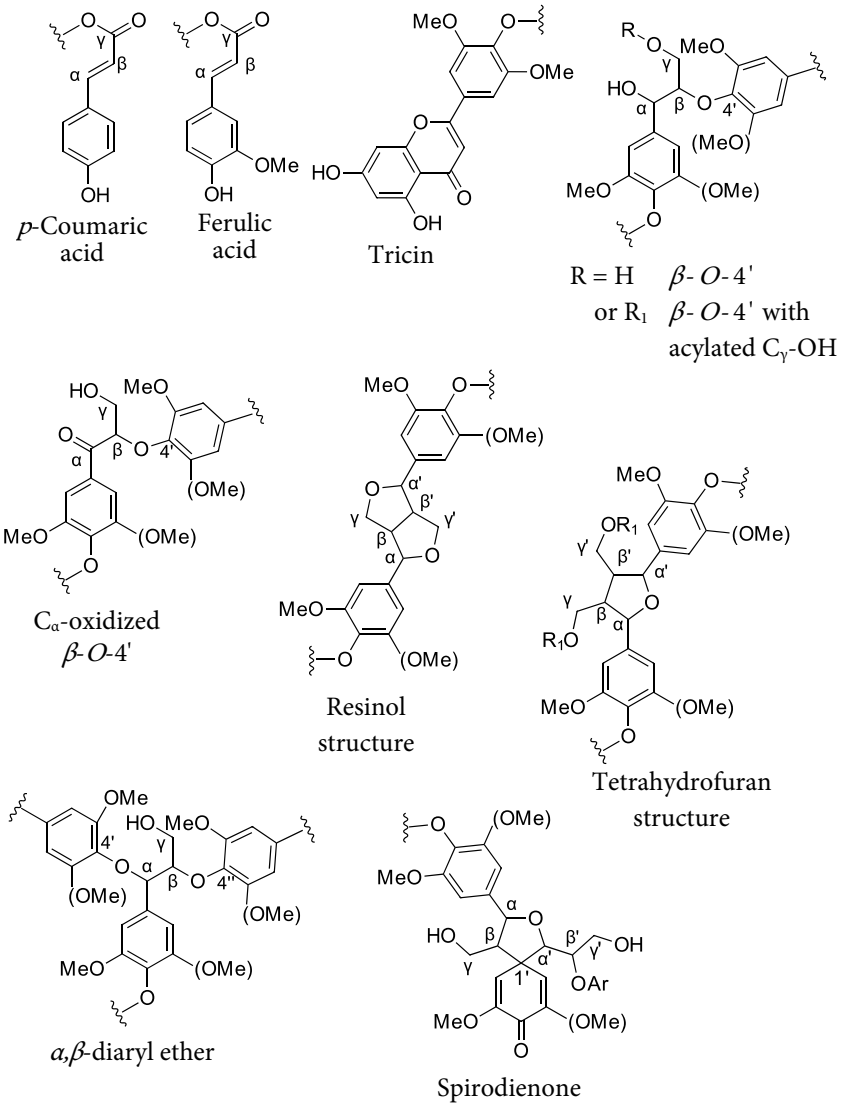

Figure 5. Main structures of sugarcane bagasse lignin. R_1 in the structures of *β-O*-4' with acylated C_γ-OH and tetrahydrofuran is acetyl or *p*-coumaroyl group (del Río et al. 2015).

2.3 Hydrotropic treatment

2.3.1 Hydrotropic agents

Hydrotropic agents or hydrotropes are chemicals that, when used in a concentrated form of the aqueous solutions, improve the solubility of water-insoluble organic substances (Hodgdon and Kaler 2007; Procter 1971). Such compounds are amphiphilic substances, meaning that they consist of hydrophobic and hydrophilic parts. Despite the similarity with surfactants in respect of the amphiphilic nature, these two groups of compounds differ from each other by the size of the hydrophobic part which is usually smaller in the case of hydrotropes (Srinivas and Balasubramanian 1998). The difference in the hydrophobic part, in turn, governs the different solubilization behavior of these two groups. Hence, a higher concentration of a hydrotrope is required to initiate solubilization of a hydrophobic compound, and the solubilization power is much superior compared to surfactants (Friberg and Blute 2006).

Many types of chemical compounds, including aromatic anionic/cationic/nonionic and aliphatic molecules, are today recognized to possess hydrotropic properties (Friberg and Blute 2006; Hodgdon and Kaler 2007). However, the most common ones used for the biomass processing were metal salts of aromatic acids (Procter 1971). Several hydrotropic agents are depicted in Figure 6 as an example.

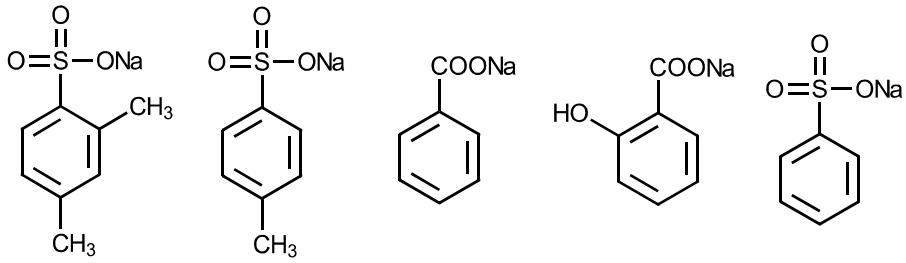

Figure 6. Examples of some hydrotropic agents used for biomass processing (Bland et al. 1978; Gromov and Odincov 1957a; Procter 1971). Sodium salts of: (A) xylenesulfonate, (B) toluensulfonate, (C) benzoate, (D) salicylate, (E) benzenesulfonate.

2.3.2 Hydrotropic treatment of biomass: a short overview

A hydrotropic process for biomass treatment was patented first time by McKee in 1943 (McKee 1943). In the patent, the author describes a process for the recovery of cellulose and lignin from various types of biomass using 30–40% aqueous solutions of the salts with the aromatic anions derived from a single benzene ring. The publication also includes an example of treatment of poplar wood chips for 12 hours at a temperature of 150 °C using 30% sodium xylenesulfonate (SXS) solution.

The author of the patent mentioned several benefits of the application of the hydrotropic method for biomass processing in comparison with the conventional cooking processes (McKee 1946, 1954, 1960). The most remarkable technological feature is a simple regeneration procedure of the cooking solution that comprises three steps: dilution of the spent solution with water, filtration of the precipitated lignin and reconcentration of the dilute hydrotropic solution to the operating concentration. Before the regeneration, the spent solution can be reused for several subsequent treatments until it becomes saturated with dissolved lignin. The saturation point is reached at about 350 g of lignin per liter of the solution. In practice that would mean, if using the conditions and the results of the conventional hydrotropic (R) or acidified (F) treatment from Paper I, one solution could be used for 5 treatments. The reuse of the spent solution for the subsequent treatments can help to save the energy that otherwise would be required to heat fresh cooking solution. After the regeneration, the hydrotropic solution does not lose its efficiency and can be re-applied for new treatments. McKee (1946) stated that the same solution could be used for 72 subsequent treatments, and it remained as effective as the fresh one. There is also a possibility to obtain several by-products, such as lignin, furfural, acetic acid, and so forth. Besides the advantages mentioned above, other benefits include the environmental friendliness of the process, a high alpha-cellulose content of the pulps, low consumption of the cooking agent and lower capital investment to erect a hydrotropic process-based mill compared to a kraft pulp mill. The latter requires more extensive evaporation, installation of a recovery boiler and a causticizing plant.

The positive aspects of the hydrotropic method motivated other scientists to become engaged in research on this process in the 1950s–1980s. The studies dealt with the investigation of hydrotropic treatment of different types of biomass, *i.e.* softwood, hardwood, non-wood, bark, and biomass species (Gromov and Odincov 1957a; Hinrichs et al. 1957; Lenz and Kurth 1963;

McKee 1946; Nelson 1978; Procter 1971); the efficiency of different hydrotropic agents, such as sodium salts of xylenesulfonic, toluenesulfonic, benzenesulfonic, benzoic, salicylic acids (Figure 6) (Bland et al. 1978; Gromov and Odincov 1957a; references in Procter 1971); the effect of the process conditions and various additives on the pulp properties (Gromov and Odincov 1957b, 1959; Gromov and Khrol 1964; Nelson 1978; references in Procter 1971; Treimanis et al. 1981). Since the original proposed treatment of wood biomass was fairly long, *i.e.* 12 h (McKee 1946), some attempts were made to shorten the treatment time. One of the suggested methods rested upon the usage of higher temperatures (Gromov et al. 1967; Kalninsh et al. 1967). At the same time, alkaline buffers were added to the cooking solution to neutralize the liberated acids and, thus, diminish their adverse effect on the pulp quality which was especially pronounced at the higher temperatures.

The research in that time was mainly focused around the manufacture of paper-grade chemical pulps and semi-chemical pulps, and the aim was to eventually develop a process that could compete with the existing kraft and sulfite pulping methods with respect to the pulp quality. However, despite the extensive research and numerous efforts, the hydrotropic process has not been realized in a full industrial scale. The reasons for this were associated with the drawbacks of the process. Hydrotropic treatment has a limited application with respect to raw materials. In particular, it cannot sufficiently delignify softwoods at the reasonable process conditions, as has been shown experimentally by several researchers (Gromov et al. 1963; Gromov and Odincov 1957a; Korpinen and Fardim 2009; Nelson 1978). In Gromov's experiments on hydrotropic pulping of spruce and pine wood chips (Gromov and Odincov 1957a), a delignification degree of only 62–64% could be reached after the treatment with 40% SXS solution for 10 h at 150 °C. For comparison, 93.7% of the original lignin could be removed from aspen chips at the same conditions (Gromov and Odincov 1957a). The explanation for such behavior is related to the structural differences in the lignins of two types of the raw materials. Lignin in softwoods is mainly composed of G units, which are less reactive and have greater proneness to condensation reactions (Nelson 1978; Pinto et al. 2005; Procter 1971). Secondly, the process has not eventually become comparable to conventional kraft or sulfite methods with regard to the pulp quality (Table 2). As to the former, hydrotropic pulps exhibited inferior mechanical properties (Gromov and Tupuraine 1960; Hinrichs et al. 1957; Nelson 1978; Procter 1971) and, with the latter, the main aspect was the lower yield and brightness of hydrotropic pulps at a given lignin content (Procter 1971). Modification of the

hydrotropic solution by addition of alkali/buffers could improve the mechanical properties of the hydrotropic pulps (Table 2), but such a method would raise the problems related to the recovery of a hydrotropic agent (Procter 1971). The added alkaline reagents will react with the acids that are liberated form the biomass during the treatment, *e.g.* acetic acid. The formed salts of these acids are well-soluble in an aqueous medium, and they will not be removed from the solution during the recovery step. Therefore, they will accumulate in the hydrotropic solution impairing its solvent efficiency (Procter 1971). In addition, it has been shown that the presence of certain salts can influence the delignification result during hydrotropic treatment (Gromov and Khrol 1964). In particular, when sodium acetate was added to the solution, the amount of lignin dissolved during the treatment was lower compared to the experiment with the pure hydrotropic solution (Gromov and Khrol 1964).

Table 2. Comparison of hydrotropic, kraft and acid sulfite pulping of eucalyptus (*E. regnans*) (data from Nelson 1978).

Parameters	Hydrotropic[a]		Kraft 1[b]	Hydro-tropic[a]	Kraft 2[c]	Acid sulfite
	No additive	+ 4% NaOH				
Temperature, °C	170	170	170	158	170	-
Duration, h	1.5	5.5	1.42	5.5	1.75	-
Yield, %	47.1	53.4	52.4	48.3	53.5	55.0
Kappa #	37.5	48.8	13.8	24.6	15.9	-
Freeness, csf	184	166	179	250	250	250
Tear index, $mN \times m^2/g$	5.8	7.6	7.3	7.7	10.8	8.1
Breaking length[d], km	8.1	10.2	13.3	7.1	11.5	8.0
Burst index, $kPa \times m^2/g$	5.3	6.4	8.6	-	-	-

[a]40% (w/v) solution of hydrotropic agent, liquor-to-solid ratio 6, 70 min to 170 °C.
[b]total alkali 15%, sulfidity 25%, liquor-to-solid ratio 3.5:1, 60 min to 170 °C.
[c]same as kraft 1 except that the heating time to 170 °C was 45 min.
[d]breaking length is the length at which a paper strip would break under its own weight if hung vertically (TAPPI T 494 om-01).

Today, upon the emergence of a biorefinery concept, the topic of a hydrotropic process has been revisited. The method has received increased attention as a potential candidate for biomass refinery applications, such as biomass fractionation or for pretreatment purposes, where the mechanical properties of the obtained products are of the least concern. Besides this thesis and related to it articles, the recent research activity on the hydrotropic method includes extraction of lignin from spruce and birch wood (Korpinen

and Fardim 2009) and bagasse (Ansari and Gaikar 2014); manufacture of pulps from miscanthus, oat hulls and intermediate flax (Denisova et al. 2015a; b) and application of a hydrotropic process as pretreatment before enzymatic hydrolysis (Mou et al. 2014a; b, Mou and Wu 2016, 2017).

2.3.3 Mechanism of lignin dissolution during hydrotropic treatment of lignocellulosic biomass

The reactions taking place during hydrotropic treatment of lignocellulosic biomass are complex, giving the fact that biomass consists of a mixture of polymers that have complex structures, especially lignin.

It is generally acknowledged that the removal of lignin from lignocellulosic material during hydrotropic treatment involves two steps (Gromov 1963; Ishikawa et al. 1970; Nelson 1978). In the first step, the native lignin is altered and fragmented due to the cleavage of the lignin-lignin and lignin-carbohydrate linkages. In the subsequent step, the lignin fragments are solubilized by the hydrotropic solution. However, the reactions do not stop at this point, and the liberated lignin is further modified in the solution (Gromov 1963; Procter 1971).

In general, hydrotropic treatment is performed under the acidic conditions. The conditions of the treatment can be alkaline as well, if alkali or alkaline salts are added. However, the alkaline pH should be avoided, because this can cause problems with the recovery of hydrotropic solution (Procter 1971). If acids are not added during the treatment, the process is autocatalyzed, and the pH drops due to the liberated organic acids, such as formic and acetic acids (Gromov 1963; Procter 1971). Therefore, one can suppose that the reactions responsible for the fragmentation of a lignin macromolecule during hydrotropic treatment are similar to those taking place during acidic organosolv pulping processes. The most important reactions are the cleavage of the α-*O*-4' and β-*O*-4' bonds (McDonough 1993; Sarkanen 1990).

The mechanism of the α-aryl ether bond cleavage is depicted in Figure 7. This cleavage mechanism occurs through the formation of the intermediate benzyl carbocation (Sarkanen 1990). The formed carbocation can further react with water to form benzylalcohol or with another nucleophilic molecule, if such is present in the system. In addition, a counterproductive coupling reaction with another lignin unit can take place resulting in lignin condensation.

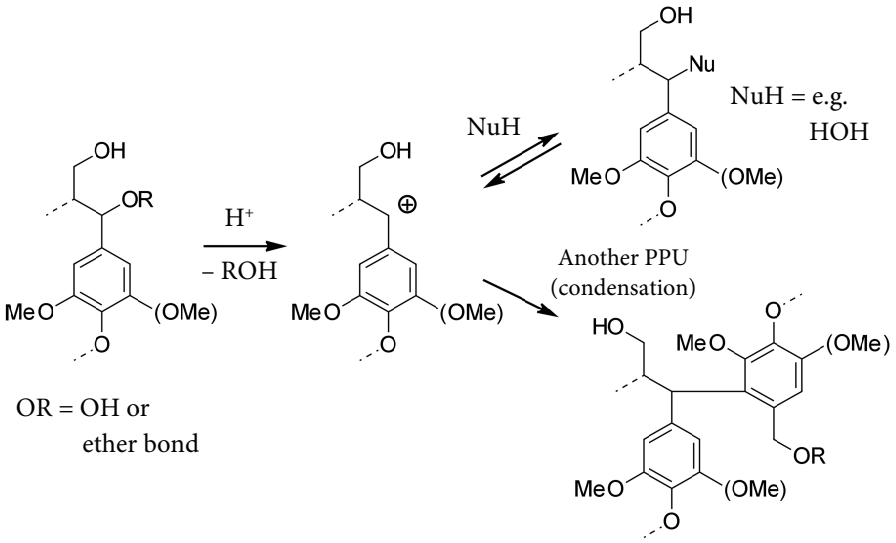

Figure 7. Cleavage of an *α*-aryl ether bond, addition of a nucleophilic molecule (Nu) and an example of a condensation reaction (Sarkanen 1990).

