LIGNOCELLULOSIC MATERIALS IN HYDROLYSIS AND SPENT LIQUORS

A Thesis Presented to the Faculty of Graduate Studies of Lakehead University

by

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DEDICATION

To my grandfather Boris Semenovich Besprozvanniy, who is always on my mind

To my family for their invaluable support

To Heidi McCue for her patience and support

ABSTRACT

Biomass pretreatment is widely used for softening biomass prior to its disintegration for value added product production. There are numerous methods for biomass pre-treatment, among which alkaline pulping and acid hydrolysis are the most widely applied. Hydrothermal treatment or autohydrolysis has a similar concept of action to acid hydrolysis, while autohydrolysis is the chemical-free and environmentally friendly technology. Hydrothermal and alkaline pulping pretreatments lead to lignin and polysaccharide dissolutions in hydrolysate and spent liquor (SL), respectively. Lignin and carbohydrates presented in hydrolysates and SL can be used for manufacturing value-added products. Lignin can be employed in carbon fiber, phenol formaldehyde, and hydrogen productions, for example, and hemicelluloses could be used for ethanol or xylitol production. However, the direct conversion of lignocellulosic materials present in hydrolysates and SL to value-added products is expensive due to their low concentrations. Lignocelluloses can be isolated from these liquors via acidification, solvent precipitation and membrane filtration. It is well known that lignocelluloses have different properties. Pulping and pretreatment processes have also great impacts on the properties of the extracted lignocellulose in liquors. Despite their effectiveness in isolating lignocelluloses, the impact of lignocelluloses properties on the efficiency of extraction processes is unknown. In this dissertation, the effect of autohydrolysis parameters on the properties, structure and composition of extracted lignocellulosic material was investigated. Also, the effect of lignocellulose's properties presented in hydrolysates and SL on the efficiency of acidification and solvent extraction was examined.

The efficiency of lignocelluloses extraction from softwood chips via flow through autohydrolysis pretreatment was investigated in this PhD study. The highest temperatures applied in the autohydrolysis process were found to yield maximum removal of lignin from wood; whereas, prolonged hydrothermal treatment increased the removal of hemicelluloses from wood. In addition, it was discovered that a low flow velocity led to higher lignocelluloses removal. However, at high liquid flow rates hemicelluloses with larger molecular weight (Mw) were extracted. Gel permeation chromatography (GPC) analysis revealed the presence of lignin-carbohydrate complexes (LCC) in the hydrolysates. The GPC analysis showed that the hydrolysate generated in autohydrolysis treatment with a high liquid to solid (L/S) ratio contained a significantly lower amount of lignin presented in the LCC form. Moreover, 2D HSQC NMR

spectroscopy revealed the existence of LCC linkages only in lignocellulosic materials obtained via lyophilisation of hydrolysate produced at the lowest autohydrolysis severity.

The material generated as the result of mixing ethanol with hydrolysates produced at low autohydrolysis intensity showed the highest sugar content and negligible lignin content. Acidification of hydrolysates generated at high severity conditions led to extraction of almost pure lignin. The isolated material produced from hydrolysates due to ethanol or acid addition showed approximately twice as much heat capacity values as that of dried hydrolysates. The ^{1}H-NMR analysis revealed that the extracted materials via acidification contained more methoxyl groups and a lesser degree of cross-linking than those present in hydrolysate. The GPC analysis also suggested the presence of LCC in the hydrolysates before and after treatment processes.

In addition, the current research studied the properties, structures, and composition of lignocelluloses in spent liquors before and after isolation. Furthermore, the effect of organic solvents (i.e., ethanol, acetone, isopropyl alcohol) on lignocelluloses removal and the properties of the isolated lignocelluloses were determined. In examining the impact of the solvent type, it was discovered that hemicelluloses isolation had the highest isolation at the lowest solvent concentrations. While acetone and isopropyl were found to be efficient for lignosulfonate extraction, the highest hemicelluloses removal was achieved with ethanol treatment. The molecular weight and charge density of isolated lignocelluloses was higher when ethanol was used in comparison with acetone and isopropyl. The examination of the heating values, ignition temperatures, and heat capacity values of the precipitates revealed the possibility of using these materials as fuel or additives in briquette production. Additionally, a direct correlation between the inorganic content of the extracted lignocelluloses and their T_g values was discovered. The results achieved in this dissertation can be used as guidelines for developing biorefining processes for generating lignocelluloses with different properties from hydrolysate or spent liquors of different pulping processes. In addition, information available in literature on the structure and properties of lignin-carbohydrate complexes (LCC) as well as their production, analysis and application were discussed and critically evaluated.

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LIST OF ABBREVIATIONS AND SYMBOLS

A.donax	Arundo donax
Ar	Aromatic unit
Ara	Arabinose
ASGPR	Asiaglycoprotein receptors
BE	Benzyl ether
C	Carbon
C_9	Phenylpropane unit
Carbohydrates-DDQ	Carbohydrates liberated from LCC due to DDQ application
CC_{50}	Cytotoxic concentration
C_p	Heat capacity
DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
DMSO	Dimethyl sulfoxide
DMSO-d6	Deuterated DMSO
EC_{50}	Effective concentration
FTIR	Fourier-transform infrared spectroscopy
G_2	Guaiacyl lignin units
Gal	Arabinogalactan (Galactan)
GC/MS	Gas chromatography–mass spectrometry
GGM-L	Galaglucomannan-lignin
GGM-L-Pectin	Galaglucomannan-lignin-pectin

GLC	Gas-liquid chromatogram
Glu	Glucan
Glu-L	Glucan-lignin
Glu-Xyl-L	Glucan-xylan-lignin
GM	Glucomannan
GM-L	Glucomannan-lignin
GM-L-Xyl	Glucomannan-lignin-xylan
GPC	Gel permeation chromatography
H	Hydrogen
$H_{2,6}$	Hydroxyphenyl lignin units
HIV	Human immunodeficiency virus
IR	Infrared spectroscopy
IV-DP	Intrinsic-differential pressure
L	Lignin
L-C bonds	Lignin-carbohydrate bonds
LCC	Lignin-carbohydrate complex
LCCs	Lignin-carbohydrate complexes
LCC-AcOH	LCC extracted with acetic acid (AcOH)
LCC-We	LCC extracted in accordance with the method developed by Watanabe et al. (1987)
LFP complex	Lignin–ferulate–polysaccharide complex

Man	Mannan
MW	Molecular weight
MWL	Milled wood lignin
N	Nitrogen
NMR	Nuclear magnetic resonance
NSSC	Neutral sulphite semichemichal process
O	Oxygen
PhyGlc	Phenyl glycosidic
PHL	Prehydolysis liquor
Rha	Rhamnose
RI	Reflective index
S	Sulphur
$S_{2,6}$	Syringyl lignin units
SI	Selective index
SL	Spent liquor
TBAH	Tetrabutylammonium hydroxide
T_g	Glass transition temperature
THF-d8	Deuterated tetrahydrofuran
T_{onset}	Degradation onset temperature
UV	Ultraviolet
Xyl	Arabino-4-O-methylglucoronoxylan (Xylan)

Xyl-Glu-L	Xylan-glucose-lignin
Xyl-L	Xylan-lignin
Xyl-L-Ara	Xylan-lignin-arabinose
Xyl-L-Gal	Xylan-lingin-galactan
Xyl-L-Glu	Xylan-lignin-glucan
Xyl-L-GM	Xylan-lignin-glucomannan
δ_c	^{13}C NMR chemical shift
δ_H	^{1}H NMR chemical shift
^{13}C NMR	Carbon NMR spectroscopy
^{1}H NMR	Proton NMR spectroscopy
2D HMBC NMR	Two-dimensional heteronuclear multiple bond coherence NMR
2D HSQC NMR	Two-dimensional heteronuclear single quantum coherence NMR
2D TOCSY NMR	Two-dimensional total correlation NMR
3D HSQC-TOCSY NMR	Three-dimensional HSQC-TOCSY NMR

LIST OF CHEMICAL COMPOUNDS

$(CH_3CO)_2O$	Acetic anhydride
AcOH	Acetic acid
$Ba(OH)_2$	Barium hydroxide
C_2H_6OH	Ethanol
$C_4H_{10}O$	Ethyl ether
C_5H_5N	Pyridine
$C_6H_8O_6$	Ascorbic acid
$CF_3SO_3CH_3$	Trifluoromethanesulphonate
CH_3I	Methyl iodide
D_2O	Deuterium oxide
H_2O	Water
H_2SO_4	Sulphuric acid
H_3BO_3	Boric acid
HCl	Hydrochloric acid
N_2	Nitrogen gas
Na_2SO_4	Sodium sulphate
$NaBH_4$	Sodium borohydride
NaH	Sodium hydride
NaI	Sodium iodide
$NaIO_4$	Sodium periodate

NaOH	Sodium hydroxide

INTRODUCTION

Biomass has revealed great potential for fuel and value-added product manufacturing (Santos et al. 2013). Biomass consists of lignin, hemicellulose, cellulose and extractives (Du et al. 2013). These polymers are widely used for manufacturing different products. Isolated hemicelluloses and lignin could be used for ethanol and carbon fiber manufacturing, for example (Pothiraj et al. 2006; Chandel et al. 2011; Lora and Glasser 2002; Vishtal and Kraslawski 2011), and cellulose is a key component in papermaking processes (Zheng et al. 2009).

Pretreatment is widely used for softening the structure of biomass prior to chemical, physical or biochemical conversion (Menon and Rao 2012). Autohydrolysis is a chemical-free, efficient, and environmentally friendly method of biomass pretreatment (Carvalheiro et al. 2008; Sixta et al. 2013). In autohydrolysis, biomass is treated with liquid hot water or steam, which causes lignin and carbohydrate dissolution in hydrolysate (Carvalheiro et al. 2008). Batch and flow-through systems are employed for autohydrolysis operations (Galia et al. 2015). Batch based autohydrolysis is widely discussed in literature (Galia et al. 2015; Kumar and Christopher 2017). Although, the impact of flow-through technology on the lignocelluloses of biomass is not well known scientifically, this technology has been widely applied in industry. This method allows for improved lignin and hemicelluloses extraction and prevents the formation of fermentation inhibitors (Lui and Wyman 2003). Available literature discusses the use of flow though autohydrolysis of herbaceous plants, whereas coniferous species results in a more condensed structure and different compositions (Fox and McDonald 2010; Sjöström 1993; Menon and Rao et al. 2012). Softwood species have widely been used in Canada in pulp and paper, and recently in hydrolysis based dissolving pulp production processes. Consequently, it is important to analyse the impact of flow-through autohydrolysis on the extraction of lignocelluloses from softwood species.

In woody biomass, the majority of lignin and carbohydrate molecules are covalently linked in lignin-carbohydrate complex (LCC) form (Lawoko et al. 2005; Du et al. 2014). It was reported that, all lignin fragments are bonded to carbohydrates in softwood species (Lawoko et al. 2005); whereas, 47-66% of lignin moieties *in vivo* anchored with hemicelluloses in hardwood species. The presence of bonds between lignin and carbohydrates negatively affects the efficiency of pre-treatment technology and the purity of the obtained material (You et al. 2015; Shevchenko and

Bailey 1996). In addition, the presence of LCC in herbaceous and forage plants limits the digesting ability of ruminates (Lam et al. 2003). The evidence of LCC in kraft pulp and various spent liquors has been recently reported (Lawoko et al. 2005; Fatehi et al. 2016). However, there are no publications discussing LCCs presence in autohydrolysis liquor and its derivatives.

Alkaline pretreatment technology involves the application of strong bases, such as sodium hydroxide, ammonia and calcium hydroxide (Kim et al. 2016). Alkaline pretreatment leads to lignin removal and pore surface enlargement, which is improving enzymatic hydrolysis performance (Kim et al. 2016). In the neutral sulfite semichemical (NSSC) process, which is widely applied for corrugated medium paper production, the biomass is treated with sodium sulphite and carbonate (Area et al. 2000), which leads to lignocelluloses dissolution in pulping spent liquor (SL). Presently, SL is treated in the wastewater systems (Sitter et al. 2014). The development of efficient, inexpensive and reliable methods for isolating lignocelluloses from SL will promote NSSC-based biorefinery development.

Acidification, organic solvents supplement, flocculation and membrane dialysis are the most widely applied technologies for lignocelluloses extraction from pretreatment liquors (Liu et al. 2011 a,b; Shi et al. 2011; Sitter et al. 2014; Rojas et al. 2006). Sitter et al. (2014) reported that 37% of lignin and 37% of hemicelluloses were removed from the SL of NSSC process via flocculation due to a supplement of polyethyleneimine (PEI) polymer. Application of membrane dialysis with a molecular weight cut-off of 1000 g/mol isolated around 20% of lignin content from the black liquor of kraft pulping process (Rojas et al. 2006). However, the removal via flocculation may be expensive, and membrane dialysis technology is limited due to a possibility of fouling (Tarasov et al. 2017; Guo et al. 2012). Considering these limitations, the extraction of lignocelluloses from SL of NSSC process via acidification, organic supplement and combined acid/solvent treatment were considered the most industrially attractive methods, which also have been applied in this study. Cave and Fatehi (2015) reported that acidification (pH 1.8) removed 6% of lignin and 53% of polysaccharides from the SL of NSSC process. In another study, it was found that acidification led to the extraction of 47% of lignin presented in the liquor produced in hydrothermal pretreatment (PHL) of hardwood chips (Liu et al. 2011a). Application of organic solvents for the lignocellulosic material extraction from various prehydrolysis liquors is widely described in the literature (Liu et al. 2011a,b; Villar et al. 1993). Caperos and Villar (1990) reported the extraction of 22% of lignin and 40% of hemicelluloses from eucalyptus kraft black liquor via ethanol supplement at ratios of

0.2-12L of ethanol per 1 L of black liquor. Mixture of kraft black liquor with large amounts of ethanol, methanol or isopropanol (solvent/black liquor 10/1 v/v) removed 60% of the lignin presented in the black liquor (Villar et al. 1993). Also, it was found that a combination of acidification and ethanol treatment resulted in the isolation of 50% of lignin and 20% of carbohydrates from hardwood PHL (Liu et al. 2011 a,b). However, information on the extraction of lignin and hemicelluloses from the SL of NSSC process via organic solvents or combined application of acidification and solvent treatment is limited.

It was found that, the methods used for lignocellulosic material precipitation affect the properties of the resulting materials (Tarasov et al. 2017; Liu et al 2011a). The properties of precipitated lignocelluloses affect the end-use application of the obtained materials. For instance, molecular weight (Mw) distribution affects reactivity and physicochemical parameters of lignin (Cao et al. 2012; Chen and Li 2000). Lignosulfonates (lignin derivatives presented in SL) with low and high molecular weights could be used for dispersants and flocculants manufacturing, respectively (Aro and Fatehi 2017). Thermal properties are also important, due to a possible application of lignocellulosic material as binders for anthracite and coal briquettes (Lumadue et al. 2012; Malhotra 2010). Ash content and calorific values are the key parameters affecting lignocellulosic material application as a fuel source. High ash content will lower the stove efficiency, while a low calorific value affects profitability by increasing fuel consumption (Tarasov et al. 2013). Additionally, ignition temperature is important too as it predetermines the energy consumption required for fuel inflammation. Heat capacity (C_p) is another important parameter for material application as a fuel or a binder. The C_p value depends on the mobility of lignin molecules and cross-linkage degree (Olsson and Salmen 1997). An evaluation of glass transition temperature (T_g) is also essential to determine the range of temperatures for transition of polymers from solid to rubbery state (Seyler 1994). Since the T_g value is affected by molecular weight, molecular mass distribution and cross-linkage degree (Hatakeyama and Hatakeyama 2010), lignin's T_g value has a major influence on the viscoelastic properties of biomass and its derivatives (Horvath et al. 2011). However, it is unclear how the proposed extraction methods will impact the properties of isolated lignocelluloses, which is one objective of this dissertation.

Rationale behind the study

- Lack of fundamental studies on correlating autohydrolysis process parameters and the properties of extracted lignocelluloses for spruce species
- Lack of fundamental studies on the presence of lignin-carbohydrate complexes in hydrolysate and its extraction from hydrolysate following different methods
- Lack of fundamental studies on the impact of isolation processes for extracting lignocelluloses from the SL of NSSC process
- Lack of fundamental studies on correlating the properties of isolated lignocelluloses and their potential end-use applications

Novelty of current research includes

- Critical review on the existence, analysis, production and application of lignin-carbohydrate complex (LCC). Investigation on the impact of flow-through autohydrolysis process conditions on lignocelluloses extraction from sprue species.
- Investigation on the existence of lignin-carbohydrate complexes (LCC) in hydrolysate and its removal following different isolation methods
- Investigation on the key properties of lignocelluloses isolated from the SL of NSSC process and suggestion of possible industry applications.

The overall objectives of this study were to

- Conduct a literature review on the existence, properties, isolation and application of LCC;
- Study the influence of flow-through autohydrolysis treatment parameters on extraction efficiency and the properties of lignocelluloses from spruce species;
- Study the existence and structure of LCC in hydrolysate;
- Study the efficiency of different processes for extracting lignocelluloses from the SL of NSSC process; and to
- Study the properties of extracted lignocelluloses from the SL for identifying their end-use applications.

In order to achieve the abovementioned objectives, the following chapters were developed. Chapter one discusses the methods employed in the current thesis.

Chapter two presents the summary of the literature relevant to the current dissertation. This part reviews publications related to the existence, analysis, production and application of LCC.

Chapter three discusses the presence of lignin-carbohydrate (LCC) in the hydrolysate produced via flow-through autohydrolysis of spruce wood chips. Additionally, the effect of flow-through autohydrolysis parameters on the efficiency of lignocellulosic material extraction and on the properties of lignin and hemicelluloses is studied. The obtained results suggest that all examined hydrolysates contain LCC. It is found out the hydrolysates produced at the highest liquid to solid (L/S) ratio contain the lowest amount of LCC. However, membrane dialysis demonstrates that these LCCs are involving large lignocellulosic fragments. Generally, hydrolysates contain a significant amount of lignin in LCC forms, but LCC has a lower molecular weight than unbound lignin. Also, it is noted that the Mw of hemicelluloses is slightly increased with the rise of liquid flow velocity in the autohydrolysis process, which can be related to its high efficiency in removing hemicelluloses with large molecular weights.

Chapter four focuses on the properties and structures of the precipitates obtained from flow-through autohydrolysis liquors of spruce species. The chapter contains two main sections. In the first section, the property of the precipitates is presented. The precipitates are extracted by ethanol or acid supplement. It was found that the precipitates produced hydrolysates produced under low process severity had large lignin molecules and small carbohydrates. The precipitates obtained via ethanol or acid treatment possess a significantly higher heat capacity in comparison with those obtained via directly drying of hydrolysates. In the second section, the existence of LCC in the precipitates is found. The LCC found in the dried hydrolysate is produced under a low hydrolysis intensity, but it is not found in the precipitates produced under other conditions.

Chapter five focuses on the lignosulfonate extraction from the SL of NSSC process. This chapter contains three main sections. The first section discusses the efficiency of lignocelluloses precipitation via mixing SL with ethanol, acetone, and isopropyl alcohol. The impact of acid pretreatment on the efficiency of solvent isolation is also studied. In the second part, the charge densities and molecular weight (Mw) of the produced precipitates are presented. In the third section, the scheme of the process for lignosulfonate extraction from the SL of NSSC pulping

process is suggested. The results of this study demonstrate that acid pretrearment hampers the efficiency of the solvent extraction in isolating lignocelluloses. Ethanol generates the lignocelluses with higher Mw and charge density than other solvents.

Chapter six focuses on the thermal properties of the lignocellulosic material extracted from SL via organic solvents and acidification. Elemental analysis of the precipitates is also presented. It was found that the calorific values of the materials obtained from untreated SL are similar to those obtained from wood pellets. The ignition temperatures of the obtained precipitates are lower than those of commercial lignosulfonates and wood. Also, it is claimed that glass transition temperature and heat capacity values are related to the inorganic compounds of the extracted materials. Based on the results, the application of the extracted materials as fuel additive seems reasonable. However, their ash content should be taken care of.

Chapter seven is comprised of the conclusions and suggestions for future research.

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Chapter 1 Methodology

1.1 Biomass autohydrolysis and membrane dialysis of hydrolysis liquors

Hydrothermal or autohydrolysis treatment of biomass for lignocellulosic material extraction is widely discussed in the literature (Song et al. 2008; Leppanen et al. 2011). In this PhD thesis, the autohydrolysis of softwood chips was conducted using a flow-through pulping digester. Figure 1.1 depicts the schematic diagram of flow-through autohydrolysis process for producing hydrolysate. In this study, wood chips were continuously treated with hot water in a closed-loop system at certain processing conditions. After each operation, the spent liquor, called hydrolysate, was collected via a drainage valve shown in Figure 1.1. Considering amounts of lignin and hemicelluloses in the initial wood material and in the hydrolysate, the efficiency of hydrolysis process was determined.

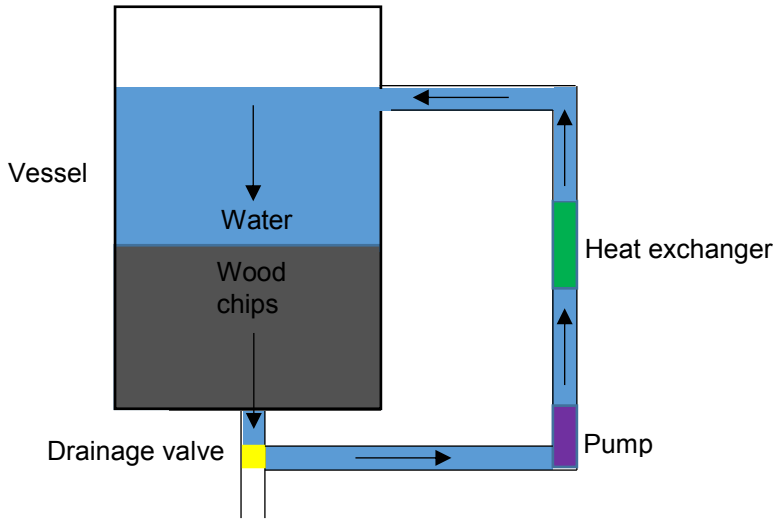

Figure 1.1 Schematic diagram of flow-through autohydrolysis process of biomass.

Membrane dialysis was described as an efficient technique for fractionating small and large molecules present in liquors (Oinonen et al. 2015; Chen et al. 2014; Fatehi and Chen 2016). Herein, semipermeable membrane tubes with particular pore sizes (molecular weight cut-off or MWCO) were filled with hydrolysate and then the tubes were placed into deionized water. Molecules with a larger size than the pore diameter of the membrane could not pass through the membrane, whereas the molecules with a smaller size diffused through the membrane. After a certain period

of time, the system reached a balance (equilibrium) and the water was required to be replaced. Frequent replacements and stirring of this system accelerated the dialysis process and led to complete removal of molecules with the size smaller than the size of the membrane pores (Venekei 2013).

1.2 Characterization of hydrolysates and spent liquors

Application of UV spectrophotometry is widely reported in the literature as an efficient method for determining lignin content of spent and hydrolysis liquors (Lee et al. 2013: Liu et al. 2011a). In the UV spectrophotometry, the wavelength of light, emitted by a lamp, was narrowed by monochromator and passed though the sample. The sample absorbed the light and the detector measured the intensity of absorbance (Skoog et al. 2007). Functional groups of lignin macromolecules demonstrated two absorbance bands at 200-230 nm and 260-280 nm, with maximum at 205 nm and 280 nm (Lee et al. 2013). For quantification of acid-insoluble lignin, the absorbance at 205 nm was chosen (Liu et al. 2011a; Fatehi et al. 2016) due to the presence of absorbance peaks for furfural at 280 nm (Stenius 2000). The amount of lignin was calculated using a calibration curve.

The ion-exchange chromatography (IC) is a well-known technology for assessing the compositions of polysaccharides and monosaccharides in liquors (Sullivan and Douek 1994; Verhaar and Dirkx 1977). As the IC technology cannot detect polysaccharides, polysugars were hydrolysed into monosugars prior to IC experiments in this thesis work (Liu et al. 2008). The basic principle of IC is the separation of molecules via ionic interactions. In this study, the CarboPacTM SA10 column was used as a stationary phase and sodium hydroxide as a mobile phase. The stationary phase in the IC column contains charged functional groups, which interact with oppositely charged ionised molecules and allows targeted moieties to bond and separate. The surrounding environment's pH affects the net charge of the molecules (Jungbauer and Hahn 2009). Sugars are weak acids, which can be ionised to anionic form at high pH levels. The pH level in the mobile phase affects the retention behaviour of sugars and at the same time, the electromechanical response of electrodes. Ionised sugars accumulate on the gold electrode, and can be recognized via oxidative desorption process (Sulsom et al. 2014).

An elemental analyzer is a powerful tool in estimation of carbon, hydrogen, sulphur and nitrogen contents of different materials (Fadeeva et al. 2008; Singh et al. 2005). In this work, the materials

were combusted in a furnace at 1000 °C, which converted carbon to carbon monoxide, hydrogen to water, nitrogen to nitrogen gas and sulphur to sulphur dioxide. Gases released during these processes allowed the estimation of organic elements of the materials (Thompson 2008).

Fourier transform infrared spectrophotometery (FTIR) is a well-known method for the investigation of functional groups available on the surface of materials (Faix 1991; Postma et al. 2014; Xu et al. 2013). In this analysis, samples are subjected to IR radiation. Molecules selectively absorb IR radiation of certain wavelengths, which leads to alteration of the electrical dipole moment of molecules and causes transformation of vibrational energy levels of molecules from the ground state to an excited state. The vibrational energy interval designates the frequency of absorption peaks. The quantity and intensity of peaks attribute to the vibration frequency and changes of the dipole moment of sample molecules, respectively (Mainka et al. 2015). IR frequency will be intensively absorbed when its photon energy coincides with the vibrational energy of the molecule. Thus, FTIR technology provides detailed information about the chemical composition of materials (Griffiths and De Haseth 1986: Mainka et al. 2015), and is used in this work.

The chemical structure and composition of biomass can be analyzed via nuclear magnetic resonance spectroscopy (NMR) technology (Capanema et al. 2004; Nagy et al. 2010; Ma et al. 2012). The main principle of NMR is based on the fact that all atomic nuclei are electrically charged and many of them have a spin moment (Shashhidher et al. 2011). The atomic nucleus in functional groups of polymers demonstrates different resonances under a magnetic field (Gerothanosis et al. 2002). Elucidation of polymers by radio waves magnetizes atomic nuclei (e.g. ^{1}H or ^{13}C) of different functional groups. Magnetized nuclei move from a ground state to an excited state and the excited nuclei absorb some energy. The transmission of energy proceeded at the wavelength that corresponds to radio frequencies and when the nuclei return to their initial state, energy is released at the same frequency (Shashhidher et al. 2011; Gerothanosis et al. 2002). This released energy is scanned and interpreted at chemical shifts and considered as footprints of functional groups, as relaxation times of nuclei are determined by surrounding chemical groups (Gerothanosis et al. 2002). This method was extensively used in this dissertation to analyze the properties of extracted biomass.

1.3 Properties of lignocellulosic materials

Thermal properties of lignocellulosic materials isolated from liquors were analysed via thermogravimetric analyzer (TGA) and differential scanning calorimetry (DSC) (Singh et al. 2005; Sammons et al. 2013). TGA technology represents change in the weight of samples as a function of temperature and/or time (Gabbot 2008). Mass lost at a certain temperature is related to the decomposition of materials. The DSC is a powerful instrument measuring the changes of thermodynamic characteristics of polymers along with temperature against time (Spink 2008; Gill et al. 2010). In the DSC experiment, a thermoelectric disc is employed for heat transfer between a sample and a reference. The tested material is placed in an aluminum pan (sample pan) and an empty pan is applied as a reference (reference pan) (Hetayothin 2010). In this analysis, both pans are heated with the thermoelectric disk at a linear heating rate to a certain temperature. The difference between the temperatures of the sample pan and control pan (heat flow) is estimated by thermocouples. The measured heat output is then plotted as a function of temperature (Gill et al. 2010). The data obtained allows us to estimate the heat capacity (C_p) of the sample, which is defined as the amount of heat required for raising the samples' temperature by one degree (Spink 2008). The T_g value is the range of temperature, at which the polymer is transferring from the solid brittle state to the soft rubbery state, or vice versa (Seyler 1994). When the temperature of the tested material reaches the T_g region, the step-change in molecular mobility occurs, which leads to the endothermic step increase and is reflected on the plot (Garcia-Fernandez et al. 2010; Thakur 2011). As the glass transition occurs over a range of temperatures, the value, which corresponds the midpoint of the slope of the function line, is considered as T_g (Garcia-Fernandez et al. 2010; Hetayothin 2010).

Heating value or calorific value of the substance is defined as the amount of energy per unit of mass liberated from combustion of biomass. Bomb calorimetry is a widely applied technology in analyzing heating values of various materials (Obernberger and Thek 2010). This technology includes combustion of the sample in an atmosphere of pure oxygen in a sealed steel container (bomb). Samples with a certain mass are ignited with electricity via fuse. Energy released due to combustion increases the temperature in the bomb and these changes are monitored with a thermometer. This data, along with initial weight of the sample, allows the equipment to determine the heating value of the testified material (Piedade 1999).

As described in the literature, gel permeation chromatography (GPC) is widely applied for analysis of molecular weight (Mw) of polymers (Kostanski et al. 2004; Tolbert et al. 2014). Molecular weight is determined by separation of molecules based on their hydrodynamic volumes using size-selective porous gel particles as a stationary phase and solvent or aqueous solution as a mobile phase (Gellerstedt 1992; Coskun 2016; Xu et al. 2013). When samples pass through the chromatographic column containing the gel, large fragments are separated first and small ones are eluted in the next stages of experiment. The time that is consumed for molecules with a certain size to elute from the column (retention time) is monitored for molecular weight analysis. Molecular weight could be estimated by comparing the elution time of samples with standard polymers (Striegel et al. 2009).

Charge density analysis of the samples was conducted using a particle charge detector (PCD) according to the method established earlier (Saeed et al. 2011). In this analysis, samples were neutralized by a solution containing standard polymers with an opposite charge density, i.e., poly diallyldimethylammonium chloride (PDADMAC) for anionic surfaces and polymers, and potassium polyvinyl sulfate (PVSK) for cationic surface and polymers (Gaudreault et al. 2009). The samples were dissolved in deionized water at 1wt.% concentration followed by separation of soluble and insoluble fractions. The charge density of the soluble and insoluble portions of lignocellulosic samples was determined via direct and back titration, respectively. The suspension containing the soluble part of the sample is placed in the measuring cell of PCD and titrated against the standard solution of PDADMAC. The volume of standard solution (vol. PDADMAC, 0.005M) and the initial mass sample in the mixture allows estimation of the charge density of soluble part of the tested material (Inwood et al. 2018).

The water-insoluble part of the sample is mixed with the standard solution of PVSK (0.005M). The volumes of PVSK in the solute before and after sample treatment are estimated via titration with PDADMAC standard solution. The charge density of the insoluble portion of the tested material is then calculated as explain in an earlier work (Inwood et al. 2018).

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Chapter 2 A review: Lignin-carbohydrate complexes: properties, applications, methods for extraction and analysis

2.1 Abstract

The complexity of lignin and hemicellulose segmentation has been known since the middle of the 19th century. Studies confirmed that all lignin units in coniferous species and 47-66% of lignin moieties in deciduous species are bonded to hemicelluloses or cellulose molecules in lignin-carbohydrate complexes (LCC). Different types and proportions of lignin and polysaccharides present in biomass lead to the formation of LCC with a great variety of compositions and structures. The nature and amount of LCC linkages and lignin substructures affect the efficiency of pulping, hydrolysis and digestibility of biomass. This review chapter discusses the structure, compositions and properties of LCC present in biomass and in the products obtained via pretreating biomass. Methods for extracting, fractionating and analyzing the LCC in biomass, pulp and spent pulping liquors are critically reviewed. The main perspectives and challenges associated with these technologies are extensively discussed. LCC could be extracted from biomass following varied methods, among which dimethyl sulfoxide or dioxane (Björkman's) and acetic acid (LCC-AcOH) processes are the most widely applied. The oxidation and methylation treatments of LCC materials elucidate the locations and frequency of binding sites of hemicelluloses to lignin. The two-dimensional nuclear magnetic resonance (NMR) analysis allows the identification of the structure and the quantity of lignin-carbohydrate bonds involved in LCC. LCC application seems promising in medicine due to its high anti-HIV, anti-herpes and anti-microbial activity. In addition, LCC was successfully employed as a precursor for preparation of spherical biocarriers, in the past.

2.2 Introduction

Biomass shows great potential for fuel and non-fuel applications. It is a mixture of cellulose, hemicellulose, lignin and extractives (Santos et al. 2013), which are considered as the most common natural polymers on earth (Lawoko 2013).

Lignin, hemicellulose and cellulose form unique and complex structures in wood. Softwood species include 33-42% cellulose, 22-40% hemicellulose, 27-32% lignin and 2-3.5% extractives (Sjostrom 1993; Nhuchhen et al. 2014). Hardwood species contain 38-51% cellulose, 17-38% hemicellulose, 21-31% lignin and 3% extractives (Nhuchhen et al. 2014; Menon and Rao 2012). The ranges of the amount of lignin, hemicelluloses, cellulose and extractives in herbaceous plants are 0-40%, 20-50%, 25-95% and 4-9%, respectively (Arsene et al. 2013; Smit and Huijgen 2017; Menon and Rao 2012).

These polymers are widely applied for manufacturing different products. For example, hemicelluloses are used for ethanol or xylitol production (Pothiraj et al. 2006; Chandel et al. 2011). Lignin is used for producing carbon fibers and dispersants (Lora and Glasser 2002; Vishtal and Kraslawski 2011). Cellulose is used in pharmaceuticals and the papermaking industry (Zheng et al. 2009; Shokri and Adibka 2013; Garcia et al. 2011).

To produce value-added products, biomass components should be separated. Hydrolysis, pulping, and bioconversion processes are considered the dominant fractionation processes of biomass. Hydrolysis aims at separating hemicelluloses from other components of biomass. Pulping processes are common methods to obtain cellulosic materials used for producing various paper grades. Bioconversion procedures can also be applied for separating cellulosic sugars from the remaining part of biomass. These methods are based on liberation of the bonds of lignin and holocellulose.

Despite its effectiveness, biomass fractionation is characterized by some challenges, one of which is the difficulty in separating lignin from carbohydrates (Balakshin et al. 2007; Kim et al. 2016). Lignin and carbohydrate moieties are chemically bonded in native biomass forming a lignin-carbohydrate complex, LCC (Du et al. 2014; Balakshin et al. 2014). LCC linkage plays a crucial role in wood structure since all lignin moieties in softwoods (Lawoko et al. 2005) and 47-66% of lignin fragments in hardwoods (Henriksson et al. 2007) are bonded to carbohydrates, mainly to

hemicellulose (Balakshin et al. 2011). Numerous studies report the presence of LCC in native biomass materials in coniferous, deciduous and non-wood plants (Lawoko et al. 2005; Dammstrom et al. 2009; Yao et al. 2016; You et al. 2015; Zhang et al. 2016). Due to its strong bonding, the presence of LCC affects the overall extraction of lignin and carbohydrates (You et al. 2015; Balakshin et al. 2014; Shevchenko and Bailey 1996). For example, a low yield of kraft pulping process is related to the challenge in breaking lignin-carbohydrate linkages in hardwood species (Silva et al. 2017), which can be attributed to the alkaline stability of LCC bonds. In addition, the formation of lignin-hemicellulose linkages in pulp during the kraft pulping process has been suggested (Gierer and Wannstrom 1986; Tenkanen et al. 1999). Several studies confirm the existence of lignin-hemicellulose linkages in softwood (Lawoko et al. 2003) and in hardwood (Henrikson et al. 2007; Li et al. 2011) kraft pulps. Chen et al. (2010) report that autohydrolysis of hardwood results in extraction of xylan during the initial stage of autohydrolysis, whereas the isolated xylan units are found to be associated with lignin in the later stage of autohydrolysis. Another study reports that the efficiency of enzymatic hydrolysis of poplar, is significantly affected by LCC linkages (Balan et al. 2009). Carbohydrates are covalently anchored and shielded by lignin in plant cell walls, which reduces the area of celluloses accessible for enzyme attacks (Laureano-Perez et al. 2005; Chandra et al. 2007). The cleavage of LCC bonds is reported to improve the enzymes accessibility to biomass (Lam et al. 2003; Zhao et al. 2016a). Also, the existence of covalent cross-linkages in forage grasses significantly affects the ability of ruminants to digest, due to the limited access of rumen fermentation microorganisms to carbohydrates in the fodders (Lam et al. 2003). Therefore, a better understanding of LCC structure may help determine appropriate processes to break lignin-carbohydrate bonds, and thus, to extract lignocelluloses from biomass effectively and selectively (Balakshin et al. 2014).

The molecular weight (MW) of lignin is reported to be an essential parameter for its application as a flocculant and dispersant (Aro and Fatehi 2017). The low content of methoxyl groups reduces the heat capacity (C_p) of lignin and increases its glass transition temperature ($T_{g)}$ (Olsson and Salmen 1997; Li and McDoland 2014). The heating values of lignocellulosic material are to a large extent dictated by the presence of inorganic compounds (Obernberger and Thek 2010), which also affect the T_g value of lignin (Tarasov et al. 2017). Lignin with a low C_p and a high heating value can be utilized as fuel or a binder for pellet production (Tarasov et al. 2017). The hydrophilicity

and structural plasticity of lignin is reported to have a positive correlation with its phenolic groups (Li et al. 2016; Olsson and Salmen 1997).

The properties of hemicelluloses and cellulose also impact their applications. It is reported that low MW sugars are favourable for biofuel production (Shevchenko et al. 2000; Liu et al. 2012), while hemicelluloses with high MW can be used in cosmetics and pharmaceutical products (Wilfor et al. 2008; Rissanen et al. 2014). Moreover, high MW polysaccharides can be employed in the food industry. For example, galaglucomannan obtained from process waters of thermomechanical pulping process demonstrates a MW between 39,000 g/mol and 46,000 g/mol (Xu et al. 2008) and can be applied as a replacement for gear or xanthan gums (Rissanen et al. 2014). However, the relatively low heating values of hemicelluloses and cellulose (Pathak et al. 2016; Kaupp and Goss 2013) limit their applications as fuel.

According to our knowledge, there is no report available to discuss the properties of LCC and their impact on its end-use applications. This chapter intends to 1) introduce LCC and its properties, 2) describe methods followed in literature to produce LCC, 3) describe the methods used to quantify and analyze the composition, structure and properties of LCC, and 4) review the proposed LCC applications.

2.3 Lignin-carbohydrate linkages

In 1838, Paymen proposed an "incrustation theory", which assumes that lignin crusted cellulosic materials. The "incrustation theory" is based on the observation that celluloses in the cell walls have different properties when non-cellulosic materials are isolated from wood (Grushnikov and Shorygina 1970). Erdman (1866) explained the complexity of disuniting lignin from carbohydrates by the fact that these polymers were associated with "glycolignose" materials (Koshijima and Watanabe 2003). Recent research has confirmed that lignin and hemicelluloses are covalently bound and form lignin-carbohydrate complexes (Santos et al. 2013; Jin et al. 2006). There is now more information about the bonds between lignin and cellulosic molecules. Eriksson et al. (1980) suggests a fractional bonding between lignin and cellulose units in softwoods. Lam and Iiyma (2000) propose lignin-cellulose linkages in rice straw. Jin et al. (2006) report that over 50% of lignin units in softwoods and 17% in hardwoods are covalently (molecularly) bound to cellulose moieties in wood. The linkages between lignin and pectin units are also suggested in wood (Meshitsuka et al. 1982; Minor 1982).

Linkages between lignin and carbohydrates are generated under the conditions of lignin biosynthesis. During nucleophiles supplement to quinone methides, the intermediate connections are developed due to p-hydroxycinnamyl alcohol oxidation (Brunow and Lundquist 2010). There are eight different types of lignin-carbohydrate (L-C) bonds, i.e., benzyl ether, benzyl ester, glyosidic or phenyl glyosidic, hemiacetal or acetal linkages, and ferulate or diferulate esters that are linked to lignin at 4-OH and 4-O positions (Kosikova and Ebringerova 1994; Lawoko 2005; Eriksson et al. 1980; Albersheim et al. 2010; Zhao et al. 2016a). Figure 2.1 presents the structures of the main types of LCC bonds. Benzyl ester bonds connect lignin and carbohydrate moieties through uronic acid of sugars and hydroxyl group of lignin; benzyl ether and phenyl glycosidic link glycosyl or manosyl residues of carbohydrates and phenolic or hydroxyl groups of lignin (Albersheim et al. 2010; Koshijima and Watanabe 2003). Glycosidic bonds link carbohydrates and side chain hydroxyl groups of lignin (Zhao et al. 2016a). Acetal bond is the linkage generated by the carbonyl groups of phenypropanes substructure fragments of lignin and hydroxyl groups of carbohydrates (Grushnikov and Shorygina 1970). Ferulate and deferulate esters present the major part of LCC linkages in grasses and other non-wood plants (Zhao et al. 2016a). It has been found that high amounts of ferulate and coumarate acids are bound to carbohydrates in cell walls in different herbaceous plants (Albersheim et al. 2010). Ferulic acid demonstrates the ability to oxidatively couple with lignin, proteins and other ferulic acids (Oliveira et al. 2015). Due to the presence of carboxylic acid groups at the end of prophenyl groups, ferulate acid is able to produce ester linkage with polysaccharides (Oliveira et al. 2015). Consequently, ferulate esters of polysaccharides are linked with lignin via oxidative coupling and form “lignin–ferulate–polysaccharide” (LFP) complexes (Ralph et al. 1995; You et al. 2015). Due to the abundance of feruic acids in herbaceous plants and lack of information about ferulic acid existence in the wood fibres, there are limited studies about ferulate linkages in wood. The bark of softwood materials is reported to possess some ferulate esters (Virgili et al. 2000). Reiter et al. (2013) propose ferulic acid formation in the kraft pulping process due to the cleavage of aryl parts of ethers.

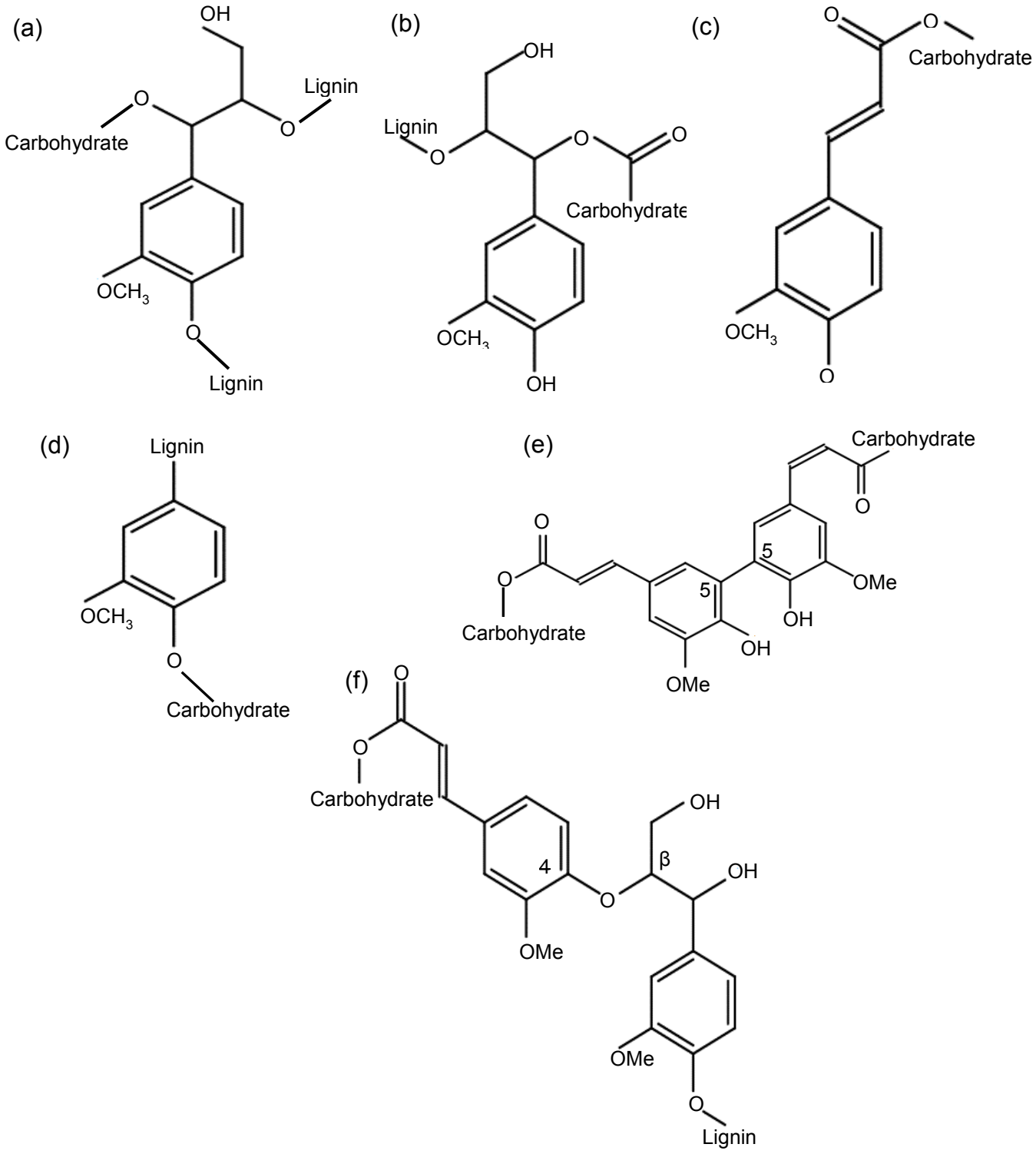

Figure 2.1 Main types of LCC linkages (a) benzyl ether; (b) benzyl ester; (c) ferulate ester; (d) phenyl glycosidic; (e) diferulate ester (5'-5' linkage) (f) diferulate ester (4-O-β linkage) (after Albersheim et al. 2010).

Benzyl ether (BE), ester and phenyl glycosidic (PhyGlc) are the most typical lignin-carbohydrate linkages (Balakshin et al. 2011; Albersheim et al. 2010), showing varying strength under different conditions. Benzyl ether bonds are reported to be alkaline stable (Koshijima and Watanabe 2003; Brunow and Lundquist 2010). However, benzyl ether linkages with phenolic hydroxyl groups are proposed to be alkali-liable (Kosikova et al. 1979).

Benzyl ester linkages are easily cleaved in alkaline conditions (Koshijima and Watanabe 2003; Brunow and Lundquist 2010). Silva et al. (2017) report that hardwoods with a high content of PhyGlc linkages show the lowest kraft pulping yield performance, which could be related to the alkaline-stable nature of the PhyGlc bonds (Takahashi and Koshijima 1988a; 1988b). In another study, it is noted that the hydrolysis of PhyGlc bond leads to only 4% cleavage in neutral aqueous conditions, whereas 96% of the bond can be cleaved via acid treatment, which is proposed to be generated during hydrothermal treatment of hardwood species (Lawoko et al. 2009). Consequently, a high acid-liability and alkali resistance of PhyGlc linkages are suggested. Benzyl ether and ester bonds are also not stable under acid hydrolysis conditions (Kosikova et al. 1979; Cheng et al. 2017). Ferulate esters are highly alkali-liable and can be cleaved by alkaline hydrolysis at room temperature (Koshijima and Watanabe 2003). However, due to the affinity of ferulate acid to generate ester bonds with hemicelluloses and ether linkages with lignin, the alkali treatment of herbaceous plants results in the extraction of lignin and ferulic acid molecules (Koshijima and Watanabe 2003; Buranov and Mazza 2008).

2.3.1 Quantity of lignin-carbohydrate bonds in wood and non-wood species

It can be postulated that the benzyl ether bonds are dominant in softwood LCC (Balakshin et al. 2011; Du et al. 2014; Giummarella et al. 2016). The phenyl glycosidic linkages are prevalent in hardwood LCC, while the amount of ester linkages greatly varies in different species (Balakshin et al. 2011; Zhao et al. 2016b). Herbaceous plants show significantly higher amounts of LCC linkages than woody materials. The quantification of lignin-carbohydrate linkages in herbaceous plants demonstrates the vast majority of phenyl glycosidic linkages (You et al. 2015; Yao et al. 2016). The detection of benzyl ester (α-ester) linkages is not reported in other studies (Balakshin et al. 2011; Du et al. 2014; You et al. 2015; Yao et al. 2016). The quantification of lignin-carbohydrate linkages in various LCC is presented in Table 2.1. Each lignin monomeric unit contains aromatic rings, which is equivalent to mol percentage of aromatic hydrogen or carbon

atoms (Balakshin and Capanema 2015; Capanema et al. 2004; Balakshin et al. 2014; Speight 2005). The estimation of lignin's substructures and LCC bonds is mostly expressed as number per 1 or 100 aromatic units (Ar).

Table 2.1 Quantification of lignin-carbohydrate linkages in LCC.

Preparation	per 100 Ar		
	Benzyl ether	PhyGlc	γ-ester
Softwood LCC			
Pine LCC-AcOH[1]	4.3	6.8	4.5
Spruce GM-L[2]	3.8	4.4	ND
Spruce Xyl-L[2]	6.1	ND[6]	ND
Hardwood LCC			
Birch LCC-AcOH[1]	0.7	5.4	5.6
Eucalypt LCC-AcOH[3]	0.3-0.9	5.0-7.9	ND
Herbaceous LCC			
A. Donax Bjorkman LCC[4]	251	42	3
Wheat Bjorkman LCC[5]	50	2	14
A.Donax LCC-AcOH[4]	4	ND	0.14

[1]Balakshin et al. 2011; [2]Du et al. 2014; [3]Zhao et al. 2016; [4]You et al. 2015; [5]Yao et al. 2016; [6] Not detected

2.3.2 Softwood LCC structure

Table 2.2 depicts the compositions of LCC fractions extracted from various biomass sources. There are a few hypotheses about the LCC structures and compositions for softwoods. Balakshin et al. (2011) state that the benzyl ether linkages in softwood LCC mainly involve mannose. In another study, Giummarella et al. (2016) suggest that xylose is the main carbohydrate with ether bonds, whereas mannose is linked by PhyGlc bonds. Lawoko et al. (2005) suggest that lignin involved in LCC has two different structures, with one type attached to xylan and the other one connected to glucomannan. It is proposed that xylan is linearly linked to lignin, while GM-L complexes have a branched structure (Lawoko et al. 2005). Another study reports that Xyl-L and Glu-L fractions isolated from spruce wood have condensed and linear structures, respectively (Du et al. 2014). Oinonen et al. (2015) hypothesize random crosslinks between galactoglucomannans, xylans and lignin in Norway spruce. It is reported that the glucomannan-lignin fraction is water-soluble, which suggests a low cross-linkage degree in this complex since extensively cross-linked polymers are typically insoluble in water (Koshijima and Watanabe 2003). Takahashi and Koshijima (1988b) also assume that softwood LCC consists of small and repeating lignin units bounded to the polysaccharide chain (1988b).

2.3.3 Hardwood LCC structure

Dammstrom et al. (2009) propose that xylan in hardwood is present in three forms, one of which is glucuronoxylan attached to cellulose units, in the second form xylan is a part of xylan-lignin complexes, and the third one is free xylan. Koshijima and Watanabe (2003) state that xylose is the main sugar for benzyl ether linkages. In other research, it is reported that 50% of glucan moieties are involved in benzyl ether linkages, with xylan contribution only 5-10% (Balakshin 2014). Takahashi and Koshijima (1988a) state that lignin and glucuronoxylan are linked by benzyl ester bonds with 30% uronic acid of the linkage. Takahashi and Koshijima (1988b) propose hardwood LCC structure in the form of a very long polysaccharide chain linked to a few large lignin moieties.

2.3.4 Non-wood LCC structure

The LCC of grasses mainly includes arabinoxylans bridged to lignin via ferulate esters (Albersheim et al. 2010; Moore and Jung 2001). Arabinoxylans play an important role in LCC linkage formation, due to the conceivable existence of covalent bonding between arabinose, xylan and lignin moieties in forage crops (Kondo et al. 1990). Also, xylan is reported as a main component binding lignin and carbohydrates in bamboo, rice straw and ryegrass (Peng et al. 2014; Azuma and Koshijima 1988; Buranov and Mazza 2008; Sipponen et al. 2013). It is proposed that lignin in wheat straw is bound to glucan and xylan moieties via ferulate acid and PhyGlc linkages, respectively (Zikeli et al. 2015; Yao et al. 2016). In another study, You et al. (2015) suggest that PhyGlc bonds exist between cellulose and lignin in herbaceous plants due to the abundance of these bonds. However, Rio et al. (2016) report that no signs of benzyl ether, ester or PhyGlc linkages are found in a glucose-lignin fraction of sisal and abaca. It is also proposed that xylan is linked to lignin via PhyGly bonds in bamboo (Zhang et al. 2016). Other work confirms that PhyGlc is bound to guaiacyl and syringyl lignin units with xylan moieties in abaca and sisal (Rio et al. 2016).

Table 2.2 Composition of LCC fractions from wood and herbaceous plants.

LCC fraction	Relative content in LCC fraction, %		Relative amount of carbohydrates, %				
	Acid-soluble lignin	Total carbohydrates	Ara	Xyl	Man	Gal	Glu
Softwood (Spruce) (Lawoko et al. 2005)[1]							
GGM-L-Pectin	39	56.9	5.6	4.2	58.9	22.1	9.1
GM-L-Xyl	56	36.8	3.3	8.4	52.2	10.9	25.3
Glu-L	7	89.8	1.1	1.0	2.8	0.22	94.9
GM-L-Xyl	41	51.8	1.3	6.2	64.7	3.5	24.1
Xyl-L-GM	65	32	9.7	58.4	13.4	7.2	11.2
Xyl-L-GM	29	59.1	6.9	59.0	24.0	7.11	2.9
Softwood (Spruce) (Du et al. 2014)							
Glu-L	19.3	80.7	1.9	2.5	8.6	1.2	85.8
GM-L	29.2	70.8	4.7	10.6	30.9	4.4	49.4
Xyl-L	42.7	57.3	13.0	65.3	3.2	3.0	15.6
Hardwood (*Eucalyptus globules*) (Li et al. 2011)[3]							
Glu-Xyl-L	14.2/15.8[3]	85.8/84.2[3,4]	0.6/0.7	18.1/18.9	2.3/2.2	1.3/1.5	77.2/76.3
Xyl-Glu-L	34.7/39.6[3]	60.4/65.3[3,4]	2.0/2.2	42.7/44.4	2.9/3.3	4.1/4.9	44.6/47.1
Hardwood (*Eucalyptus globules*) (Henriksson et al. 2007)[4]							
Xyl-Glu-L	29.0	71	1.2	40.8	10.4	8.0	39.6
Glu-Xyl-L	53.2	46.8	5.4	19.2	6.7	6.7	62
Glu-L	9.5	90.5	0.8	4.8	0.6	0.5	94.1
Xyl-L	37.5	62.5	1.1	91.3	2.7	1.9	3.0
Xyl-Glu-L	13.8	86.2	0	58.7	12.1	2.3	26.9
Hardwood (*Betula verrucosa*) (Henriksson et al. 2007)[5]							
Glu-Xyl-L	29.2	70.8[4]	0	41.7	11.8	10.0	36.6
Glu-Xyl-L	15.9	84.1[4]	0.9	16.2	0.5	0.7	81.7
Xyl-L-Gal	35.8	64.2[4]	1.0	75.9	2.2	2.7	18.2
Herbaceous plant (Maize stem) (Sipponen et al. 2013)							
Xyl-L-Ara	38.3	50.3	10.5	83.0	ND[6]	0[7]	6.4
Xyl-L-Glu	18.7	70.7	5.9	82.6	ND[6]	0[7]	11.5
Herbaceous plant (Sisal) (Rio et al. 2016)							
Glu-L	7.8	92.2[4]	1.5	9.0	0.9	0.2	88.4
Xyl-L	24.1	75.9[4]	0.6	89.4	2.6	0.3	7.1
Herbaceous plant (Abaca) (Rio et al. 2016)							
Glu-L	4.4	95.6[4]	0.3	4.1	0.5	0.1	95.0
Xyl-L	29.4	70.6[4]	3.4	75.5	13.0	0.3	7.8
Not fractionated LCC from herbaceous plants							
LCC-AcOH (Bamboo)[7]	19.9	48	3.8	32.9	3.1	1.0	59.2
Björkman LCC (A.donax)[8]	34.8	65.2	6.2	59.2	0.1	3.3	28.9
Björkman LCC (Wheat straw)[9]	16.03	75.11[1]	9.3[1]	75.1[1]	2.2[1]	0[1]	13.4[1]
LCC-WE (Rice straw)[10]	27.7	63.9	13	80.1	0.4	2.3	13

[1]Converted to % based the data presented in the source; [2]Enzymatically treated LCC fractions (Du et al. 2014); [3]12/24 h of ball milling; [4]Calculated by authors by subtraction the portion of lignin (%) presented in the LCC fraction from 100%; [5]LCC fractions were prepared in accordance with method described by Lawoko et al. (2005); [6]Not detected (Sipponen et al. 2013); [7]Zhang et al. 2016; [8]You et al. 2015; [9]Yao et al. 2016; [10]Azuma and Koshijima 1988.

2.3.5 LCC in softwood pulp

As stated earlier, LCC can exist in pulp, and it can also be extracted from spent liquor. The analysis of pulp reveals that 85-90% of lignin remains in softwood kraft pulp linked to carbohydrates in LCC forms (Lawoko et al. 2003; 2004); whereas, in oxygen-delignified pulp all lignin units are involve in LCC (Lawoko et al. 2004). Due to the galaglucomannan decomposition during kraft pulping, LCC is present in kraft pulp as Glu-L, GM-L-Xyl and Xyl-L-GM fragments with 12, 45 and 27% of total relative amounts of lignin found in pulp, respectively (Lawoko et al. 2005). It is apparent that hemicellulose-lignin complexes contain both xylan and glucomannan in different proportions, implying that lignin crosslinks with xylan and glucomannan in softwood kraft pulp (Lawoko et al. 2003). Tenkanen et al. (1999) report that the degradation of xylan during the enzymatic hydrolysis of pine kraft pulp significantly enhances the decomposition of glucose; while, the hydrolysis of glucomannan does not improve the decomposition of cellulose in the same manner as that of xylan. Also, it is found that the hydrolysis of glucomanan increases after a considerable removal of xylan. These results imply that xylan is partially covered by glucomannan, whereas in pine kraft pulp, xylan is entrapped by glucose (Tenkannen et al. 1999). The increment in the degree of oxygen delignification leads to the degradation of Glu-L complex from, whereas the GM-L-Xyl and Xyl-L fractions obtained from oxygen-delignified softwood pulp included 80% and 20% of total lignin content in pulp, respectively (Lawoko et al. 2004; 2005). Therefore, it is proposed that GM-L-Xyl complex is resistant to oxygen delignification, which might be attributed to the high alkali-resistance of phenyl glycosidic and benzyl ether bonds present in this complex.

In addition, with the increment of oxygen delignification intensity, the relative amount of xylan in the GM-L-Xyl fraction decreases, while the relative content of lignin in these complexes increases. At the highest severity of oxygen delignification, almost all lignin moieties in pulp have been found to be linked to glucomannan units. This leads to the conclusion that the main issue of oxygen delignification of softwood species is attributed to glucomanann LCC (Lawoko et al. 2004).

2.3.6 LCC in hardwood pulp

LCC fractionation of eucalyptus kraft pulp shows that Glu-Xyl-L and Xyl-L fractions include 8% and 12% of total lignin content present in the eucalyptus pulp, respectively. The compositional analysis of LCC fractions present in birch kraft pulp shows that Xyl-L-Glu contains 34% of total

pulp lignin and 16% of lignin units in Xyl-L fraction (Henriksson et al. 2007). Table 2.3 lists the composition of LCC fractions extracted from softwood and hardwood kraft pulps.

Table 2.3 Composition of LCC fractions from softwood and hardwood kraft pulp.

LCC fraction	Relative content in LCC fraction, %		Relative amount of carbohydrates, %				
	Acid-soluble lignin	Total carbohydrates	Ara	Xyl	Man	Gal	Glu
Softwood Kraft pulp (Spruce) (Lawoko et al. 2005)							
Glu-L	2.4	96.0	0.52	3.65	4.17	0.00	91.67
GM-L-Xyl	35	41.7	2.88	26.86	48.44	4.08	17.75
Xyl-L-GM	23	75.6	5.82	75.13	8.33	0.53	10.19
Oxygen-delignified softwood pulp (Spruce) (Lawoko et al. 2005)							
GM-L-Xyl	22.8	77.0	1.36	11.48	62.84	4.23	20.09
Xyl-L	5.1	97.9	2.5	20.2	58.8	3.3	15.3
Hardwood kraft pulp (Eucalyptus globules) (Henriksson et al. 2007)							
Glu-Xyl-L	0.5	99.52	0.7	10.6	0.3	0.1	88.4
Xyl-Glu-L	0.83	99.22	0.4	92.4	0.9	0.7	5.6
Glu-L	1.48	98.52	1.5	0.8	0.2	0.2	97.3
Hardwood kraft pulp (Betula verrucosa) (Henriksson et al. 2007)							
Xyl-Glu-L	1.2	98.82	0.2	86.9	0	0	12.9
Xyl-L	0.33	99.72	0	97.7	1.2	0	1.1
Glu-L	0.6	99.42	0.5	0.6	0.2	0	98.8

[1]Converted to % based the data presented in the source; [2]Calculated by authors by subtraction the portion of lignin (%) presented in the LCC fraction from 100%;

2.3.7 LCC in spent liquors

It is suggested that lignin-carbohydrate complexes can be extracted and dissolved along with lignin and hemicelluloses in the spent liquors of biomass pretreatment (Lawoko et al. 2005; Tunc et al. 2010). Tamminen et al. (1995) propose the existence of xylan-lignin and galactan-lignin complexes in the spent cooking liquor (black liquor) of the kraft pulping process. It is proposed that some of xylan and glucomannan moieties are dissolved in black liquor along with lignin (Tenkannen et al. 1995). In another study, Fatehi et al. (2016) propose LCC's presence both in prehydrolysis liquor (PHL) generated during pretreatment of hardwood chips with saturated steam and in the spent liquor (SL) of neutral sulfite semichemical pulping (NSSC) process, in which hardwood biomass is treated with sodium sulphite and carbonate. Also, Tarasov et al. (2017) reported the existence of LCC in the hydrolysate obtained via flow-through autohydrolysis of softwood chips. It was found that the hydrolysate produced with a high liquid to solid (L/S 10/1 wt./wt.) ratio contains 19% of lignin in the LCC form. Furthermore, 89% of lignin moieties bound

to carbohydrates in the hydrolysate are generated under a high temperature and lower L/S ratio (Tarasov et al. 2018).

2.4 LCC properties

The MW of LCC is not widely discussed in the literature. Table 2.4 depicts the properties of LCCs extracted from various sources. The MW of LCC prepared from poplar is reported to range from 9800 g/mol to 17,500 g/mol (Zhao et al. 2017a). LCC extracted from non-wood species features a very wide range of molecular weights. The MW of LCC from *Prunella vulgaris* is estimated to be 8500 g/mol (Zhang et al. 2007), whereas the MW of wheat straw LCC is around 38,700 g/mol (Yao et al. 2016). The molecular weights of xylan (Xyl) and glucan (Glu) rich LCC complexes obtained from softwood kraft pulp are estimated to be in the range of 8000-35,000 g/mol and 15,000-45,000 g/mol, respectively (Lawoko et al. 2004). It is reported that the MW of lignin and hemicelluloses present in these LCC fractions is related to the relative lignin content of kraft pulp. The Xyl-L fraction obtained from pulp with lower lignin content possesses lignin and hemicelluloses with a higher MW (Lawoko et al. 2004). Also, hardwood PHL and SL of NSSC process are reported to contain LCC with the molecular weights of 2000 and 1500 g/mol, respectively (Fatehi et al. 2016).

The thermal stability of LCC is affected by various factors, such as interunit structures, functional groups, degree of condensation and molecular weights (Chen et al. 2016). Experimental conditions also impact LCC's thermal stability. In one study, Nassar and MacKay (1984) report that LCC from spruce starts to decompose in the temperature range from 220 to 260 °C, whereas the degradation onset temperature (T_{onset}) of spruce lignin is reported to be 210-220 °C. This difference could be related to the hemicellulose presence in LCC, as hemicelluloses contain more inherent moisture than lignin does (Nassar and MacKay 1984). Another study states that softwood LCC possessed T_{onset} of 236 °C, whereas LCC and carbohydrate-free lignin from bagasse started to decompose at 277 and 268 °C, respectively (Singh et al. 2005). At 590 °C, the weight of softwood LCC decreases by 45.7%, whereas LCC isolates from bagasse and carbohydrate-free lignin experiences 55.6 and 52.5% weight loss, respectively (Singh et al. 2005). A lower degradation of softwood LCC could be related to their bonding extent, which decomposes slowly at elevated temperatures (Singh et al. 2005). Molecular weight, molecular weight distribution and degree of crosslinking impact T_g value of LCC (Hatakeyama and Hatakeyama 2010). In the past, lignin was

reported to have T_g between 80 and 193 °C (Trajano et al. 2013), while hemicellulose and cellulose had T_g in the range of 200-250 °C and 150-220 °C, respectively (Stokke et al. 2014). The T_g of LCC from bamboo is reported to be 166 °C (Youssefian and Ruhbar 2015).