The cleavage of the *β*-*O*-4' bond is an important reaction, because this linkage is the most abundant in a lignin macromolecule (sections 2.1 and 2.2). Therefore, the cleavage of this bond plays an especially important role in the fragmentation of the lignin macromolecules. The pattern of how this bond is cleaved depends on the pH of the process (Li et al. 2000; Westermark et al. 1995). Li et al. (2000) studied the cleavage of a *β*-aryl ether bond under acidic and neutral conditions using guaiacylglycerol *β*-guaiacyl ether (G-*O*-G'), syringylglycerol *β*-syringyl ether (S-*O*-S') and syringylglycerol *β*-guaiacyl ether (S-*O*-G') model compounds. The author has shown that the cleavage of the *β*-*O*-4' bond at an elevated temperature takes place via a homolytic and/or acidolytic cleavage mechanism depending on the pH of the reaction. The former (Figure 8) occurs in neutral and slightly acidic media, and the latter prevails at higher acidity. In particular, when the model compound of G-*O*-G' type was heated in EtOH/water (1:3) at 175 °C and a pH of 3 (acetic acid/ammonia buffer solution) for 1 h, only the products of the homolytic cleavage were found after the reaction (Li et al. 2000). A similar result was obtained after heating of the model compounds in neutral conditions at 160 °C for 2 h (Li et al. 2000). For comparison, the cleavage of the *β*-aryl ether linkage via the acidolysis took place when syringylglycerol *β*-guaiacyl ether was heated at 160 °C for 2 h in the presence of 0.1 M sulfuric acid, thus at more acidic conditions (Li et al. 2000). However, both mechanisms can also occur simultaneously. In the cited study (Li et al. 2000), this was observed

when the reaction was carried out in 0.2 M acetic acid/dioxane (10:1) at 160 °C for 2 h. Based on the results of the study (Li et al. 2000) one can summarize that if the pH of hydrotropic treatment is not extremely acidic, as for example in the experiments in Papers I–III where the lowest pH at the end of the treatments was around 3.5, the predominant route for the *β-O*-4' bond cleavage would be the hemolytic cleavage. However, if the pH of the process is lowered more, both mechanisms can play a role in the cleavage of the *β-O*-4' bond. At last, if strong acids are used for the acidifications, the acidolytic cleavage will prevail.

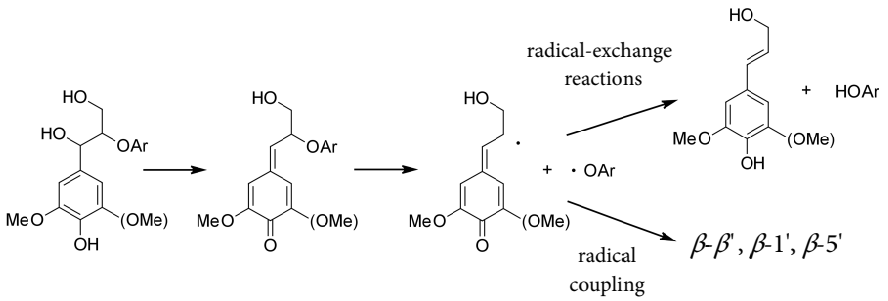

Figure 8. Homolytic cleavage of a *β-O*-4' bond. Ar denotes a phenylpropane unit without phenolic hydroxyl (Li et al. 2000).

After the cleavage of the lignin macromolecule, the lignin fragments are dissolved in the hydrotropic solution. The mechanism of lignin dissolution was reviewed and explained elsewhere (Gromov 1963). It has been proposed that the dissolution takes place through the intermolecular interaction of lignin with the hydrophobic part of a hydrotropic agent. It should be noted that the molecules of the hydrotropic salt do not chemically attach to the lignin. This conclusion was made based on the absence of any sulfur in the hydrotropic lignins isolated from lignocellulosic materials with sodium xylenesulfonate solution. Possible interaction could be Van der Waals forces between the aromatic rings of lignin and molecules of hydrotropes.

The author (Gromov 1963) has also speculated that a certain number of hydrotropic molecules must be associated with a lignin macromolecule to prevent its interaction with other lignin macromolecules and, thus, keep it dissolved. Upon dilution of the solution, the concentration of hydrotropic molecules becomes small, and lignin molecules interact with each other and precipitate from the solution. However, it has also been established that the concentration of a hydrotrope in the solution must reach a characteristic value, so-called minimal hydrotrope concentration (MHC), beyond which

the hydrotropic solution acquires the solubilization power (Srinivas et al. 1997 and references therein). At this concentration, the molecules of a hydrotropic salt form non-covalently self-associated layered structures with non-polar regions that accommodate hydrophobic molecules (Srinivas et al. 1997). The existence of MHC can well explain the solubilization of lignin and its precipitation upon the dilution of the hydrotropic solution.

2.3.4 Hydrotropic lignin

A great deal of the research related to the hydrotropic process has been focused on the pulp properties. However, several papers have been devoted to the investigation of lignin isolated with this process as well as to its applications.

It has been stated by McKee that hydrotropic lignin is unaltered or only slightly different from the original lignin of biomass (McKee 1943, 1946, 1960). Other researchers have pointed to the opposite, though, *i.e.* lignin isolated by this method is modified and is different from the corresponding protolignin (Ishikawa et al. 1970; Kreicberg and Grabovskij 1960; Zoldners and Surna 1969). Thus, hydrotropic lignin extracted from aspen wood at 160 °C and 120 min was shown to be more condensed compared to dioxane lignin, which was considered as a representative of the protolignin (Zoldners and Surna 1969). The conclusion was made based on the yield of aromatic aldehydes formed upon nitrobenzene oxidation (8.1 and 24.3%, respectively) and on the quantity of phenol reacted with the lignins (47 and 99%, respectively). The authors, however, also mentioned that the treatment conditions had a great effect on the extent of the modification and the reactivity of hydrotropic lignin.

Several researchers and research groups worked on the utilization of hydrotropic lignin. The inventor of the process, McKee, suggested that lignin upon addition of formaldehyde can produce thermoset plastic of a medium quality without usage of phenol (McKee 1954). Similar application of hydrotropic lignin was discussed by Kalninsh et al. (1962), who performed experiments on the substitution of phenol in phenol-formaldehyde resins with hydrotropic lignin. In the cited publication, the authors make an example of resin with 55% of phenol being replaced by the hydrotropic lignin. Other researchers carried out hydrogenation of lignin dissolved during hydrotropic treatment over a Ni catalyst to obtain phenolic compounds (Gromov and Pormale 1961). With the conditions employed in the study, the yield of phenols was 12% of the dissolved lignin. In another

study, hydrotropic aspen lignin was subjected to nitrobenzene oxidation in an alkaline medium, and the reaction yielded 8.8% of aromatic aldehydes based on Klason lignin of the hydrotropic lignin (Kreicberg and Grabovskij 1960). Telysheva et al. (1966) obtained 39.3% (based on Klason lignin of hydrotropic lignin) of low molar mass products from aspen hydrotropic lignin by oxidative degradation with oxygen in an alkaline media in the presence of copper oxide. The authors further fractionated the obtained products into aldehyde, acidic, phenolic and neutral fractions.

2.4 Lignin application: antimicrobial properties

Lignin is generally considered as a waste by-product of the pulp and paper and ethanol industry (Espinoza-Acosta et al. 2016). Despite that a small percentage of lignin extracted from biomass at a commercial scale is utilized for making low added value products, most of it is burned in the form of spent solution, so-called black liquor, to produce energy and to recover the pulping chemicals (Espinoza-Acosta et al. 2016). Given the fact that lignin is renewable, available in high quantities and has a unique structure and composition, many researchers have tried to find a way to utilize this polymer for high added value applications, for example as an antioxidant (Dizhbite et al. 2004; Dong et al. 2011; Pan et al. 2006). One of the potential fields of high added value applications of lignin is related to its antimicrobial properties. Several research articles are devoted to this area. Different types of lignins have been tested for their antimicrobial activity against Gram-positive and Gram-negative bacteria as well as against fungi.

Nada et al. (1989) studied the antimicrobial properties of lignins isolated from bagasse by soda and kraft pulping methods and lignin isolated from cotton stalks by a soda process. The obtained lignins did not show any antimicrobial activity against fungi, *Aspergillus niger*, and Gram-negative bacterium, *Escherichia coli*. Better performance was shown by the lignins against Gram-positive types of bacteria, *Bacillus subtilis* and *Bacillus mycoids.* However, the activity against these bacteria was also dependent on the raw material and the pulping conditions which were used for the extraction of the lignins.

Lignins in the form of spent solutions from Organocell pulping of spruce wood and neutral sulfite semichemical pulping (NSSC) of a mixture of hardwood as well as organocell lignin and prehydrolysis beech lignin were subjected to the antimicrobial activity test against several types of yeasts (Slavikova and Kosikova 1994). Some lignin and spent solution samples were

also applied after oxidation treatment. The results of the study showed that the tested lignin samples could greatly inhibit the growth of *C. albicans, T. cutaneum and S. roseus*. Based on the inhibition efficiency, the authors arranged the lignin samples in the following order: organocell spent solution<organocell lignin<NSSC spent liquor. Beech prehydrolysate lignin showed good efficiency against *C. albicans*, but its performance was inferior to other samples with respect to other yeasts. Interestingly, the oxidation of the organocell and prehydrolysis lignins mitigated their antimicrobial efficiency. It was also mentioned in the article that the antimicrobial efficiency could correlate with the surface tension, which was explained in terms of better ability of compounds with a lower surface tension to penetrate through the cell wall. The NSSC spent solution, which showed the highest inhibition in the study, had the lowest surface tension.

The antimicrobial activity tests of several types of lignins against microorganisms were conducted by Telysheva et al. (2005). The authors concluded that the antimicrobial properties strongly depended on the lignin prehistory, the type of the microorganism and the concentration of lignin in a cultural medium. For example, it was shown that the inhibition efficiency of *E. coli* growth differed among the soda lignins isolated from different non-wood raw materials, and it was strongest for sisal, abaca and flax soda lignins.

Based on these research cases (Nada et al. 1989; Slavikova and Kosikova 1994; Telysheva et al. 2005), it is clear that lignins can behave as antimicrobial agents, and it is also evident that the antimicrobial performance depends considerably on a type of microorganisms and lignins tested. From this point of view, the botanical origin of lignin and the method used for its production are the crucial factors that determine its antimicrobial behavior. The antimicrobial properties of lignins are apparently connected to its nature via the structure, meaning that the lignin prehistory affects the lignin structure and this, in turn, reflects its antimicrobial properties. It has been shown by Zemek et al. (1979) using various lignin-derived low molar mass compounds and different types of microorganisms that the chemical structure of such compounds greatly influences the antimicrobial efficiency. In particular, a structure with a double bond at the C_α, C_β position and a methyl group at C_γ showed the highest efficiency. Contrarily, the compounds with a carbonyl group in C_α, C_β positions or with a carboxyl and hydroxyl group in the side chain were less efficient.

Despite the fact that many types of lignins have been tested, there is no information about the antimicrobial properties of hydrotropic lignins in the literature. Therefore, investigation of the antimicrobial behavior of this

special type of lignin isolated from birch wood presented a great scientific interest.

2.5 Objectives of the study

The aim of the research presented in this thesis was to explore hydrotropic treatment as a process for biomass refinery and to generate knowledge that would allow further development of biorefinery technology based on the hydrotropic treatment. Despite the fact that the hydrotropic process has been extensively researched, and many scientific papers about this process can be found, a major part of the performed earlier research had a papermaking context. However, application of the hydrotropic treatment as a biorefinery tool instead of a pulping process for papermaking requires additional knowledge, and other process streams must also be considered. Therefore, the study on this process is still worthwhile.

In the scope of the study, the objectives were set to investigate the feasibility of hydrotropic treatment for fractionation of different raw materials. This was accomplished by application of the hydrotropic method for processing of birch wood from Finland and sugarcane bagasse from Brazil and characterization of the obtained fractions, mainly cellulose and lignin. In addition, the lignin obtained from the fractionation of the birch wood chips was utilized for the preparation of a shaped product, lignin-cellulose beads, and these beads together with the lignin itself were tested for their antibacterial properties.

3 Experimental part

3.1 Materials

3.1.1 Biomass and hydrotropic agent

Birch wood chips used for the experiments in Papers I and II were collected from a Finnish pulp and paper mill. The chips were air-dried and screened to isolate 4–6 mm thick fractions.

Sugarcane bagasse (Paper III) was obtained from Centro de Tecnologia Canavieira (Piracicaba, Brazil). Upon arrival in Finland, it was placed into a freezer at -20 °C. Before the experiments, the bagasse was defrosted, air-dried and screened using a vibratory sieve shaker AS 200 (Retsch, Germany) employing 4, 2, 1, 0.71, 0.5 and 0.25 mm trays (Figure 9). The fractions collected from the trays >0.5 mm were combined, mixed and used for the experiments.

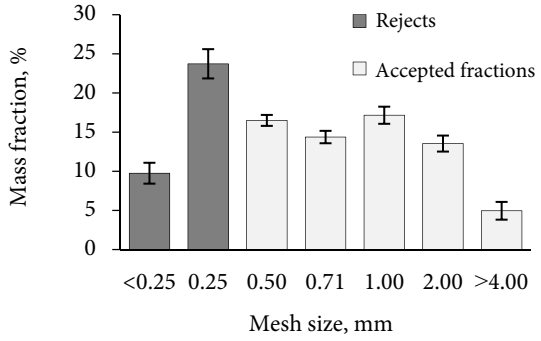

Figure 9. Weight distribution of sugarcane bagasse fractions.

A technical grade of sodium xylenesulfonate (SXS) with purity of >90% was used as a hydrotropic agent.

3.1.2 Other materials and chemicals

In Paper IV, cellulose in the form of sulfite dissolving-grade pulp (cellulose plus) was obtained from Domsjö Fabriker. For the experiments, the pulp was treated in a mixture of ethanol and hydrochloric acid for 2 h at 75 °C (Trygg and Fardim 2011). Intrinsic viscosity of the treated pulp was 138.6 mL/g.

In the antibacterial activity assays in Paper IV, Gram-positive *Staphylococcus aureus* control strain ATCC 25923 and Gram-negative *Escherichia coli* laboratory strain XL-1 Blue (Stratagene) were used. The

strains were cultured in conical, sterile, loosely capped 50-mL polypropylene tubes in tryptic soy broth (TSB) at 37 °C, 250 rpm. TSB was purchased from Sigma-Aldrich.

Other chemicals were obtained from the chemical companies and were used without further purification.

3.2 Methods

3.2.1 Hydrotropic treatment

The treatments of birch wood (Paper I) were performed batch-wise in stainless steel reactors. One treatment was carried out using 36% SXS aqueous solution (R_{bir}). For the other three, the hydrotropic solution was modified either by addition of hydrogen peroxide at the dosage of 2.5% based on wood (H_{bir}) or by acidification with formic acid to the pH of 3.5 (F_{bir}), or by addition of both hydrogen peroxide, 2.5% based on wood, and formic acid to lower the pH to 3.5 (HF_{bir}). Before placing the chips into the digester, they were impregnated with the hydrotropic solutions under reduced pressure at room temperature. The time-temperature profile of the treatments was as follows: heating rate of 1.5 °C/min, dwell time of 120 min and dwell temperature of 170 °C. The liquor-to-solid ratio was 4 (w/w).

For Paper II, hydrotropic extraction of lignin from birch wood was performed using the solutions and the conditions of R_{bir} and HF_{bir} treatments.

Sugarcane bagasse (Paper III) was treated using 1 L reactors placed into a rocking digester. R_{bag} and F_{bag} treatments were carried out using unmodified and acidified 30% SXS solutions, respectively. The liquor-to-solid ratio was 10 (w/w) and the ramp was 1.5 °C/min. Other parameters are specified in Table 3. When the treatments ended, the reactors were transferred into buckets with cold tap water and were kept there for 10 minutes.

Table 3. Conditions of hydrotropic treatments of sugarcane bagasse.

Treatment	Dwell temperature, °C	Dwell time, min	Initial pH
$R1_{bag}$	150	120	9.5
$F1_{bag}$	150	120	3.5
$R2_{bag}$	170	60	9.6
$F2_{bag}$	170	60	3.5
$R3_{bag}$	170	120	9.6
$F3_{bag}$	170	120	3.5

The obtained treated material (Papers I, II and III) was disintegrated to produce fibers (pulps), pre-washed with tap water to remove remained spent solution, centrifuged and soaked in 0.5% NaOH solution at a consistency of about 7.5% for 10 minutes. Subsequently, the NaOH-treated pulps were thoroughly washed with tap water until the filtrates became colorless. Screening of the pulps was carried out using a Valmet TAP03 rotary screen with a 0.06 mm slit basket. The screened yield was estimated by subtracting the rejects from the total yield.

3.2.2 Bleaching of birch hydrotropic pulps

Selected birch hydrotropic pulps (Paper I) were oxygen delignified (O) and bleached with a chelation–pressurized peroxide bleaching–chelation–peroxide bleaching sequence (Q-PO-Q-P) (Table 4). The parameters and the chemical dosages of the Q and PO stages were chosen based on the literature (Anderson and Amini 1996; Wackerberg et al. 1997), respectively, and for the P stage based on trial experiments. The unpressurised stages were carried out in plastic bags heated in a thermostatic water bath. The oxygen delignification and the PO stage were performed in a Quantum Mark IV mixer. Washing of the pulps after the bleaching was done using distilled water until the neutral pH.

Table 4. Conditions of oxygen delignification and bleaching of birch hydrotropic pulps.

Conditions/ chemicals	Stages[a]				
	O	Q	PO	Q	P
Temperature, °C	95	70	90	70	90
Time, min	60	60	30/120[b]	60	270
Consistency, %	10	10	10	10	10
pH	-	6-7	-	6-7	-
Pressure, bar	6-7	-	5	-	-
DTPA[c], %[d]	-	0.2	-	0.2	-
H_2O_2, %[d]	-	-	3.5	-	3
NaOH, %[d]	2	-	3	-	3
$MgSO_4$, %[d]	0.3	-	0.3	-	0.3

[a]O, oxygen delignification; Q, chelation; PO, pressurized hydrogen peroxide bleaching stage; P, peroxide bleaching stage.
[b]30 min for HF_{bir} pulp and 120 min for R_{bir} pulp.
[c]DTPA, diethylenetriaminepentaacetic acid.
[d]based on oven-dry pulp.