The elemental analysis of softwood and bagasse LCC is reported by Singh et al. (2005). Carbon and hydrogen contents of both LCCs are estimated as 63-60% and 5.4-6.3%, respectively. These values are similar to carbohydrate free bagasse and softwood kraft lignin (Singh et al. 2005; Lourencon et al. 2015).

Table 2.4 LCC properties.

LCC source	Mw, g/mol	Thermal properties[5,6,7]			Elemental analysis, %[5,8]				
		$T_{onset,}$ °C	Degradation at 590°C, wt.%	$T_{g,}$ °C,	C	H	N	S	O
Hardwood	9800-17,600[1]	N/A[9]	N/A	N/A	51.9	5.8	N/A	N/A	42.4
Softwood	12,000[2]	220-260	45.7	N/A	59.6	6.3	1.1	2.2	N/A
Non-wood	8500-38,700[3,4]	277	55.6	166	62.8	5.4	N/A	N/A	N/A

[1]Zhao et al. 2017; [2]Koshijima et al. 1989; [3]Zhang et al. 2007; [4]Yao et al. 2016; [5]Singh et al. 2005; [6]Nassar and MacKay 1984; [7]Youssefian and Ruhbar 2015; [8]Merewether et al. 1972; [9] Not applicable

Another important parameter of LCC is anti-UV activity. The anti-UV activity is determined by selective index (SI) (Nanbu et al. 2013; Sakagami et al. 2016). SI is defined as the ratio of two parameters: 50% cytotoxic concentration (CC_{50}), which is defined as the amount of the compound (μg/mL) required for the reduction of the number of living cells by 50%; and 50% effective concentration (EC_{50}) that raises the viability of UV-irradiated cells by 50% (Kato et al. 2014; Ueki et al. 2013; Abid et al. 2012). In other words, SI quantitatively expresses the ability of the compound (LCC) to defend cells from UV-induced damage (Sakagami et al. 2016).

It is reported that the LCC produced from pine cone and pine seed shell extracts shows anti-UV activity with SI of 24.8-38.1 and 25.6, respectively. A similar anti-UV activity (SI=38.5) is reported for herbaceous LCC (Sasa senanensis Rehder leaves), whereas the lignin extracted via alkali treatment shows a significantly higher anti-UV activity with an SI of 61.5 (Nanbu et al. 2013).

2.5 LCC exctraction

In 1935, Hibbert and coworkers proposed the presence of lignin-xylan complexes in spent liquor obtained from extraction of spruce saw meal treated with a mixture of anhydrous ethylene alcohol glycol and hydrogen chloride (Gray et al. 1935). In a later study, Merewether (1954) reported the presence of xylan-lignin complexes in the spent liquor produced via ethanolysis (in the presence of sodium bicarbonate) of eucalyptus wood meal. In 1953, Traynard and co-workers reported the extraction of LCC from poplar species via water hydrolysis at 140 °C (Koshijima and Watanabe 2003).

The methods for LCC extractions established by Björkman (1954; 1956) became a milestone in the investigation of lignin and LCC's structure and composition. Figure 2.2 outlines Björkman's procedure for LCC preparation. In this method, the biomass is saturated with toluene prior to milling for 48 hours (Björkman 1956; Holtman et al. 2006). Afterward, the milled material is mixed with 1,4-dioxone/water (96/4 vol/vol) solution in a wood/solvent ratio of 1/10 wt./wt. and is stirred for 24 h at ambient temperature under a nitrogen atmosphere (Holtman et al. 2006). Then, the solution is centrifuged. The evaporation of its solvent from supernatants will help separation of milled wood lignin (MWL) (Holtman et al. 2006). It is reported that MWL represents up to 50% of total lignin content in wood (Björkman 1956; Grushnikov and Shorygina 1970). The precipitates of the centrifugation are then extracted with dimethylformamide (C_3H_7NO) or dimethyl sulfoxide ($(CH_3)_2SO$)). The obtained material is purified by dissolution in 50/50 vol/vol acetic acid/water ($AcOH/H_2O$) mixture followed by precipitation via washing with a dichloromethane/ethanol (CH_2Cl_2/C_2H_6O) mixture. The produced precipitates are considered as Bjorkaman LCC. This product is comprised of 16-34 wt.% lignin and 66-84 wt.% carbohydrates (Grushnikov and Shorygina 1970; Koshijima and Watanabe 2003).

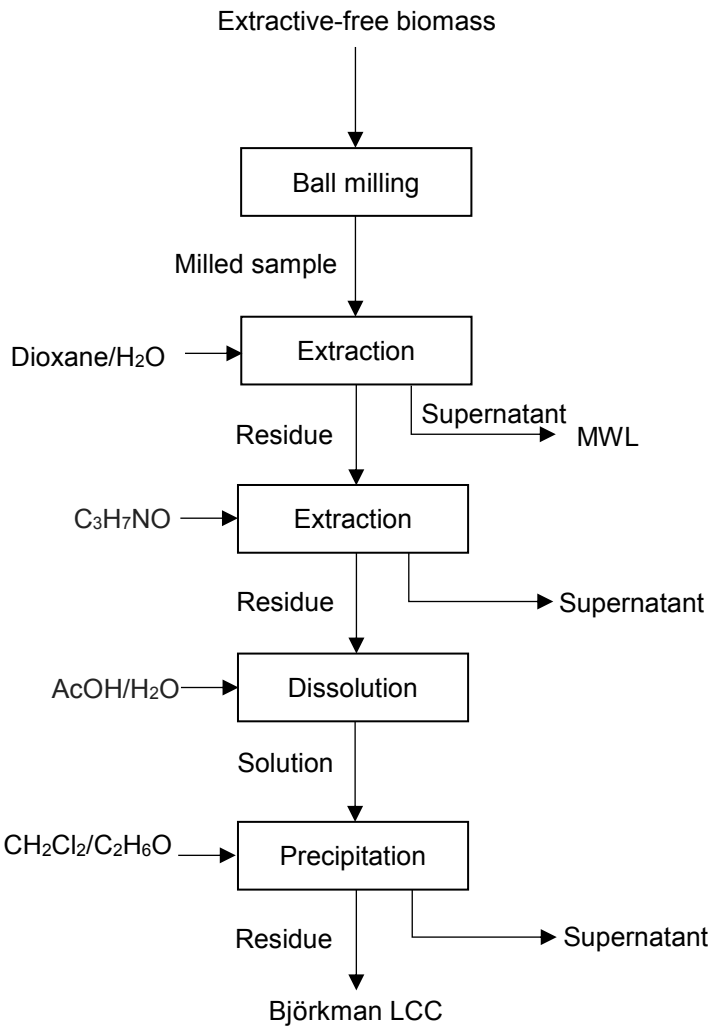

Figure 2.2 Björkman's method for LCC preparation (Björkman 1956; Holtman et al. 2006).

Björkman's procedure uses solvents with high boiling points, such as dimethylformamide or deimethysulfoxide (Koshijima and Watanabe 2003). Balakshin et al. (2011; 2007) and You et al. (2015) reported procedures for LCC extraction from softwood (pine), hardwood (birch) and herbaceous biomass (*A. donax*). Figure 2.3 represents the process of LCC preparation using acetic acid (AcOH). In this method, extractive-free wood sawdust is ground by planetary ball milling for 5 h and 600 rpm (Balakshin et al. 2011). In case of herbaceous biomass, the extractive-free material is subjected to planetary ball milling for 12 h and 450 rpm (You et al. 2015). Then, the produced material is extracted by 96/4 vol/vol 1,4-dioxane/water mixture in accordance with the Bjorkman's procedure. Afterward, the solvent is evaporated in vacuum and then a few drops of deionized water

are added to the solid material to remove traces of dioxane followed by rotary evaporation. The dried material is considered as MWL. Then, MWL is dissolved in 90% aqueous AcOH (at 20 mL/g ratio). The addition of water to the mixture leads to precipitations of purified MWL. Then, the supernatant of this process is collected and lyophilised. Furthermore, the dried material is treated with a few drops of water for AcOH removal. After repeating the purification procedure three times, the dried material is considered to be LCC-AcOH (Balakshin et al. 2011; You et al. 2011).

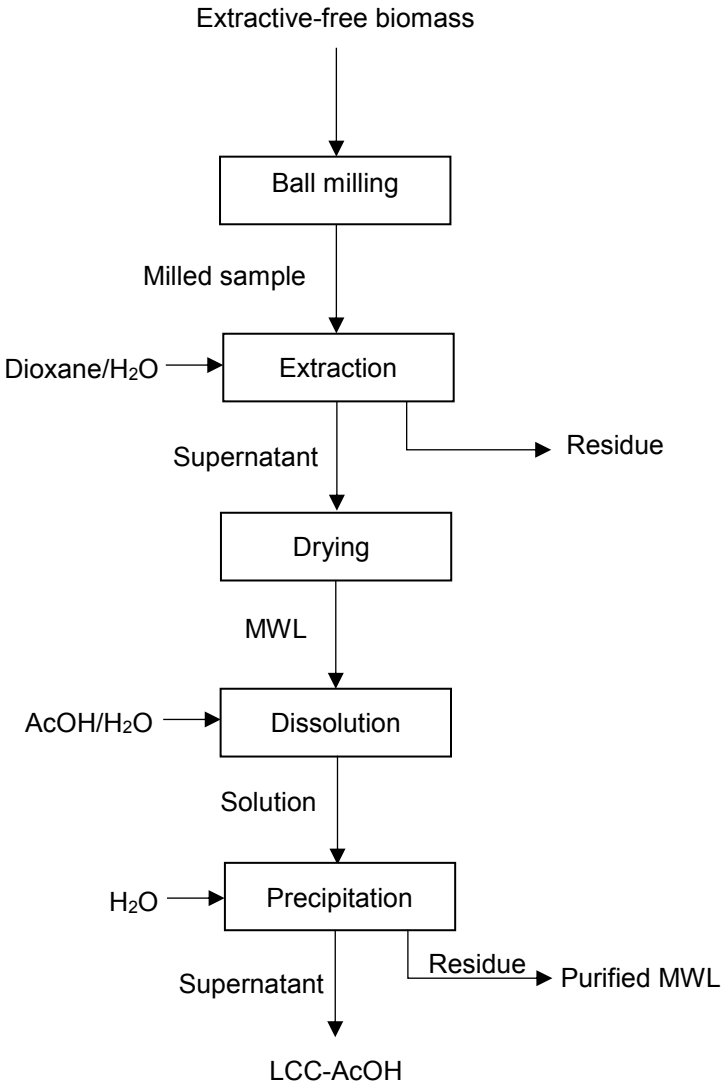

Figure 2.3 LCC-AcOH preparation procedure (Balakshin et al. 2011; You et al. 2015).

Another method for LCC extraction was developed by Watanabe and co-workers in 1987. Figure 2.4 outlines the procedure for LCC-WE preparation. In this method, woody materials are ground

and then MWL is extracted using an 80/20 vol/vol dioxane/water solution. The obtained residue is first treated with cold water (20 °C), washed and then treated again with hot water (at 80 °C). The dissolved materials of these processes are precipitated with ethanol (C_2H_6O) and considered as LCC-WE (Koshijima and Watanabe 2003).

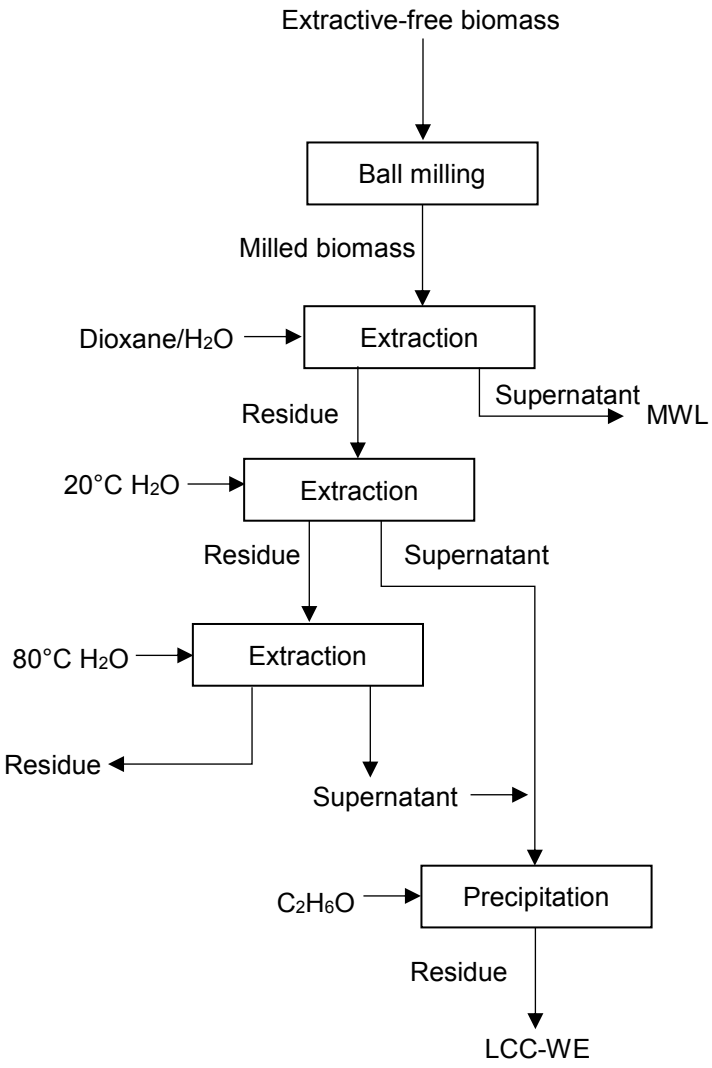

Figure 2.4 LCC-WE preparation procedure (Watanabe et al. 1987).

2.6 LCC fractionation

Softwoods, hardwoods and non-woods have different morphologies and compositions of lignin and carbohydrates, which results in variations in their LCC properties. LCC extracted from softwood is claimed to have hemicelluloses, such as galactoglucomannan (GGM), glucomanan (GM), arabino-4-O-methylglucoronoxylan (Xyl) and arabinogalactan (Gal), bound to lignin (L) moieties (Azuma and Koshijima 1988). Sugars of LCC in hardwoods consist of 4-O-methylglucoronoxylan, whereas LCC from non-woods are composed of arabino-4-O-methylglucoronoxylans (Azuma and Koshijima 1988). LCC fractionation procedures reveal more detailed information regarding the structure and composition of various LCCs presented in biomass and pulps.

2.6.1 LCC fractionation via enzymatic hydrolysis and barium hydroxide

Lawoko proposes a combination of ball milling, enzymatic hydrolysis and treatment with barium hydroxide ($Ba(OH)_2$) for fractionating LCC (Lawoko et al. 2003; 2004; 2005; 2006). The procedure for LCC fractionation via this method is shown in Figure 2.5. First, the extractive-free spruce wood species are ball-milled for 3 h. Next, the milled substance is treated with endoglucanase enzymes (Novozyme 476) followed by centrifugation. Then, the hydrolysate of this enzymatic treatment is treated with 5% aqueous barium hydroxide (($Ba(OH)_2$) for 2 h after centrifugation, which leads to the precipitation of solid material (Lawoko et al. 2006; Du et al. 2013). The generated precipitate is dissolved in 1/1 $AcOH/H_2O$ solution and then precipitated again via ethanol supplement. After dialysis and drying, the produced material is considered GGM-L-Pectin fraction (Lawoko 2013; Lawoko et al. 2006). The precipitates of enzymatic hydrolysis are swollen in urea for 24-48 h at room temperature. Afterward, the soluble part of the urea mixture is mixed with $Ba(OH)_2$, resulting in the formation of two LCC fractions; GM-L-Xyl is found in residue due to its poor solubility, and highly soluble Xyl-L-GM fraction dissolves in supernatant. Next, GM-L-Xyl and Xyl-L-GM fractions are separated by centrifugation. Then, GM-L-Xyl fraction is purified with $AcOH/H_2O$ solution and re-precipitated in ethanol as described above. Xyl-L-GM portion, remaining in the barium hydroxide solution, is also dissolved in 50% aqueous AcOH solution and precipitated in ethanol. Afterward, the obtained fractions are dialysed and freeze-dried (Lawoko et al. 2006; Lawoko 2013).

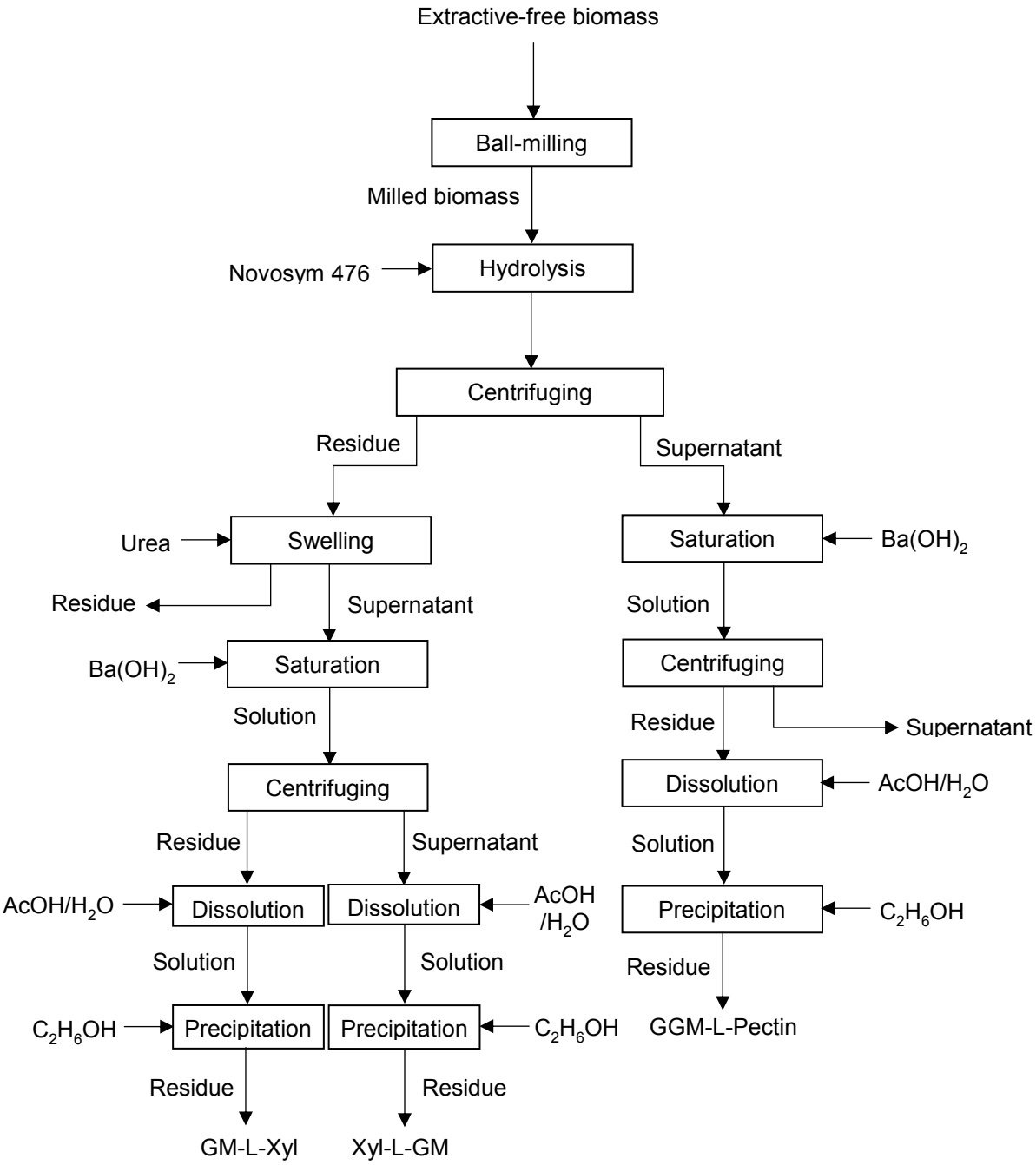

Figure 2.5 LCC fractionation with combined application of enzymatic hydrolysis and barium hydroxide solution (Lawoko et al. 2006; Lawoko 2013).

2.6.2 LCC fractionation via DMSO/tetrabutylammonium hydroxide (TBAH) mixture

The degradation of β-O-4 interunits of syrigyl lignin and high solubility of these lignin segments in water after endogluconase hydrolysis and urea treatment indicates the fact that the procedure

should be modified for hardwood LCC preparation (Henriksson et al. 2007). Li et al (2011) propose a method for fractionation of hardwood LCC, which includes ball milling for 12-24 h and dissolution in 50/50 vol/vol DMSO/tetrabutylammonium hydroxide (TBAH) mixture. This leads to the dissolution of cellulose components of hardwood but the lignin structures in LCCs remain intact (Li et al. 2011). Mixing the product with water results in the precipitation of LCC. The lyophilisation of the precipitates generates Glu-L fraction in the precipitates, and Xyl-L in the solution.

2.6.3 LCC fractionation via alkaline extraction and enzymatic hydrolysis

Sipponen et al. (2013) report an efficient method for isolation of alkali-soluble LCC fractions from non-wood plants (Maize stem) via combined application of alkaline extraction and enzymatic hydrolysis. Figure 2.6 depicts the procedure for alkali-soluble LCC fractions isolation. This procedure describes the extraction of extractive-free biomass with 0.5M sodium hydroxide (NaOH) solution for 24 h at room temperature under nitrogen (N_2) atmosphere. This treatment leads to the formation of precipitates and dissolved substances. The product is centrifuged, and the generated residue and supernatant are separated. Then, the pH level of the obtained supernatant is adjusted to 2 by hydrochloric acid (HCl) supplement. The acidified solution is kept for 16 h in the dark and then centrifuged for collection of the insoluble part. The generated suspension is lyophilised, and the obtained solid material is considered as Xyl-L-Ara complex. The precipitate produced from the initial NaOH solutions is treated with Novozym 476 enzyme, which leads to the generation of hydrolysed suspension. The suspension is centrifuged, and hydrolysed solids are precipitated, washed and lyophilised. Then, the dried residue is extracted with 2M NaOH at ambient temperature under N_2 atmosphere. Afterward, Xyl-L-Glu complex is isolated from alkaline extract via acidification, as described above (Sipponen et al. 2013).

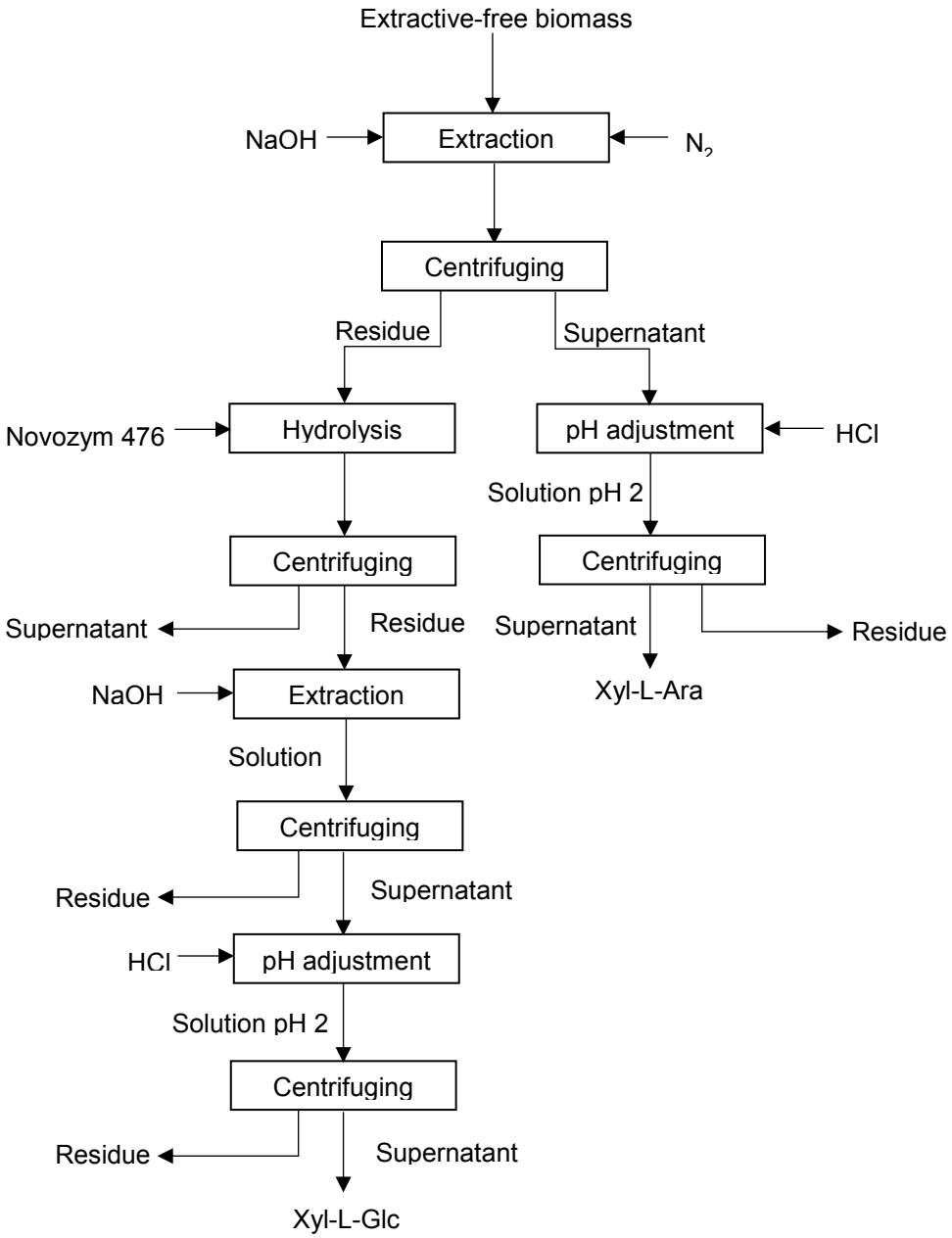

Figure 2.6 The procedure for isolation of alkali-soluble LCC fractions (Sipponen et al. 2013).

2.6.4 LCC fractionation via universal method

Du et al. (2013) report a universal fractionation process for LCC from lignocellulosic biomass, which combines the procedures developed by Lawoko et al. (2005) and Li et al. (2011). Figure 2.7 depicts the procedure for universal fractionation of LCC. In this method, the extractive-free

biomass is first ground by ball milling and then dissolved in DMSO/TBAH solution in accordance with the method developed by Li et al. (2011). It is reported that after 12 h of ball milling, the milled materials of hardwood, softwood and herbaceous species entirely dissolve in DMSO/TBAH mixture (Li et al. 2011; Du et al. 2013). The obtained solution is diluted with water, generating two phases of residue and supernatant. The generated residue is washed with water and lyophilised to obtain the Glu-L fraction. The produced supernatant is saturated with $Ba(OH)_2$, which leads to the aggregation of barium ions with GM-L fraction and its further precipitation. Both the precipitate and the supernatant are neutralized with HCl, then purified and dried. The materials collected from the precipitate and supernatant are considered as GM-L and Xyl-L fractions of LCC, respectively (Du et al. 2013). It is also reported that the molecular weight of LCC generated in this process is extremely high. Glu-L, GM-L and Xyl-L fractions have the MW of 490,000 g/mol, 63,000-160,000 g/mol and 18,000 g/mol, respectively (Du et al. 2013). Considering the complete dissolution of the examined wood and herbaceous species in DMSO/TBAH mixture, it is suggested that this method can be also applied for the fractionation of LCCs present in pulp and other processed lignocellulosic materials (Du et al. 2013).

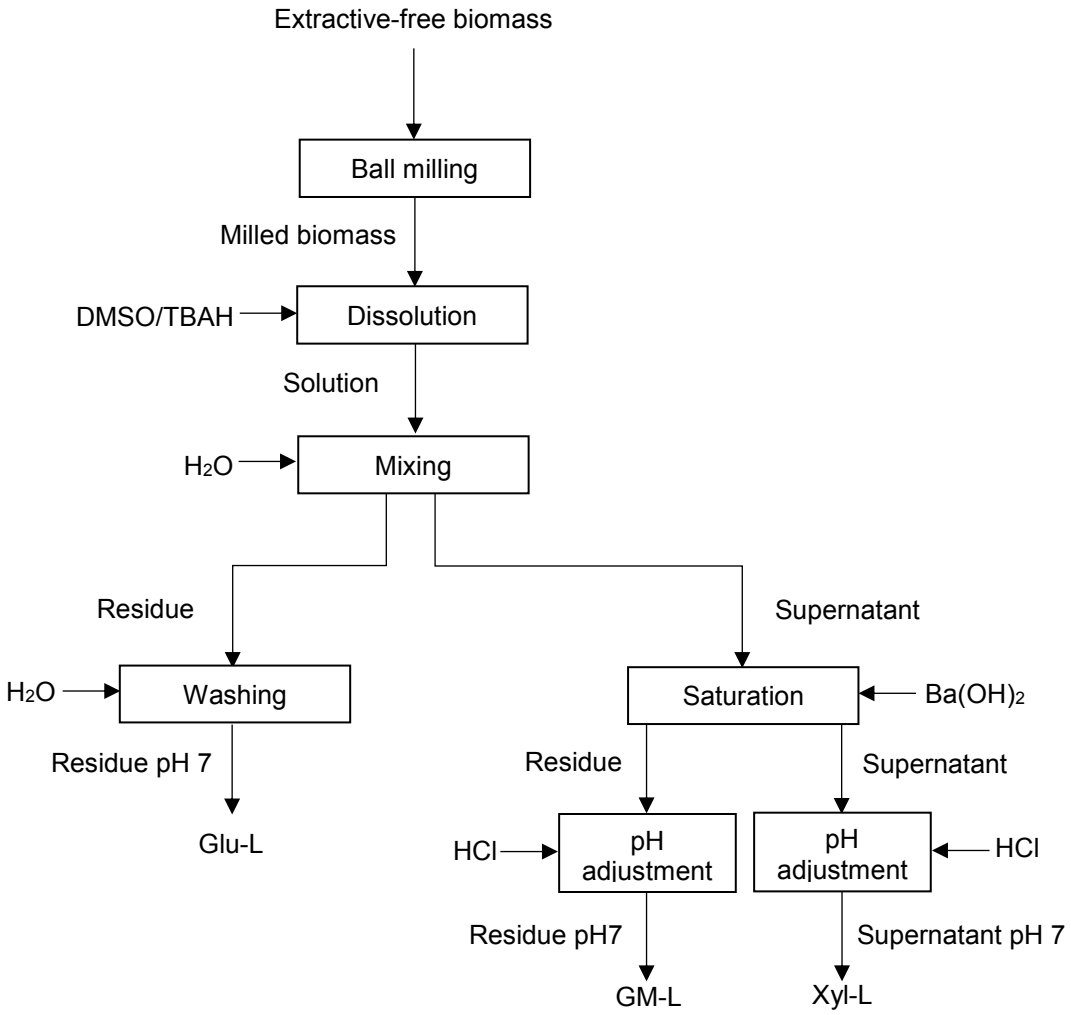

Figure 2.7 The procedure for universal LCC fractionation (Du et al. 2013).

2.6.5 Fractionation of pulp LCC

It is well known that the pulping process leads to a significant delignification of biomass. As stated earlier, benzyl ether and phenyl glycosidic lignin-carbohydrate linkages are alkali-stable, and hence will persist through the pulping process (Lawoko et al. 2003). Gierer and Wannstrom (1986) suggest the formation of new LCC bonds during the pulping process. In another study, Tenkanen et al. (1999) investigated the existence of linkages between lignin and carbohydrates in softwood and hardwood kraft pulp via selective enzymatic hydrolysis of cellulose, xylan and mannan units. The association between lignin and xylan, glucomanan and glucose units in pine kraft pulp and lignin-xylan bonding in birch kraft pulp is proposed (Tenkanen et al. 1999). To quantify LCC formation in these processes, Lawoko and colleagues (2003) designed a protocol for fractionation

of the LCC obtained from softwood kraft pulp and oxygen delignified pulp. Figure 2.8 outlines the protocol for fractionation process of LCC material presented in pulp.