3.2.3 Isolation of hydrotropic lignin from the spent solutions

In Paper III, the spent solutions were filtered through glass fiber filters GF/C before lignin precipitation.

Extracted lignins were isolated from the spent solutions by 10-fold (Paper II) or 3-fold (Paper III) dilution with hot water and subsequent filtration through a 125 mL sintered glass funnel no. 2 (Figure 10). The temperature of the spent solutions after the dilution was 75 °C. The first portion of the filtrates was recirculated back onto the funnels to recover lignin particles passed through the filter at the beginning of the filtration. After that, the filtrates were clear. The filtered lignins were further washed with hot water.

In Paper II, the funnels were heated to maintain a temperature of 75–80 °C, and the lignin suspensions were stirred during the whole washing. The amount of washing water was 8-fold (v/v) of the spent solution taken for the precipitation.

In the experiments for Paper III, the amount of wash water was 10-fold (v/v) of the spent solution. The stirring of the lignin suspensions inside the funnels and heating of the funnels to maintain a temperature of 80 °C was done only during the last washing step.

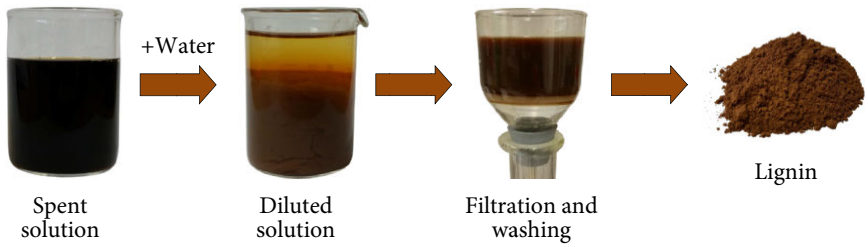

Figure 10. Isolation of lignin from the spent solutions.

The amounts of dissolved, precipitated and non-precipitated lignins were estimated using Eq. 1–Eq. 4:

Lignin removed from the raw material (delignification degree), % based on lignin in raw material

$$L_{dis} = \frac{L_{RM} - L_{pulp} \times \frac{m_{pulp}}{m_{RM}}}{L_{RM}} \times 100 \quad (1)$$

The yield of lignin obtained by precipitation, % based on raw material

$$L_{precip/RM} = L_{precip\ SS} \times \frac{m_{SS}}{m_{RM}} \times 100 \quad (2)$$

The same as above, % based on lignin in raw material. For the estimation of the lignin yield based on the lignin in raw material, the purity of the isolated lignins was taken into account, and it was estimated using the content of non-lignin compounds in the lignins.

$$L_{precip/lignin} = L_{precip\ SS} \times \frac{m_{SS}}{m_{lignin\ RM}} \times 100 \quad (3)$$

Lignin that did not precipitate upon dilution of spent solution, % based on lignin in raw material

$$L_{non\text{-}precip} = L_{dis} - L_{precip/lignin}, \quad (4)$$

where L_{RM} and L_{pulp} are the content of lignin in the raw material and pulp, %; m_{RM} and m_{pulp} are the amount of the raw material used for the treatment and the amount of obtained pulp, g; $L_{precip\ SS}$ is the amount of lignin obtained from spent solution by precipitation, g/g of spent solution; m_{SS} is the amount of spent solution estimated as a sum of the raw material dissolved during the treatment, water present in the raw material and hydrotropic solution used for the treatment, g; $m_{lignin\ RM}$ is the amount of lignin in the raw material, g.

3.2.4 Preparation of lignin-cellulose beads

Lignin-cellulose beads were prepared by co-dissolution of cellulose and lignin and shaping of the resultant solution. The beads were designed to have different mass ratios of cellulose and lignin: 100/0 (100C), 90/10 (90C10L), 75/25 (75C25L) and 60/40 (60C40L). Lignin R isolated from birch (Paper II) was used as a raw material.

Cellulose-lignin solutions were prepared using 7% NaOH/12% urea/water solvent, and the concentration of cellulose was always 5% (based on cellulose and solvent). The cellulose was first dispersed in the solvent at room temperature. Then, the desired amount of lignin was added, and stirring continued until all lignin clumps disappeared. Subsequently, the beaker was transferred into a cooler jar and kept there at -12 °C for about 45 min. The dissolution of cellulose was examined by light microscopy. Before shaping, the solutions were centrifuged at 3500 rpm for 5 min to remove air bubbles.

The obtained solution was extruded through a 5 mL Eppendorf combitip into 10-fold (v/v) 2M HCl. The obtained beads were left overnight in the acid.

On the following day, they were washed with running tap water for 30 min and distilled water for 15 min (Trygg et al. 2013).

3.3 Analyses

3.3.1 Characterization of biomass and hydrotropic pulps

Before the analyses, birch wood chips (Papers I and II) and the bagasse (Paper III) were ground with a cutting mill employing a sieve cassette with 1 mm openings.

Lignin content

Lignin content was analyzed according to TAPPI T222 om-02 with some modifications (Schwanninger and Hinterstoisser 2002) and TAPPI UM 250. In Paper III, the lignin content was corrected for the silica content with the assumption that Si-containing compounds remained in Klason lignin as SiO_2 during the lignin determination. The silicon content was measured from the pressed ash of the bagasse or the pulps by energy-dispersive X-ray spectroscopy (EDS) using a JEOL JSM-6335F (Japan) SEM-EDS. The accelerating voltage of 20 kV was applied, and several spots (3–5) were analyzed. INCA Suit (v. 4.04) software was used to process the spectra.

Carbohydrates

Carbohydrate composition was determined by both acid methanolysis using 2M HCl in MeOH or hydrolysis with 72% sulfuric acid followed by gas chromatography (GC) as described elsewhere (Sundberg et al. 1996).

For the analysis of residual sucrose in the bagasse (Paper III), 10 mg of the material was extracted batch-wise three times for 90–120 min with 2 mL of distilled water. The extractions were performed at room temperature under stirring, and the solutions were withdrawn after centrifuging at 3500 rpm for 5 min. The supernatants from each extraction were combined and freeze-dried. Subsequently, the dry residues were dissolved in 150 µL of pyridine and silylated using 150 µL of hexamethyldisilazane (HMDS) and 70 µL trimethylchlorosilane (TMCS). The silylated samples were analyzed with GC using the method for the analysis of sugars after the hydrolysis (see above).

Other analytical methods

Other methods used include kappa number (SCAN-C 1:00), brightness (ISO 3688), intrinsic viscosity (ISO/FDIS 5351) and ash content (TAPPI T 211 om-02). Extractives were determined following SCAN CM 49:03 using a 95:5 (v/v) acetone-water mixture.

3.3.2 Chemical and elemental composition of lignins

The methods were used for the characterization of birch and bagasse hydrotropic lignins isolated in the studies for Papers II and III.

Elemental composition

CHNS elemental composition was carried out using a Thermo Scientific Flash 2000 series elemental analyzer (Vega et al. 2013). Oxygen (250 L/min, 6 min) was used as an oxidizer. Helium at a flowrate of 140 L/min was used as a mobile phase. The temperature of the reactor was 950 °C.

Residual hemicelluloses

Carbohydrates in the lignins were determined using the acid methanolysis and GC (section 3.3.1) with several exceptions. The methanolysis time was reduced to 3 h and, in addition to the column HP-1 (25 m×0.200 mm i.d., 0.11 μm, Agilent Technologies), the results were also obtained using an HP-5 column with the dimensions of 25 m×0.199 mm i.d., 0.11 μm (Agilent Technologies, USA). Two columns were necessary to use, because some sugar peaks overlapped with the peaks of unknown compounds on the HP-1 column chromatograms causing difficulties with the integration, and these peaks were better resolved with the HP-5 column.

Low molar mass aliphatic and aromatic compounds

The analysis was carried out using GC after silylation with N,O-bis(trimethylsilyl)-trifluoroacetamide and TMCS. The chromatography was performed with Clarius 500 (PerkinElmer) and AutosystemXL (PerkinElmer) gas chromatographs equipped with short (6–7 m) and long (25 m) columns, respectively (Strand et al. 2011). The former was used for the analysis of the groups of the compounds, and the latter allowed analysis of the individual compounds except steryl esters and triglycerides. The peaks in the chromatograms were identified using gas chromatography-mass spectrometry (GC-MS) as described elsewhere (Smeds et al. 2012).

3.3.3 Analysis of lignin structure

FTIR (Paper II)

FTIR spectra were collected from a KBr lignin pellet with a Thermo Scientific Nicolet iS50 spectrometer using 32 scans and the resolution of 4 cm^{-1}. Lignin concentration in the pellet was around 1% (w/w). OMNIC spectra (Thermo Scientific) software was used to process the spectra.

NMR methods (Paper II)

^{31}P NMR was performed as described elsewhere (Gosselink et al. 2010) after derivatization with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane.

A semiquantitative ^{1}H-^{13}C HSQC NMR spectroscopy was performed on a Bruker AVANCE III 400 MHz instrument equipped with a 5 mm BBI probe (5 G/cmA). For the analysis, about 50 mg of each lignin was dissolved in 1 mL of DMSO-d_6. The details of the method are described in Paper II.

Analytical pyrolysis (Paper II and III)

Hydrotropic lignins were analyzed with conventional Py-GC-MS using a Pyrola 2000 pyrolyzer (Pyrol AB, Lund, Sweden) and a GC-MS instrument. Before the pyrolysis, wood was milled as described in section 3.3.1 and sieved to isolate fractions <250 μm and freeze-dried. Ground bagasse was also additionally milled to improve sampling.

The procedures were essentially based on those described elsewhere (Smeds et al. 2012). The pyrolysis temperature was 600 °C (Paper II) or 580 °C (Paper III). In the case of the bagasse study, a different column and conditions were used in the GC as specified in Paper III.

TMAH/Py-GC-MS (Paper III) was performed according to the literature (Smeds et al. 2016) using a pyrolysis temperature of 360 °C. The GC column and the conditions were the same as in the conventional pyrolysis in Paper III.

The identification of the released compounds was done mainly with the help of the MS library created in the Laboratory of Wood and Paper Chemistry at Åbo Akademy University and partly using the Wiley 10/NIST 2012 mass spectral libraries.

Size-exclusion chromatography (Paper II and III)

For Paper II, SEC was performed as described in the literature (Gosselink et al. 2010).

For Paper III, SEC was performed using X-stream H_2O 1000 Å columns connected in a series: 50×10 mm i.d. guard column and 250×10 mm i.d. main column (Jordi Labs, USA). Before the analysis, lignins were dissolved in DMSO. DMSO/0.05M LiBr was used as an eluent. The signal from the UV (DAD) detector was collected at 300 nm. Phenol and a series of pullulan standards were used for calibration.

3.3.4 Characterization of the spent solutions

The following methods were used for characterization of the spent solutions in Paper III.

Furfural and HMF

The content of furfural and 5-hydroxymethylfurfural (HMF) was analyzed by high-performance liquid chromatography (HPLC) (Agilent 1260 series, Waldbronn, Germany) using a UV detector. Before the analysis, 0.2 mL of the spent solutions was diluted with distilled water in 50 mL volumetric flasks. The parameters of the chromatography including the column and the eluent are specified elsewhere (Korpinen et al. 2014). Diluted solution of SXS (62 mg/L) was run to verify that the hydrotropic agent did not interfere with the analyzed compounds. Quantification of furfural and HMF was done using the signals at 276 and 284 nm, respectively, with the help of calibration curves.

Acetic and formic acids

The content of the acids was determined with the same instrument as above using a 250 mm×4.6 mm i.d. Synergi 4 μm Hydro-RP 80A HPLC column (Phenomenex®, CA, USA). For the analysis, the spent solutions were first diluted with distilled water (2–3:10 v/v) and then with 40 mM KH_2PO_4 in a ratio of 1:1. 20 mM KH_2PO_4 was used as an eluent. Dilute solutions of acetic and formic acids were used to determine the retention times. The acids were quantified using calibration curves and the signals at 210 nm in the case of acetic acid and 220 nm for formic acid.

Total dissolved solids

Total dissolved solids were measured by freeze-drying 10 mL of the spent solutions. The values were used for calculations. The freeze-dried samples were converted to fine powder by crushing and were used for the determination of the content of carbohydrate monomers and polymers.

Carbohydrates

The total content of carbohydrates was determined by the acid methanolysis and GC (section 3.3.2). The calibration solutions were modified by addition of SXS, the amount of which corresponded roughly to that in the samples.

The content of sugar monomers was analyzed by silylation and GC in the same way as it was done for the determination of total carbohydrate content but without methanolysis. 0.1 mg/mL xylitol in MeOH was added as an internal standard. The GC conditions were the same as those used in the

determination of carbohydrates by the hydrolysis (section 3.3.1). The amount of carbohydrate polymers was estimated by subtraction of sugar monomers from the total amount of carbohydrates.

SEC

The same set up was used as described in section 3.3.2. Before the analysis, the spent solutions and approximately 30% SXS solution were diluted 4-fold with distilled water, mixed well and centrifuged at 3500 rpm for 5 min. Subsequently, about 2.7 mL of the supernatants was freeze-dried, and the dried residue was dissolved in DMSO.

3.3.5 Characterization of lignin-cellulose beads

Lignin content

Lignin content in the beads and in the birch hydrotropic lignin was analyzed as described in section 3.3.1. The acid-soluble lignin was measured from the combined filtrates and washing water.

ATR-FTIR

The measurements were performed with an ATR module of the FTIR instrument (section 3.3.3). The spectra were collected using 64 scans with the resolution of 4 cm^{-1}.

Weight, size, shape and porosity

The weigh was determined with an ordinary procedure. The excess of water from never-dried beads was removed with a paper towel. The dimensions of the beads and the circularity were determined as described in the literature (Trygg et al. 2013). The porosity was calculated according to the equation:

$$P = \frac{V_{bead} - V_{solid}}{V_{bead}} \times 100\%, \quad (5)$$

where V_{bead} is a volume of a bead calculated as described elsewhere (Trygg et al. 2013); V_{solid} is a volume of the solid matter in a bead estimated from the weight of oven-dry (OD) beads and the density of the solid matter, assuming the densities of lignin and cellulose to be 1.3 (Feldman 2002; Ni and Hu 1995) and 1.5 g/cm^3 (Ettenauer et al. 2011), respectively.

Imaging

The microstructure of the beads and the distribution of lignin in the beads were studied by Field Emission Scanning Electron Microscopy (FE-SEM) and laser scanning confocal fluorescence microscopy, respectively. For both

methods, the beads were frozen in liquid nitrogen and freeze-dried. The SEM was done using a LEO Gemini 1530 (Zeiss (LEO), Germany) microscope and an In-Lens detector. The accelerating voltage was 2.7 kV. The beads were sputter coated in Temcarb TB500 (Emscope Laboratories, Ashford, UK).

A Leica TCS SP5 STED (Leica Microsystems GmbH, Germany) microscope with a GaAs-hybrid detector (Leica) and a 10.0x N.A. 0.30 dry objective was used for the confocal fluorescent microscopy. Fluorescence images were obtained with the excitation wavelength of 488 nm and the emission bandwidth of 600–650 nm. In the case of the reflection channel, the corresponding settings were 476 nm and 450–484 nm. About 20 images were collected with a step of approximately 5 μm. ImageJ was used to process the images.

Lignin leaching

Lignin leaching from the air- and never-dried beads as well as from the lignin itself was studied by keeping the beads and the lignin in distilled water for several 20–24 h periods and analyzing the solutions by UV spectroscopy. At the end of each 20–24 h period, the solutions were withdrawn, and a fresh portion of distilled water was added. In the case of the lignin, the beakers were centrifuged at 3900 rpm for 30 min to sediment the lignin particles, and before the UV spectroscopy, the supernatants were additionally filtered through 0.2 μm PTFE Acrodisc membrane filters. The amount of leached lignin in the solution was determined by UV spectroscopy as described in TAPPI UM 250.

3.3.6 Antibacterial activity of lignin-cellulose beads

Prepared lignin-cellulose beads in Paper IV were tested for the antibacterial activity against two common pathogens, namely Gram-negative *E. coli* and Gram-positive *S. aureus*.

The assay was performed for all never-dried beads, air-dried 60C40L beads and hydrotropic lignin. The load of the beads corresponded to 50 mg of the dry weight, and the dosage of lignin was 20, 50 and 100 mg (dry weight). The beads and the lignin were kept in 5 mL of inoculated TSB for 24 h at 37 °C under shaking at 250 rpm. The number of bacteria in TSB before (1–3×10^{-6} CFU/mL) and after the incubation period was determined by viable counting.

The second part of the study dealt with the determination of half inhibitory (IC50) and 90% inhibitory (IC90) concentrations for 60C40L never-dried beads using *S. aureus* only. A similar experimental set up was

used, and the number of the beads per tube was increased from 2 to 64 in 2-fold increments.

3.3.7 Other analyses

Residual hydrogen peroxide in the spent bleaching solutions after the peroxide bleaching stages in Paper I was analyzed by a titration with 0.2 M sodium thiosulfate solution. Before the titration, 5–10 mL of the spent solutions was mixed with 20 mL of 0.2 N sulfuric acid, 10 mL of 10% potassium iodide solution, 100 mL of distilled water and 5 drops of 3% ammonium molybdate solution.

4 Results and discussion

4.1 Fractionation of birch wood using conventional and modified hydrotropic processes

Birch wood chips were treated with 36% aqueous SXS solution at the temperature of 170 °C and the dwell time of 120 min. The hydrotropic solution was modified by addition of hydrogen peroxide or formic acid, or both of them simultaneously. The objective of the study was to examine the feasibility of hydrotropic treatment for birch wood fractionation and also the effect of the additives on the process performance.

4.1.1 Hydrotropic treatment of birch wood and its modification

Ordinary hydrotropic treatment (R_{bir}) removed slightly over 90% of the lignin from birch wood (Table 5), and the treated chips could be easily converted to fibers by mechanical disintegration. All the additives used for the modification of 36% SXS solution greatly improved the delignification, and the resultant pulps had lower kappa numbers than the reference one (Table 5). Addition of hydrogen peroxide at the applied dosage showed a somewhat more pronounced effect on the kappa number reduction compared to the acidification of the solution with formic acid, which could be related to the oxidizing effect of hydrogen peroxide and the corresponding reactions that it triggered in the acidic media (Gierer 1982; Lachenal 1996). When both chemicals were added, the result in terms of a delignification degree and a kappa number was even better (Table 5).

Table 5. Initial and end pHs of hydrotropic solutions and properties of birch hydrotropic pulps.

Pulp[a]	pH		Yield, %		Kappa number (K)	Intrinsic viscosity (V), mL/g	V/K	Degree of delignification, %
	Initial	End	Total	Screened				
R_{bir}	9.3	3.5	47.6	47.3	26.6±0.2	1136±7	42.7	91
H_{bir}	8.9	3.6	47.5	46.8	13.6±0.2	958±3	70.5	95
F_{bir}	3.4	3.4	45.1	45.0	17.7±0.0	849±1	48.0	95
HF_{bir}	3.5	3.5	43.5	43.2	8.2±0.1	660±1	80.1	97

[a] R_{bir}, 36% SXS; H_{bir}, 36% SXS+H_2O_2; F_{bir}, 36% SXS+formic acid; HF_{bir}, 36% SXS+H_2O_2+formic acid.

The addition of the reagents resulted in lower intrinsic viscosity values of the pulps (Table 5). Generally, intrinsic viscosity correlates with the molar mass of pulp cellulose (da Silva Perez and Van Heiningen 2002) and, consequently, cellulose of the hydrotropic pulps that had lower viscosity values was more degraded. The more extensive degradation of cellulose upon addition of formic acid was caused by the intensification of the hydrolytic reactions. In the case of hydrogen peroxide, besides the autocatalyzed hydrolysis, the reduction of cellulose DP was additionally caused by harmful radicals formed upon the high temperature and/or due to the presence of transition metals in the wood (Lachenal 1996). In contrast to the kappa number, formic acid exhibited a more drastic effect on the viscosity than did hydrogen peroxide.

The modification of hydrotropic solution had both a positive and a negative effect on the treatment performance. However, if calculating the ratio of viscosity to a kappa number, which is a way of expressing the process selectivity (Patt et al. 2002), one could observe that the values were higher for the treatments that used the modified hydrotropic solutions. In particular, hydrogen peroxide-assisted processes exhibited higher viscosity/kappa number ratios.

4.1.2 Chemical composition of birch hydrotropic pulps

Hydrotropic treatments also removed, together with lignin, a great deal of hemicelluloses, and the pulps were enriched in cellulose. The residual hemicelluloses consisted mainly of xylan, which was also abundant in the original birch wood. The pulps obtained with the hydrotropic solutions containing formic acid had a higher cellulose content (Figure 11). Such a result could be attributed essentially to the enhancement of the hydrolytic reactions by formic acid, which led to the greater removal of the hemicelluloses. Hydrogen peroxide was not as effective as formic acid with respect to the pulp purity, as can be observed from the cellulose content for, for example, R_{bir} and H_{bir} vs R_{bir} and F_{bir} pulps (Figure 11). The pulp produced with HF_{bir} treatment had the highest cellulose content.

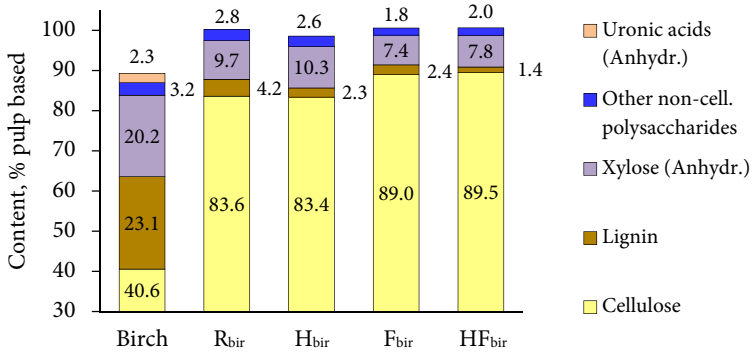

Figure 11. Content of cellulose, lignin, xylose (anhydrous), uronic acids (anhydrous) and non-cellulosic polysaccharides in birch wood based on wood and in birch hydrotropic pulps based on pulp.

It should be mentioned here that in the original paper (Paper II), the analysis of hemicelluloses in the pulps and the raw material was performed using the acid methanolysis procedure only. However, this method does not give true results, at least for certain hemicellulose units, in the case of modified materials, such as chemical pulps. Such samples contain a more resistant part of hemicelluloses that cannot be fully depolymerized by the methanolysis mixture (Sundberg et al. 1996; Willför et al. 2009). Therefore, the carbohydrates in the samples were additionally analyzed using the acid hydrolysis method. In the case of the birch wood, both methods gave comparable results with respect to the neutral hemicellulose units. However, greater discrepancy was observed for the pulps. In particular, the yields of xylose and mannose in the acid methanolysis were only 60–70% and 40–45%, respectively, of the yield of these sugars from the hydrolysis. Therefore, the results for these units from the hydrolysis procedure are reported in this thesis. The difference between the acid methanolysis and hydrolysis has also been discussed in relation to other types of treated residues/pulps, namely birch kraft pulp (Willför et al. 2009) and SO_2-ethanol-water residues/pulps obtained from spruce wood (Iakovlev and van Heiningen 2012).

Analyses of the chemical composition revealed that the low yields (Table 5) of F_{bir} and HF_{bir} pulps compared to the reference one (R_{bir}) and H_{bir} was due to the content of the non-cellulosic compounds, as the content of cellulose in the pulps based on wood was about the same, and it was slightly lower for HF_{bir} pulp (Figure 12).

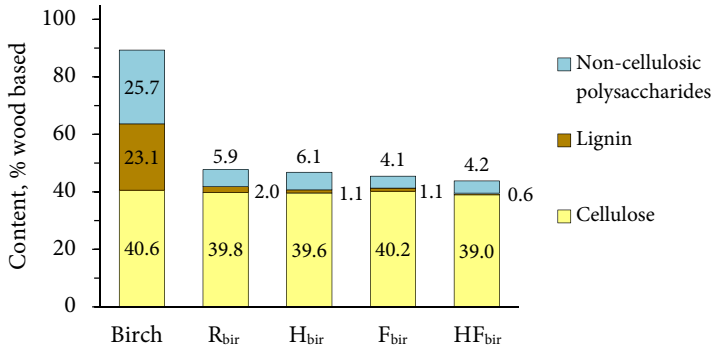

Figure 12. Content of cellulose, lignin and non-cellulosic polysaccharides in birch wood and birch hydrotropic pulps based on original wood.

The cellulose content in the pulps (wood based) was also close to that of the original wood, which implied that there was no loss of cellulose in the course of the hydrotropic treatments or it was minor. This is an important advantage of a hydrotropic process over the alkaline cooking methods. In the alkaline processes, a significant amount of cellulose is lost due to the peeling reactions. These reactions remove one by one reducing glucose units of the cellulose chains converting them into carboxylic acids (Alen 2000b). For example, in the studies related to prehydrolysis and soda anthraquinone pulping (Soda-AQ) of birch wood (Borrega et al. 2013; Testova et al. 2014), the loss of the cellulose comprised about 27% based on cellulose after the Soda-AQ pulping, and it was 17% and higher after the prehydrolysis and pulping depending on the conditions. In a kraft process, the loss of cellulose upon pulping of birch wood (*Betula verrucosa*) is 15% based on cellulose (Sjöström 1993a). In contrast to the alkaline pulping methods, the peeling reactions do not take place during hydrotropic treatment and, therefore, the recovery of cellulose from birch wood was high, if not quantitative.

Summarizing this part, the addition of hydrogen peroxide and formic acid or both improved performance of hydrotropic treatment in terms of delignification and, in the case of formic acid, pulps with a higher cellulose content could be obtained. This suggested that F_{bir} and HF_{bir} pulps were potentially suitable for the application as dissolving-grade pulps, where high content of cellulose is important, because it is a principal compound of dissolving pulps. In addition, relatively low viscosity values would not be very critical, as dissolving-grade pulps have intrinsic viscosity values starting from

450 mL/g and higher depending on a target application (Schild and Sixta 2011; Sixta 2006; Sixta et al. 2004).

4.1.3 Bleaching of birch hydrotropic pulps

Birch hydrotropic pulps having the highest and lowest kappa numbers, yields and viscosity values, R_{bir} and HF_{bir} pulps, respectively, were oxygen-delignified and then bleached with a TCF sequence.

HF_{bir} pulp consumed less hydrogen peroxide compared to the reference pulp to achieve a similar brightness level (Table 6). More severe conditions, in particular longer retention time (Table 4), had to be applied for the reference pulp during the PO stage due to the considerably higher starting kappa number (Table 5) and, consequently, a higher amount of the bleaching chemical was consumed in this stage (Table 6). The PO-bleached pulps became comparable with each other with respect to the kappa number, brightness and intrinsic viscosity. In the last bleaching stage, the consumption of hydrogen peroxide was nearly the same (Table 6).

Table 6. Properties of birch hydrotropic pulps after different bleaching stages, and hydrogen peroxide consumption in the PO and P stages, and total in the bleaching.

Stages and pulp properties	Pulp	
	R_{bir}	HF_{bir}
Oxygen delignification		
Kappa number	16.3±0.2	4.4±0.1
Viscosity, mL/g	1002±1	617±1
PO stage		
Kappa number	2.4±0.1	1.3±0.1
Viscosity, mL/g	588±6	578±4
Brightness, %ISO	76.0±0.3	77.9±0.7
H_2O_2 consumption, kg/t OD pulp[a]	32.7	10.4
P stage		
Viscosity, mL/g	562±15	542±11
Brightness, %ISO	87.5±0.1	87.9±0.1
H_2O_2 consumption, kg/t OD pulp[a]	20.1	21.3
Total H_2O_2 consumption, kg/t OD bleached pulp	56.3	33.2

[a]pulp before the bleaching stage.

The bleaching additionally removed lignin and hemicelluloses from the pulps and lowered further an overall yield. The final yields were 41.4 and 38.7% for

R_{bir} and HF_{bir} pulps, respectively. The residual hemicelluloses consisted of anhydroxylose and glucomannan. Their quantity was determined with the help of the hydrolysis procedure, and it was 6.8 and 1.8%, respectively, for the reference pulp. In the case of HF pulp, the corresponding values were 5.1 and 1.3%. Hence, the pulp obtained with the modified process had higher purity than the reference one, and the lower yield of HF_{bir} bleached pulp was partly offset by the lower content of hemicelluloses.

Based on the intrinsic viscosity values and the purity, one could suggest the obtained pulps could be used as dissolving grade pulps, which generally have a low content of hemicelluloses and intrinsic viscosities above 450 mL/g (Schild and Sixta 2011; Sixta 2006; Sixta et al. 2004). However, in order to make more accurate conclusions about the suitability of the hydrotropic pulps for the dissolving-grade pulp applications other properties should be taken into account. Also, the actual manufacturing process of a particular product should be simulated (Sixta 2006).

4.1.4 Isolation of birch hydrotropic lignin

Lignin from birch wood was extracted with the conditions of R_{bir} and HF_{bir} treatments, *i.e.* with pure 36% SXS solution (R_{bir}) and 36% SXS solution with added formic acid and hydrogen peroxide (HF_{bir}). The pulp yield, the content of residual lignin and the degree of delignification are show in Table 7. The extracted lignins were precipitated from the spent solutions by dilution with 10-fold of hot distilled water.

Table 7. Results of the hydrotropic treatments used for the extraction of lignin from birch wood.

Treatment	Pulp yield, %	Residual lignin, %	Degree of delignification, %
R_{bir}	48	4.5	91
HF_{bir}	43	1.7	97

The yield of crude lignins from both treatments was 16.1% based on oven-dry wood or about 160 kg from 1 t of wood. This number was lower than the amount of lignin dissolved during the treatments, which was 21.9 and 23.3% based on oven-dry wood for R_{bir} and HF_{bir} treatments, respectively. Thus, it was not possible to recover all the lignin that was dissolved during the treatments. Some amount stayed in the diluted spent solution forming a non-precipitating fraction. It could also be possible that some amount was

removed during the washing step. However, it should have been small, as lignin is poorly soluble in water.

Presence of the non-precipitating fraction during the isolation of hydrotropic lignin from the spent solution has been reported previously in connection to the hydrotropic treatment of aspen wood (Gromov and Odincov 1957b; c). The authors reported that 10 to 50% (based on lignin in wood) of the lignin did not precipitate upon the dilution (Gromov and Odincov 1957b). It was presumed that this fraction consisted of low molar mass lignin fragments, and it was shown that its amount depended on the treatment conditions (Gromov and Odincov 1957b; c). The authors also partially isolated this fraction by lowering the pH of the diluted spent solution and heating (Gromov and Odincov, 1957b).

Besides the hydrotropic process, non-precipitating lignin has also been observed in other processes. For example, in the study of ethanol-water pulping of hardwoods, 20 to 40% of the original lignin present in wood remained dissolved in the spent solution during the isolation of lignin from the spent solution by dilution (Hergert et al. 1999).

4.2 Characterization of birch lignins isolated with conventional and modified hydrotropic treatments

4.2.1 Chemical composition of birch hydrotropic lignins

The chemical and elemental composition of the lignins was studied with the purpose to obtain information about their purity. Possible non-lignin compounds originated either from the raw material or from the hydrotropic agent. The former included carbohydrates, extractives and other substances present in birch wood, and the latter could be contaminants in the hydrotropic agent.

Both lignins contained very small quantities of residual carbohydrates (Figure 13, A). Such a result could be explained by the acidic nature of the hydrotropic treatments, which resulted in extensive hydrolysis of hemicelluloses and their removal. In the case of the modified process, these reactions were intensified by the added formic acid and, therefore, HF lignin contained less residual carbohydrates compared to the lignin from the reference process. Xylose (anhydrous form) was the most abundant among the hemicellulose units. This sugar unit was also present in a high amount in the birch wood (section 4.1.1). The residual hemicelluloses were bound to the

lignins and, because of this, they could not be removed by washing with water.

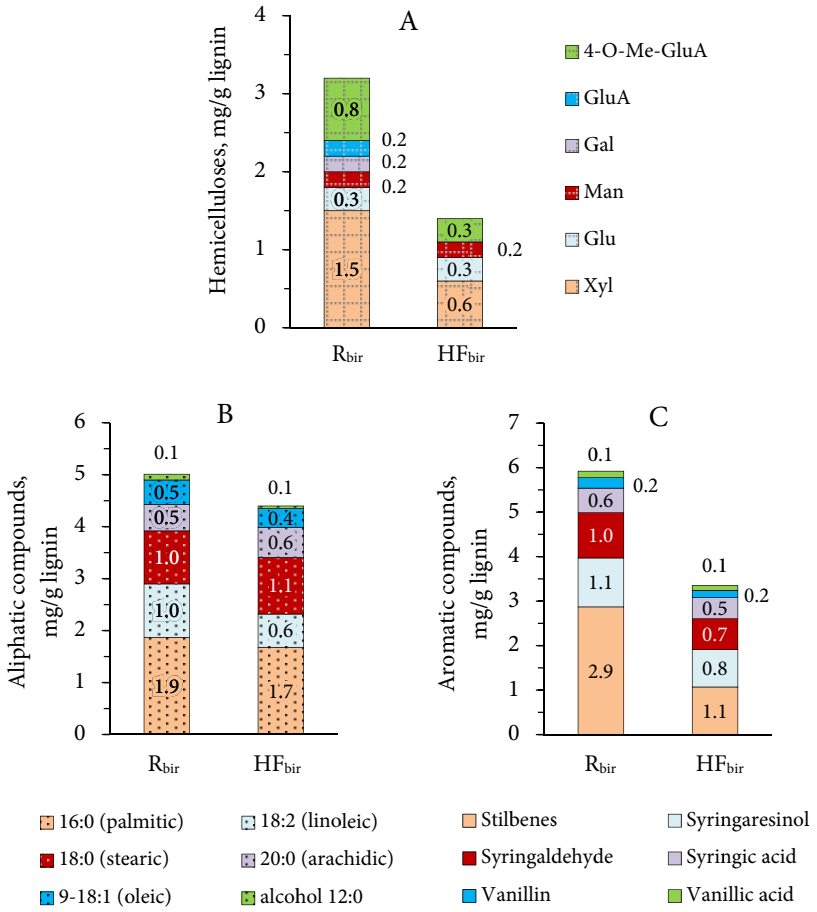

Figure 13. Residual hemicelluloses (A) and aliphatic (B) and aromatic (C) low molar mass compounds in birch hydrotropic lignins. The content of hemicelluloses is expressed in an anhydrous form.