In this process, pulp is hydrolysed with endoglucanase (Novozym 476) enzyme for 48 h. Then, the produced hydrolysate is centrifuged and the precipitated material is separated. The residue is swollen in urea solution overnight at room temperature. Afterward, the material is centrifuged in order to separate the insoluble part from the supernatant. The insoluble part is then washed with water for removal of urea and is dissolved in alkaline (18%NaOH) borate (4%H_3BO_3) solution for 4 h at ambient temperature for dissolution of mannose polysaccharides (Henriksson et al. 2007). The precipitate generated via centrifuging of the alkaline borate solution, is washed and recycled to enzymatic hydrolysis stage. The supernatant obtained via centrifugation of alkaline borate solution is subjected to pH adjustment via acetic acid supplement causing precipitation of Glu-L fraction at pH 12. Glu-L fraction is separated with centrifugation; where the pH of the solution drops to 7. Since no precipitate is formed at pH 7, the solution is dialyzed and lyophilised, and then the dried material is dissolved in 0.2 M NaOH. The produced solution is treated with 5% aqueous $Ba(OH)_2$, which leads to the precipitation of glucomannan rich material. The obtained fraction is collected and re-dissolved in 0.2 M NaOH and again is precipitated with barium hydroxide solution. Further, the precipitate is mixed with 1/1 AcOH/H_2O vol/vol solution and then precipitated via ethanol supplement. After the generated solid material is dialyzed and freeze-dried, it is considered GM-L-Xyl fraction. The suspension obtained after the second treatment with aqueous $Ba(OH)_2$ and GM-L-Xyl fraction separation is mixed with 50% aqueous AcOH solution and then precipitated as described above. After the produced substance is purified, it is considered Xyl-L-GM portion. The application of this procedure for fractionation of LCC presented in oxygen-dignified softwood pulp results in Xyl-L-GM fraction (Lawoko et al. 2005).

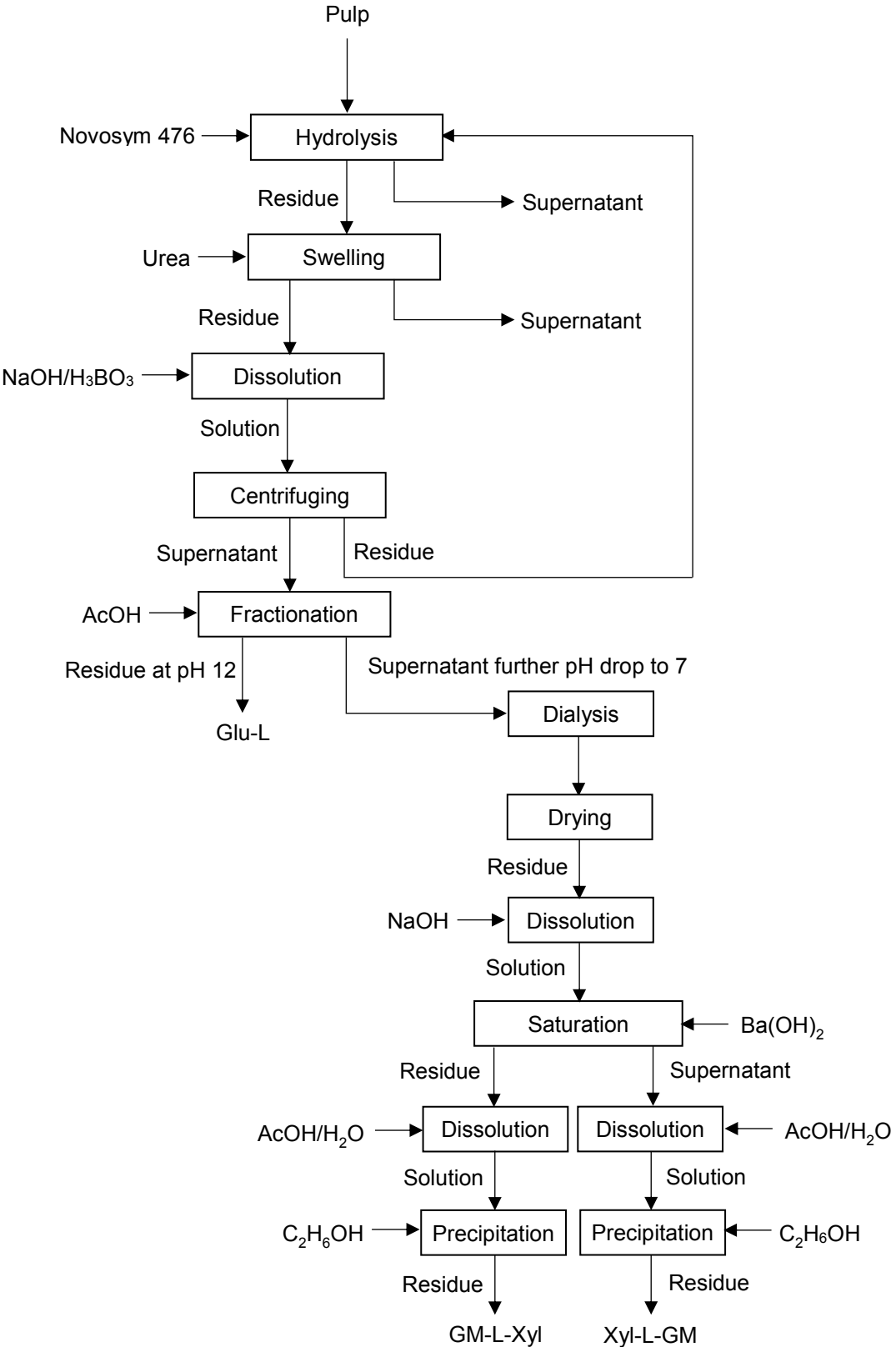

Figure 2.8 The procedure for pulp LCC fractionation (Lawoko et al. 2003; 2004).

2.7 Analysis of LCC

NMR has primarily been used for analyzing the structure of LCC. However, other methods such as alkaline, acidic degradation, oxidation, methylation and enzymatic analysis are also useful for LCC structure analysis (Iversen 1985; Azuma et al. 1985; Balakshin et al. 2014).

2.7.1 Ester linkages analysis via alkali degradation of LCC

Alkali degradation is widely applied for ester bonds identification in hardwood (Takahashi and Koshijima 1988a) and softwood (Obst 1982) LCC. The analysis is based on ester bonds saponification, lignin and polysaccharides disassociation (Bolker, 1963; Obst 1982). The procedure for alkali degradation of LCC is presented in Figure 2.9a. According to this methodology, LCC is dissolved in sodium hydroxide (0.1M NaOH) solution for 1.5-2 h at room temperature (Obst 1982; Takahashi and Koshijima 1988a). The solution is then neutralized with AcOH and centrifuged. The precipitate is washed with water and lyophilised (Obst 1982). The comparative IR analysis of untreated LCC and alkali-treated LCC demonstrates the absence of 1730 cm-1 in the spectrum of alkali-treated LCC, which confirms complete saponification of ester bonds in alkali-treated LCC preparation (Takahashi and Koshijima 1988a; Obst 1982). Obst (1982) reports that 10-20% of linkages in LCC are present in the form of esters in the alkali degradation method.

Moreover, the existence of ester linkages can be estimated via sodium borohydride (NaBH4) reduction method (Takahashi and Koshijima 1988a; Obst 1982). In this method, LCC is dissolved in water with the addition of $NaBH_4$ and NaOH (Obst et al. 1982). This treatment leads to the reduction of esters to the neutral sugars (Takahashi and Koshijima 1988a). In one analysis, the comparison of glucuronic acid concentration before and after borohydride reduction reveals that approximately 30% of linkages in beech LCC are ester-type (Takahashi and Koshijima 1988a). However, the linkages degraded due to the alkali treatment are not necessary ester bonds, as benzyl ethers with hydroxyl groups are also alkali-liable (Kosikova et al. 1979).

2.7.2 Ether linkages analysis via acid degradation of LCC

The existence of ether bonds in LCC is investigated via the combined application of sodium borohydride reduction, followed by acid treatment of reduced LCC (Eriksson and Lindgren 1977; Eriksson et al. 1980). This method is based on the analysis of new phenolic and benzyl alcohol

hydroxyl groups generated due to the hydrolysis of ether linkages (Eriksson and Lindgren 1977; Eriksson et al. 1980). The procedure for the combined acid and alkali degradation of LCC is shown in Figure 2.9b. In this method, enzymatically (enzyme Hemicellulase 680) treated spruce LCC is first subjected to the sodium borohydride treatment for the reduction of esters as described above (Eriksson et al. 1980; Takahashi and Koshijima et al. 1988a). Then, the reduced LCC is subjected to the selective hydrolysis of linkages between arabinose side chains and xylan (arabinofuranosidic bonds) by dissolution of the material in aqueous mixture of H_2SO_4/AcOH 1/1 vol/vol at 90 °C for 2 h. Hydrolyzed LCC is precipitated from the obtained solution via treating with ethyl ether ($C_4H_{10}O$) followed by centrifugation. Afterward, the produced residue is washed with ethyl ether and dried (Eriksson and Lindgren 1977; Eriksson et al. 1980). The obtained material of acidified LCC is dissolved in 1 M NaOH under N_2 conditions at ambient temperature for 40 h (Eriksson et al. 1980; Eriksson and Lindgren 1977). Then, the pH of the obtained solution is adjusted to 4 by supplement of 2 M HCl solution and the material is precipitated as described above (Eriksson and Lindgren 1977). This treatment is supposed to saponify all remaining glucuronic acid ester bonds between glucuroxylan and lignin (Takahashi and Koshijima et al. 1988a; Eriksson et al. 1980). It is suggested that xylan units remaining in the LCC after this treatment are bound to lignin by ether bonds to xylose moieties (Eriksson and Lindgren 1977; Eriksson et al. 1980).

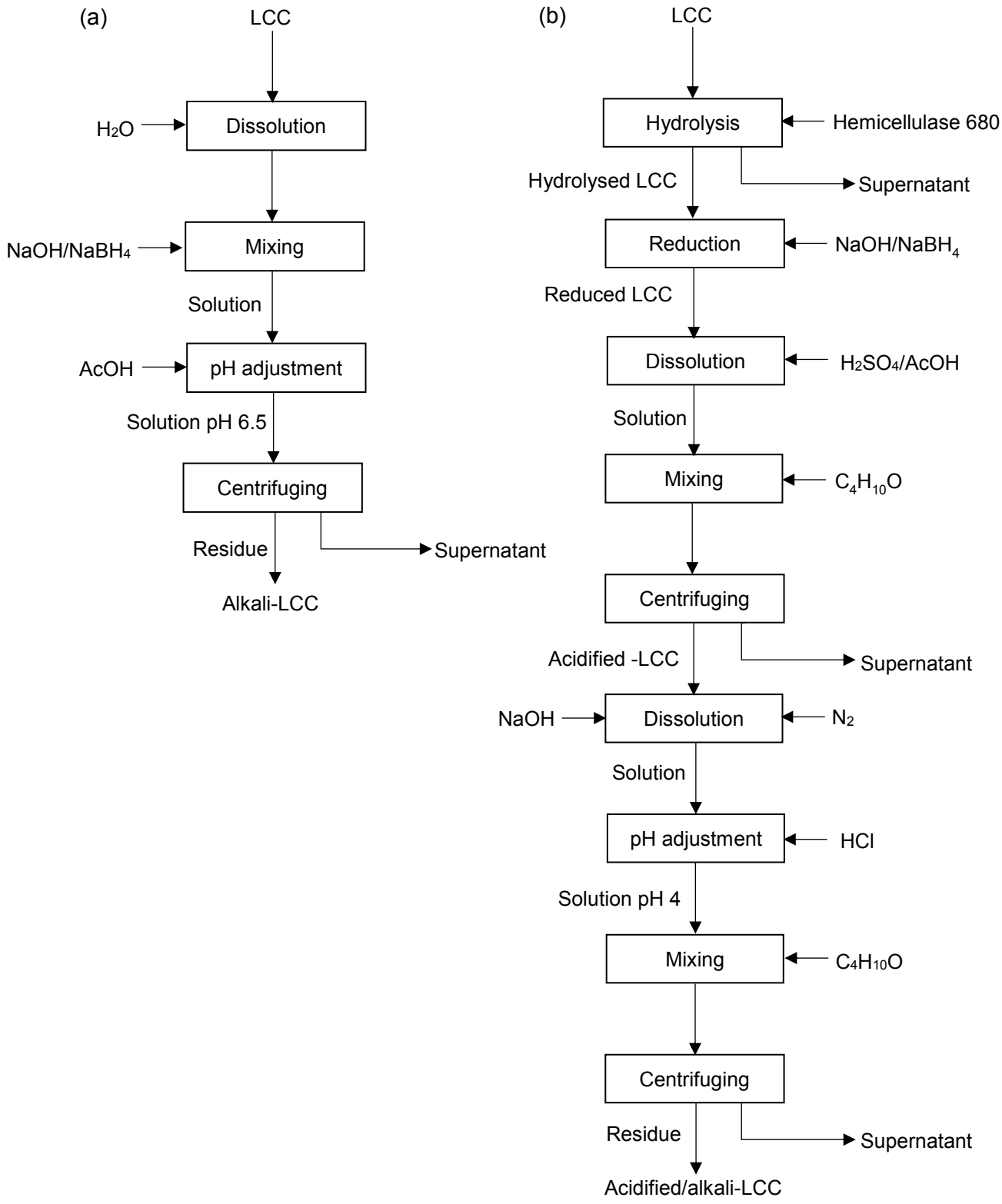

Figure 2.9 (a) Alkali-LCC (Obst 1982; Takahashi and Koshijima 1988a) and (b) acid/alkali-LCC degradation procedure (Eriksson and Lindgren 1977; Eriksson et al. 1980).

2.7.3 Phenyl glycosidic linkages analysis via smith degradation of LCC

The glycosidic linkages in softwood LCC are studied via the degradation method developed by Smith and colleagues (Dryhurst 2015). This method allows the conversion of glycosidic linkages into acyclic acetal bonds, which are liable to acid hydrolysis and can be decomposed via mild acid hydrolysis treatment (Eriksson et al. 1980). The procedure for Smith degradation of LCC is presented in Figure 2.10. This method involves periodate oxidation, borohydride reduction and acid hydrolysis stages. It is proposed that sugar moieties, remained in LCC after these treatments, are bound to lignin (Eriksson et al. 1980; Yaku et al. 1981). The LCC is first dissolved in 1/1 vol/vol of H_2SO_4/AcOH solution at the approximate LCC/solution ratio of 10/1 wt./wt. The solution is then mixed with sodium periodate ($NaIO_4$) and kept for 72 h at 5 °C in a dark place. Afterward, the insoluble material generated in the oxidation is collected and subjected to treatment with sodium borohydride ($NaBH_4$) in water for 12 h. Then, the treated material is suspended in H_2O/AcOH mixture and then hydrolyzed with 0.25 M H2SO4 solution for 8 h at ambient temperature. The precipitates of this process are collected, washed with water and treated with sulfuric acid at 100 °C for 12 h (Eriksson et al. 1980). In a related work (Yaku et al. 1981), the oxidation of LCC is performed for 220 h. Then, the solution is purified via dialysis and treated with $NaBH_4$ for 15 h; after which the solution is treated with H_2SO_4 for 15 h followed by centrifugation for precipitate separation. The collected precipitate is washed with water and ethanol, and then dried (Yaku et al. 1981).

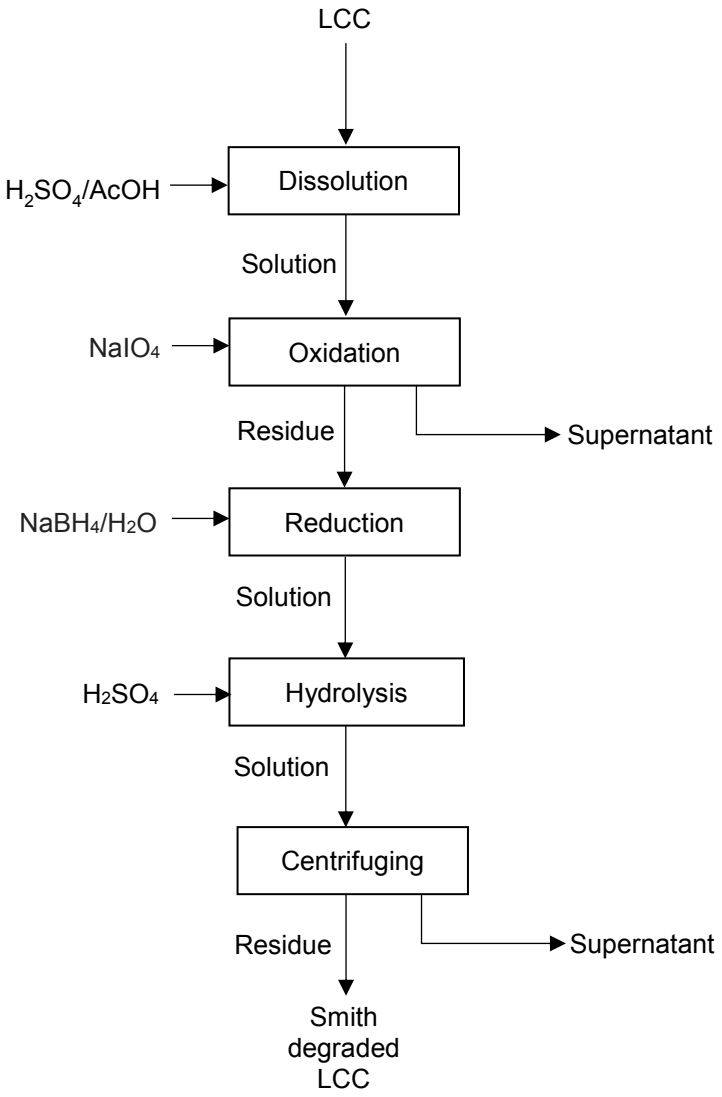

Figure 2.10 The procedure for Smith degradation of LCC (Eriksson et al. 1980; Yaku et al. 1981).

Enzymatic treatment is used as the preparation procedure prior to analysis. This method is widely applied for sugar content reduction of wood and non-wood LCC (Eriksson et al 1980; Eriksson and Lindgren 1977, Du et al. 2014; Kondo et al. 1990). Also, glucoronoyl and feruloyl esterases enzymes could be applied for benzyl ester and furulic acid ester bond studies of LCC (Sunner et al. 2015; Crepin et al. 2003).

2.7.4 Paper electrophoresis

LCC structure was studied via paper electrophoresis in 1958 by Lindgren (Koshijima and Watanabe 2003; Grushnikov and Shorygina 1970). Electrophoresis is the method of separating ionic particles and its migration with certain velocity as a result of the application of the external electric field (Lubran 1966; Frutsch and Krause 2003). In the paper electrophoresis system, two chambers (anode and cathode) are filled with a conductive medium (electrolyte) and connected by paper strip, which is soaked in the electrolyte at the opposite ends (Lubran 1966). The velocity of migration is determined by the mobility of charged particles and field strength. The electrophoretic mobility depends on charge density, size, and shape of particles (Fritsch and Krause 2003). In this analysis, Bjorkman LCC and MWL isolated from fir wood are pre-coloured with Procoin dye and then deposited on the glass-fiber paper strips near the anodic side at two different spots. Then the ends of the paper strips are placed in the 0.05 N sodium hydroxide solution and the current of 1.8 kV is applied for 45 min (Azuma et al. 1981; Grushnikov and Shorygina 1970; Koshijima and Watanabe 2003). In this work, the LCC was separated into two parts, the slower moving part composed of carbohydrates and the faster part containing both lignin and hemicellulose moieties. LCC spot was found to move slower than the MWL spot, which confirmed the hypothesis that MWL is a product of dissociation of L-C bonds (Koshijima and Watanabe 2003; Grushnikov and Shorygina 1970).

2.7.5 Determination of LCC structure via methylation

Methylation analysis could be employed as an alternative to NMR spectroscopy for structural analysis of poorly soluble carbohydrates and LCCs (Laine et al. 2002, 2004; Takahashi and Koshijima 1988b). Methylation is also used for studying lignin-carbohydrate linkages (Morrison 1974; Laine et al. 2002, 2004; Takahashi and Koshijima 1988b). The protocol for methylation of LCC preparations obtained from biomass or pulp is presented in Figure 2.11a. LCC produced from ryegrass is methylated in accordance with the procedure described by Hakomori (1964). In this method, LCC is dissolved in dimethyl sulphoxide (DMSO) in a nitrogen atmosphere. Afterward, a mixture of sodium hydride (NaH) and methyl iodide (CH_3I) is added to the solutions. Then, the solution is mixed with chloroform and filtered for sodium iodide removal (NaI). Finally, DMSO is removed from the solution via extraction with water and chloroform is evaporated via drying over anhydrous sodium sulfate (Na_2SO_4) (Morrison 1974).

The methylation of LCC generated from kraft pulp can be performed via the method developed by Ciucanu and Kerek (1984). In this process, LCC is dissolved in DMSO and mixed with NaOH and CH_3I solution. The mixture is sonicated for 30 min at ambient temperature for carbohydrate suspension. Then, the sample is mixed with water until a pH of 2.5 is reached. The suspension is then centrifuged at 6000 g for 20 min (Laine et al. 2004). The generated precipitate can be lyophilised and considered as methylated LCC.

The success of the methylation process is confirmed by reduction of absorption in hydroxyl-group region at 3400 cm-1 (Morrison 1974) and increment of absorption in methyl group regions, 2930 cm-1 in FTIR analysis (Laine et al. 2004).

After the methylation, an acid methanolysis of the methylated samples is performed (Laine et al 2002; 2004) to yield methylated monosugars (Balakshin et al. 2014). The solution is then acetylated, and the obtained alditol acetate mixture is subjected to GC/MS and gas-liquid chromatogram (GLC) analysis (Balakshin et al. 2014) for identification of carbohydrates bonding in acetylated LCCs (Takahashi and Koshijima 1988a). The nature of carbohydrate bonding is specified in accordance with unmethylated (but acetylated) sites of monosaccharides (Balakshin et al. 2014).

2.7.6 Determination of LCC structure via combined oxidation and methylation

Methylation analysis allows studying the nature of LCC bonds. The relative amount of carbohydrates and the specification of linkage in sugars involved in benzyl-ether can be determined following another approach. Watanabe and coworkers (1986) designed a procedure that included methylation and oxidation with of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) for identifying the location and frequency of the bonding sites of sugars to lignin. It is also reported that DDQ particularly attacks nonphenolic benzyl ether linkages, but neutral to glycosidic bonds in LCC (Koshijima et al. 1984, 1989).

Figure 2.11b outlines the procedure of combined application of methylation and DDQ oxidation for LCC analysis. In this process, methylation is conducted with methyl trifluoromethanesulphonate ($CF_3SO_3CH_3$) (Prehm 1980) along with cleavage of ether linkages by DDQ oxidation (Watanabe et al. 1986; Watanabe and Koshijima 1988). The softwood LCC is first acetylated with acetic anhydride ($(CH_3CO)_2O$)) and pyridine (C_5H_5N) at 40 °C for 18 h for

hydroxyl group protection. Then, the acetylated material is treated in solution of 50% aqueous dioxane and 50% of DDQ at 40 °C for 24 h. This treatment does not affect the acetyl group and glycosidic bonds between sugars, but it separates benzyl ethers from electron-donative benzene skeleton due to oxidation. In this analysis, carbohydrates bonded to lignin by ether and ester linkages at the α- and γ-conjugated positions are released due to disruption of lignin-carbohydrate linkages caused by DDQ oxidation (Balakshin et al. 2007). The new hydroxyl groups of carbohydrates liberated from the cleaved LCC bonds are subjected to a methylation procedure for further analysis. For this purpose, the effect of DDQ-oxidation is terminated by ascorbic acid ($C_6H_8O_6$) supplement. Then, the obtained carbohydrates are methylated, in accordance with Prehm (1980), by $CF_3SO_3CH_3$ at 50 °C for 3 h and methylated carbohydrates are recovered via centrifugation (Prehm 1980). Afterward, the obtained methylated samples are hydrolysed, reduced with sodium borohydride and acetylated (Prehm 1980; Watanabe et al. 1986). This product contains partially methylated alditol acetates, which are subjected to GC/MS analysis (Watanabe 1986). The position of methoxyl group specifies the location of monosaccharide bonding to lignin (Balakshin et al. 2014). Monosaccharides are indicated in accordance with their retention times by mass spectrometry (Watanabe et al. 1986; Prehm 1980). Thus, a combined application of methylation and DDQ oxidation allows for identification of positions and types of carbohydrate bonds to lignin in LCC by α- and γ- ether and ester linkages (Watanabe et al. 1986; Takahashi and Koshijima 1988a).

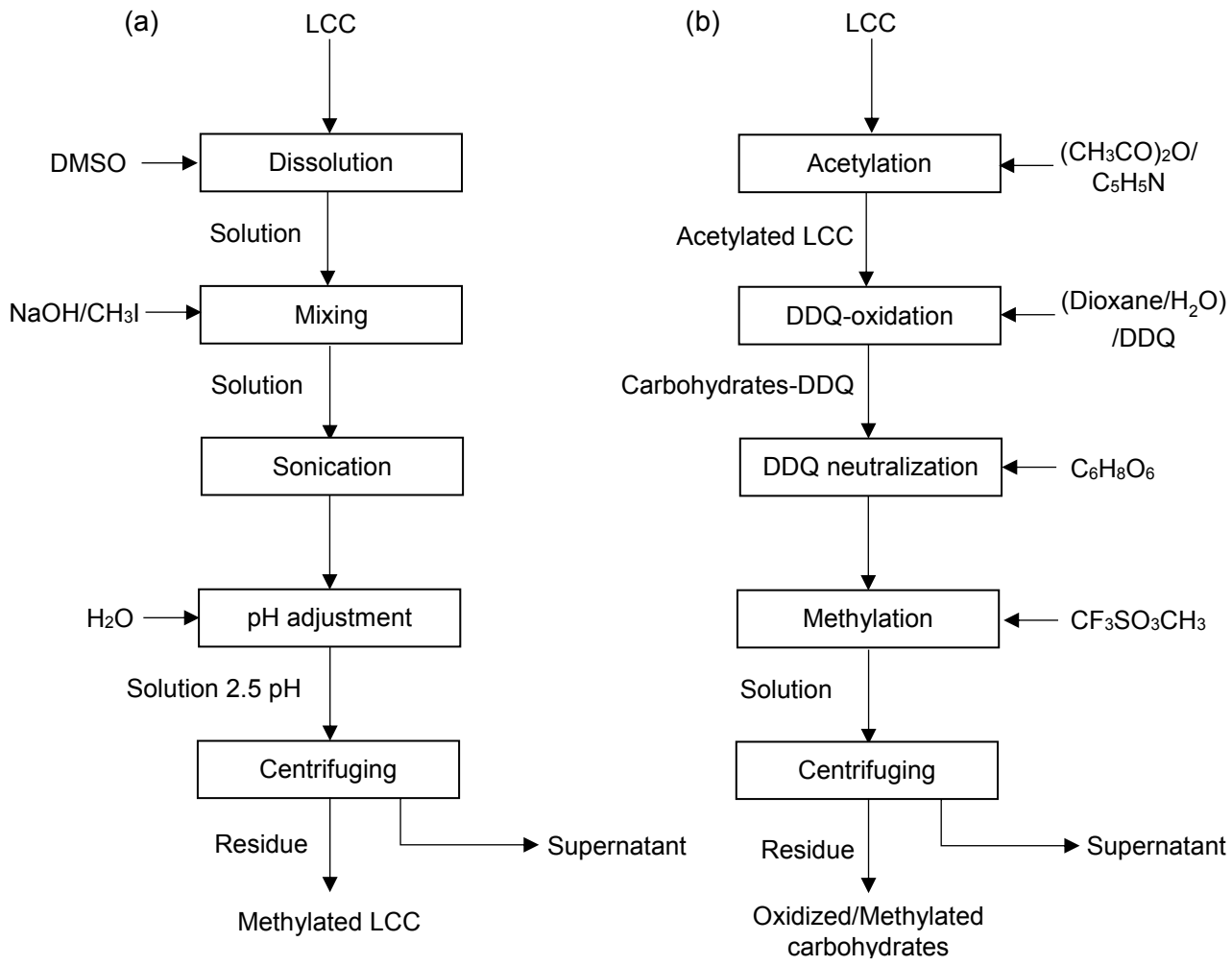

Figure 2.11 Procedures for LCC analysis by (a) methylation (Laine et al. 2004) and (b) DDQ/methylation techniques (Watanabe et al. 1986).

2.7.7 Determination of LCC presence with gel permeation chromatography (GPC) analysis

In other work, the existence of LCC is identified with gel permeation chromatography (GPC) via applying the triple detection technique (Tunc et al. 2010; Fatehi et al. 2016; Tarasov et al. 2018). The molecular weight of lignin is estimated via UV detector at 280 nm wavelength; the MW of carbohydrates is analyzed using reflective index (RI) and intrinsic-differential pressure (IV-DP) detectors (Fatehi et al. 2016). Similar retention times obtained for UV, IR and IV-DP pulses suggest the inter-linkages between lignin and hemicelluloses, and hence the presence of LCC (Tunc et al. 2010; Fatehi et al. 2016). This method is employed for investigation of LCC presence in SL of NSSC process, PHL, autohydrolysis liquor, and hydrothermally treated biomass (Fatehi

et al. 2016; Tarasov et al. 2018; Tunc et al. 2010). Also, the application of this method allows the estimation of the MW of LCC and carbohydrate free lignin (Tarasov et al. 2018; Fatehi et al. 2016).

2.8 Application of NMR technology for LCC analysis

An evaluation of LCC structure and composition via NMR technology is a major landmark in LCC analysis (Balakshin et al. 2014). Different LCCs have different affinity to dissolve in solvents; some LCCs could be dissolved in deuterium oxide (D2O), deuterated dimethyl sulfoxide (DMSO-d6) or 50/50 vol/vol D_2O/THF-d8 solutions, but some are insoluble in organic solvents, such as tetrahydrofuran-d8 (THF-d8) and chloroform (Uraki et al. 2006). Generally, the high solubility of LCC in DMSO-d6 makes the application of this solvent attractive for NMR analysis (You et al. 2015).

^{1}H NMR, ^{13}C NMR and 2D HSQC NMR are widely used for lignin and hemicelluloses analysis (Capanema et al. 2004; Nagy et al. 2010; Ma et al. 2012). Recently, due to the superimposition of signals in ^{1}H and ^{13}C NMR frequency resources, 2D HSQC (^{1}H-^{13}C) NMR technology has been widely applied for structural analysis of LCC bonds (Balakshin et al. 2011; Yuan et al. 2011).

^{1}H-NMR is reported to provide information on the presence and compositions of the hydroxyl groups in LCC. In one study, Skurikin (1968) suggests the existence of carbohydrate units in lignin extracted from oak wood (via ethanol treatment) based on the higher signal intensity of aliphatic hydrolxyl protons in LCC than in other lignin samples (Kosikova et al. 1973). Merewether et al. (1972) report that the amount of aliphatic, phenolic hydroxyl groups and free carboxylic groups in hardwood LCC is 0.6, 0.9 and 0.1 per phenylpropane unit (C9), respectively. The lignin present in hardwood contains around 0.32 phenolic hydroxyl groups per C9 unit (Freudenberg and Neish 1968). The excess of phenolic groups in LCC could be related to the cleavage of alkali-liable linkages during LCC formation/extraction (Merewether et al. 1972). In another study, Kosikova et al (1973) investigate the effect of alkaline and acid hydrolysis on the composition of LCC isolated from beech wood via ^{1}H-NMR spectroscopy. It is found that both types of hydrolysis do not affect the structure of insoluble lignin in LCCs as no significant changes in distribution of protons in aromatic region are observed (Kosikova et al. 1973). Prior to the ^{1}H-NMR studies, LCC samples could be acetylated (Merewether et al. 1972) for a better resolution in NMR analysis (Balakshin et al. 2011; Du et al. 2013). However, acetylation may cause chemical modifications of lignin, which is undesirable (Pu et al. 2013). ^{1}H NMR analysis takes only a few minutes (You

and Xu 2016), but the data obtained during ^{1}H-NMR assessment may be indistinct due to signal overlapping (Koshijima and Watanabe 2003) originating from the short chemical shift diapason (δ_H 12-0 ppm) of ^{1}H NMR spectra (Pu et al. 2013).