Low molar mass aliphatic and aromatic compounds were also detected in the lignins by silylation and GC (Figure 13, B, C). These compounds were not chemically bound to the lignins. This could be deduced from the method of their detection, which did not include any destructive steps, such as methanolysis or hydrolysis. The source of the aliphatic and, perhaps, some of the aromatic compounds could be the extractives of the birch wood. However, it was also possible that the aromatic compounds were derived from the birch lignin itself as a result of the fragmentation reactions. The

presence of the low molar mass aromatic and aliphatic compounds in the lignin can be explained by their co-precipitation during the lignin precipitation due to the poor solubility in water and/or good affinity for lignin.

Proteins and amino acids were another type of compounds that were transferred into the lignins from the raw material. Their content was estimated from the nitrogen content (Table 8) using a conversion factor of 6.25 that assumes that proteins contain 16% (w/w) of nitrogen (Browning 1967). Both lignins showed the same content of proteins/amino acids, about 1.4%. The proteins were most probably chemically associated with the lignins, as in the case of acetic acid lignin obtained from wheat straw (Pan and Sano 2000).

Table 8. Elemental composition of the birch hydrotropic lignins.

Lignin	Elements, wt%			
	C	H	S	N
R_{bir}	65.5±0.1	5.9±0.0	0.16±0.01	0.23±0.01
HF_{bir}	64.5±0.2	5.5±0.1	0.39±0.01	0.22±0.00

Both lignins contained sulfur (Table 8). However, its content in the lignins was still much lower than that in kraft lignins (1.0–3.0%) or lignosulfonates (3.5–8.0%) (Vishtal and Kraslawski 2011). It was quite unlikely that the sulfur originated from sodium xylenesulfonate. If SXS had reacted with lignin, the content of sulfur would have been much higher, taking into account that the amount of SXS used for the treatments was quite high. In addition, free SXS was removed from the lignins by the extensive washing. Therefore, the most satisfactory explanation for the source of the sulfur in the lignins would be possible contaminants of the hydrotropic salt, especially considering the fact that the hydrotropic agent was of a technical grade with the purity >90%. One of such contaminants was 2,2',5,5'-tetramethyldiphenylsulfone (and its isomers). This compound was detected in the Py-GC-MS analysis. Later, it was also found in the gas chromatograms from the analysis of low molar mass aliphatic and aromatic compounds. It is quite likely that this compound is poorly soluble in water, and it co-precipitated together with the lignins during the recovery procedure, when the spent solutions were diluted with water. The contribution of this compound to the total sulfur content was 0.1%-units.

The purity of the lignins could be roughly estimated taking into account all the non-lignin compounds and assuming that the rest of the sulfur (0.06% for R_{bir} and 0.3% for HF_{bir} lignins) originated from SXS. It was 96 and 95% in the case of R_{bir} and HF_{bir} lignins, respectively.

Lignin obtained by the modified process showed a slightly lower content of carbon than the reference lignin (Table 8). Both lignins with respect to the CHO composition were similar to some technical lignins, for example birch ethanol/H_2O lignin and Alcell lignin (Fengel and Wegener 1984c; Hergert et al. 1999; Ni and Hu 1995).

4.2.2 Structure of birch hydrotropic lignins

The ratio of the lignin units was determined with the help of Py-GC-MS. Both lignins had nearly the same ratio of H, S and G units per 100 phenylpropane units (PPU); albeit, the lignin from the reference treatment exhibited a slightly higher S/G ratio than the lignin from the modified process (Table 9). However, in general, it could be concluded that the modification of the hydrotropic solution did not result in a significant change of the lignin unit ratio. The unit ratio in the hydrotropic lignins was also similar to that of the original birch lignin.

Table 9. Molar ratios of the lignin units in the birch hydrotropic lignins and birch wood determined with Py-GC-MS.

Lignin	Units per 100 PPU			S/G
	H	G	S	
Birch	4	29	66	2.3
R_{bir}	2	27	71	2.6
HF_{bir}	2	29	69	2.3

A somewhat similar result in respect to the predominance of S units in the structure of the lignins was obtained with a 2D NMR technique. However, the S/G ratio estimated with the NMR method was higher than that obtained with the Py-GC-MS (Table 10).

Table 10. Content of the main linkages and lignin units in the birch hydrotropic lignins determined with 2D NMR. Mass (M_w) and number (M_n) average molar masses and a polydispersity index (PDI) of the lignins.

Lignin	Main linkages, per 100 PPU[a]			Units, %					Molar mass, g/mol		
	*β-O-*4' (A)	*β*-5' (B)	*β-β'* (C)	H	G	S	S'[a]	S/G	M_w	M_n	PDI
R_{bir}	15.0	3.3	3.2	1	19	78	2	4.2	5306	1035	5.1
HF_{bir}	11.8	3.2	3.0	2	22	73	3	3.4	5910	1143	5.2

[a]structures A–C and also S' unit are shown in Figure 14.

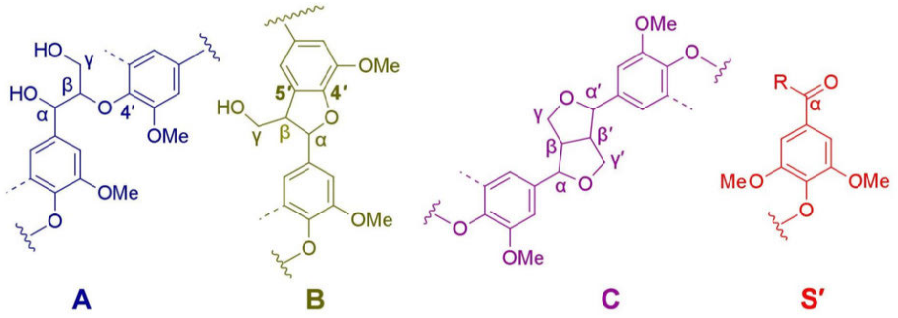

Figure 14. Lignin structures listed in Table 10.

More *β-O-*4' linkages were cleaved in the course of the modified process compared to the reference one (Table 10). This was apparently connected to the better delignification capability (Table 7) of the modified solution, which was owing to the presence of hydrogen peroxide and formic acid. However, the quantities of other main linkages, *β*-5' and *β-β',* were quite similar for both lignins.

The content of *β-O-*4', *β*-5' and *β-β'* linkages in birch wood lignin is 40–55, 2–5 and 7–10 per 100 PPU (section 2.1). Hence, it was clearly seen that the amount of *β-O-*4' and *β–β'* linkages decreased during the treatments, and *β*–5' was not much affected. The mechanism responsible for the cleavage of the *β-O-*4' bond is described in section 2.3.3. At a high temperature and a moderately acidic pH, the *β*-aryl ether linkage is broken due to the homolysis of a C_β-*O* bond via the quinone methide intermediate (Li et al. 2000; Westermark et al. 1995). This reaction leads to the formation of radicals that can recombine to give origin to *β*-1', *β*-5' and *β-β'* structures (Figure 8) (Li et al. 2000). The *β*-1' and *β*-5' structures can be further converted to stilbenes loosing at the same time C_γ-OH (Hallac et al. 2010; Li et al. 2000). As to the

other two linkages, *β*-5' and *β*-*β*', the information on possible transformations of these linkages under moderately acidic conditions at elevated temperatures is not available in literature. Therefore, it is difficult to say what kind of reactions involving these linkages could take place during the hydrotropic treatments. Besides the aforementioned reactions, other types of reactions triggered by hydrogen peroxide took place in the course of the modified process. These reactions are listed in Paper I.

The cleavage of the bonds in the native birch lignin led to the formation of lignin fragments. The average molar masses and a polydispersity index of the lignin obtained by the modified process were slightly higher compared to the reference lignin (Table 10).

Another consequence of the chemical reactions was the change in the phenolic and aliphatic OH ratio. According to the literature (Rauhala et al. 2011), the original lignin in birch wood (*Betula pendula*) contains 5.4 mmol/g of aliphatic OH groups and 1.2 mmol/g of phenolic ones. The opposite was observed for the hydrotropic lignins, in which phenolic OH groups dominated over the aliphatic ones (Table 11). The increase in the content of phenolic OH groups occurred due to the cleavage of the aryl ether bonds, *e.g.* *β*-*O*-4', which resulted in the conversion of the non-phenolic lignin units into the phenolic ones. The reduction in the content of the aliphatic hydroxyls was also related to the aryl ether cleavage mechanism and the accompanying reactions, as has been shown by the studies performed by Li et al. (2000). Based on the products formed upon heating of the arylglycerol *β*-aryl ether lignin model compounds in buffer solution at a pH of 3, one could clearly observe that a part of the aliphatic hydroxyls was lost or they were converted into ethers, such as in the structure of syringaresinol/pinoresinol (Li et al. 2000). Such changes in the contents of both types of hydroxyl groups have been reported for other autocatalyzed or moderately acidic processes and raw materials: hydrothermal treatment of eucalyptus wood (Leschinsky et al. 2008), organosolv treatment of *Buddleja davidii* (Hallac et al. 2010) and hydrothermal treatment of birch wood (Rauhala et al. 2011).

Table 11. Content of hydroxyl and carboxyl functional groups in the birch hydrotropic lignins (mmol/g) determined with the help of ^{31}P NMR.

Lignin	Aliphatic OH	Phenolic OH				Carboxyl
		Total	5-substituted (condensed+S)	G-OH	H-OH	
R_{bir}	1.38	3.22	2.43	0.64	0.15	0.30
HF_{bir}	1.13	3.16	2.39	0.60	0.17	0.32

In relation to the OH-containing functional groups determined with ^{31}P NMR, both lignins were comparable to each other, except the aliphatic OH groups (Table 11). The lower content of these groups in the lignin from the modified process was apparently related to the higher severity of this treatment due to the added reagents, which resulted in more extensive removal of the aliphatic OH groups.

In summary, both isolated lignins differed in the contents of the non-lignin compounds, *β-O*-4' bonds and aliphatic hydroxyl groups. However, they were also similar in many aspects despite the significant difference in the performance of the conventional and modified treatments. The close structure of the lignins could also be seen from their FTIR spectra, which were identical (Paper II).

4.3 Fractionation of sugarcane bagasse using a hydrotropic process

Screened sugarcane bagasse (>0.5 mm) was treated at the temperatures of 150 and 170 °C and dwell times of 60 and 120 min using 30% SXS aqueous solution with and without addition of formic acid (Table 3).

4.3.1 Hydrotropic treatment of sugarcane bagasse

The hydrotropic process efficiently delignified the sugarcane bagasse at most of the chosen conditions, except the autocatalyzed process performed at 150 °C and dwell time of 120 min (Table 12). The residue obtained under these conditions ($R1_{bag}$) still contained a high amount of lignin (Figure 15), and it could not be defibrated with an ordinary disintegrator. Other treatments reached a sufficiently high degree of delignification, and the treated bagasse residues were easily converted to fibers. The higher severity of the processes, *i.e.* a higher temperature, longer treatment time and lower pH, resulted in a

higher degree of delignification, which could be followed from the decrease in the kappa number (Table 12) and lignin content (Figure 15, A).

Table 12. Some properties of the pulps produced from sugarcane bagasse with hydrotropic treatments.

Pulp	End pH	Yield, %		Kappa number	Intrinsic viscosity, mL/g	Degree of delignification, %
		Total	Screened			
$R1_{bag}$	4.6	66.8	–	–	–	50
$F1_{bag}$	3.7	50.7	49.4	42.3±0.5	807±1	84
$R2_{bag}$	4.2	49.7	48.1	39.6±0.3	886±0	85
$F2_{bag}$	3.5	46.1	45.1	22.4±0.0	636±1	92
$R3_{bag}$	3.9	46.2	45.0	25.2±0.0	687±1	91
$F3_{bag}$	3.5	44.7	43.7	22.1±0.1	500±0	93

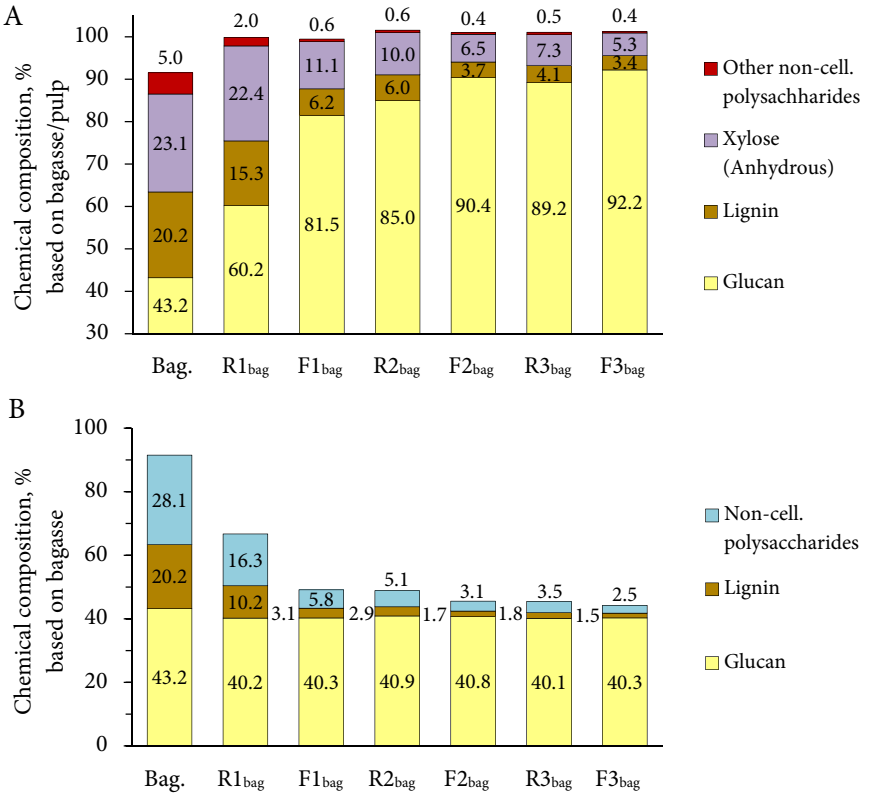

Figure 15. Content of main constituents in sugarcane bagasse and in the hydrotropic pulps based on bagasse/pulp (A) and based on bagasse (B).

Intrinsic viscosity also decreased, as the process severity increased (Table 12). As was mentioned before (section 4.1.1), intrinsic viscosity is associated with the molar mass or DP of cellulose in pulp. The longer treatment time and the lower pH during the treatments resulted in more extensive hydrolysis, *i.e.* cleavage of the cellulose chains, which led to the greater reduction of cellulose DP. Although some pulps had low intrinsic viscosity values, they were still in the range of viscosities of certain grades of dissolving pulps (Schild and Sixta 2011; Sixta 2006; Sixta et al. 2004).

The chemical composition of the residues also varied depending on the severity of the processes (Figure 15, A). More severe conditions during the treatments increased the rate of delignification and hydrolytic reactions, and as a result, more lignin and hemicelluloses were removed from the bagasse. $F3_{bag}$ pulp, which was produced at the most severe conditions, showed the highest purity amongst the pulps, and $R1_{bag}$ pulp was the least pure. Generally, all pulps were enriched in cellulose and, therefore, could be regarded as cellulose fractions with a different degree of purity. The most abundant non-cellulosic component present in the pulps was hemicelluloses, mainly anhydroxylose, followed by lignin.

The content of glucan in the residues based on the original bagasse was lower than that in the bagasse (Figure 15, B). It is important to mention here that the yield of glucan based on bagasse was determined using the screened yields. Therefore, it does not include glucan (cellulose) of the reject fraction. However, if it had also been taken into account, the content of glucan based on the bagasse in the case of the pulps would have been still lower than that of the bagasse. The difference could be explained by the presence of non-cellulosic glucan. Since residual sucrose was not detected in the bagasse, it could be a part of the hemicelluloses or another carbohydrate constituent such as, for example, mixed-linkage (1→3), (1→4)-β-glucan (Morais de Carvalho et al. 2017). Such glucan was easily soluble, and it was removed from the bagasse even at the mildest conditions. Notably, dissolved glucan was also found in all the spent solutions (section 4.3.5). Therefore, the residues had a lower amount of glucan in % based on the original bagasse. It is quite likely that most of it, if not all, originated from the cellulose.

The lower yield of the residues (Table 12) obtained with the more severe processes was mainly due to the lower contents of the non-cellulosic compounds, and there were no additional significant losses of cellulose in such treatments (Figure 15, B). However, during soda pulping of sugarcane bagasse after a prehydrolysis step, the loss of cellulose can be as high as 25–27% based on the original cellulose, as has been shown in the study on the

manufacturing of dissolving pulps from Brazilian sugarcane bagasse (Andrade and Colodette 2014). This beneficial feature of the hydrotropic process over the alkaline ones has already been mentioned and explained in relation to the birch wood (section 4.1.2).