^{13}C-NMR is reported to provide information on the composition of lignin or carbohydrate parts of LCC (Balakshin et al. 2014; You et al. 2015) and positions of lignin-carbohydrate bonds on lignin's side chains (Xie et al. 2000). In one study, lignin-carbohydrate bonds are reported to be located at the Cα-position of lignin moieties in the LCCs isolated from Ginkgo wood (Xie et al. 2000). In another work, ^{13}C-NMR analysis of the LCC isolated from oat wheat confirms sugar bonding with lignin at α-position of lignin (Barakat et al. 2007). ^{13}C NMR technology has a significantly wider shift range (δ_C 0-200 ppm) than ^{1}H-NMR spectroscopy. However, in the LCC analysis, intensive signals of carbons present in carbohydrates could impede the accurate designation of signals from carbons present in lignin moieties of LCC (Xie et al. 2000). Xie et al. (2000) apply a method where LCCs are enriched with ^{13}C lignin precursors. This modification allows for increasing the intensity of signals produced from LCC linkages (Xie et al. 2000). Barakat et al. (2007) also observe a peak at 81 ppm of ^{13}C-NMR spectrum of non-wood LCC, which is assigned to benzyl ether groups. However, these results are not conclusive, as these signals could also be attributed to aryl glucerol and spiro-dienone substructures of lignin and carbohydrates (Zhang and Gellerstedt 2001; Balakshin et al. 2007). The ^{13}C NMR analysis of lignin material isolated via ball-milling can be used for structural analysis of LCC (Min et al. 2014). It is reported that phenyl glycosidic, benzyl ether and ester linkages can be indicated by the clusters at δ_C 103-96 ppm, 90-78 ppm and 65-58 ppm in ^{13}C NMR spectra, respectively (Min et al. 2014). However, Balakshin et al. (2014) state that ^{13}C-NMR analysis cannot be considered as a dependable method for LCC linkage analysis as its signals overlap the signals from lignin or carbohydrate units. Also, ^{13}C-NMR is a very time-consuming analysis, as it takes more than 24 h to obtain a reliable spectrum (You and Xu 2016).

2.8.1 Qualitative analysis using NMR technology

For the accurate investigation and detection of lignin carbohydrate bonds, a combined analysis of signals from both protons and carbons should be conducted. This analysis can be executed with the use of 2-dimensional ^{1}H-^{13}C NMR technology. The main advantage of 2-dimensional technology over 1-dimensional technology is a significantly better dispersion of lignin and

carbohydrates impulses (Balakshin et al. 2003), which is attributed to its ability to avoid the overlapping of signal from ^{1}H nucleus by correlating it with signals from ^{13}C nucleus (Ralph and Landucci 2010). This leads to a noticeably improved resolution of NMR spectra (Ralph and Landucci 2010) and more accurate allocation of lignin and carbohydrates signals (Balakshin et al. 2003). Numerous 2-dimensional NMR techniques are applied for investigating LCC's structure and composition, such as heteronuclear multiple bond coherence (HMBC) (Balakshin et al. 2007), total correlation (TOCSY) (Evtuguin et al. 2005) and heteronuclear single quantum coherence (HSQC) (Yuan et al. 2011; Balakshin et al. 2011; Du et al. 2014). The HSQC technology is the most widely employed method due to its diversity in representing structural features and modifications of lignin and carbohydrate units (You and Xu 2016). The HSQC NMR is reported to provide information on lignin carbohydrate linkages in LCC obtained from various softwoods (Balakshin et al. 2011; Giummarella et al. 2016; Du et al. 2014), hardwoods (Yuan et al. 2011; Balakshin et al. 2011) and herbaceous plants (You et al. 2015; Yao et al. 2016; Zhang et al. 2016). Balakshin and colleagues report the direct detection of phenyl glycosidic linkages in the LCC of eucalyptus (Balakshin et al. 2014) and pine (Balakshin et al. 2007). Also, 2-dimensional NMR analysis of pine LCC indicates the presence of benzyl ether linkages (Balakshin et al. 2007), which confirms the findings reported by Watanabe et al. (1986) via DDQ oxidation technique. The absence of signals from α-ester linkage is reported in 2-dimensional NMR studies of LCC (Du et al. 2014; Yuan et al. 2011; Balakshin et al. 2007). Balakshin et al. (2007) observe a significant presence of γ-ester in pine LCC, whereas Yuan et al. (2011) report that the signals of γ-esters on the 2 dimensional NMR spectra of poplar LCC are indistinct. The 2-dimensional NMR technology is also applied for investigating the presence of γ-ether in LCC linkages. However, the region, where these linkages may be located (δ_C/δ_H 65-75/3.0-4.5 ppm), extensively overlapped other areas of the spectrum (Balakshin et al. 2014). Three-dimensional (3D) NMR technology could be employed for deeper investigation of lignin and carbohydrates structural features (Ammalahti et al. 1998; Balakshin et al. 2014). 3D HSQC-TOCSY technology provides a combined analysis of ^{1}H-^{1}H and ^{1}H-^{13}C correlations. HSQC spectroscopy demonstrates the interconnectivity of protons and carbons spectroscopy, while TOCSY projection associates these with other hydrogen nuclei of the ^{1}H-^{1}H spin system (Liitia et al. 2003). Thus, the 3-dimensional NMR analysis allows collection of more accurate information regarding certain structural features of lignin and carbohydrates. However, long experimental period (24-48 h) (Ralph and Landucci 2010) and

ability to collect the majority of structural data of lignin and carbohydrates via 1-dimensional and 2-dimensional NMR spectroscopies impedes the application of 3-dimensional NMR technology (You and Xu 2016).

The concentration of LCC for 2-dimensional HSQC NMR analysis seems to be different in different experiments. Yuan et al. (2011) solubilise 90 mg of Björkman LCC sample in 0.5 mL of DMSO-d6, whereas Du et al. (2014) dissolve 20 mg of enzymatically treated LCC in 0.75 mL of DMSO-d6. Also, the number of collected complex points is 1024, and 1.5 s is the relaxation delay for ^{1}H-dimension (Yuan et al. 2011). For 2-dimensional HSQC NMR analysis of enzymatically treated LCC, the collected complex points 2048 and 1s recycle delay are recorded for ^{1}H-dimension (Du et al. 2014). Furthermore, 64 transients and 256 time increments are documented in ^{13}C-dimension in both studies. The signal of DMSO-d6 at δ_C/δ_H 39.5/2.49 ppm is considered as a reference (Yuan et al. 2011; Du et al. 2014). Table 2.5 lists 2 dimensional HSQC NMR signal assignments for LCC linkages with DMSO-d6 used as a solvent.

Table 2.5 Two-dimensional HSQC NMR shift in DMSO-d6 for LCC linkages.

LCC linkage	δ_C/δ_H, ppm
Benzyl ether	
C1-α lignin-C-6 of Glu, Gal, Man and C-5 of Ara	80-81/4.5-4.7[1]
C2-α lignin-Xyl	80-81/5.1-4.7[1]
C2--α lignin-Xyl	81.2/5.1[2]
Ester	
α-ester	75/6.1[1]
γ-esters	65-62/4.0-4.5[1,2]
Phenyl glycosidic	
$PhyGlc_1$	100.2/5.03[3]
$PhyGlc_2$	100.3/4.85[3]
$PhyGlc_3$	101.9/4.86[3]

[1]Wen et al. 2013; [2] Balakshin et al. 2011; [3]Du et al. 2014

2.8.2 Quantitative analysis of LCC linkages using NMR

A quantitative evaluation of LCC linkages in NMR analysis became possible with a method developed by Zhang and Gellerstedt (2007). This procedure involves a combined application of ^{13}C and HSQC NMR technologies. The main aspect of this approach is the use of certain clusters of ^{13}C spectra as internal references for conversion of the corresponding signals present in 2-dimensional spectra into absolute values (Balakshin et al. 2014; Yuan et al. 2011). The region

between 102 and 162 ppm in ^{13}C NMR spectrum is considered as reference since the peaks belong to 6 aromatic carbon rings and 0.12 vinylic carbons (Capanema et al. 2004). To obtain a number of substructures present in the region of interest per 1 aromatic unit, the integral areas of these peaks should be divided by 6.12 (Capanema et al. 2004). Integration of clusters at 103.6-96.0 ppm, 90.0-78.0 ppm and 64.5-58.5 ppm in a ^{13}C NMR spectrum should be applied for quantitative analysis of PhyGlc, BE and ester linkages, respectively (Yuan et al. 2011; Balakshin et al. 2011). Amounts of PhyGlc, BE and ester linkages in Björkman LCC (Yuan et al. 2011) and LCC-AcOH (Balakshin et al. 2011) per 100 Ar can be estimated in accordance with the following equations:

$$\boldsymbol{PhyGlc} = \frac{\boldsymbol{2D_{PhGlc}}}{\boldsymbol{2D_{103-96/5.5-3.8}}} \times \frac{\boldsymbol{13C_{103-96}}}{\boldsymbol{13C_{163-106}}} \times \boldsymbol{600} \quad (2.1)$$

$$\boldsymbol{BE} = \frac{\boldsymbol{2D_{BE}}}{\boldsymbol{2D_{90-78/5.7-3.0}}} \times \frac{\boldsymbol{13C_{90-78}}}{\boldsymbol{13C_{163-106}}} \times \boldsymbol{600} \quad (2.2)$$

$$\boldsymbol{Ester} = \frac{\boldsymbol{2D_{Est}}}{\boldsymbol{2D_{65-85/5.0-2.5}}} \times \frac{\boldsymbol{13C_{65-58}}}{\boldsymbol{13C_{163-106}}} \times \boldsymbol{600} \quad (2.3)$$

where, $2D_{PhyGlc,}$ $2D_{BE}$ and $2D_{Est}$ are the volumes of the signals assigned to the PhyGlc, BE, and ester linkages, respectively (Table 2.5); $2D_{103\text{-}96/5.5\text{-}3.8}$, $2D_{90\text{-}78/5.7\text{-}3.0}$ and $2D_{65\text{-}85/5.0\text{-}2.5}$ are the total resonance of signals in the corresponding areas of the 2D spectra; $^{13}C_{103\text{-}96}$, $^{13}C_{90\text{-}78}$ and $^{13}C_{65\text{-}58}$ are the volume of specified clusters signals in the ^{13}C spectra and 600 (or 612) is the number of aromatic carbons in 100 monomeric lignin moieties (Balakshin et al. 2011).

Another strategy for LCC linkages and lignin inter-unit quantification is to use the data from HSQC spectra and aromatic units (C_9) as an internal standard (Wen et al. 2013). This approach uses the specific clusters of signals in HSQC spectra, which include all aromatic units. The total amount of C_9 units in softwood, hardwood and non-wood species can be quantified by integration values, in accordance with equations 2.4, 2.5 and 2.6, respectively (Sette et al. 2011; Wen et al 2013):

$$\boldsymbol{IC_9} = \boldsymbol{G_2} \quad (2.4)$$

$$\boldsymbol{IC_9} = \boldsymbol{0.5IS_{2,6}} + \boldsymbol{G_2} \quad (2.5)$$

$$\boldsymbol{IC_9} = \boldsymbol{0.5IS_{2,6}} + \boldsymbol{G_2} + \boldsymbol{0.5IH_{2,6}} \quad (2.6)$$

where IG_2 is the integration value of cluster assigned to guaiacyl lignin (G_2) units in HSQC spectra (Sette et al. 2011). The correlations of the C2-C6 position of syringyl units ($S_{2,6}$) are twice the amount of syringyl units (S-type lignin); hence, to prevent an overestimation of C_9 units, half of $S_{2,6}$ integration value ($IS_{2,6}$) was used for C_9 quantification (Sette et al. 2011). Aromatic units in herbaceous species, in addition to G and S units, also include hydroxyphenyl units (H-type lignin). Therefore, for the quantification of C_9 units presented in grasses, half of the integral value of the clusters assigned to C2-C6 hydroxyphenyl moieties ($H_{2,6}$) is also included (Wen et al. 2012). Then, the amount of LCC linkages per 100 Ar could be estimated following equation 2.7 (Zhang et al. 2016):

$$\boldsymbol{AX = \frac{IX}{IC_9} \times 100} \tag{2.7}$$

where AX is the amount of LCC linkages per 100 Ar; IX is the integration value of the objective bond. Zhang et al. (2016) applied this method for the estimation of LCC linkages in LCC-AcOH preparation of bamboo.

Another method for the evaluation of LCC linkages involves the integration of a corresponding region of 2-dimensional spectra assuming the sum of identified substructures to be 100% (Ibarra et al. 2007). The main disadvantage of this method is its inability to evaluate LCC linkages and other substructures in absolute values (Balakshin et al. 2011). The combined application of data from ^{13}C and HSQC NMR provides more reliable information about LCC linkages due to the more accurate quantification of aromatic units with ^{13}C NMR spectroscopy.

2.9 LCC application

2.9.1 Anti-microbial and anti-HIV effects of LCC

The LCCs isolated from *Lentinus edodes* mycelia, pinecone and pine nut shells via alkaline extraction and acid precipitation show a high anti-UV effect, which could be applied for manufacturing a sunscreen (Vinardell and Mitjans 2017). It is articulated that LCC from *Sasa senanesis* Rehder leaves have a higher anti-UV activity comparing with natural polyphenols (Sakagami et al. 2016).

In addition, different medicobiologic applications of LCC are suggested (You et al. 2015). Zhang et al. (2007) note a high anti-herpes activity of LCC of pine cone and Prunella plant. Sakagami et

al. (2010) reported anti-HIV, anti-influenza virus and anti-herpes effects of LCC extracted from pine cones. The antiviral and immunostimulatory effects of LCC from herbaceous plant (*P.anisum*) are also reported by Lee et al. (2011). It is assumed that the mechanism of anti-HIV activity is related to the ability of LCC to inhibit the HIV adsorption and penetration into cells (Sakagami et al. 2010). It is found that the lignin units are more important for anti-HIV activity than sugar moieties. However, the application of phenylpropenoid monomers did not demonstrate any anti-HIV activity, which implies the significance of highly polymerized structure of LCC (Sakagami et al. 2010). Different LCCs from softwood cone or seed shells demonstrate the ability to stimulate anti-microbial activity. It is reported that the anti-microbial activity of LCC is considerably reduced with the carbohydrate unit degradation, which indicates the importance of sugar units for anti-microbial activity of LCC (Sakagami et al. 2010).

2.9.2 LCC-based biological carriers

Lignin-carbohydrate complexes demonstrate good biological compatibility and mechanical resistance (Kai et al. 2016; Erakovic et al. 2009). These properties are attributed to a combination of water-repellent, inelastic lignin units, and hydrophilic, flexible carbohydrates in LCCs (Zhao et al 2017a). Zhao et al. (2017a,b) applied LCC fractions from poplar wood for the preparation of spherical biocarriers. Biocarriers are inactive compounds able to attract, keep and biomagnify certain microorganisms (Fliermans 1996). Zhao et al. (2017a,b) estimate the proliferation of liver cells after application of biocarriers prepared from various species of hardwood (Poplar) and softwood (*Ginkgo biloba* L). Galactose units of LCC are able to recognize liver cells due to the presence of asiaglycoprotein receptors (ASGPR) on hepatocytes, as galactose functions as ligand and bind hepatic cells with these receptors. LCC-hepatocytes complexes are proposed to be able to culture hepatocytes due to the interaction of galactose and ASGPR (Zhao et al. 2017b). The effect of LCC biocarriers on metabolic activity of hepatocytes was evaluated; the results demonstrate the improvement of liver cells proliferation. It was reported that the cell number of human hepatocytes, cultured in hardwood LCC carriers and control groups (without application of LCC biocarriers) in a definite time period is 1.84-1.68×10^5 cells/mL and 1.32×10^5 cells/mL, respectively (Zhao et al. 2017a). The implementation of softwood LCC biocarriers increases the number of cells cultured to 6.5×10^4 cells/mL, whereas the control group shows cell numbers of 5.5×10^4 cells/mL (Zhao et al. 2017b). The hepatocytes cultured in the LCC biocarriers show significantly higher values of albumin secretion and blood urea nitrogen released from the

hepatocytes, which indicates a better biocompatibility and higher metabolic activity of cells cultured in LCC biocarriers (Zhao et al. 2017a). The results show that LCC biocarriers are highly biocompatible and could be applied as a precursor of biomaterial for culturing human liver cells (Zhao et al. 2017a,b).

2.9.3 Other applications

Due to the abundance of hydroxyl groups, LCC can also be applied in composite production as a component in polyurethane polymers and epoxy resins (Singh et al. 2005). LCC could be used in polymer composites, as carbohydrate moieties are able to adhere to other ingredients in the polymer system (Singh et al. 2005).

2.10 Summary

The composition, structure and properties of LCC present and extracted from different biomass sources are described in this review chapter. In softwoods, all lignin moieties are involved in LCC, whereas in hardwoods and herbaceous plants, LCC constitutes 47-66% and 16-35% of total lignin, respectively. The predominance of benzyl ether linkages is reported in softwood LCC, whereas esters and phenyl glycosidic bonds were found to be dominant in deciduous species. Likewise, in the case of non-wood plants, phenyl glycosidic linkages are dominant. A high amount of benzyl ether and phenyl glycosidic bonds negatively affect kraft pulping and the delignification performance due to alkali resistance of these linkages; while, the ester bonds are alkali liable. Softwood species contain LCC with two different structures of lignin, namely lignin-xylan and lignin-glucomannan. However, hardwood species contain xylan-lignin and cellulose-lignin complexes. Herbaceous LCC mainly contains arabinoxylan linked with lignin moieties via ferulate esters. The application of DMSO/TBAH mixture with $Ba(OH)_2$, followed by an enzymatic hydrolysis, allows for the separation of three LCC fractions and is considered to be a universal method of LCC fractionation from biomass. Alkali and combined acid/alkali degradation strategies could be employed for ester and ether linkages analysis. The Smith degradation method is applied for the estimation of phenyl glycosidic bonds of LCC. The application of DDQ and methylation allows the identification of the bonding sites of sugars involved in benzyl ether and ester linkages. GPC analysis reveals LCC existence in black liquor, hydrolysates, PHL and SL of NSSC process. ^{1}H -NMR technology is applied to research alkali-liable and acid-liable bonds existence in LCC. ^{13}C-NMR spectroscopy analysis elucidates the bonding sites of sugar units to lignin moieties.

However, for accurate determination and quantification of lignin-carbohydrate linkages, a combined application of 2D HSQC and ^{13}C-NMR technologies is required. Last but not least, LCC materials show promising results as anti-HIV agents due to their ability to inhibit HIV adsorption and to penetrate into cells. Moreover, LCCs seem to be efficient as precursors for biocarrier production.

2.11 References

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Chapter 3 Flow through autohydrolysis of spruce wood chips and lignin carbohydrate complex formation

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3.1 Abstract

Autohydrolysis technology is widely used for extracting lignocellulose from wood chips. In this chapter, the flow through autohydrolysis of spruce wood chips was studied fundamentally. In addition to rising the temperature to 185 °C and prolonging the time of hydrolysis to 75 min, a flow rate of 2L/min led to more lignocellulose removal from wood chips. However, the lower temperature of 170 ºC, 15 min and flow rate of 6 L/min led to lignocelluloses with a larger molecular weight in the hydrolysis liquor. Gel permeation chromatography analysis (GPC) confirmed the existence of lignin-carbohydrate complexes (LCC) in the hydrolysis liquors. The composition of lignin, hemicelluloses and LCC in hydrolysis liquors were related to autohydrolysis conditions. Nineteen percent of lignin moieties were in LCC form in the hydrolysis liquor produced under the conditions of liquid/solid (L/S) ratio of 10 (wt./wt.), 180 ºC and 15 min. Furthermore, the liquor produced under the conditions of L/S ratio of 5/1, 190 °C and 15 min contained 89% of lignin in LCC forms. Moreover, the efficiencies of membrane dialysis, acidification and ethanol extraction in extracting lignocellulose were different for different hydrolysis liquors implying that the properties of hydrolysis liquors (and thus the hydrolysis conditions) would significantly affect the performance of downstream processes for isolating lignocellulose from hydrolysis liquors.

3.2 Introduction

Lignocellulosic materials consist mainly of cellulose, hemicellulose, lignin and extractives (Ayeni et al. 2015; Sarip et al. 2016). They have great potential for use in the production of value-added products. Cellulose is widely used for ethanol production as well as in pharmaceutical and papermaking industries (Garcia et al. 2011; Shokri and Adibka 2013; Zheng et al. 2009). Hemicelluloses could be used for ethanol, xylitol and furfural productions (Chandel et al. 2011; Pothraj et al. 2006). Lignin could be applied in the production of carbon fibers, car brakes, polyurethane foams and dispersants (Lora and Glasser 2002; Vishtal and Kraslawski 2011).

To use these lignocellulosic materials in value-added productions, they should be extracted from woody biomass effectively. Autohydrolysis technology is a chemical-free, efficient, cost-effective and commercially applied method for extracting hemicelluloses from woody biomass (Carvalheiro et al. 2008; Sixta et al. 2013). The autohydrolysis process could be performed using hot water or steam (Galia et al. 2015; Pienkos and Zhang 2009; Santos Muguet et al. 2013). However,

hemicellulose extraction is associated with lignin extraction in the autohydrolysis processes (Carvalheiro et al. 2008).

In the past, research on the autohydrolysis process was conducted in batch systems (Galia et al. 2015; Kumar and Christopher 2017). The flow through technology may have advantages over batch systems. For example, more lignin and hemicellulose extraction and less fermentation inhibitors were claimed to be produced in the flow through process compared to the batch process (Galia et al. 2015; Liu and Wyman 2003).

It was reported in the literature that autohydrolysis conditions impacted the isolation of lignocellulose from wood chips. The effects of temperature and residence time on autohydrolysis were studied for batch systems (Kapu and Trajano 2014). Ando et al. (2000) stated that the majority of lignin and hemicelluloses could be extracted from bamboo and chinquapin in a flow through process in the temperature range of 180 and 285 °C. However, Japan cedar lignin showed high resistance against isolation at the temperature range of 180 and 250 °C (Ando et al. 2000). It was reported that the flow through autohydrolysis of corn stover under the conditions of 0-10 mL/min flow rate, 180 °C and 16 min treatment time isolated 20-57.5 wt.% of xylan and 14.7-34 wt.% of lignin (Liu and Wyman 2003).

Water consumption is an important economical factor in the hydrolysis process (Jansson et al. 2014), which makes the liquid to solid (L/S) ratio a crucial factor at determining the feasibility of the autohydrolysis process. Tunc (2014) reported that an increase in the L/S from 3/1 to 50/1 resulted in an improvement in removing lignin from 2 wt.% to 4 wt.% from *Eucalyptus globulus* in a batch system. The increase in L/S ratio also decreased the polysaccharide and monosaccharide extractions from 3 wt.% to 1.6 wt.% and from 1.2 wt.% to 0.3 wt.% from wood chips, respectively. In another batch system studied by Testova and coauthors (2009), lignin and xylan concentrations increased with the L/S ratio rise in the hydrolysis liquor for birch wood species. These results depicted that the amount of water was a major factor in separating lignocellulose in the autohydrolysis process. However, research on the flow through autohydrolysis was mainly conducted on agro-based biomass. It is well known that softwood has more condensed structure than agro-based biomass (Fox and McDonald 2010). Thus, available literature results may not be conclusive for predicting the performance of flow through autohydrolysis of softwood species. The first objective of this study was to investigate the impact of flow through autohydrolysis process conditions on the extraction of lignin and hemicelluloses from spruce wood chips.

As stated above (Ando et al 2000; Liu and Wyman2003), the amounts of lignin and hemicellulose extractions in hydrolysis processes were correlated implying that 1) the separation of lignocellulose from hydrolysis liquor may not produce pure hemicelluloses or lignin and 2) lignin and hemicelluloses may have chemical bonds and be present in lignin-carbohydrate complexes (LCC) in hydrolysis liquors. Lawoko et al. (2006) claimed that almost all of lignin and polysaccharides are bound in spruce wood species. In the autohydrolysis process, lignin and polysaccharides may be separated as individual segments or still present in LCC form in hydrolysis liquors. Recently, the presence of LCC in the hardwood-based hydrolysis liquor and neutral sulfite semichemical (NSSC) spent liquor were reported (Fatehi et al. 2016). The second objective of this study was to investigate the presence and properties of LCC in softwood-based hydrolysis liquor produced in an autohydrolysis process.

It is well known that the concentration of lignocellulose is low in hydrolysis liquors. To develop economically feasible process for the end-use application of lignocelluloses, they should be extracted from the hydrolysis liquors. It was reported that lignin and hemicelluloses could be effectively isolated from prehydrolysis liquor (PHL) and black liquor of kraft pulping processes via acidification or organic solvent extraction (Liu et al. 2011a,b; Tarasov et al. 2015). Membrane technology has been widely used for isolating lignin and hemicelluloses from hydrolysis liquors (Oinonen et al. 2015). Also, Du et al. (2013) applied membrane dialysis technology for LCC fractions separation. The third objective of this work was to assess the efficiency of different methods in isolating lignocellulose from hydrolysis liquors that were produced under different conditions.

In this research work, the autohydrolysis of softwood chips (spruce) was comprehensively studied in a flow through system. The main purpose of this work was to evaluate the effect of process conditions on the performance of autohydrolysis in extracting lignocellulose from spruce. The presence of LCC and the impact of autohydrolysis on the properties of LCC in the hydrolysis liquors was investigated for the first time. Based on the concentration, compositions and molecular weights of lignin and hemicelluloses in hydrolysis liquors, the properties of LCC in hydrolysis liquor was proposed. Furthermore, the efficiency of different extraction processes in isolating lignocellulose from hydrolysis liquors was investigated in detail.

3.3 Materials and methods

3.3.1 Materials

Spruce wood chips were obtained from a pulp and paper mill located in Northern Ontario, Canada. The wood chips were stored in sealed plastic bags at 4 °C prior to use. Ethanol (95 vol. %), sulfuric acid (98 wt. %) and sodium hydroxide powder (97 wt. %) were obtained from Fisher Scientific and used as received. $NaNO_3$ powder, analytical grade, was purchased from Sigma Aldrich. Cellulose acetate membrane tubes (molecular weight cut-off of 1000 10,000 and 25,000 g/mol) were purchased from Wako Chemicals, Japan. Also, 3-(trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt (TSP) was purchased from SigmaAldrich company.

3.3.2 Autohydrolysis

The process of autohydrolysis was conducted using a 2 L pulping digester (Greenwood Instruments, 2200). In this analysis, liquid is collected from the bottom of the digester and circulated to the top to provide a flow through process. In each experiment, 300±5g of wood chips were loaded in the pulping digester and distilled water was poured into the vessel. The impact of liquid/solid (L/S) ratio on the autohydrolysis of softwood chips is not reported in the literature for flow through systems. In the past, the autohydrolysis experiments of softwood species was only conducted in batch systems with the L/S ratio ranging from 4/1 to 10/1 wt./wt. (Kapu and Trajano 2014). In the present work, the L/S ratios of 4/1, 5/1, 6/1, 8/1 and 10/1 (based on the dry weight of wood chips) were carried out.

Song et al. (2008) conducted hot water treatment of spruce chips and sawdust in a batch system. It was reported that the temperature rise from 160 °C to 180 °C resulted in a significant lignin extraction from both wood chips and sawdust. In the current study, the experiments were conducted at temperatures of 170, 175, 180, 185 and 190 °C for the process time range of 15 and 75 min. The residence time study was conducted after optimizing the hydrolysis temperature. In another work, flow through autohydrolysis of spruce sawdust was conducted with only fresh water (without liquid circulation) for preventing hemicellulose degradation (Leppanen et al. 2001). In the present study, hydrolysis liquor was circulated in the digester to resemble the process applied in industry.

Furthermore, a liquid flow rate ranging between 2 and 6 L/min was used in this work. For temperature effect assessment, experiments were conducted at 15 min residence time; for residence

time effect they were carried out at 180 °C; both sets of experiments were carried out at the L/S ratio of 5 wt./wt. and liquid flow rate of 6 L/min. The experiments for L/S ratio effect was conducted at 180 °C and 6 LPM flow rate for 15 min. The experiments for the flow rate effect were performed at 180 °C for 15 min residence time and L/S ratio of 5 wt./wt. After each experiment, the produced hydrolysis liquor was collected and kept at 4°C in a refrigerator prior to analysis.

3.3.3 Separation of lignocelluloses from hydrolysis liquors

The lignocelluloses were separated from hydrolysis liquors using different methods. (1) The hydrolysis liquor was concentrated using membrane dialysis with three molecular weight cuts-off of 1000, 10,000 and 25,000 g/mol. The dialysis tubes were filled with approximately 50±1 g of hydrolysis samples and placed into distilled water for 48 h. The distilled water was changed every 8-12 h. Then, the samples were transferred from dialysis tubes to plastic containers and stored at 4 °C as previously discussed. (2) Lignin isolation via acidification of spent liquors has been widely reported in the literature (Liu et al. 2011a; Shi et al. 2011). In one set of experiments, the pH of 100±5g hydrolysis liquor was decreased to 1.5 by adding 20 wt.% sulfuric acid to hydrolysis liquors at room temperature (Liu et al. 2011a; Shi et al. 2011). After acidification, the acidified samples were placed in containers and centrifuged at 4000 rpm for 5 min. Afterward, the supernatants and the precipitants were separated. The supernatants were stored in a refrigerator 4°C. The precipitants were transferred to glass containers and placed in an oven at 60°C for 48 h. (3) Hydrolysis liquors were mixed with ethanol at the ethanol/hydrolysis ratio of 4/1 wt./wt. as described by Liu and coworkers (2011a). Due to limited solubility of lignocellulose in ethanol (Tarasov et al. 2015), lignocellulose aggregate via hydrogen bonding in the mixture and precipitate (Fatehi and Chen 2016).

3.3.4 Lignin, hemicelluloses, acetic acid and furfural analyses

The acid-insoluble and acid-soluble lignin contents of wood chips were analyzed in accordance with TAPPI 222 and TAPPI Method 250, respectively (Dashtban and Qin 2012). The cellulose content of wood chips was measured via applying the Kurschner-Hoffer method (Reid and Lynch 1937). The hemicellulose content of wood chips was determined in accordance with TAPPI T223. The extractive content of wood chips was determined via solvent (i.e. ethanol) extraction following TAPPI T280.

The lignin content of hydrolysis liquor was analysed in accordance with TAPPI UM 250 using UV spectrophotometry at 205 nm (GENESYS 10S UV/vis, Thermo Scientific) (Liu et al. 2011a). In the autohydrolysis process, monosaccharides and oligosaccharides isolated from biomass and dissolve in hydrolysis liquors (Leppanen et al. 2011). The amount of monosugars was measured using an ion chromatography (Dionex, ICS 5000, ThermofisherScientific), which had a CarboPacTM SA10 column and an electrochemical detector (ED) (Dionex-300, Dionex Corporation, Canada). This instrument cannot detect polysugars. For determining polysugar concentration, the hydrolysis liquors were pretreated with 4 wt. % sulfuric acid at 121 °C for 1 h in accordance with the procedure described by Liu (2008). This process leads to the conversion of polysugars to monosugars (Liu 2008), implying that all sugars in the hydrolysis liquors will be in monomeric forms. This analysis leads to the determination of total sugars (i.e., hemicelluloses) present in the hydrolysis liquor. The amount of monosugars in the hydrolysis liquor was determined using ion chromatography and the results were considered as total sugars of hydrolysis liquors. The concentrations of acetic acid (Ac) and furfural (Fur) in hydrolysis liquors were assessed using a nuclear magnetic resonance (NMR) spectroscopy (Varian Unity Inova 500 MHz) in accordance with methods applied in the past (Saeed et al. 2012). The TSP was applied as an internal standard for measuring the concentrations of acetic acid and furfural in hydrolysis liquor. To determine the mass of lignocellulose isolated from wood chips, the concentrations of lignocellulose in hydrolysis liquors were determined as described above. Considering the volume of hydrolysis liquors and the concentration of lignocellulose in hydrolysis liquors, the total mass of lignocellulose removed from wood chips were calculated. Considering the original content of lignocellulose in wood chips, the removal of lignocellulose from wood chips was identified for hydrolysis liquors produced under different conditions.

3.3.5 Molecular weight

Approximately, 5 g of the hydrolysis liquor samples were diluted in 0.1 mol/L $NaNO_3$ to generate a 5 g/L concentration. Then, the measurements were conducted using a gel permeation chromatography, Malvern GPCmax VE2001 Module + Viscotek TDA305 with multi-detectors using PolyAnalytic PAA206 and PAA203 columns. A solution of 0.1 mol/L $NaNO_3$ was utilized as solvent and eluent. In the measurements, column temperature was set at 35 °C and the flow rate was set at 0.70 mL/min. The molecular weight of lignin was analysed via UV detector at 280 nm

wavelength. The molecular weight of hemicelluloses was determined using reflective index (RI) and intrinsic-differential pressure (IV-DP) detectors (Fatehi et al. 2016). This technique was previously used for evaluating the molecular weight of lignin, hemicelluloses and LCC in various spent liquors (Fatehi et al. 2016; Lawoko et al. 2005).

3.4 Results and discussion

3.4.1 Wood compositions

The compositions of spruce wood chips are listed in Table 3.1. It contained 26 wt.% acid insoluble lignin, 17 wt.% hemicelluloses, 45 wt.% cellulose and a balance of extractives. Mannose was the main sugar of spruce. These results are well in agreement with literature results (Sjostrom 1993).

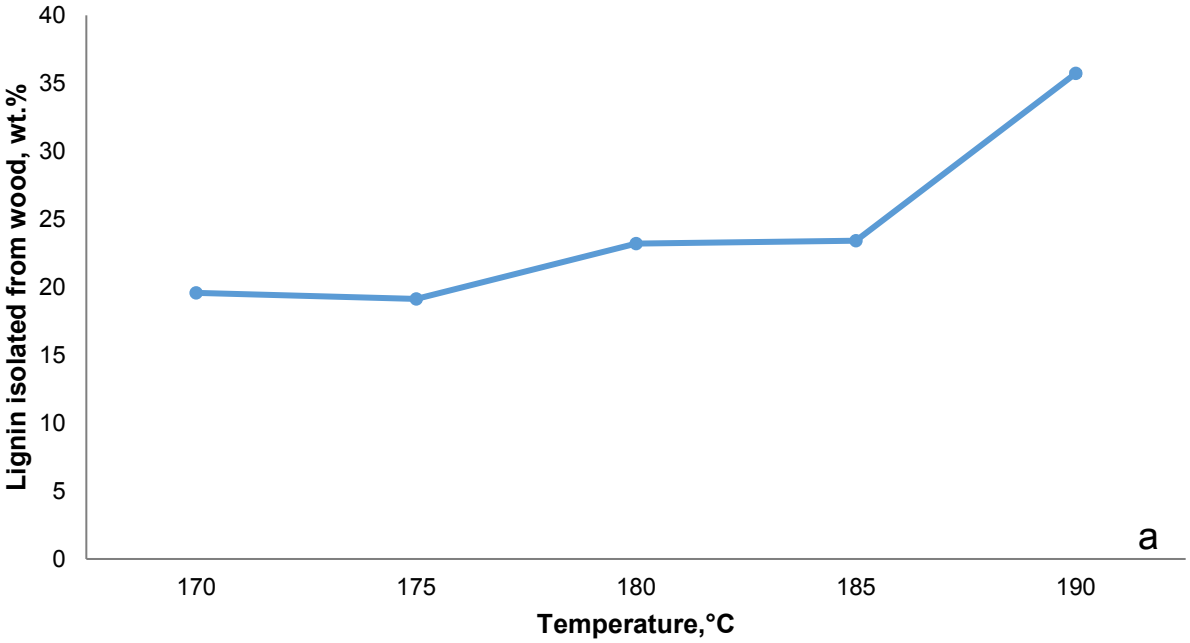
Table 3.1 Compositions of spruce wood chips.

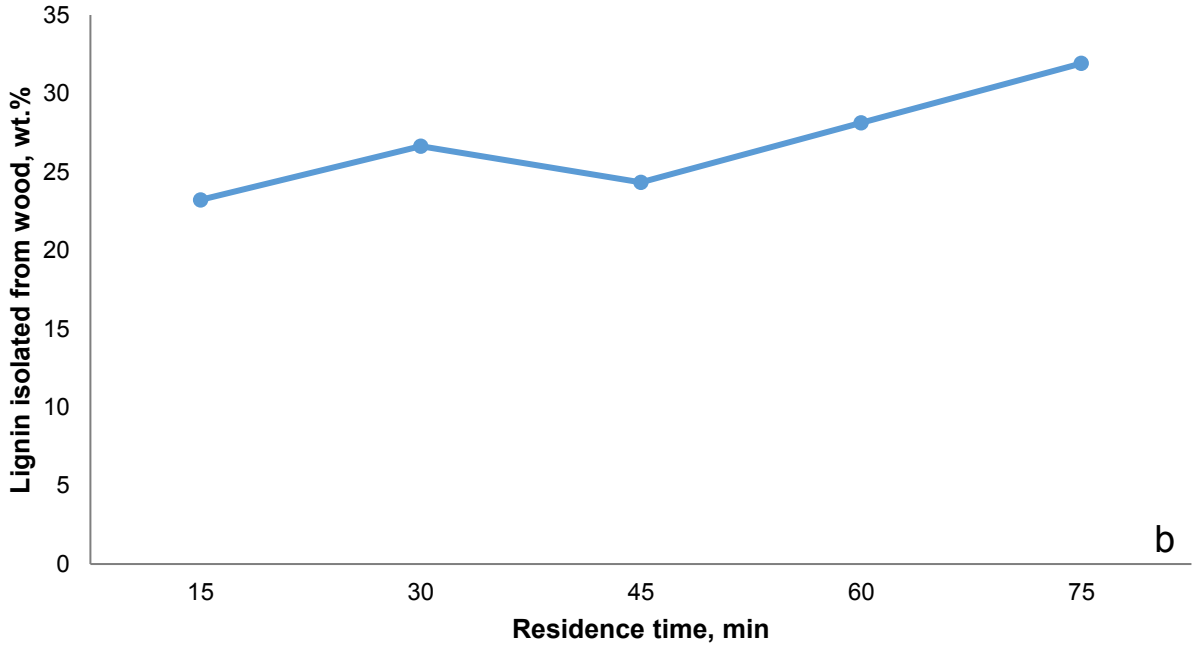
Component	wt. %, oven-dried wood
Acid-insoluble lignin	26.54
Acid-soluble lignin	2.76
Arabinose (Ara)	1.86
Galactose (Gal)	2.46
Rhamnose (Rha)	0.27
Xylose (Xyl)	5.98
Mannose(Man)	6.40
Total hemicelluloses	16.96
Cellulose	45.12
Extractives	2.89

3.4.2 Hydrolysis process

Figure 3.1 depicts isolation efficiency of lignin from wood chips obtained under different conditions of hydrolysis. Relatively high rate of lignin extraction in the first 15 minutes of treatment was probably attributed to the more intensive cleavage of acetyl groups at a short residence time and hence higher presence of acetic acid in the hydrolysate (Khazraie et al. 2017). However, it was found that lignin extraction rate decelerated at residence times of 30 and 45 min, which could be related to lower intensity of acetic acid formation. As seen, lignin was isolated more from wood chips at higher temperature and L/S ratio as well as longer residence time. Other researchers have observed similar trends in the hydrolysis of different biomass species (Leppanen et al. 2011; Song et al. 2008; Tunc 2014). Table 3.2 lists the properties of hydrolysis liquors produced under different conditions. At a higher temperature or longer residence time, more acetic acid was generated, which increased the acidity of the system, improving the lignin isolation from

wood chips (Leppanen et al. 2011; Song et al. 2008). Leppanen et al (2011) obtained 21% lignin isolation via flow through autohydrolysis of Norway spruce sawdust at 240 °C and noted that the amount of lignin in the hydrolysis liquor was increased at L/S ratio of above 6/1. Tunc (2014) and Testova et al. (2009) also noted that the higher ratio of L/S increased lignin isolation in the autohydrolysis of hardwood chips. The higher amount of liquor led to a higher possibility for lignin dissolution from wood chips in hydrolysis liquors (Tunc 2014). Flow rate showed insignificant effect on lignin extraction (Figure 3.1).


40
35
30
25
20
15
10
5
0
Lignin isolated from wood, wt.%
170
175
180
185
190
Temperature,°C
a


35
30
25
20
15
10
5
0
Lignin isolated from wood, wt.%
15
30
45
60
75
Residence time, min
b

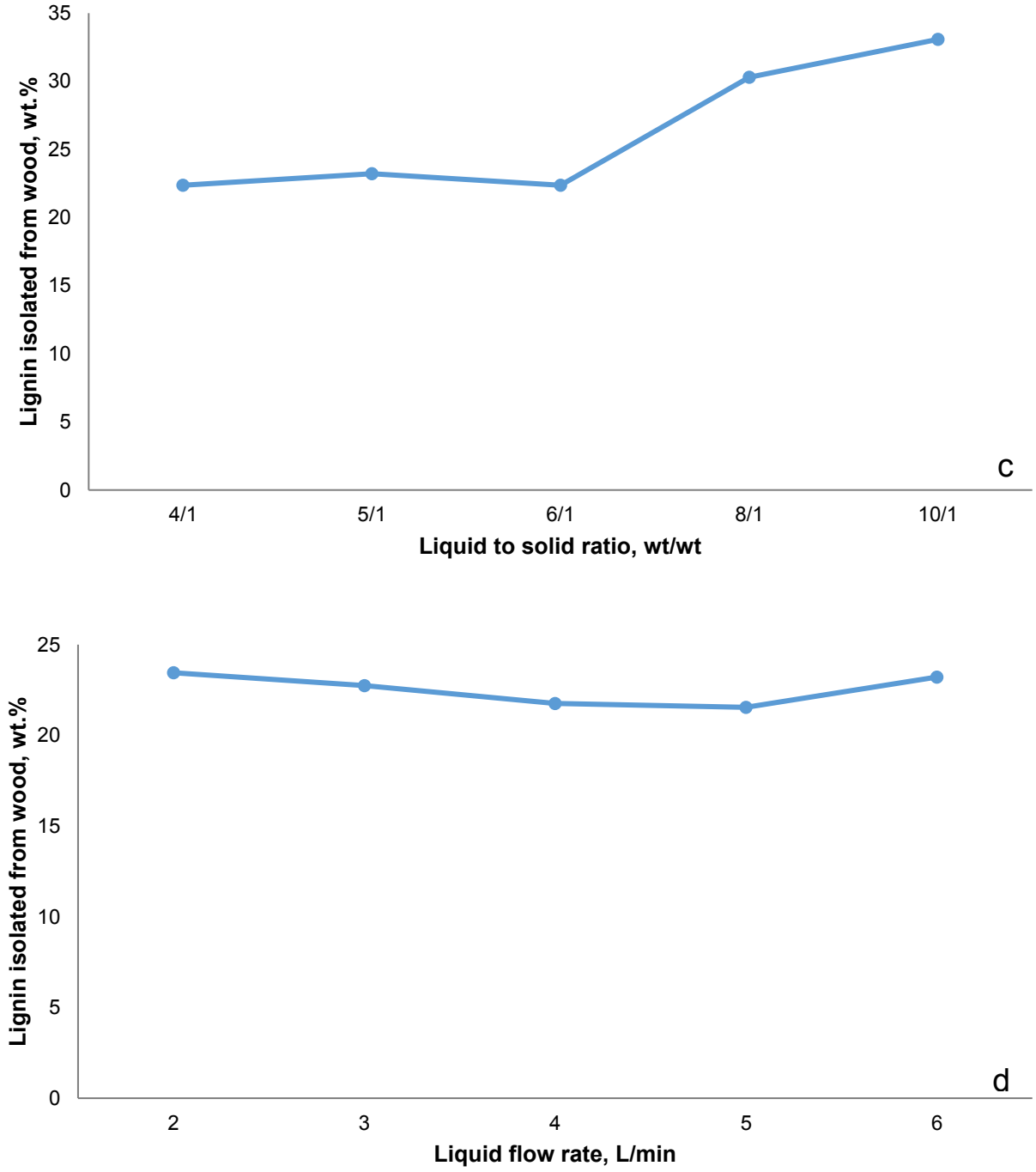


Figure 3.1 Amount of lignin isolated from spruce wood chips under different autohydrolysis conditions (experimental conditions: a) 15 min, L/S ratio of 5 wt./wt. and flow rate of 6 L/min, b) 180 °C, L/S ratio of 5 wt./wt. and flow rate of 6 L/min; c) 180 °C, 15 min and flow rate of 6 L/min and d) 180 °C, 15 min and L/S ratio of 5 wt./wt.).

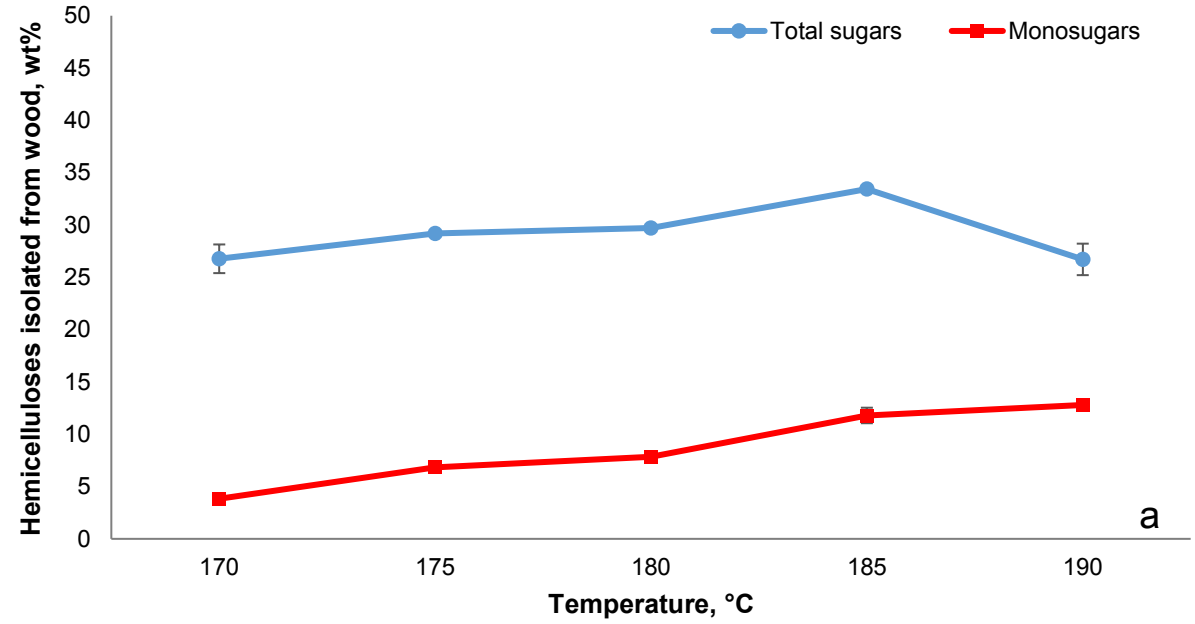
Figure 3.2 shows the amounts of hemicelluloses in the hydrolysis liquors extracted from the wood chips under different conditions. Relatively fast extraction of hemicelluloses in the first 15 minutes of hydrolysis, but relatively slow at longer residence times, was probably related to enhanced formation of acetic acid at short residence time, as it was discussed previously. The concentration of hemicelluloses in the hydrolysis liquor increased when the temperature increased to 185 °C and then decreased at a higher temperature. This reduction is attributed to the degradation of hemicelluloses into furfural and other products at elevated temperatures (Table 3.2) (Kapu and Trajano 2014; Song et al. 2008). The concentration of monosaccharides in the hydrolysis liquor was between 3.8 and 12.8% of total mass of hemicelluloses in wood chips. The removal of monosaccharides increased with rising temperature and reached the maximum at the highest temperature studied (190 °C). Other researchers also observed a rise in the monosaccharides concentration with temperature increase in the autohydrolysis process (Leppanen et al. 2011; Song et al. 2008). This phenomenon can be explained by the acidity increase in the system as acetic acid was generated more greatly in the hydrolysis at a higher temperature (Table 3.2) (Song et al. 2008).

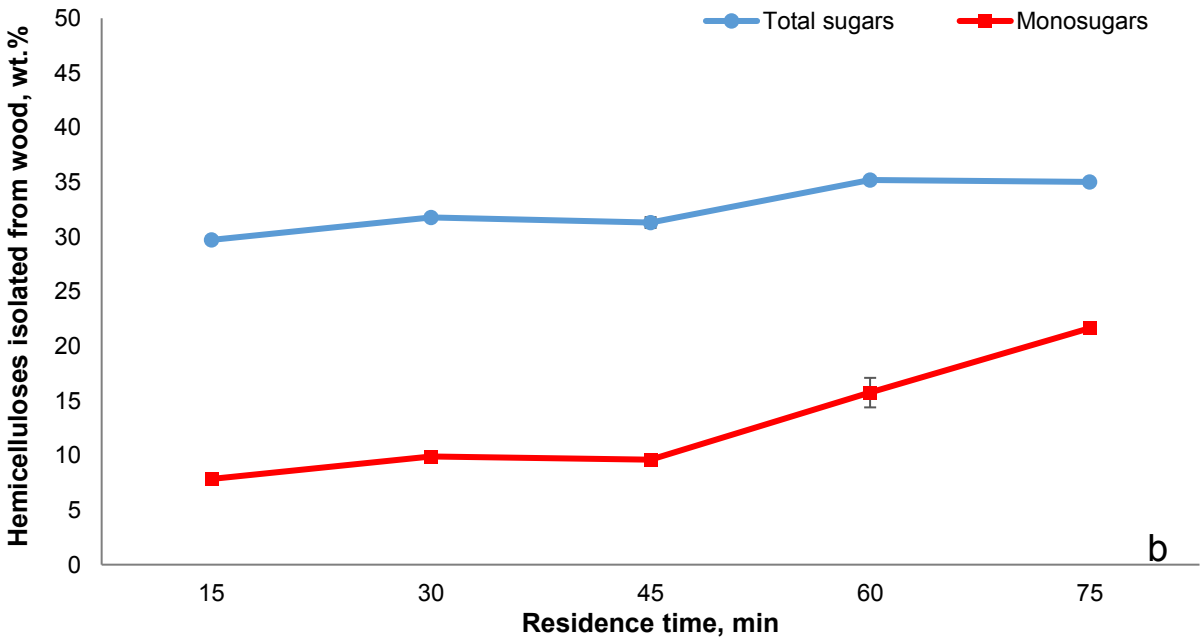
Furthermore, hemicellulose concentration reached the maximum at 60 min residence time. Further time elongation did not affect the hemicelluloses extraction from wood chips. Leppanen et al. (2011) reported that half of hemicelluloses were extracted from biomass at 180 °C after 30 min of water hydrolysis. However, the mass of monosaccharides extracted from wood chips gradually increased with prolonging the residence time (Figure 3.2), which is ascribed to the degradation of hemicelluloses under acidic conditions at a prolonged time (Song et al. 2008). Song et al. (2008) conducted hot water hydrolysis of spruce in a batch system at 180 °C and obtained 39.7% and 65.4% removals for monosaccharides after 60 and 100 min of residence times, respectively.

It was observed that the extraction of total sugars (30%) and monosaccharides (7.5%) were insignificantly affected by L/S ratios (Figure 3.2). Tunc (2014) and Testova et al. (2009) also reported an insignificant change in the hemicellulose extraction with changing the L/S ratio in the autohydrolysis of hardwood chips. This could be explained by better dissolution performance of carbohydrates at a higher liquid mass in the hydrolysis process (Testova et al. 2009).

Interestingly, the flow rate of the liquid circulation affected the hemicellulose removals in that the maximum removal was obtained at the minimum liquid flow rate of 2 L/min. Monosugars also had the highest concentration in the hydrolysis liquor at the lowest flow rate of 2 L/min. This could

be related to the higher acetic acid concentration in lower liquid flow rate as explained earlier (Table 3.2).





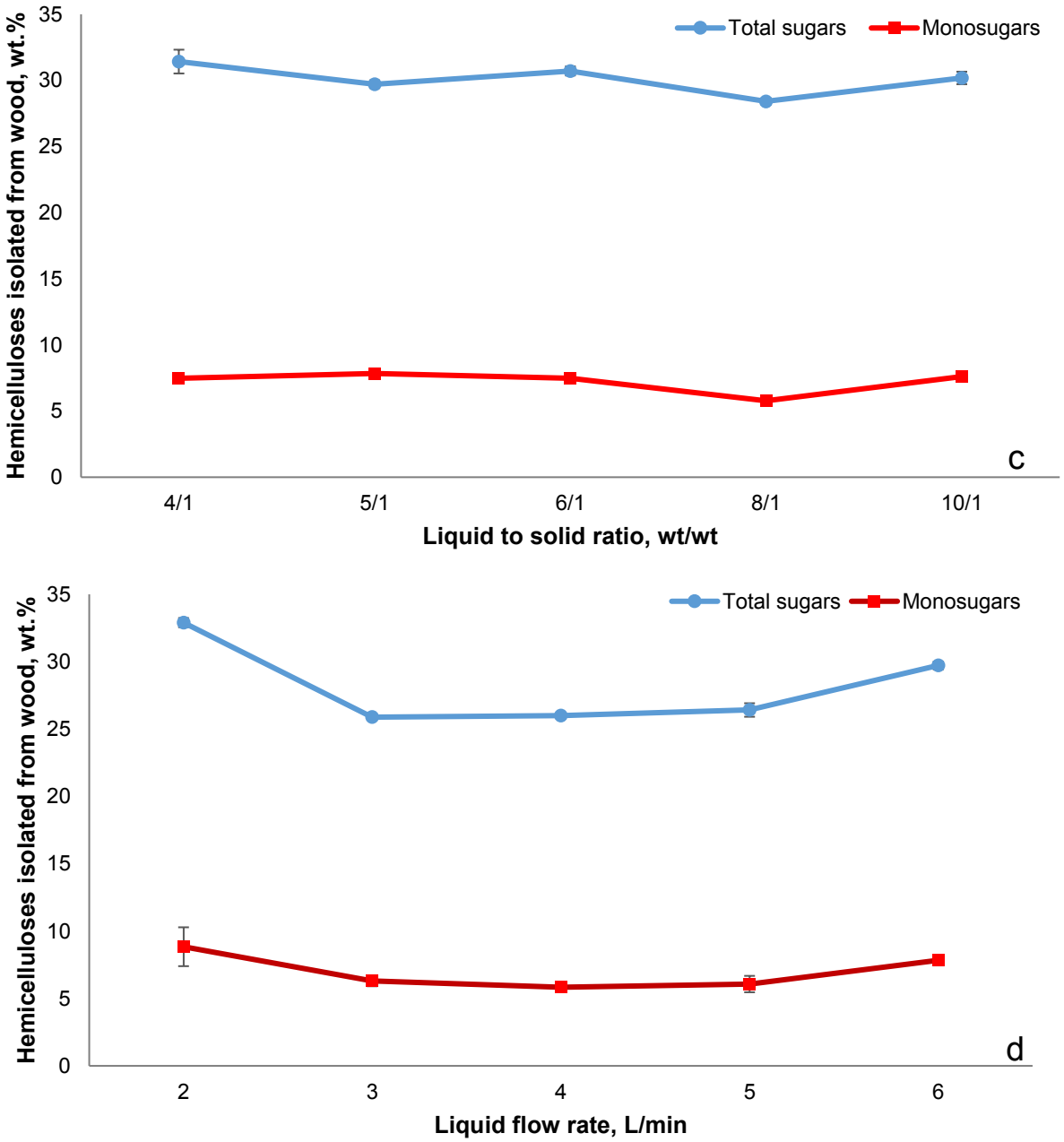


Figure 3.2. Total sugar and monosugar amounts isolated from spruce wood chips under different autohydrolysis conditions: a) 15 min, L/S ratio of 5 wt./wt. and flow rate of 6 L/min, b) 180 °C, L/S ratio of 5 wt./wt. and flow rate of 6 L/min; c) 180 °C, 15 min and flow rate of 6 L/min and d) 180 °C, 15 min and L/S ratio of 5 wt./wt.

Table 3.2 also lists the compositions of hydrolysis liquors obtained under different conditions. It was observed that the amount of galactose and mannose in the hydrolysis liquor reached the

maximum at 180 °C and 60 min. In the past, xylose and mannose represented major parts in sugar of softwood species (Leppanen et al. 2011; Song et al. 2008). The glucose concentration in the hydrolysis liquor increased at a temperature above 180 °C. This phenomenon could be related to the cellulose degradation at this temperature (Thoorens et al. 2014; Yu and Wu 2010). The cleavage of bonds between galactose and galactoglucomannan backbone under acidic hydrolysis conditions may generate galactose, but harsh hydrolysis conditions may decompose it (Sjostrom 1993). This can be the reason for the maximum concentration of galactose at 185 °C. The maximum concentration of arabinose in hydrolysis liquors obtained at the lowest hydrolysis intensity (i.e., low temperature and time), which might be due to liberation of arabinose linkage from arabinoglucuronoxylan chain under acidic conditions of hydrolysis (Sjostrom 1993). However, arabinose degraded under harsher experimental conditions due to its instability under acidic conditions (Song et al. 2008). It is seen in Table 3.2 that at temperature of 190 °C, furfural content increased, while polysaccharides amount decreased. This behavior is attributed to the sugars decomposition to furfural and other by-products (Kapu and Trajano 2014; Song et al. 2008).

Table 3.2. Hemicellulose compositions in the hydrolysis liquors obtained under different experimental conditions.

Experiment conditions		Poly/monosaccharides content in autohydrolysis liquors, wt.% based on wood								
		Arb	Gal	Rha	Glu	Xyl	Man	Total	Ac	Fur
L/S ratio 1/5; 15 min; 6 L/min	°C	Temperature effect (measurement error ±0.7%)								
	170	0.64/0.35	0.84/0.11	0.02/0.02	0.46/0.01	0.79/0.12	1.79/0.04	4.54/0.65	0.04	ND[1]
	175	0.32/0.32	0.99/0.29	0.04/0.04	0.75/0.05	0.64/0.30	2.22/0.17	4.95/1.16	0.22	ND
	180	0.30/0.33	0.96/0.32	0.04/0.04	0.79/0.07	0.68/0.34	2.26/0.24	5.04/1.33	0.37	ND
	185	0.23/0.30	1.05/0.51	0.04/0.05	0.96/0.15	0.68/0.49	2.71/0.50	5.67/2.00	0.52	0.25
	190	0.11/0.13	0.85/0.52	0.02/0.03	1.03/0.37	0.46/0.40	2.06/0.72	4.53/2.17	0.98	1.61
L/S ratio 1/5; 180°C; 6 L/min	min	Time effect (measurement error ±0.45%)								
	15	0.30/0.33	0.96/0.32	0.04/0.04	0.79/0.07	0.68/0.34	2.26/0.24	5.04/1.33	0.37	ND
	30	0.27/0.29	1.15/0.48	0.05/0.04	0.83/0.10	0.63/0.44	2.47/0.33	5.39/1.68	0.35	ND
	45	0.21/0.23	0.96/0.4	0.04/0.04	0.89/0.11	0.65/0.45	2.56/0.40	5.31/1.63	0.38	ND
	60	0.22/0.24	1.14/0.73	0.03/0.03	1.07/0.26	0.68/0.64	2.83/0.78	5.97/2.67	0.36	0.39
	75	0.19/0.25	1.12/0.92	0.03/0.04	1.15/0.45	0.62/0.75	2.83/1.25	5.94/3.67	0.46	0.39
180°C;15 min; 6 L/min	L/S ratio	L/S ratio effect (measurement error ±0.45%)								
	4/1	0.28/0.31	1.04/0.31	0.04/0.03	0.89/0.06	0.71/0.38	2.38/0.20	5.33/1.27	0.47	ND
	5/1	0.30/0.33	0.96/0.32	0.04/0.04	0.79/0.07	0.68/0.34	2.26/0.24	5.04/1.33	0.37	ND
	6/1	0.34/0.38	1.00/0.29	0.04/0.03	0.77/0.04	0.73/0.38	2.33/0.15	5.21/1.27	0.34	ND
	8/1	0.31/0.32	0.98/0.24	0.04/0.03	0.71/0.03	0.73/0.26	2.05/0.11	4.82/0.98	0.36	ND

	10/1	0.32/0.37	1.05/0.32	0.04/0.03	0.72/0.04	0.70/0.36	2.28/0.16	5.12/1.29	0.33	ND
	LPM	Flow rate effect (measurement error ±0.61%)								
L/S ratio 1/5; 180°C;15 min	2	0.29/0.21	1.19/0.42	0.04/0.03	0.85/0.07	0.69/0.48	2.51/0.28	5.58/1.50	0.21	ND
	3	0.31/0.34	0.81/0.24	0.04/0.02	0.65/0.03	0.62/0.31	1.97/0.13	4.39/1.07	0.21	ND
	4	0.32/0.36	0.83/0.22	0.04/0.02	0.64/0.03	0.60/0.26	1.97/0.10	4.41/0.99	0.23	ND
	5	0.29/0.33	0.81/0.23	0.04/0.02	0.67/0.03	0.60/0.3	2.07/0.12	4.48/1.03	0.28	ND
	6	0.30/0.33	0.96/0.32	0.04/0.04	0.79/0.07	0.68/0.34	2.26/0.24	5.04/1.33	0.37	ND

[1]Not detected

3.4.3 Impact of process conditions on molecular weight

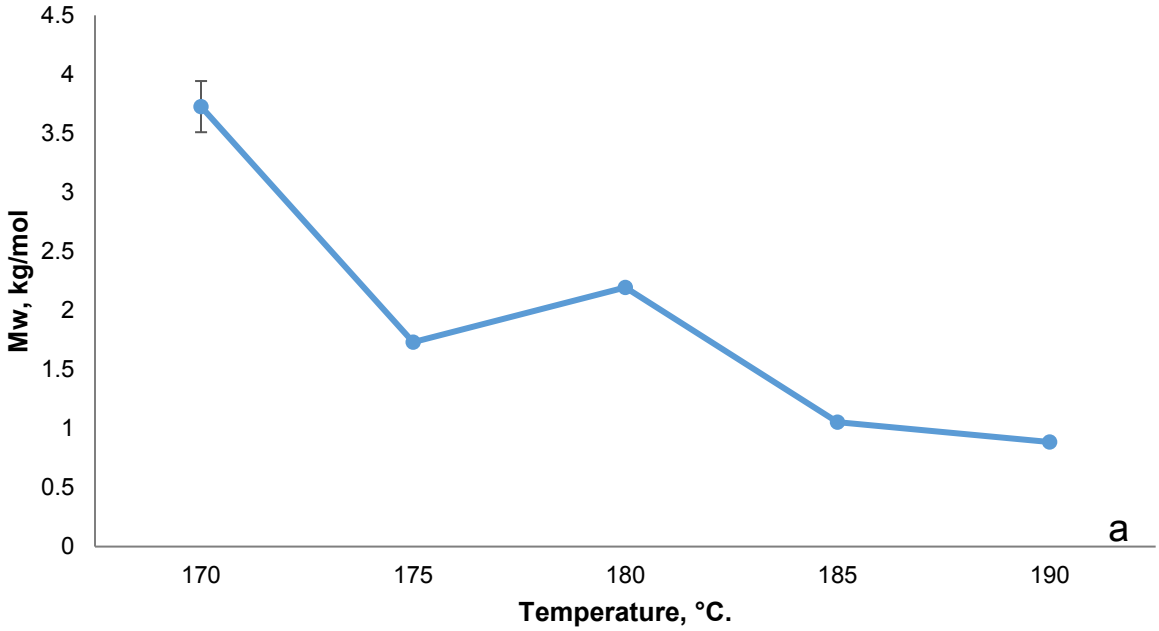
Lignin with two different molecular weights of 800-1200 and 55,000 g/mol was found in hydrolysis liquors, and they remained unchanged, regardless of the hydrolysis conditions (i.e., time, temperature, L/S and liquid flow rate). In another work, the molecular weight of lignin extracted from milled wood and enzyme treated Norway spruce were 23,500 g/mol and 53,850 g/mol, respectively (Tolbert et al. 2014).