Comparing the treatments of bagasse and birch wood performed at the same conditions (R_{bir} *vs* $R3_{bag}$ and F_{bir} *vs* $F3_{bag}$) except the concentration of the hydrotropic agent, which should not have had any effect, one could observe that the raw materials were not considerably different regarding the proneness to delignification, although the birch wood showed a slight advantage in this respect (Table 5 and Table 12). The pulps obtained from the birch wood also had higher intrinsic viscosities than the corresponding bagasse pulps (Table 5 and Table 12). However, the bagasse hydrotropic pulps had a lower content of hemicelluloses, so they were purer (Figure 12 and Figure 15).

4.3.2 Isolation of bagasse hydrotropic lignins

The dissolved lignins were isolated from the spent solutions by dilution with hot water and filtration. The yield of crude lignins and the yield of lignins in a pure form ('pure' lignin) were dependent on the treatment conditions, and both yields were higher for the more severe processes (Figure 16, A). The 'pure' yields were estimated from the crude yields by taking into account the lignin purity, which was estimated indirectly from the contents of the non-lignin compounds (Table 13). It should be noted here that the yields of the crude hydrotropic lignins from the bagasse were lower in comparison to the lignin yields from the birch wood. On the one hand, it could be because of the difference in the recovery protocol applied in both cases. On the other hand, it could also be influenced by the structural differences in the lignins of these two raw materials.

Similar to the study on hydrotropic treatment of birch wood (Paper II), it was not possible to isolate all the lignin dissolved during the treatments. The lignin yields in a pure form varied in the range of 50–70% based on lignin in the bagasse, being higher for the more severe processes (Figure 16, B). A certain part of the lignins remained dissolved upon the dilution of the spent solution with hot water and, thus, could not be recovered. It constituted 25–50% of the bagasse lignin, and it was higher for the milder processes. In the case of the mildest process ($R1_{bag}$), none of the dissolved lignin could be recovered by the method used in the study.

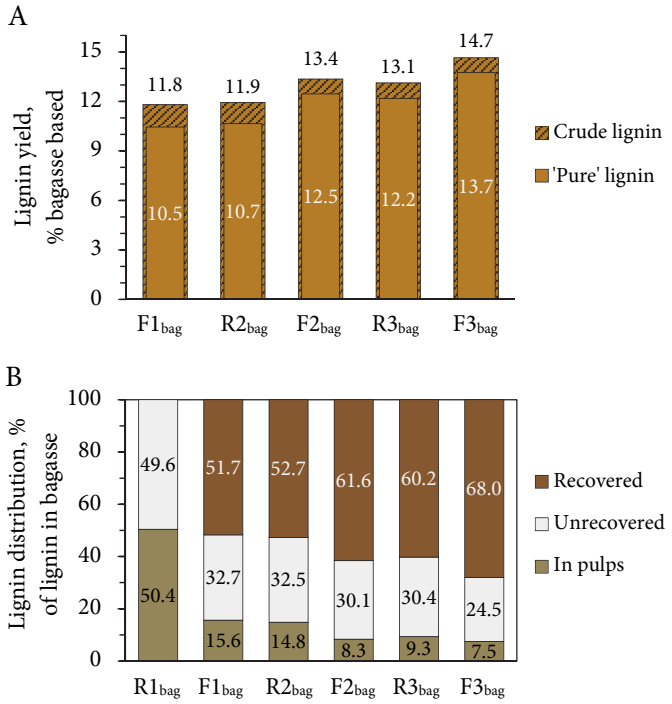

Figure 16. (A) The yield of crude lignin and lignin in a pure form based on bagasse. (B) Distribution of bagasse lignin between different process streams, *i.e.* residual lignin in the pulps, recovered in a pure form and remained in the spent solution during the recovery of lignin.

The material remained dissolved in the diluted spent solutions after the lignin precipitation was analyzed with SEC. To minimize the interference of the non-lignin compounds, the signal was collected at 300 nm (UV detector), and the calculation was performed using only the part eluted before phenol, *i.e.* 94 g/mol and above. It was presumed that the results obtained with a such set up would reflect the average molar mass of the unrecovered lignin. The chromatograms of the non-precipitated lignin fractions had several maxima at about 100–120, 250 and 500 g/mol (pullulan calibration). Generally, these fractions had relatively low molar masses (Figure 17). With the exception of the $R1_{bag}$ process, they varied from 330 to 580 g/mol (pullulan standards) corresponding to 2–3 lignin units. The average molar masses were lower for the more severe processes. In the case of $R1_{bag}$ spent solution, the fraction that remained dissolved upon the dilution exhibited much higher average M_w, 4110 g/mol. Such a result could be explained by the fact that none of the

dissolved lignin precipitated upon the dilution with water, and the diluted spent solution still contained high molar mass lignin fractions. It is important to mention that the average molar masses of the recovered lignins ranged between 2220 and 7710 g/mol (Figure 17), thus being considerably higher than the average molar masses of the corresponding unrecovered lignins.

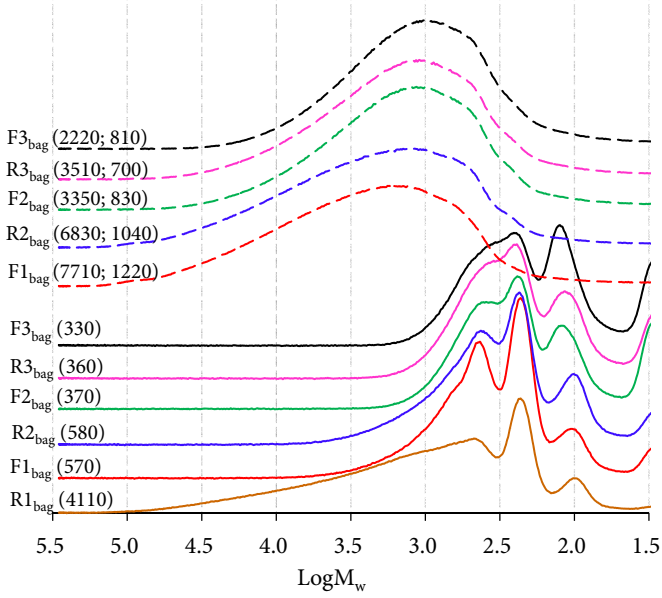

Figure 17. Size exclusion chromatograms of the unrecovered lignins (solid lines, normalized to the areas below the curves) and sugarcane bagasse hydrotropic lignins (dash). The signals were obtained with a UV detector, 300 nm. Average M_w and M_n (lignins only) are shown in brackets. The calibration was performed with a set of pullulan standards and phenol.

The solubility of the unrecovered lignins can be elucidated by several factors. A higher molar mass part of the unrecovered lignins could be more hydrophilic than the precipitated lignin. One could hypothesize that this fraction was connected to carbohydrates. This guess could be supported by the fact that upon increase in the process severity, the amount of the residual carbohydrates in the recovered lignins and the amount of the carbohydrates in the spent solution became lower (Table 13) and, at the same time, the yields of the lignins increased. In the case of the $R1_{bag}$ process, the entire lignin extracted from the bagasse could be in the form of lignin-carbohydrate complexes. Lower molar mass fractions of the unrecovered lignins could be soluble in the diluted spent solution (in fact, warm) as such without being

connected to more hydrophilic moieties, because the concentration of these fractions in the diluted spent solutions was quite low, *i.e.* the total concentration of the unrecovered lignins in the diluted spent solutions was 1–2.5 g/L and, in general, phenolic compounds are soluble in water to a certain extent.

4.3.3 Properties of bagasse hydrotropic lignins

The recovered lignins differed in purity. The difference was mainly affected by the content of residual hemicelluloses (Table 13). Similar to the pulps, the purity was higher for the lignins isolated from the more severe treatments owing to more extensive hydrolytic reactions and, thus, more complete removal of the hemicelluloses.

Table 13. Chemical composition of sugarcane bagasse hydrotropic lignins. Only the most abundant individual compounds are shown.

Compounds, mg/g of lignin	Lignin				
	$F1_{bag}$	$R2_{bag}$	$F2_{bag}$	$R3_{bag}$	$F3_{bag}$
Aliphatic compounds					
total	4.0	3.8	4.2	4.3	4.2
acid 16:0	2.0	1.9	2.2	2.2	2.2
acid 18:2	0.2	0.2	0.2	0.2	0.2
acid 18:1	0.6	0.6	0.6	0.7	0.6
acid 18:0	0.2	0.2	0.2	0.3	0.2
sitosterol	0.6	0.4	0.6	0.4	0.5
Aromatic compounds					
total	2.0	3.8	3.4	3.9	4.2
x,x,4-trihydroxyacetophenone	1.3	2.4	2.2	2.5	2.9
stilbenes	0.6	1.1	0.8	0.9	0.9
Hemicelluloses (anhydrous units)					
total	48.2	42.3	7.0	6.1	2.1
arabinose	10.8	6.4	1.4	0.7	0.2
xylose	28.5	28.5	2.4	2.2	0.5
mannose	0.3	0.2	0.1	0.2	0.1
galactose	0.9	1.1	0.1	0.3	0.0
glucose	4.3	4.0	1.9	2.1	1.0
4-O-Me-glucuronic acid	3.3	2.0	0.9	0.6	0.3

Generally, the bagasse hydrotropic lignins contained the same groups of compounds as the birch hydrotropic lignins (section 4.2.1), although at

different quantities, and there were also some differences regarding the presence of the individual constituents. Besides hemicelluloses, among these compounds were low molar mass aliphatic and aromatic compounds, nitrogen-, *e.g.* proteins, and sulfur-containing compounds (Table 13). A possible source of these compounds has also been explained for the birch hydrotropic lignins (section 4.2.1).

Different treatment conditions also affected the elemental composition of the lignins (Table 14). One could observe the correlation between the amount of carbon and the process severity. Such a relation could be attributed to the formation of the condensed structures in the lignins upon the increased process severity or/and to the amount of residual carbohydrates, because they have a higher O/C ratio than lignin.

Table 14. Elemental composition of the sugarcane bagasse hydrotropic lignins.

Lignin	Elements, wt%			
	C	H	N	S
$F1_{bag}$	63.2±0.2	6.1±0.0	0.58±0.01	0.31±0.04
$R2_{bag}$	64.1±0.1	6.1±0.0	0.57±0.00	0.31±0.01
$F2_{bag}$	65.6±0.0	6.1±0.0	0.54±0.00	0.26±0.02
$R3_{bag}$	65.6±0.0	6.1±0.0	0.59±0.01	0.29±0.02
$F3_{bag}$	66.6±0.0	6.0±0.0	0.48±0.00	0.29±0.01

Sulfur in the bagasse hydrotropic lignins was mainly derived from the contaminant of the hydrotropic agent, namely tetramethyldiphenylsulfone (Table 14). Its contribution to the sulfur content was 0.2–0.25 %-units. It is worth mentioning that tetramethyldiphenylsulfone was also found in the hydrotropic agent. This was done by repeated extraction of 4-fold diluted 30% SXS solution with methyl tert-butyl ether (MTBE) and analysis of the combined MTBE extract with GC. The obtained chromatograms, besides the peaks of the standards, contained peaks characteristic of tetramethyldiphenylsulfone (Figure 18, the peaks 1–5 shown on the enlarged part). Apart from this compound, some part of sulfur, 0.05–0.1%-unit, could also originate from the bagasse itself, as this raw material can contain up to 0.1% of sulfur (Hassuani et al. 2005; Seabra and Macedo 2011).

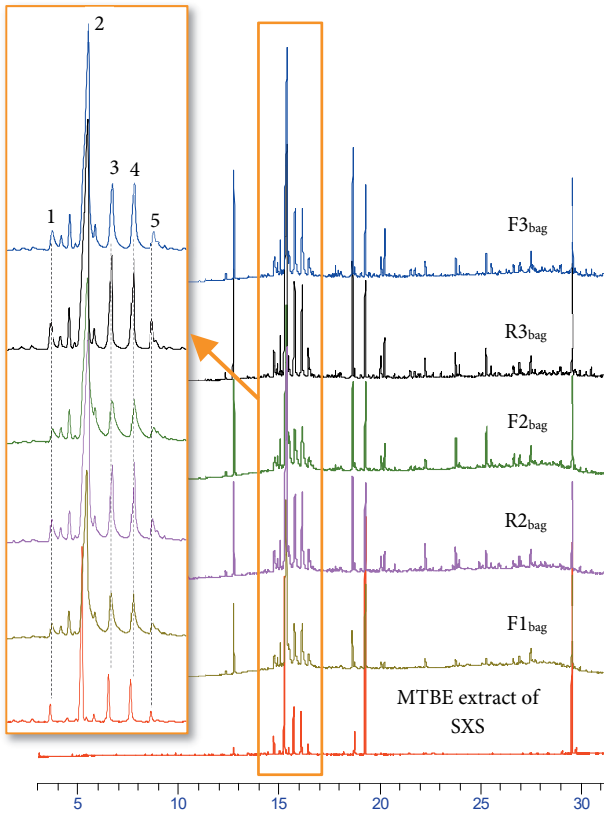

Figure 18. Gas chromatograms from the analysis of low molar mass compounds in the lignins and a chromatogram of the MTBE extract of the hydrotropic agent.

A distinct feature of the bagasse hydrotropic lignins was a relatively high content of nitrogen compared to the birch hydrotropic lignins (Table 8 and Table 14). This amount of nitrogen could be translated into 3–3.7% of protein content. Such difference was reasonable given the fact that the bagasse originated from sugarcane, which is an herbaceous plant and, in general, lignins isolated from such raw materials contain higher amounts of proteins than lignins isolated from wood (Gosselink et al. 2004; Pan and Sano 1999, 2000).

Besides the chemical and elemental composition, the process conditions also had an effect on the macromolecular properties of the lignins. The lignins extracted at the milder conditions had higher mass average molar masses and were more polydisperse than the lignins obtained at the more severe conditions (Figure 17). In the more severe treatments, the native bagasse lignin underwent more extensive cleavage of the lignin linkages

forming the fragments with lower average M_w. The results also implied that, at the most severe conditions, the condensation reactions were not very extensive, and repolymerisation of the dissolved lignin fragments did not take place.

4.3.4 Analytical pyrolysis

Analytical pyrolysis included two methods, namely conventional pyrolysis and pyrolysis with chemical treatment with tetramethylammonium hydroxide. The former was mainly used to determine the ratio of the lignin units in the bagasse hydrotropic lignins and in the bagasse lignin. The latter gave a possibility to gain information about the presence of the hydroxycinnamic acids.

Obtained bagasse hydrotropic lignins contained a high proportion of S units, and the S/G ratios varied in the narrow range of 1.5–1.7 (Table 15). An S/G ratio in the original bagasse lignin was lower, or 1.0. Such results could be explained by the redistribution of the lignin units between different lignin fractions, *i.e.* in pulp/unrecovered lignin/recovered lignin. For example, it was shown that after kraft pulping of different hardwoods the residual lignins in the pulps had higher relative content of G units compared to the lignins in the raw materials (Pinto et al. 2005). The authors explained this result by better chemical reactivity of S units and greater susceptibility of G units towards the condensation reactions.

Table 15. Relative abundances (mol%) of aromatic compounds released during Py-GC-MS of sugarcane bagasse and bagasse hydrotropic lignins.

Phenolic compounds	Lignin					
	Bagasse	$F1_{bag}$	$R2_{bag}$	$F2_{bag}$	$R3_{bag}$	$F3_{bag}$
H unit derivatives	3.2	10.8	5.4	10.3	9.1	11.7
G unit derivatives	13.5	17.9	13.6	15.3	14.1	13.4
S unit derivatives	13.8	26.0	21.9	24.9	23.5	22.5
4-vinylphenol (H/*p*-CA)	54.4	31.8	41.5	34.5	36.1	35.9
4-vinylguaiacol (G/FA)	12.5	9.4	12.7	10.4	12.2	11.8
4-vinylsyringol (S)	2.6	4.0	5.0	4.5	5.1	4.7
H:G:S	10:44:45	20:33:48	13:33:54	20:30:49	19:30:50	25:28:47
S/G	1.0	1.5	1.6	1.6	1.7	1.7

A relatively high content of H units in the hydrotropic lignins as well as in the original bagasse lignin could be false, because some of *p*-substituted phenolic compounds that were used for the estimation of H units could actually

originate from other sources, for example proteins (Ralph and Hatfield 1991; Rencoret et al. 2015). Moreover, 2D NMR (HSQC) analysis of 'milled wood' lignin isolated from Brazilian sugarcane bagasse and also of the whole bagasse itself has shown a low content of H units in the samples (del Río et al. 2015).

Upon the pyrolysis, the lignins also produced significant amounts of 4-vinylphenol and 4-vinylguaiacol. These compounds originated primarily from hydroxycinnamic acids, namely *p*-coumaric (*p*-CA) and ferulic (FA) acids, respectively (del Río et al. 2015). Because of this, 4-vinylphenol and 4-vinylguaiacol were not used for the estimation of the lignin unit ratio. Since other lignin units could contribute to the abundance of 4-vinylphenol and 4-vinylguaiacol, the bagasse hydrotropic lignins and the bagasse were subjected to pyrolysis with chemical treatment with tetramethylammonium hydroxide (TMAH/Py-GC-MS) to better distinguish the hydroxycinnamates. This procedure is performed at milder conditions, and it liberates methylated derivatives of the lignin units and the hydroxycinnamic acids. In addition, it prevents decarboxylation, so the compounds of interest could be clearly differentiated as methyl 4-methoxycinnamate (*p*-CA) and methyl veratrylpropenoate (FA). TMAH/Py-GC-MS proved the high abundance of these compounds in the isolated hydrotropic lignins and in the original bagasse lignin (Table 16). The *p*-CA/FA ratio in the case of the bagasse was similar to the value reported for Brazilian sugarcane bagasse elsewhere (del Río et al. 2015). In the bagasse hydrotropic lignins this ratio was higher, 9.4–12.7, due to the lower abundance of methyl ester of veratrylpropenoic acid (FA). This result could be explained by the low content of residual carbohydrates in the lignins and by the fact that ferulic acid is mainly linked to the carbohydrates, whereas *p*-coumaric acid is primarily connected to lignin (del Río et al. 2015).

Table 16. Relative molar abundances of phenolic compounds released during TMAH/Py-GC-MS analysis of the bagasse and bagasse hydrotropic lignins.

Phenolic compounds	Lignin					
	Bagasse	$F1_{bag}$	$R2_{bag}$	$F2_{bag}$	$R3_{bag}$	$F3_{bag}$
H unit derivatives	13.0	3.9	2.6	4.4	4.2	8.1
G unit derivatives	14.2	19.1	16.2	15.9	12.6	15.1
S unit derivatives	12.3	31.5	33.8	33.7	27.9	30.6
methyl 4-methoxycinnamate (*p*-CA)	51.0	41.8	43.1	42.3	49.9	42.8
methyl veratrylpropenoate (FA)	9.5	3.6	4.4	3.7	5.3	3.4
S/G	0.9	1.6	2.1	2.1	2.2	2.0
p-CA/FA	5.4	11.7	9.8	11.3	9.4	12.7

4.3.5 Characterization of the spent solutions

The spent solutions from the hydrotropic treatments of the bagasse contained dissolved bagasse constituents and products of their degradation. Besides the lignins, another dissolved bagasse constituent was hemicelluloses. During the analysis of the spent solutions, special attention was paid to pentosans, because they were present in the bagasse in much higher quantities than hexosans and uronic acids.

As can be observed for the autocatalyzed processes (R treatments, Table 12), the pH of the hydrotropic solutions became acidic towards the end of the treatments. This happened due to the enrichment of the treatment solutions with acids. Among these acids, acetic and formic acids were detected by HPLC analysis (Figure 19). Formation of the acetic acid can be elucidated by the cleavage of the acetyl groups, the content of which in sugarcane bagasse is 2.5–4% (Andrade and Colodette 2014; de Carvalho et al. 2015). More acetyl groups were split off from the hemicelluloses upon the increase in the process severity, so the concentration of acetic acid in the spent solutions was higher for the more severe treatments. The source of the formic acid was not that apparent. However, it has been shown for organosolv and hydrothermal processes that formic acid can be produced from xylan/xylose at elevated temperatures (Gosselink et al. 1995; Oefner et al. 1992). Similar routes could be expected for the hydrotropic process. A path that comprises formation of hydroxymethylfurfural (HMF) and the following degradation of HMF to levulinic and formic acid can be disregarded, because HMF was not present in the spent solutions. Generally, the yield of formic acid in the autocatalyzed processes, $R1_{bag}$–$R3_{bag}$, was smaller than the yield of acetic acid. However, it followed the same trend, meaning it was higher for the more severe treatments. The acidified treatments, $F1_{bag}$–$F3_{bag}$, had a higher concentration of formic acid compared to the autocatalyzed processes, because formic acid was added to the hydrotropic solutions before the treatments to lower the pH.

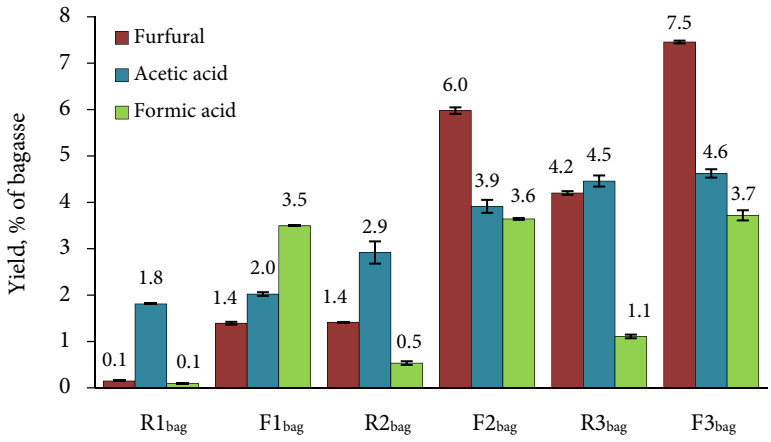

Figure 19. Yield of acetic and formic acids and furfural in the hydrotropic treatments of sugarcane bagasse.

Dissolved hemicelluloses were present in the spent solutions as polymers and monomers (Table 17). In addition, the C5 sugars also gave origin to such degradation product as furfural, which was detected in all the spent solutions (Figure 19). It is necessary to mention here that the degradation product of C6 carbohydrates, hydroxymethylfurfural, was not detected in the spent solutions. The ratio between the polymers and monomers depended on the treatment conditions leaning generally towards the monomers in the more severe treatments. Also, the yield of furfural increased as the process conditions became more severe. The main dissolved carbohydrate unit was xylose (Table 17), which was present predominantly in a polymeric form in the spent solutions of $R1_{bag}$, $F1_{bag}$ and $R2_{bag}$ treatments. In other spent solutions, the monomeric form dominated over the polymeric one.

Table 17. Carbohydrate monomers and polymers dissolved in the spent solutions.

Units	Spent solution/Treatment					
	$R1_{bag}$	$F1_{bag}$	$R2_{bag}$	$F2_{bag}$	$R3_{bag}$	$F3_{bag}$
Polymers/oligomers[a], g/100g bagasse						
Arabinose	0.4	0.3	0.5	0.2	0.1	0.0
Xylose	7.0	11.2	9.9	1.2	1.4	0.4
Mannose	0.2	0.3	0.2	0.1	0.2	0.0
Galactose	0.3	0.4	0.3	0.0	0.1	0.0
Glucose	0.8	1.1	1.0	0.9	0.9	0.3
Galacturonic acid	0.4	0.5	0.3	0.2	0.1	0.0
4-O-Me-Glucuronic acid	0.2	0.3	0.5	0.3	0.4	0.3
Monomers[a], g/100g bagasse						
Arabinose	1.0	1.3	0.3	0.3	0.1	0.1
Xylose	0.2	3.8	2.1	4.1	2.2	0.8
Mannose	0.0	0.0	0.0	0.1	0.0	0.1
Galactose	0.0	0.2	0.1	0.4	0.2	0.2
Glucose	0.0	0.1	0.0	0.3	0.2	0.5

[a]rhamnose and glucuronic acid were present in the bagasse in very small amounts and therefore are not shown.

Upon the increase in the process severity, the total content of dissolved C5 hemicelluloses and C5 sugar monomers decreased. The polymeric carbohydrates were hydrolyzed to monomers, and the monomers were further dehydrated to furfural, and the rate of these reactions increased at a higher temperature or lower pH or longer treatment time. However, besides the named products, it seemed that the pentosans or their degradation products were also converted further into some other products that were not analyzed in the study. Such observation was based on the material balance for the C5 carbohydrates (Figure 20). The sum of the pentosans left in the treated residue and the pentosans present in the spent solutions as polymers, monomers and furfural was not equal to the total content of pentosans in the bagasse. This could be partly explained by the errors during the analyses and also determination of the pulp yield. In particular, such explanation could be true in the case of $R1_{bag}$ and, possibly, $F1_{bag}$ processes. However, the analytical errors could not be a reason for the greater discrepancy in the case of the severe treatments. Therefore, it became apparent that the pentosans or their degradation products were further converted into other compounds in the course of the treatments. Among such compounds could be pyruvic, glycolic and lactic acids (Gosselink et al. 2004; Oefner et al. 1992). Furfural could also participate in the condensation reactions (Zeitsch 2000). Generally, the

discrepancy in the balance was higher for the more severe processes implying that more considerable changes happened to the C5 carbohydrates during the treatments.

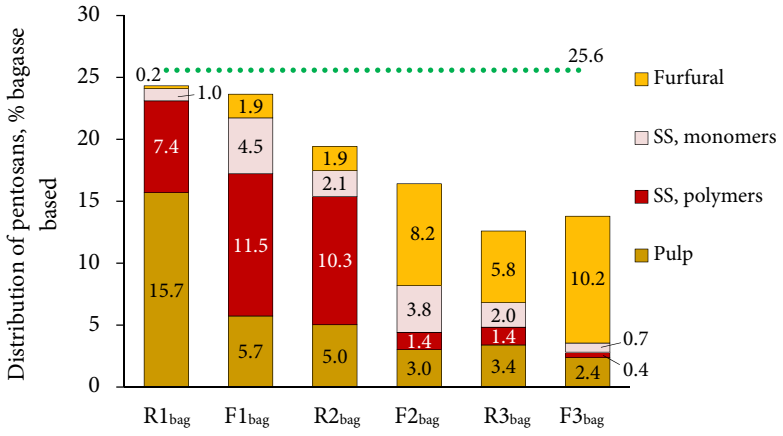

Figure 20. Distribution of pentosans between the different streams of the hydrotropic process: in the pulps, dissolved in the spent solutions as polymers (SS, polymers) and monomers (SS, monomers), and converted to furfural. Furfural and monomers were recalculated to a polymeric form. The amount of pentosans in the bagasse is shown by a dotted line.

Some compounds dissolved in the spent solutions were valuable products. In general, they can be recovered and sold to increase the revenue of the process. For example, in addition to the cellulose fraction and lignin, 60 and 75 kg of furfural, and 39 and 46 kg of acetic acid can be potentially obtained from 1 t of sugarcane bagasse using the processes $F2_{bag}$ and $F3_{bag}$, respectively.

4.4 Utilization of hydrotropic lignin for the preparation of lignin-cellulose beads

4.4.1 Preparation of lignin-cellulose beads

Lignin-cellulose beads were prepared by co-dissolution of birch hydrotropic lignin R_{bir} and HCl/EtOH-pretreated dissolving pulp (cellulose) in 7%NaOH/12% urea aqueous solution and extrusion of the solution through a syringe.

It should be mentioned here that it was not possible to obtain homogeneous solutions in all cases of the studied lignin/cellulose ratios. Both

polymers could be dissolved completely only up to 25% lignin concentration based on the weight of lignin and cellulose. At 40% lignin concentration, a part of the lignin remained in the form of particles evenly dispersed in the lignin-cellulose solution.

4.4.2 Characterization of lignin-cellulose beads

The obtained never-dried beads had a spherical shape with a diameter of about 3 mm. The lignin-cellulose beads also had tails, which became more pronounced as the content of lignin in the beads increased (Figure 21). The tailing phenomenon reflected the ratio of the major/minor axes (Table 18), *i.e.* the axes of a hypothetical ellipse, to which the beads were fitted during the processing of the scan images (Trygg et al. 2013). The structural features remained after air-drying. However, the beads shrunk considerably (Figure 21 and Table 18).

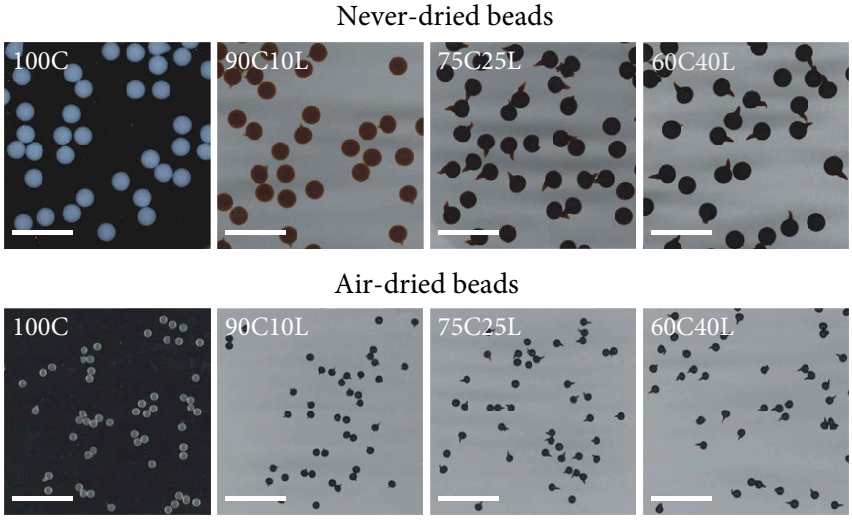

Figure 21. Scan images of the cellulose and lignin-cellulose beads. The scale bars correspond to 1 cm.

The distribution of the lignin in the beads was studied with the help of confocal fluorescence microscopy by examining the surface and the cross sections of the beads (Paper IV). The images from the reflection channel, which showed all the bead constituents, were compared with the corresponding images from the fluorescence channel. The fluorescence images were composed of the fluorescence signal originated from the lignin only, *i.e.* the pure cellulose beads were almost invisible in this channel. The

images from both channels showed the same structural features in the beads, suggesting that the lignin was evenly distributed in the beads.

Table 18. Weight, dimensions, porosity and lignin content of the air-dried and never-dried beads.

Beads	State	Weight, mg	Major/Minor axis, mm	Porosity[a] %	Lignin content, %
100C	never-dried	15.3	3.1/3.0	93.9	n.d.[b]
	air-dried	1.36	1.3/1.3	17.6	
90C10L	never-dried	15.2	3.1/3.0	93.3	9.6
	air-dried	1.43	1.3/1.2	–	
75C25L	never-dried	14.9	3.3/2.8	91.3	25.1
	air-dried	1.57	1.4/1.2	–	
60C40L	never-dried	15.2	3.4/2.9	90.7	39.2
	air-dried	1.86	1.6/1.3	–	

[a]values for air-dried lignin-cellulose beads were below zero.
[b]not determined.

It is important to mention that the lignin content measured according to the standard method corresponded well to the designed values (Table 18). This implied that the lignin was not essentially lost during the bead-making process.

4.4.3 Morphology of the beads

The microstructure of the beads was analyzed using a scanning electron microscope (Figure 22). The beads exhibited the difference between each other with respect to the microstructure of the surface and the interior. The cellulose beads and the beads with 10% of lignin (Figure 22, A and B) had bigger pores on the surface, whereas the surface of the beads with the higher lignin content was more closed (Figure 22, C and D). All beads also had a skin. This could be deduced from the different appearance of the surface (Figure 22, A–C) and the interior of the beads (Figure 22, E–J). The skin could not always be distinguished for the pure cellulose and 90C10L beads when the edges of the beads were examined under the microscope, which could be attributed to the varying thickness. In the case of other beads, it was about 5 μm.

The analysis of the cross sections showed that the beads had highly porous structures (Figure 22, E–J), which correlated well with the porosity values (Table 18). Similar to the skin, the interior of the beads also differed between the bead types. The inner surfaces of 100C and 90C10L were smoother, and

the pores were generally smaller compared to the beads with 25 and 40% of lignin. The surface of the pores in the case of 60C40L beads was very rough, perhaps due to the lignin particles (section 4.4.1).

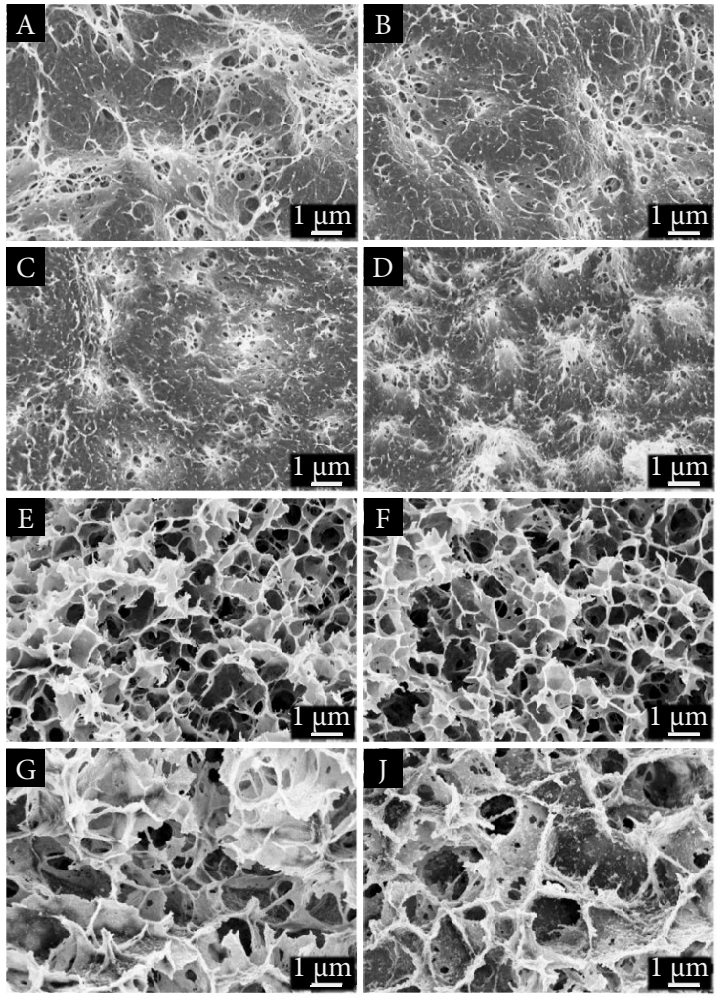

Figure 22. SEM images of liquid nitrogen-frozen and subsequently freeze-dried cellulose and lignin-cellulose beads. Magnification was 10,000. Surface: (A) 100C, (B) 90C10L, (C) 75C25L, (D) 60C40L. Cross-cut sections: (E) 100C, (F) 90C10L, (G) 75C25L, (H) 60C40L.