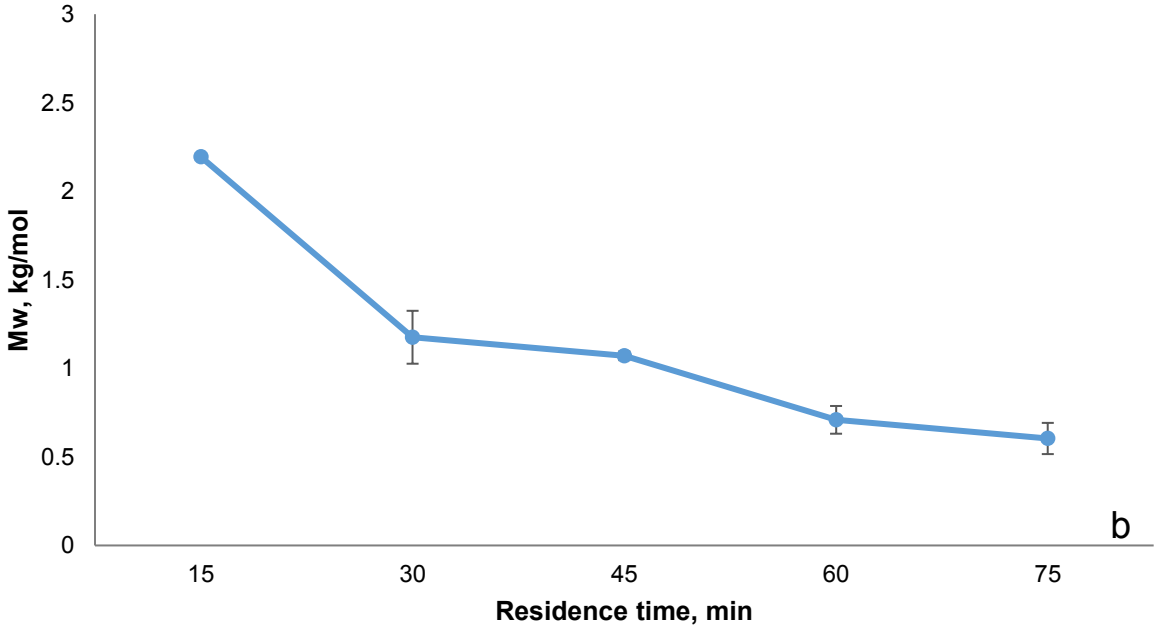
Figure 2.3 presents the molecular weight of hemicelluloses in hydrolysis liquor. It is seen that the molecular weight of hemicelluloses decreased as temperature increased (Leppanen et al. 2011; Song et al. 2008), which provided evidence for the degradation of hemicellulose at an elevated temperature (Leppanen et al. 2011). Apparently, the molecular weight of hemicelluloses was higher at 180 °C than at 175 or 185 ºC. It was reported that cellulose started to degrade at 180 °C (Yu and Wu 2010). It is possible that the molecular weight of the cleaved glucan originated from cellulose was higher than other hemicelluloses available in the hydrolysis liquors and that contributed to the overall increase in the Mw of hemicelluloses at 180 °C. Santucci et al. (2015) observed a significant increase in the cellulose removal from sugarcane bagasse via autohydrolysis at 180 °C. However, further temperature increase (>180 °C) led to the degradation of the dissolved lignin and hemicelluloses (Figure 3.2 and Table 3.2) and thus a decrease in molecular weight (Figure 3.3a).

The results also depicted that the molecular weight of hemicelluloses in hydrolysis liquors decreased with time extension (Figure 3.3b). A similar phenomenon was observed by Leppanen et al. (2011) on the flow through autohydrolysis of saw meal Norway spruce. This behavior is also related to the degradation of hemicelluloses at an extended time under acidic conditions. The L/S ratio insignificantly affected the molecular weight of hemicelluloses, which was also reported by Tunc (2014).

Interestingly, the molecular weight of hemicelluloses slightly increased as the liquid flow rate increased. Liu and Wyman (2003) hypothesized that long-chain LCC became isolated from biomass in a flow-through process and a high flow rate probably helped the isolation process.

At a higher flow rate, the chemistry of hydrolysis was not changed, thus the total amounts of hemicelluloses extracted would not change (Figure 3.3 and Table 3.2), but a more turbulent system (and thus a higher shear rate) was provided at a higher flow rate within wood chips in the digester, which may accelerate the removal of larger hemicelluloses (than smaller one) from wood chips.


4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Mw, kg/mol
170
175
180
185
190
Temperature, °C.
a


3
2.5
2
1.5
1
0.5
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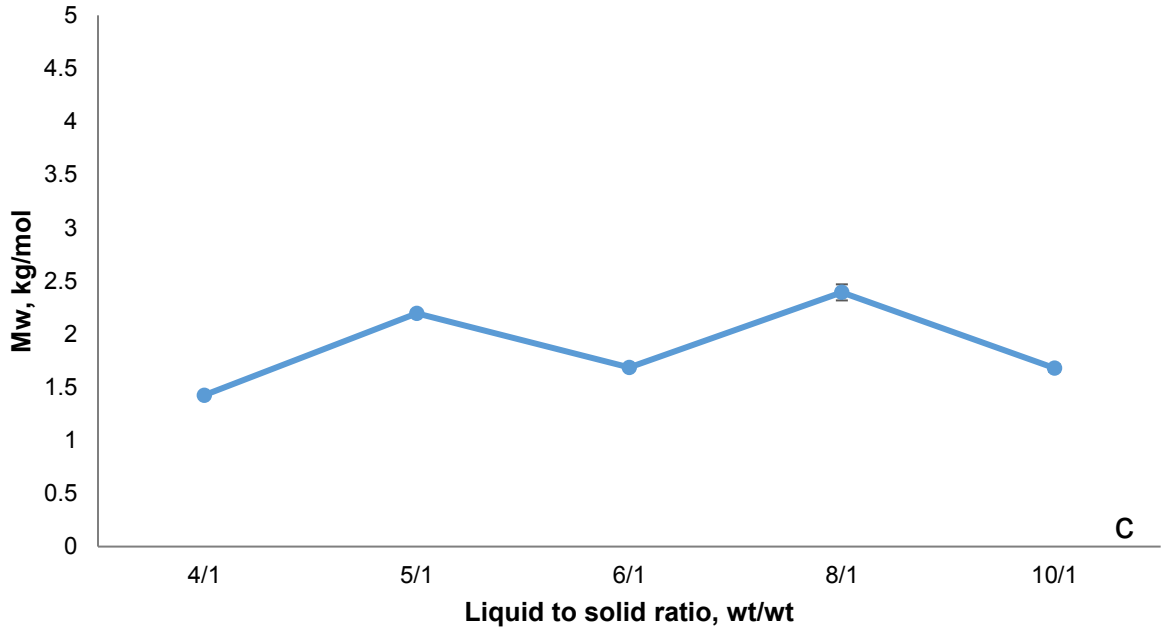
Mw, kg/mol
15
30
45
60
75
Residence time, min
b



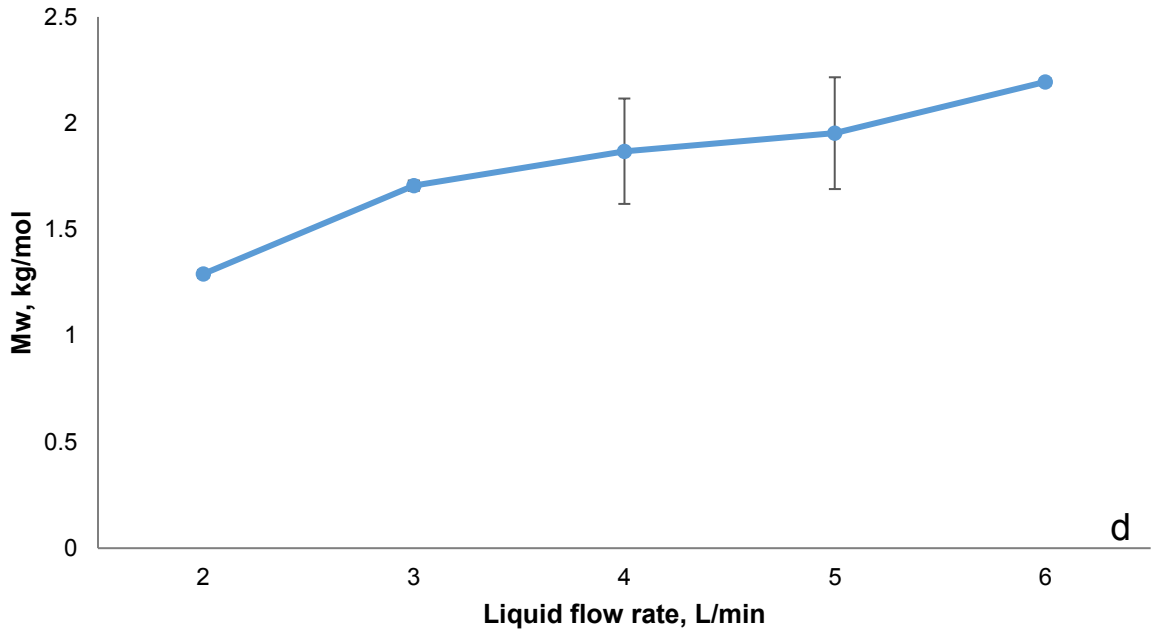


Figure 3.3 Molecular weight of hemicelluloses in the hydrolysis liquors obtained under different conditions: a) 15 min, L/S ratio of 5 wt./wt. and flow rate of 6 L/min, b) 180 °C, L/S ratio of 5 wt./wt. and flow rate of 6 L/min; c) 180 °C, 15 min and flow rate of 6 L/min and d) 180 °C, 15 min and L/S ratio of 5 wt./wt..

3.4.5 Selection of hydrolysis liquor

Based on the results, four samples were chosen for further analysis. These samples were produced at the L/S ratio of 5/1, 180 °C and 15 min (sample 1); L/S ratio of 5/1, 180 °C and 45 min (sample 2); L/S ratio of 5/1, 190 °C and 15 min (sample 3); and L/S ratio of 10/1 and 180 °C for 15 min (sample 4). The flow rate was 6 LPM for producing these samples. Samples 1 and 2 had relatively high hemicellulose contents and Samples 3 and 4 had higher lignin concentrations.

3.4.6 Characteristics of lignin-carbohydrate complexes in hydrolysis liquors

Figure 3.4 shows the detectors' responses in molecular weight analysis of sample 1. It is observable that the low molecular weight lignin (800-1,200 g/mol) had a similar retention time with that of hemicelluloses. Similar results were observed for other samples (see Appendix). However, there was no RI and IV-DP responses for lignin with the higher molecular weight (Figure 2.4). Similar retention times for UV, IR and IV-DP signals were reported as evidence of LCC's presence in spent liquors in previous studies (Tunc et al. 2010; Fatehi et al. 2016). Therefore, it can be assumed that lignin moieties with low and high molecular weights were present in the hydrolysis liquors in LCC and carbohydrate-free forms, respectively. Fatehi and coworkers (2016) reported an LCC with the molecular weight of 1500 g/mol in hydrolysis liquor produced from mixed hardwood. Based on the area under the peaks in Figure 3.4, it was proposed that 68, 59, 89 and 19% of lignin in hydrolysis liquors of samples 1, 2, 3 and 4 were in LCC forms, respectively. The rest of lignin moieties in the hydrolysis liquors were in carbohydrate-free forms.

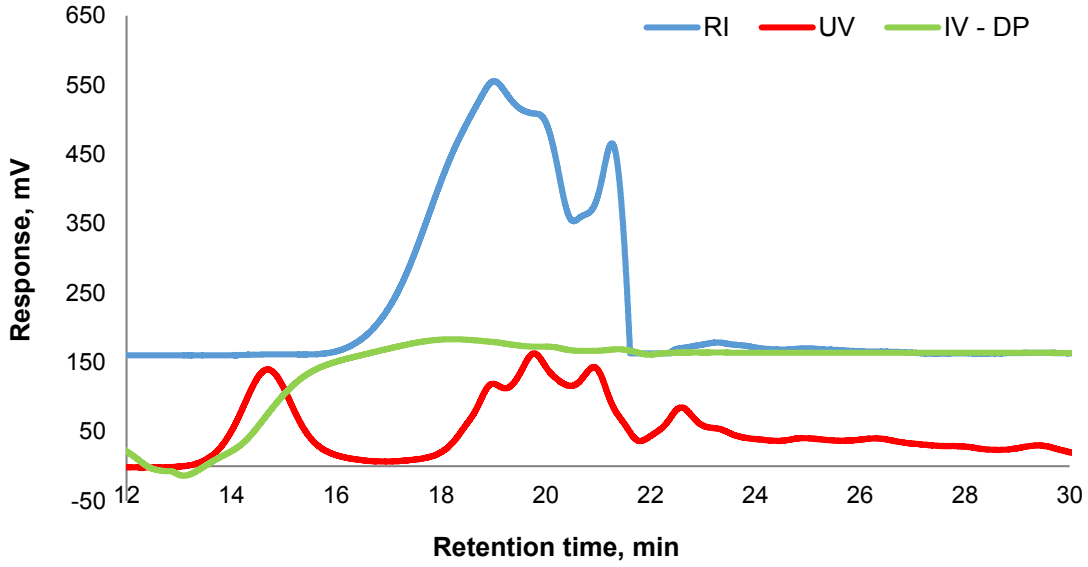


Figure 3.4 Response of RI, UV and IV-DP detectors of GPC in analyzing the molecular weight of lignocelluloses in the hydrolysis liquor of sample 1.

Figure 3.5 shows the amount of lignin remained in hydrolysis liquors after membrane dialysis with different molecular weights. Interestingly, more than 85% of lignin was removed via using membrane with the molecular weight cut off of 1000 g/mol implying the significant removal of low molecular weight lignin compounds. Evidently, more than 80% of hemicelluloses were removed from hydrolysis liquors using membrane with the same molecular wegiht cut off (1000 g/mol), and these hemicelluloses were probably in monomeric and oligomeric forms. Logically, with using membrane with larger pore opening (e.g. 10,000 and 25,000 g/mol), less lignin and hemicellulose remained in the hydrolysis liquors, but some of these lignin and hemicelluloses were larger than the pore opening of the membranes.

As the molecular weight of hemicelluloses was smaller than 4,000 g/mol (Figure 2.3), they could be effectively removed from hydrolysis liquors when membrane with molecular weight cut off of 10,000 g/mol (or larger) was used. The presence of hemicelluloses in the hydrolysis liquors dialyzed with membranes with large pores (i.e., 10,000 and 25,000 g/mol) can indirectly illustrate that these hemicelluloses were attached to lignin in LCC form so that they were sufficiently large to remain in the hydrolysis liquors after dialysis.

The use of membrane with different sizes facilitated information about the size distribution of LCC in hydrolysis liquors. Sample 1 had more lignocellulose with molecular weights larger than 1000 g/mol. Also, samples 1-3 lost significant amounts of lignocellulose (probably in LCC forms) when the pore opening of the membrane increased to 25,000 g/mol. These results suggest that the samples 1, 2 and 3 contained a considerable amount of LCC with sizes smaller than 25000 g/mol so that they were removed from the hydrolysis liquors via membrane dialysis. As stated earlier, more of lignin in hydrolysis liquors were in LCC forms in these samples, and these LCCs were smaller than carbohydrate free lignin (Figure 3.4). Therefore, more of these LCCs were removed via dialysis of these samples than sample 4. The concentration of lignocelluloses in sample 4 insignificantly dropped when the molecular weight of membrane increased to 25,000 g/mol, implying that this sample had more lignocelluloses larger than 25,000 g/mol. The presence of hemicelluloses in the samples dialyzed with the membrane, with the pore opening of larger than 25,000 g/mol, depicts that some LCC were larger than 25,000 g/mol.

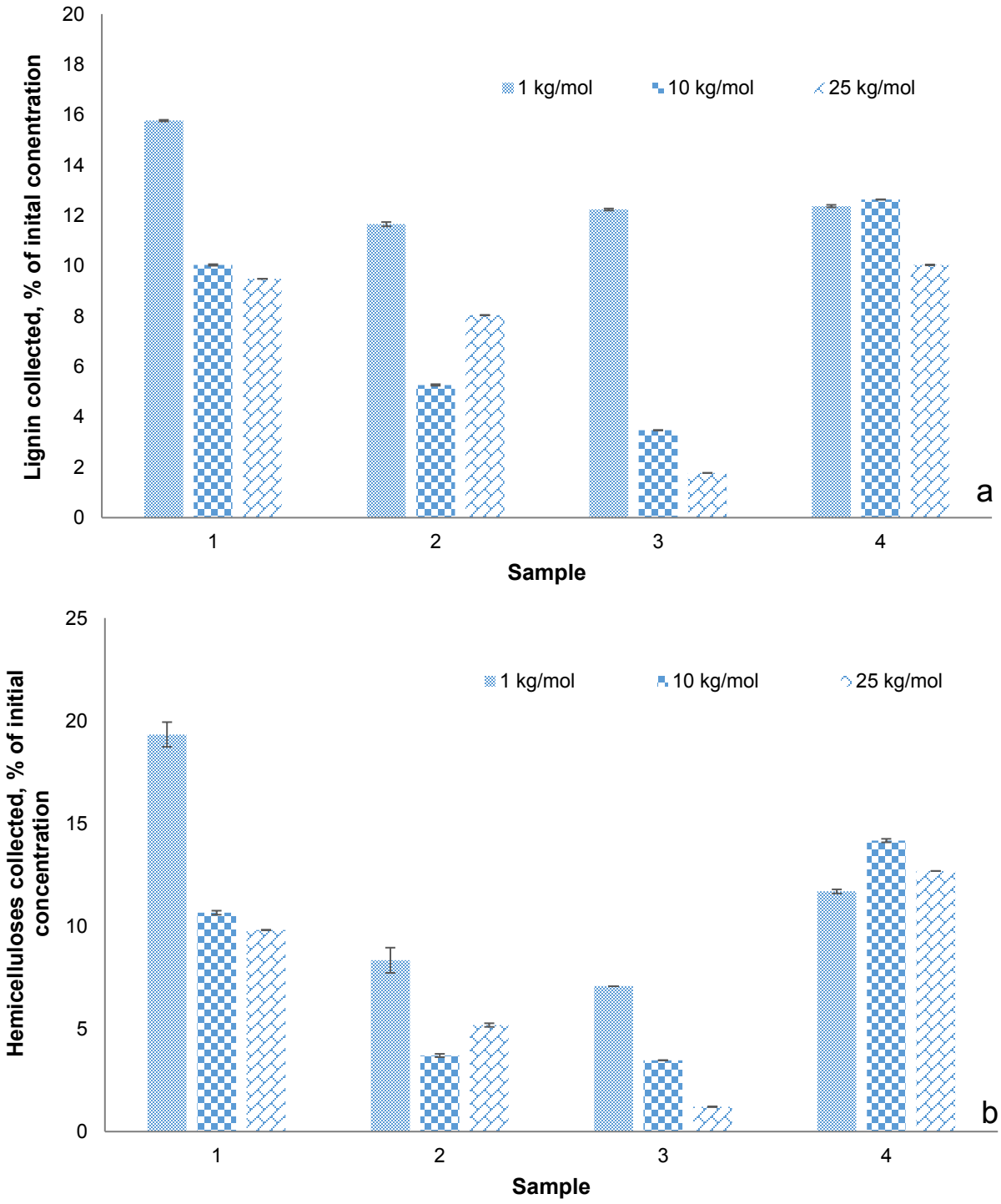


Figure 3.5 Lignin (a) and hemicelluloses (b) remained in hydrolysis liquors (% of initial amounts in hydrolysis liquors) via using dialysis membrane with different sizes.

The types of hemicellulose content of LCC can also be identified. Table 3.3 presents the compositions of hemicelluloses after membrane dialysis in the samples. It is apparent that xylan

was significantly removed from hydrolysis liquor. Also, arabinose and rhamnose were removed from the samples, regardless of the pore opening of dyalysis membrane. Lawoko et al. (2005) reported four LCC types of galactoglucomannan-lignin-pectin (GalGlcMan-L-P), glucan-lignin (Glu-L), glucomannan-lignin-xylan (GluMan-L-Xyl) and xylan-lignin-glucomannan (Xyl-L-GluMan), which contains 8, 4, 40 and 48% of total lignin of spruce wood species, respectively. Eriksson et al. (1980) proposed that lignin is bound to xylan with arabinose substituent groups in spruce wood species. Therefore, the removal of arabionose would result in the cleavage of xylose from LCC. Since galactose, glucose and mannose were present after dialysis (Table 3.3), it is suggested that LCC compounds were mainly GalGlcMan-L complexes.

Table 3.3 The compositions of the hemicelluloses isolated via dialysis with different membrane pore openings.

Sample ID	Membrane with molecular weight cut off of 1000 g/mol, % (error ±0.1-0.6%)					
	Ara and Rha	Galactose	Glucose	Xylose	Mannose	Total
1	1.3	19.03	24.75	4.52	24.76	19.33
2	0	6.74	11.99	0.81	10.4	8.34
3	1.95	5.15	7.67	1.26	9.22	7.08
4	0	10.97	17.2	2.02	15.1	11.69
Membrane with molecular weight cut off of 10,000 g/mol, % (error ±0.1-0.2%)						
1	0.81	10.91	14.88	2.08	13.18	10.66
2	1.23	3.51	5.34	0.42	4.36	3.7
3	2.14	2.57	3.95	0.48	4.46	3.47
4	1.06	15.25	20.2	4.15	16.96	14.17
Membrane with molecular weight cut off of 25,000 g/mol, % (error ±0.1-0.2%)						
1	5.83	10.03	13.66	1.84	12.1	9.81
2	0.34	4.99	7.55	0.6	6.1	5.19
3	0.29	0.93	1.42	0.12	1.53	1.21
4	3.71	13.7	18.14	3.8	15.05	12.69

3.4.7 Extraction via acidification and ethanol treatments

Figure 3.6 depicts lignin isolation from the hydrolysis liquor samples obtained via acidification and ethanol treatments. The acidification of hydrolysis liquors resulted in 16%-21% lignin isolation from the liquors. In addition to protonation of lignocellulose under acidic conditions, lignin may be condensed under strong acidic conditions, which facilitates its removal.

Comparison of samples revealed that the acidification of the sample 4 produced at the higher L/S ratio led to slightly lower lignin extraction, which might be due to its higher acid-soluble lignin (Tunc 2014). It is also noted that ethanol treatment resulted in significantly lower lignin isolation compared to acidification.

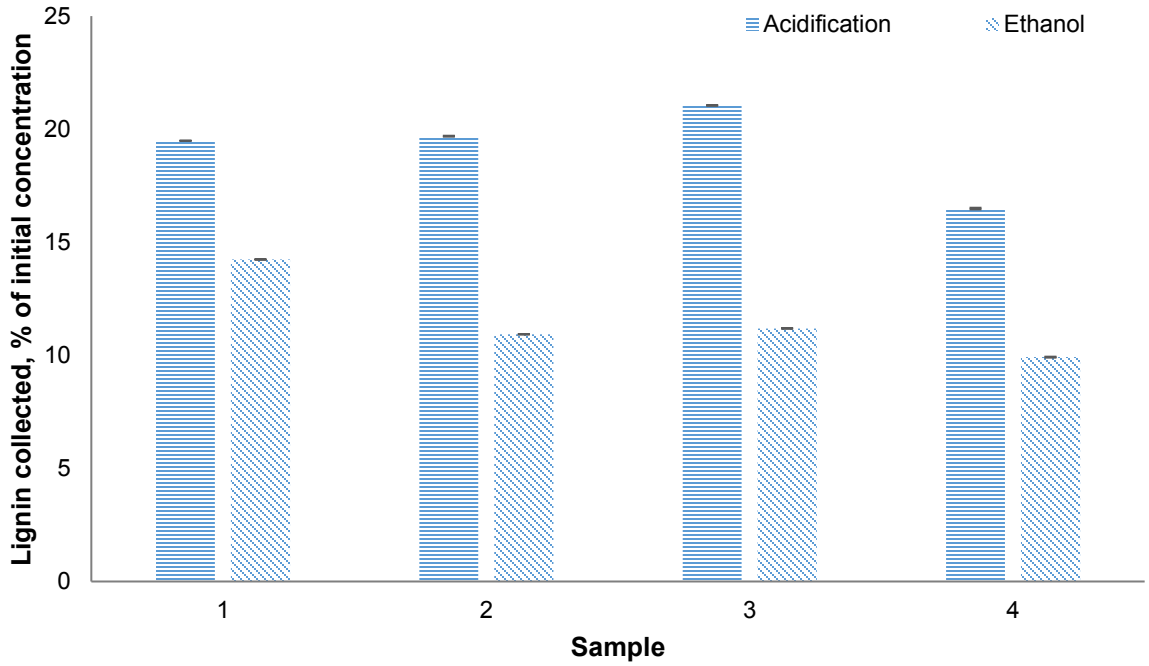


Figure 3.6 Lignin isolation from hydrolysis liquors via acidification and ethanol extraction.

Figure 3.7 shows the impact of acidification and ethanol treatment in isolating hemicelluloses from the hydrolysis liquors. It is apparent that the acidification led to 16-38% isolation of hemicelluloses. In the literature, the acidification of hardwood-based hydrolysis liquor resulted in 17% and 40% hemicellulose isolations at pH 2 and 1.5, respectively (Fatehi et al. 2016; Liu et al. 2011a). It is also possible that the hemicellulose reduction via acidification is attributed to sugar degradation, but further analyses are required for supporting this hypothesis, which is out of the scope of this work. Ethanol treatment significantly improved the isolation of hemicellulose (35-45% isolation of initial contents). The removal of hemicelluloses under acidic conditions is another indication of the linkage of hemicelluloses to lignin in LCC, as hemicelluloses are generally acid soluble, thus the ones that were linked to lignin should have been removed. Also, sample 1 had a higher lignin removal via ethanol treatment, which can be associated with the removal of LCC.

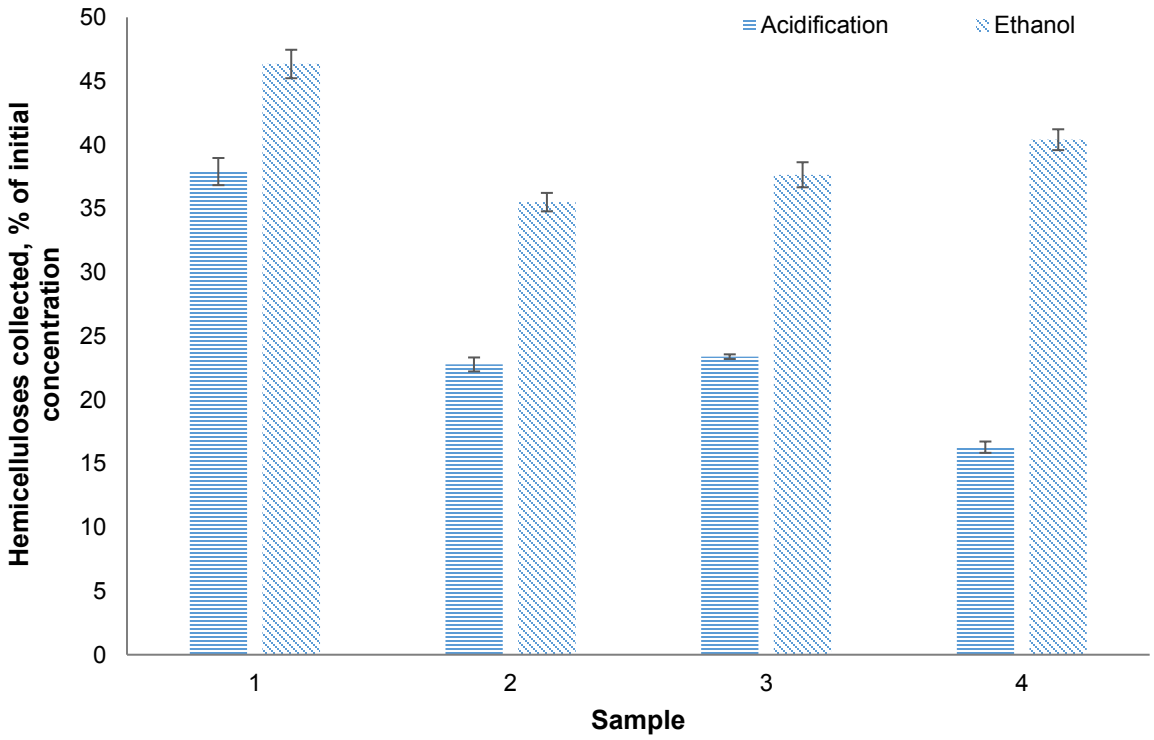


Figure 3.7 Hemicelluloses isolated from hydrolysis liquors via acidification and ethanol extraction.

Comparing the results of Figures 3.5, 3.6 and 3.7 depicts that membrane dialysis collected 10-16% of lignin and 8-20% of hemicelluloses. Acidification led to approximately 16-21% lignin removal and 16-38% hemicellulose removal for all samples. Ethanol treatment yielded in 35-45% of hemicellulose isolation and 10-14% of lignin isolation. The properties of samples also impacted the performance of purification process, as the efficiency of extraction processes was different for different samples. Lignocellulose was more efficiently isolated for sample 1 than other samples, regardless of the process. Sample 4 was less sensitive to the membrane pore opening, but more sensitive to acidification and ethanol treatment, than other samples, as it contained more lignin in carbohydrate free form.

3.4.8 Molecular weight of hydrolysis liquors after acidification and ethanol treatment

The separation methods insignificantly affected the molecular weight of lignin in the hydrolysis liquors. After acidification and ethanol treatments, the molecular weights of lignin were 50,000-54,000 and 51,000-60,000 g/mol, respectively. A slight increase in the molecular weight of lignin could be related to the removal of low molecular weight lignin and LCCs from the solution via

acidification and ethanol treatment or condensation of lignin under acidic conditions, as stated earlier.

The molecular weight of hemicelluloses in the hydrolysis liquor after different treatment methods are presented in Figure 3.8. The molecular weight of hemicellulose insignificantly reduced via acidification, implying that the hemicelluloses in lignin-free and LCC forms had similar molecular weights that the removal of LCC did not affect the overall molecular weight of hemicelluloses. Ethanol reduced the molecular weight of hemicelluloses in hydrolysis liquors, implying that high molecular weight hemicelluloses were removed, and this removal reduced the overall molecular weight of hemicelluloses after ethanol extraction.

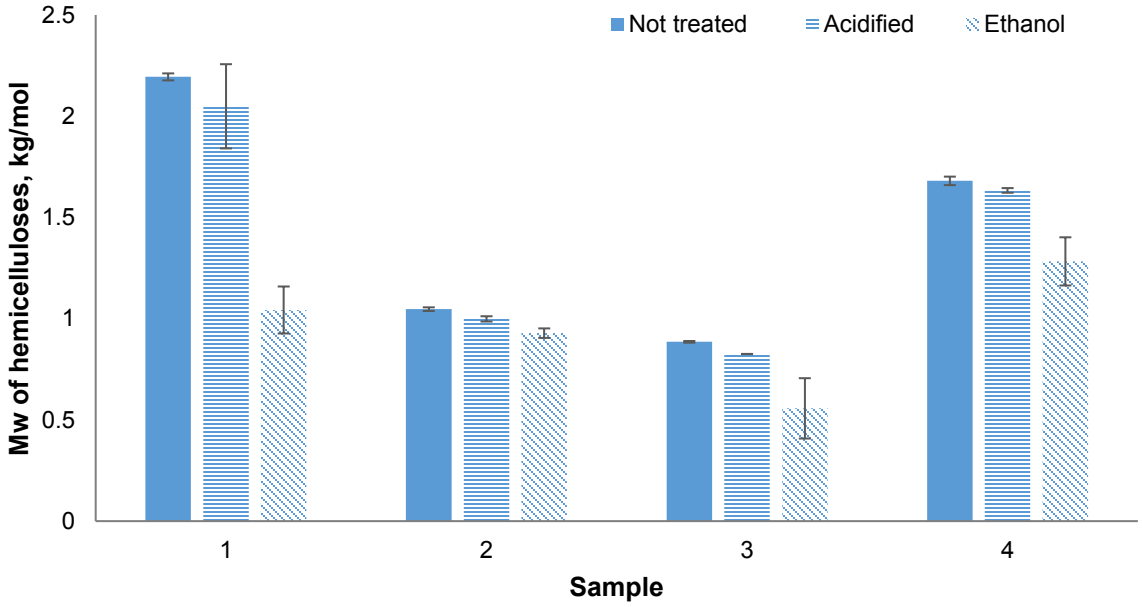


Figure 3.8 Molecular weight of hemicelluloses in hydrolysis liquors after acidification and ethanol extraction.

3.5 Application

Autohydrolysis is commercially used in industry. The results of this study showed that the yield and molecular weight of the extracted lignocellulose would be significantly affected by the conditions of autohydrolysis. If lignin were to be used for producing value-added products; higher temperature, longer time and larger liquid to wood ratio could be applied in the autohydrolysis process. Within the scope of this study, the extraction yield of hemicelluloses from wood chips

seems to be affected less significantly than lignin by autohydrolysis process conditions. However, if hemicelluloses with larger molecular weight were to be used in value-added applications, the temperature of 170°C and 15 min residence time and flow rate of 6 L/min might be used in the autohydrolysis process. The generation of LCC is unavoidable in the hydrolysis liquor, but the compositions of LCC can be affected by the hydrolysis conditions, which impact the efficiency of acidification and ethanol treatment in isolating lignin and hemicelluloses from the hydrolysis liquor. As lignin and hemicelluloses cannot be produced in pure forms via the processes investigated, end-use applications that include both lignin and hemicelluloses as raw materials may be feasible options for the use of lignocellulose of autohydrolysis liquor. These applications can be the use of extracted lignin and hemicelluloses as additives in unbleached paper board, corrugated medium, composites and plywood productions.