4.4.4 ATR-FTIR studies

The change in the lignin content in the beads could be clearly observed from the FTIR spectra (Figure 23), especially in the wavenumber range of 1450–

1750 cm^{-1}, where pure cellulose beads showed only a band at 1640 cm^{-1} originated from water (Larkin 2011) and a shoulder at about 1460 cm^{-1}. In the case of the hydrotropic lignin and the lignin-cellulose beads, several bands could be distinguished in this region, and their intensity became higher as the lignin content in the beads increased. The bands were located at 1702 cm^{-1}, carbonyl group stretching; 1601 cm^{-1}, aromatic skeletal vibration (more pronounced in syringyl-type lignin) and C=O stretch; 1513 cm^{-1} aromatic skeletal vibrations (more pronounced in guaiacyl-type lignin) and 1457 cm^{-1}, C-H asymmetric deformations in methyl and methylene groups (Faix 1991; Hergert 1971).

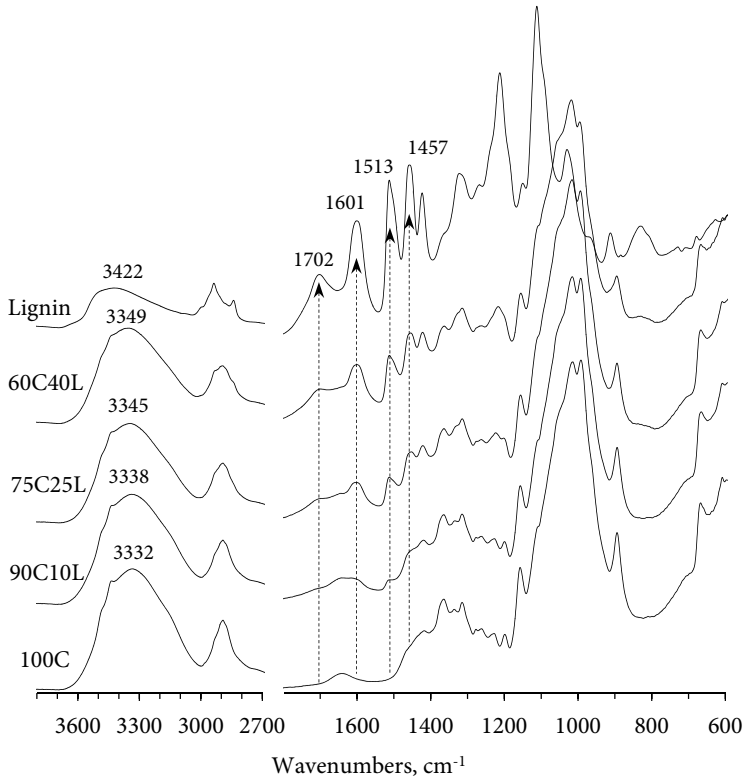

Figure 23. ATR-FTIR spectra of the lignin and lignin-cellulose beads. Note the break in the horizontal axis and the change of the scale.

The results of the FTIR spectroscopy also helped to gain information about the interaction of the lignin and cellulose in the beads by applying the subtraction method (Cañavate et al. 2000; Kondo et al. 1994; Moskala et al. 1985). After the subtraction of the lignin spectrum from the spectra of the lignin-cellulose beads, the resultant spectra looked very similar (Paper IV),

and no significant lateral shifts of the bands were observed, except the hydrogen bonded OH band at 3300–3350 cm^{-1} (Larkin 2011). Such results implied that the lignin and cellulose interacted with each other via the hydrogen bonds. The shift from 3332 cm^{-1} in the case of the pure cellulose beads to 3348 cm^{-1} for 60C40L beads was attributed to the formation of new hydrogen bonds between the lignin and cellulose. The lateral shift of the hydrogen bonded hydroxyl band towards the higher wavenumbers implied the increase in the energy of the OH group and decrease in the energy of the hydrogen bonds. The absence of the shifts of other bands could suggest that there were no other types of interactions between the polymers, besides the hydrogen bonding. However, it was also possible that they were not detected by the FTIR.

4.4.5 Leaching of lignin in water

When the lignin-cellulose beads were placed into water and kept there for several hours, some amount of lignin leached from the beads. This phenomenon was also accompanied by the transition of the solution color from colorless to yellow. Figure 24 shows cumulative leaching of lignin from the beads as a function of immersion time.

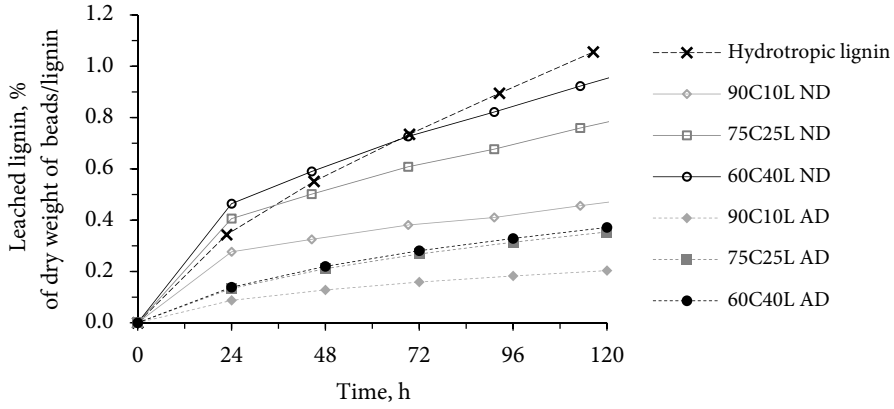

Figure 24. Cumulative leaching of lignin from the never-dried (ND), air-dried (AD) beads and birch hydrotropic lignin into distilled water at room temperature.

As can be observed from the chart (Figure 24), the extent of the leaching estimated on the bead weight basis showed a direct correlation with the lignin content in the beads. For all the lignin-cellulose beads, the rate of the leaching

was higher during the first 24 h and, after that, it slowed down and remained on nearly the same level at least during the studied period. In general, the amount of the leached lignin was still not very high even for the never-dried beads. For instance, the maximum of the leached lignin among the studied samples was found in the case of 60C40L beads after 24 h, and it was 0.46% based on the dry weight of the beads.

A greater quantity of the lignin leached from the never-dried beads than form the air-dried ones (Figure 24). The collapsed structure and the limited accessibility of water to the lignin in the case of the air-dried beads hindered the diffusion of the lignin molecules into the solution.

The birch hydrotropic lignin (R_{bir}) used for the preparation of the beads also released aromatic compounds into the solution, when it was submerged in distilled water (Figure 24). It was determined by the silylation and GC that the lignin contained low molar mass substances, such as syringaldehyde, syringic acid and vanillin, among others (Paper II). They are soluble in water to a certain degree and, therefore, they could gradually leach from the lignin upon contact with water. It was also possible that these aromatic compounds were translated together with the lignin into the beads when the beads were prepared, and they could contribute to the leached material in the case of the beads as well.

4.4.6 Antibacterial properties of the beads

Antibacterial activity studies were performed for different types of never-dried beads, the air-dried beads with 40% of lignin as well as for the birch hydrotropic lignin, which was applied at different dosages. Two types of pathogenic bacteria were used, namely Gram-positive *S. aureus* and Gram-negative *E. coli*.

The beads and the lignin at the dosages of 25 and 50 mg/5 mL of broth did not show any activity against the Gram-negative bacteria. Only slight inhibition could be achieved when the lignin dosage was raised to 100 mg/5 mL of broth (Paper IV). The obtained results were consistent with the results of other studies which reported low antibacterial efficacy of different types of lignins against *E. coli* (Dong et al. 2011; Nada et al. 1989). Besides the experimental conditions and, in particular, the applied dosages, such results could be explained by the chemical composition of the lignin. It has been shown that the antimicrobial properties depend on a type of lignin used for the studies (Telysheva et al. 2005). Furthermore, studies on the antimicrobial properties of different lignin-related model compounds have revealed a

strong relationship between the type of the model compound and the antimicrobial efficacy (Zemek et al. 1979).

Better results were obtained for Gram-positive *S. aureus*. The inhibition correlated with the lignin content in the never-dried beads and with the lignin dosage in the case of the birch hydrotropic lignin (Figure 25). Contrarily, the pure cellulose beads exhibited a positive effect on the growth of *S. aureus*. Surprisingly, air-dried 60C40L beads also promoted the growth of *S. aureus* (Figure 25, A).

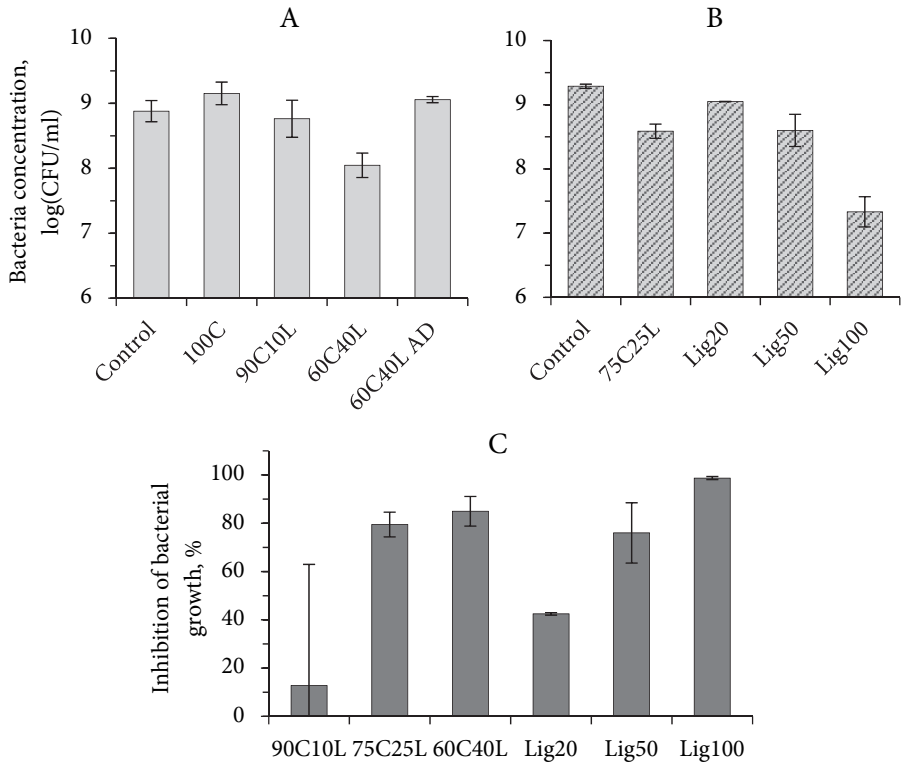

Figure 25. (A) and (B), concentration of *S. aureus* in the broth after the incubation for 24 h at 37 °C with the hydrotropic lignin (Lig20, Lig50 and Lig100) at different loadings (20, 50 and 100 mg, respectively, per 5 mL of broth) and with different types of the beads. (A) and (B) charts show the results of the tests performed on two different days. (C) Inhibition of *S. aureus* growth. AD designates air-dried beads. Initial concentrations of bacteria were 6.38 and 6.26 log(CFU/ml) for (A) and (B) assays, respectively.

The mechanism responsible for the antibacterial properties against *S. aureus* is not evident from the generated results. One could suggest that the mode of

the action could be related to the adsorption of the bacteria onto the surface of the beads (Telysheva et al. 2005) via the interaction with the lignin in the beads. Alternatively, the lignin leached into the solution could interact with the bacteria, or both mechanisms could contribute to the antibacterial action. The hypothesis about the leached lignin was supported by the positive correlation of the antimicrobial efficiency with the results of the leaching experiment (see also Figure 24). In that case, the inhibition of the bacterial growth would resemble the one that is exhibited by phenolic compounds (Cetin-Karaca and Newman 2015b; Cueva et al. 2010; Vigil et al. 2005; Zemek et al. 1979). Notably, in the mentioned studies (Cetin-Karaca and Newman 2015a; Cueva et al. 2010; Zemek et al. 1979), the phenolic compounds were applied in a form of solutions, which could also support the hypothesis related to the interaction of the bacteria with the leached lignin.

An interesting observation made during the experiments was that the pure lignin was not as efficient in the inhibition as the lignin incorporated in the never-dried beads. Comparing the inhibition for the same lignin loading, *e.g.* 20 mg of the hydrotropic lignin and 50 mg (dry weight) of 60C40L beads, it was clear that the beads were superior to the lignin (Figure 25, C). Even the lignin at the dosage of 50 mg/5 mL of TSB did not show superiority over 75C25L never-dried beads despite the 4-fold difference in the lignin loading per tube. Such a result could be connected to the lignin leaching. After keeping the beads and the lignin in water for 24 h, more lignin leached from 60C40L and 75C25L never-dried beads than from the lignin (Figure 24). The corresponding values were 0.46%, 0.41% and 0.34% based on the weight of beads or lignin. It was also possible that the original hydrotropic lignin was modified during the bead-making process that facilitated its antibacterial properties.

A more detailed study on the inhibition of *S. aureus* growth was performed with never-dried 60C40L beads. The load of the beads was increased in each test tube from 2 to 64 pieces stepwise by a factor of two (Figure 26). IC_{50} and IC_{90}, *i.e.* concentrations (or dosages) at which a 50 and 90% inhibition of bacterial growth is reached, were estimated using the obtained results. The IC_{50} was approximately 3 beads per 5 mL of TSB or, in a dry weight, 1.06 mg of beads per 1 mL of the broth. A 90% inhibition could be achieved with 16 beads/5mL TSB or 5.3 mg (dry weight) of the beads per 1 mL of TSB. Both dosages of the beads corresponded to 425.4 μg and 2.13 mg of the lignin in the beads per 1 mL of TSB.

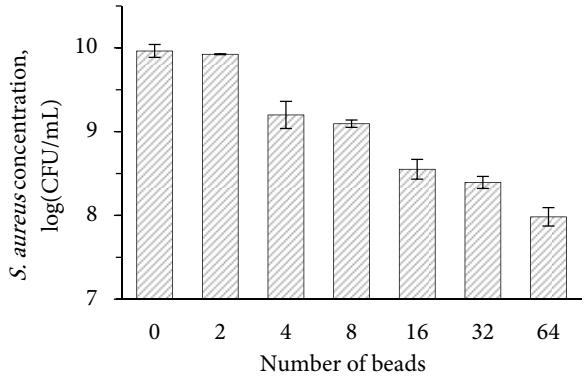

Figure 26. Concentration of *S. aureus* in the broth with a different quantity of never-dried 60C40L beads after the incubation for 24 h at 37 °C. Initial concentration of *S. aureus* was 6.48 log(CFU/mL).

5 Conclusions

Birch wood was successfully delignified with hydrotropic treatment. Addition of formic acid, or hydrogen peroxide, or both simultaneously to the hydrotropic solution increased the delignification degree from 91% for the reference process to 95–97% for the modified treatments. The modification of the solutions also resulted in higher overall selectivity estimated as a ratio of intrinsic viscosity to a kappa number. The selectivity was higher in particular for the hydrogen peroxide-assisted processes. The total yields of the obtained pulps were in the range of 43–48% being lower for the formic acid-aided treatments. However, the pulps produced with the formic acid-aided treatments had higher purity or cellulose content, 89–90% *vs* 83–84% for other pulps. Bleaching of the pulps obtained with the unmodified solution and solution with added formic acid and hydrogen peroxide improved further the purity of the pulps. The content of the residual hemicelluloses was 8.6% and 6.4%, respectively. In general, based on the intrinsic viscosity values, 540–560 mL/g, and the high content of cellulose, the bleached hydrotropic pulps could be potentially used as dissolving grade pulps.

Lignin from birch wood was extracted with the unmodified and modified with formic acid and hydrogen peroxide hydrotropic solutions. The yield of the crude lignin from both treatments was 160 kg/t of wood, and their purity was 95–96%. Both lignins contained a high proportion of S units, and they were similar to each other in many aspects. However, lignin obtained with the modified solution had lower content of *β-O*-4' bond and aliphatic hydroxyl groups, which was related to the higher severity of the modified process.

A hydrotropic process was also used for the fractionation of Brazilian sugarcane bagasse. The yield of main fractions, namely a cellulosic one (pulp) and crude lignin, was 44–67% and 11.8–14.7% based on bagasse. Their purity varied in the ranges of 60–92% and 88–94%, respectively. The purity of the fractions and the yield of lignin were higher at more severe process conditions, *i.e.* a higher temperature, longer treatment time and/or lower starting pH. Isolated bagasse hydrotropic lignins had a ratio of S/G units of 1.5–1.7, and they also contained a high amount of *p*-coumaric acid residues. Analyses of the spent solutions showed that, besides dissolved lignin, they contained dissolved hemicelluloses, sugar monomers, acetic and formic acids, furfural and other compounds. The composition of the spent solutions varied greatly depending on the process conditions.

Lignin isolated by a hydrotropic method from birch wood was used for the preparation of spherical particles via co-dissolution with cellulose in 7% NaOH/12% urea aqueous solution and shaping. Prepared beads in a never-dried state were highly porous particles. In contrast to the pure cellulose beads, the lignin-cellulose beads inhibited growth of Gram-positive bacterium *Staphylococcus aureus*, and the inhibition efficiency was higher for the beads with a higher lignin content.

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