3.6 Conclusions

Autohydrolysis conditions affected the amount and molecular weight of lignocellulose present in hydrolysis liquors. The molecular weight of lignin was stable regardless of hydrolysis conditions. The molecular weight of hemicelluloses decreased with increasing the severity of hydrolysis conditions (i.e., time and temperature) due to polysaccharide degradation. Based on GPC analysis, only 19% of lignin involved in LCC form in sample 4 that was produced at L/S ratio of 10, 180 °C and 15 min. In samples produced under the conditions of 180 °C, L/S ratio of 5 and residence times of 15 min and 45 min, 68% and 59% of lignin seemed to be in LCC forms, respectively. Membrane dialysis assessment revealed that sample 4 contained more of high molecular weight lignin, but other samples had more of LCC with the molecular weight smaller than 25,000 g/mol. Acidification resulted in 16-21% of lignin and 16-40% of hemicellulose isolations, respectively. Acidification did not change the molecular weight of hemicelluloses significantly. The ethanol extracted 10-14% of lignin and 35-45% of hemicelluloses from hydrolysis liquors, and the molecular weight of hemicelluloses was slightly smaller in the ethanol pretreated hydrolysis liquor.

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Chapter 4 Properties and structure of lignocellulosic material extracted from spruce wood chips via flow through autohydrolysis pre-treatment

4.1 Abstract

Autohydrolysis is a widely used technology for extracting hemicelluloses from wood chips. In addition to hemicelluloses, hydrolysates produced in this process contain lignin. These materials could be isolated from hydrolysates and used for generating value-added products. In this chapter, hydrolysates were produced via autohydrolysis of spruce wood chips. Then, acidification and ethanol precipitation were employed to isolate lignocelluloses from hydrolysates. Acidification of hydrolysates produced at 190 °C resulted in precipitates with 88 wt.% lignin, while those produced via ethanol treatment of this hydrolysate had 33.7 wt.% hemicelluloses. The 2-dimensional HSQC NMR analysis provided the evidence of lignin-carbohydrate linkage for lignocelluloses of hydrolysate produced under the hydrolysis conditions of 180 °C and 15 min residence time. In addition, the heat capacity (Cp) of the dried hydrolysates was 0.41 J/g°C, while the precipitates of acidification and ethanol treatment possessed the Cp of 1.18 J/g°C and 1.36 J/g°C, respectively. This increment could be related to a higher molecular mobility of lignin units in these precipitates due to degradation of Hα and Hβ interlinkages and a higher amount of methoxyl groups in the precipitates as confirmed by ^{1}H-NMR analysis.

4.2 Introduction

Lignocellulosic materials have been proposed to be used for manufacturing value-added products or fuel. Lignin can be converted to carbon fibers, phenols, flocculants, hydrogen and dispersants, for example (Lora and Glasser 2002; Stewart 2008; Vishtal and Kraslawski 2011; Fatehi et al. 2016). Hemicelluloses are widely used for ethanol production, while cellulose is vastly used in pulp and paper industry (Pothiraj et al. 2006; Garcia et al. 2011).

For use in industry, lignocelluloses need to be isolated from biomass. Autohydrolysis is a cost-effective, efficient, environmentally friendly, and widely applied technology for hemicelluloses extraction from biomass (Carvalheiro et al. 2008; Sixta et al. 2013). The application of autohydrolysis technology mainly leads to the isolation of oligomeric sugars with various degrees of polymerisation from biomass (Pu et al. 2013a; Sipponen et al. 2013) However, along with hemicelluloses, this treatment leads to the dissolution of lignin in hydrolysates of this process (Carvalheiro et al. 2008). In this work, the hydrolysates produced via autohydrolysis of spruce

wood chips were considered for further investigation, as spruce spices have recently been considered as a wood resource in dissolving pulp production process.

The hydrolysates are very dilute, which impairs the direct use of hydrolysate for value-added production. Acidification and solvent extraction have been reported to be efficient methods for lignocelluloses isolation from prehydrolysis liquor (PHL), black liquor of kraft pulping process, and spent liquor (SL) of neutral sulfite semichemichal (NSSC) process (Liu et al. 2011a,b; Shi et al. 2011; Tarasov et al. 2015). However, the isolation process influences the properties of extracted materials (Tarasov et al. 2017; Liu et al. 2011a).

The key parameters affecting the industrial application of the extracted materials are molecular weight (Mw), charge density, ash content, thermal stability, glass transition temperature (Tg), and heat capacity (Cp) (Aro and Fatehi 2017; Tarasov et al. 2017, Olsson and Salmen 1997). Lignocelluloses with a high or low Mw can be applied for production of flocculants or dispersants, respectively (Aro and Fatehi 2017). The possibilities for lignocellulose utilization as fuel or fuel additive are directly affected by the thermal stability, Tg and Cp values (Lumadue et al. 2012; Malhotra 2010). The first objective of this work was to assess the properties of precipitates produced via acidification or ethanol treatment of hydrolysates.

It was reported that the amounts of phenolic, aliphatic, carboxylic acid and methoxyl groups influenced the properties of lignocellulosic materials, e.g., phenolate hydroxyl groups improved lignin's hydrophobicity and structural flexibility (Li et al. 2016; Olsson and Salmen 1997). Methoxyl groups impeded the covalent crosslinking in lignin, and hence the mobility of lignin moieties increases (Olsson and Salmen 1997). This led to a lower glass transition temperature (Olsson and Salmen 1997) and a higher heat capacity of lignin (Li and McDonald 2014). To recognize a feasible application for the precipitates, the functional groups of the generated precipitates were evaluated as the second objective of this research.

The evidence of lignin-carbohydrate complexes (LCC) in native biomass of coniferous, deciduous and non-wood plants were reported in numerous studies (Lawoko et al. 2005; Dammstrom et al. 2009; Yao et al. 2016; You et al. 2015; Zhang et al. 2016). The presence of LCC was also reported in the black liquor of kraft pulping process (Tamminen et al 1995; Lawoko et al. 2004; 2005). Fatehi et al. (2016) confirmed the LCC presence in hardwood-based PHL and SL of NSSC. In our

previous chapter, the existence of LCC in softwood-based hydrolysate was discussed (Tarasov et al. 2018). However, Uraki et al. (2006) suggested the self-aggregation of LCC in water due to the hydrophobic interactions of lignin part of LCC. Also, it was reported that LCC linkages decomposed under acidic conditions (Kosikova et al. 1979; Zoia et al. 2008). Therefore, the third objective of this study was to investigate the presence of LCC in the precipitates generated via acidification and ethanol treatment of hydrolysates at ambient temperature.

In the present chapter, the properties of lignocelluloses precipitated from softwood-based hydrolysate were analyzed. The main purpose of this research was to evaluate the effect of autohydrolysis intensity and extraction method on structure and parameters of the precipitated materials from hydrolysates. The existence of LCC in precipitates obtained from hydrolysates was evident for the first time. Also, the compositions and functional groups of LCC were determined.

4.3 Materials and Methods

4.3.1 Materials

Ethanol (95 vol.%) and sulphuric acid (98 wt.%) were purchased from Fisher Scientific and used without purification. Dimethyl sulfoxide-d_6 (DMSO, 99.9 % purity), 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt (TSP) and chromium(III) acetylacetonate (Cr(acac)3) were purchased from Sigma Aldrich company for nuclear magnetic resonance (NMR) analysis and used as received.

4.3.2 Precipitate production

At first, hydrolysates were produced via flow through autohydrolysis of spruce wood chips under the conditions stated in Table 4.1. In this study, 300±5 g of wood chips was added to a 2L pulping digester (Greenwood, TX) along with the certain amount of distilled water. The heating rates were set as 4.5 °C/min and 2.5 °C/min when the temperature in the vessel of the digester was below and above 100 °C, respectively. The residence time of the hydrolysis treatment was adjusted when the temperature in the vessel achieved the set-point value. The flow rate of liquid circulation was set at 6 L/min in all autohydrolysis reactions. Afterward, obtained hydrolysis liquors were collected and stored at 4 °C prior to experiments. The impacts of processing temperature and residence time on the lignocellulose removals in the autohydrolysis can be considered by a severity factor (S_0) (Overend and Chornet 1987). The S_0 of autohydrolysis process could be evaluated via Eq. (4.1) (Pedersen and Meyer 2010):

$$S_o = \log(t \times e^{\frac{T-100}{14.75}}) \quad (4.1)$$

where, T is the temperature (°C); t is the residence time (min.) of hydrolysis; 14.75 is the arbitrary constant related with activation energy and temperature (Pedersen and Meyer 2010; Carvalheiro et al. 2009). The compositions of lignocellulosic material in obtained hydrolysates are presented in Table 4.1.

Table 4.1 The properties of hydrolysis liquors (Tarasov et al. 2018).

Sample ID	Autohydrolysis conditions				Lignocelluloses composition in hydrolysates, wt.% based on wood				
	Temperature, °C	Residence time, min	L/S ratio	S_0	Lignin	Total sugars	Monosugars	Acetic acid	Furfural
1	180	15	5	3.5	6.8	5.0	1.3	0.4	ND[1]
2	180	45	5	4.0	7.1	5.3	1.6	0.4	ND
3	190	15	5	3.8	10.5	4.5	2.2	1.0	1.6
4	180	15	10	3.5	9.7	5.1	1.3	0.3	ND

[1]Not detected

Three sets of experiments were conducted for lignocellulosic material extraction from hydrolysates. In one set of experiments, the pH of hydrolysates was decreased to 1.5 via using 20 wt.% sulphuric acid (Liu et al. 2011a; Shi et al. 2011). Then, acidified hydrolysates were centrifuged at 4000 rpm for 5 min. After centrifuging, the precipitates were collected and placed in an oven at 60°C for 48 h. In another set of experiments, ethanol was added to hydrolysates at the ratio of 4/1 wt./wt. (Liu et al. 2011a). Afterward, hydrolysate/ethanol mixtures were centrifuged and dried as previously described. In another set of experiments, the hydrolysates in the amounts of 25±2g were placed in an oven at 60°C for 48 h to determine the solid content of hydrolysates. The dried materials were used in the subsequent experiments. Figure 4.1 shows the process of precipitates production.

Precipitates generated via acidification of samples 1, 2, 3 and 4 were labeled as samples 1-A, 2-A, 3-A and 4-A, respectively. Precipitates produced due to ethanol treatment of samples 1, 2, 3 and 4 were indicated as samples 1-E, 2-E, 3-E and 4-E, respectively. Materials obtained via evaporation from samples 1, 2, 3 and 4 were labeled as samples 1-D, 2-D, 3-D and 4-D, respectively.

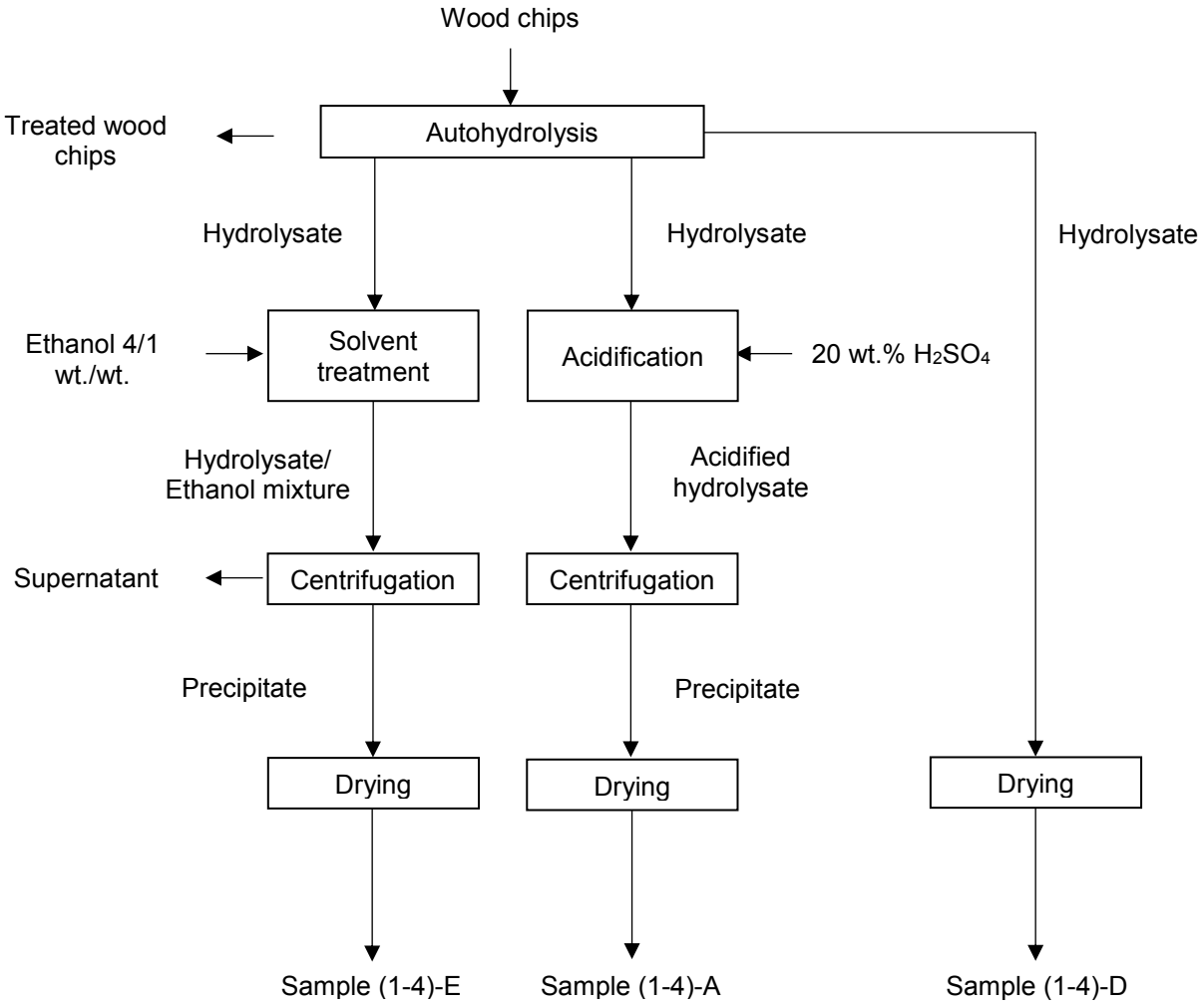

Figure 4.1 The process of precipitates production.

4.3.3 Analysis of lignin, hemicelluloses of the precipitates

For lignin and hemicellulose analysis, certain amounts of precipitates were mixed with deionized water and placed in a water bath at 30 °C and 50 rpm for 12 h. Lignin content of these mixtures was evaluated using UV spectrophotometry at 205 nm in accordance with TAPPI UM 250 (GENESYS 10S UV–vis, Thermo Scientific) (Liu et al. 2011). The concentration of polysaccharides was estimated in accordance with the method presented by Liu (2008). Ion chromatography was used for analyzing hemicellulose content of the mixtures. In one set of experiments, 5 mL of the mixtures was used for determining the monosaccharide content of the samples by ion chromatography (Dionex, ICS 5000, Thermofisher Scientific), which was equipped with a CarboPacTM SA10 column and an electrochemical detector (ED) (Dionex-300, Dionex

Corporation, Canada). Since, ion chromatography is unable to determine polysaccharide concentration, the mixtures were treated with 4 wt.% sulfuric acid and placed in a reactor at 121 °C for 1 h to convert polysaccharides to monosaccharides (Liu 2008). Then, the amount of monosaccharides in the mixture was determined by ion chromatography as stated earlier. As all hemicelluloses were converted to monosaccharides after acid treatment, this analysis showed the total sugars of the samples. Lignin and hemicellulose concentrations were determined in weight percentage of the dried mass of samples.

4.3.4 Molecular weight

The precipitates were diluted in 0.1 mol/L $NaNO_3$ to obtain an approximate concentration of 5 g/L and placed in the water bath at 30 °C and 50 rpm overnight. For molecular weight estimation, the gel permeation chromatography (Malvern GPCmax VE2001 Module + Viscotek TDA305) equipped with multi-detectors and PolyAnalytic PAA206 and PAA203 columns were used. In this experiment, 0.1 mol/L $NaNO_3$ was used as solvent and eluent. The column temperature was adjusted at 35 °C and the flow rate in GPC was adjusted at 0.70 mL/min. The molecular weight analysis of lignin was determined using a UV detector (at 280 nm wavelength) and that of hemicelluloses was determined using reflective index (RI) and intrinsic-differential pressure (IV-DP) detectors of the GPC instrument (Fatehi et al. 2016).

4.3.5 Thermal properties analysis

The thermal stability of the samples was investigated via a thermogravimetric analysis (TGA), Instrument Specialist, i1000. In TGA analysis, the precipitates were heated from 25 °C to 700 °C with a 10 °C/min heating rate. Experiments were performed in a nitrogen atmosphere at a 35 mL/min flow rate.

4.3.6 DSC analysis

Glass transition temperature (T_g) and heat capacity (C_p) values of the samples were evaluated by a differential scanning calorimetry (DSC, TA Instruments Q2000). Oven dried samples (2-5 mg) were placed in DSC pans and the samples were treated in the temperature range of 0 °C and 200 °C with a heating rate of 3 °C/min (Persson et al. 2012). The samples were heated to 200 °C, then cooled down to 0 ºC temperature and reheated to 200 °C. In the second heating cycle, the T_g and heat capacity of the samples were analyzed (Sammons et al. 2013).

4.3.7 NMR experiments

Application of ^{1}H NMR technology for estimation of functional groups of lignin was widely described in the literature (Patil et al. 2015; Li and Lundquist 1994). The advantage of ^{1}H-NMR technology is the possibility to obtain spectra with a high signal-to-noise ratio with a short processing time due to the density of ^{1}H atomic nucleus (Pu et al. 2013b). However, the shift expansion in ^{1}H NMR technology is short, ranging from 0 to12 ppm, which could result in signal overlap (Pu et al. 2013b). For better resolution of NMR spectrum, the lignin samples can be acetylated (Balakshin et al. 2011; Du et al. 2013). However, acetylation may cause chemical modifications of lignin, which is undesired (Pu et al. 2013b). Considering this, ^{1}H-NMR experiments were conducted without any modifications in this work.

For ^{1}H-NMR spectroscopy experiments, the Varian Unity Inova 500 MHz NMR instrument was employed. The samples were prepared in accordance with the procedure described by Nagy et al. (2010). Approximately, 25 mg of the dried samples were dissolved in 0.5 mL of deuterated DMSO-d_6. Then, 0.5 wt.% of TSP was added to the samples and served as an internal standard. Hydroxyl groups were identified by signals in assigned areas of ^{1}H-NMR spectrum (Nagy et al. 2010; Li and Lundquist 1994).

The investigation of LCC present in the precipitates was conducted with 2-dimensional HSQC NMR spectroscopy (Du et al. 2014; Balakshin et al. 2011; Yuan et al. 2011). Approximately, 50 mg of each sample was dissolved in 0.5 mL of deuterated DMSO-d_6. Then, Cr(acac)3 (0.013M) was added to the mixtures and employed as a relaxation agent (Balakshin et al. 2011). The mixtures were stirred overnight at 60 rpm for complete dissolution. The specifications of NMR instrument for 2-dimensional HSQC NMR experiments were set in accordance with a previous work (Yuan et al. 2011). The relaxation delay for ^{1}H-dimension was set at 1.5s with 1024 number of collected complex points. The number of transients for ^{13}C-dimension was 64, and 256 time increments were recorded. Afterward, the presence of LCC was identified in accordance with findings reported in the literature (Du et al. 2014; Balakshin et al. 2011; Yuan et al. 2011). The DMSO peak at δ_C/δ_H 39.5/2.49 ppm/ppm served as an internal reference point (Du et al. 2014).

4.4 Results and discussion

The total solid contents of hydrolysates were 13.5, 16.7, 17.9 and 16.1 wt.% for sample 1, 2, 3 and 4, respectively. The higher solid content of hydrolysates is attributed to more extraction of lignin from wood chips in the hydrolysates at elevated temperatures and longer residence times for samples 2 and 3 (Table 4.1) (Tarasov et al. 2018). The increases in the autohydrolysis temperature or residence time extension escalated the concentration of acetic acid in the hydrolysates, which leads to a higher acidity and thus elevation in the lignin and hemicellulose extraction (Leppanen et al. 2011; Song et al. 2008). The relatively high solid content in sample 4 is attributed to better lignin dissolution in hydrolysis liquor at a high L/S ratio (Tunc 2014).

Figure 4.2 presents the lignin content of the precipitates. It can be seen that lignin comprises 55 wt.% of the solid content of hydrolysates in all samples. Acidification changed the lignin content of the precipitates to 64, 78, 88 and 55 wt.% for samples 1, 2, 3 and 4, respectively. The acidification seemed to be ineffective in increasing the lignin content of sample 4. As explained earlier, sample 4 was produced at a high L/S ratio of 10 (Table 4.1). It is possible that the produced lignin was more acid soluble (Tunc 2014). Samples produced from mixing hydrolysates and ethanol had lower lignin content than original hydrolysates. It was previously reported that the sample 3 contained lignin moieties with a small molecular weight (Tarasov et al. 2018), which could be the reason for its higher removal. Recently, it was observed that the precipitates, generated via mixing SL of NSSC process with ethanol with the ratio of 20/80 or 67/33, had the Mw of 10,000 g/mol and 70,000 g/mol, respectively (Tarasov et al. 2015). It was proposed that a high amount of the solvent reduced the overall polarity of the mixture, which resulted in a higher precipitation of small polymers along with large ones. Therefore, a higher concentration of polymers with a low Mw in the precipitates led to the reduction of the molecular weight of the precipitates (Tarasov et al. 2015).

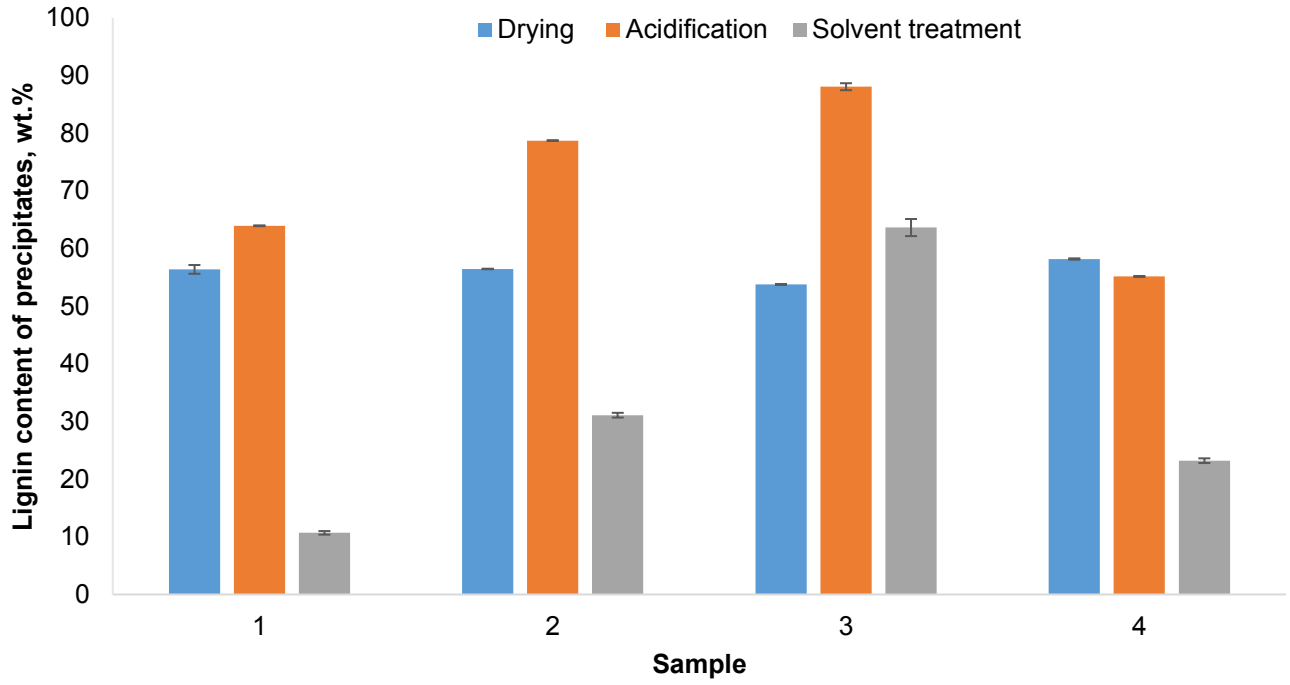

Figure 4.2 Lignin content in the precipitates.

Figures 4.3 depicts total sugars content of the samples. It is seen that hemicelluloses are comprised of 25 wt.% of dried hydrolysates. Acidification of samples 2 and 3 led to the precipitation of material with a relatively lower concentration of hemicelluloses compared with dried hydrolysates. It is well known that hemicelluloses are acid soluble, thus acidification was ineffective in isolating hemicelluloses. The precipitates obtained via acidification of samples 1 and 4 had higher hemicelluloses than dried hydrolysates. The extraction of hemicelluloses could be related to the extraction of LCC from hydrolysates.

As it was expected, the precipitates produced with ethanol treatment were richer than other samples in hemicelluloses. The precipitates generated via mixing ethanol with samples 1, 2, 3 and 4 had 42.5, 27.9, 33.7 and 41.8 wt.% hemicelluloses, respectively. Thus, the variation could be related to difference in the molecular weight of hemicelluloses. Our recent findings showed that ethanol treatment of hydrolysates led to precipitation of hemicelluloses with a high Mw (Tarasov et al. 2018). Also, it was found that the Mw of hemicelluloses in samples 1 and 4 were larger than those in samples 2 and 3 (Tarasov et al. 2018).

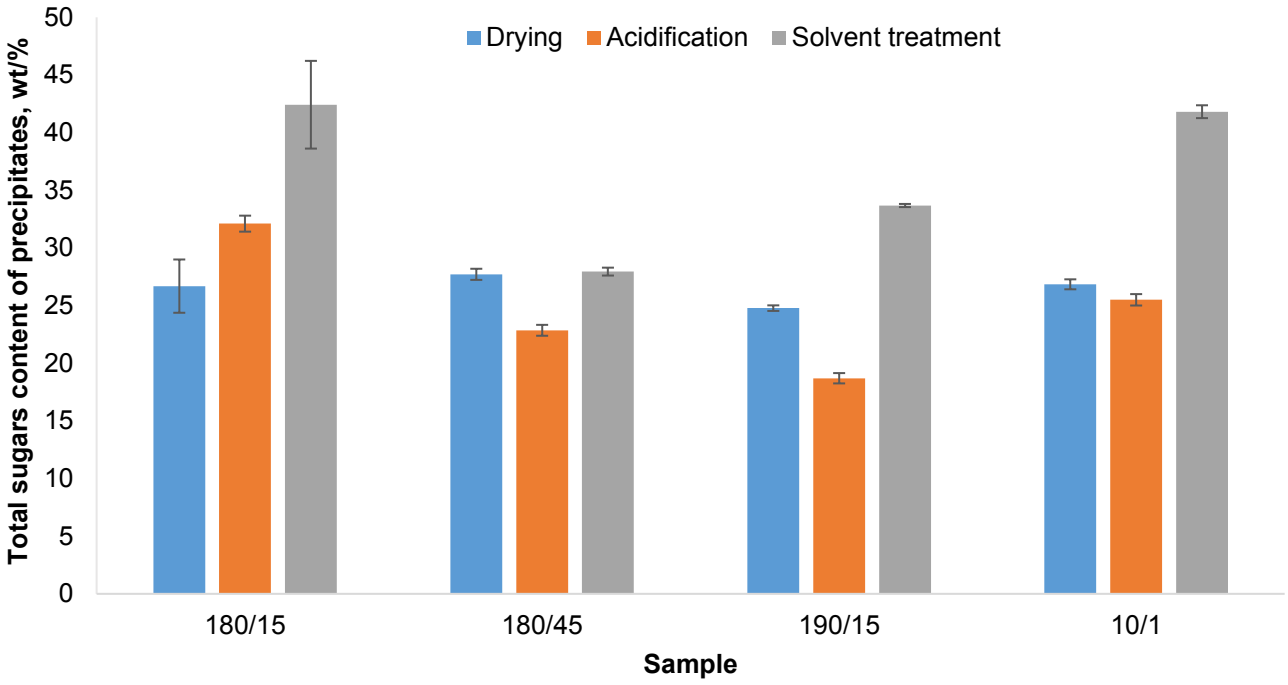

Figure 4.3 Total sugars content in the precipitates.

Table 4.2 lists the compositions of hemicelluloses in the precipitates. It is seen that the amounts of glucose (Glu), galactose (Gal) and mannose (Man) changed, which could be related to the existence of galactoglucomannan (GGM) in the precipitates. Similar trends were observed for xylan (Xyl) and arabinose (Ar); hence it is possible to assume that these monosaccharides precipitated as arabinoglucoroxylan (ArXyl). In the past, Lawoko et al. (2005) suggested that lignin in softwoods existed in the form of two different structures. One type surrounded by xylan (Xyl) and the other by glucomannan (GM). These lignin types are bonded to carbohydrates, forming four different LCCs, i.e., GGM-lignin, Glu-lignin, GM-lignin-Xyl, and Xyl-lignin-GM, which contain 8, 4, 40 and 48% of total lignin in wood (Lawoko et al. 2005).

The precipitates generated by acidification of sample 1 and 4 possessed slightly higher GGM and ArXyl compared with dried hydrolysates. This increment could be attributed to hemicelluloses precipitation along with lignin in the form of GGM-lignin and ArXyl-lignin complexes. Precipitates of samples 2 and 3 obtained after the acidification had slightly lower amounts of sugars than dried hydrolysates. This may be related to precipitation of carbohydrate-free lignin under strong acidification conditions.

The precipitates generated via mixing samples 1 and 4 with ethanol appeared to have a lower lignin (Figure 3.2) and ArXyl content (Table 3.2) compared to the dried samples. However, the relative amount of GGM in precipitates was increased from 20.6 wt.% to 40.2 wt.% and from 21.6 wt.% to 39.9 wt.% in samples 1 and 4, respectively. Therefore, it is possible that the ethanol treatment of sample 1 and 4 led to the precipitation of material with high amounts of GGM-lignin and Glu-lignin complexes. Moreover, it was noted that ethanol treatment of samples 2 and 3 slightly increased the relative content of GGM and ArXyl in comparison with that of dried samples, which could be related to the precipitation of LCC.

Table 4.2 Hemicellulose compositions of precipitates.

Compositions of total/mono saccharides in dried hydrolysates[1], wt.%/wt.%							
Sample ID	Arabinose	Galactose	Rhamnose	Glucose	Xylose	Mannose	Total
1-D	1.89/2.69	5.71/1.56	0.29/0.21	3.67/0.17	3.91/1.69	11.21/0.65	26.69/6.96
2-D	1.02/1.15	6.14/2.14	0.23/0.20	4.83/0.46	3.15/2.20	12.33/1.70	27.70/7.85
3-D	0.67/0.76	5.48/2.68	0.17/0.16	4.72/0.87	2.44/2.27	11.28/2.84	24.78/9.59
4-D	1.46/1.57	6.50/1.56	0.27/0.18	4.06/0.20	3.49/1.58	11.06/0.78	26.84/5.88
Compositions of total/mono saccharides in precipitates obtained via acidification, wt.%							
1-A	2.26/0.29	6.94/0.42	0.34/0.01	4.56/0.16	4.63/0.69	13.38/0.66	32.11/2.22
2-A	0.83/0.09	4.41/0.29	0.12/0.01	4.00/0.29	2.72/0.42	10.78/0.94	22.86/2.05
3-A	0.51/0.04	4.15/0.16	0.08/ND[2]	3.50/0.19	1.78/0.17	8.67/0.41	18.70/0.98
4-A	1.67/0.13	5.97/0.24	0.25/ND	3.70/0.19	3.41/0.34	10.51/0.54	25.50/1.45
Compositions of total/mono saccharides in precipitates obtained via ethanol treatment, wt.%							
1-E	0.18/0.12	12.22/0.08	0.10/ND	8.16/0.08	1.99/0.02	19.78/ND	42.42/0.31
2-E	0.55/0.56	6.54/0.97	0.10/0.09	5.28/0.21	1.79/0.94	13.69/0.87	27.95/3.63
3-E	1.00/0.93	6.38/1.95	0.21/0.15	6.26/0.50	3.06/1.59	16.78/1.90	33.68/7.02
4-E	0.18/0.10	11.14/0.08	0.05/ND	7.76/0.01	1.66/0.05	21.00/0.06	41.78/0.30

[1]Total sugars/ monosugars concentration in precipitates, wt.%/wt.%; [2]Not detected

Among the samples reported in Table 3.2, four samples were selected for further analysis. Sample 1-E showed a relatively higher hemicellulose content, and sample 3-A had the highest lignin content (Figure 3.2). For evaluation of the changes caused by the acid or ethanol supplement, samples 1-D and 3-D were also analyzed.

4.4.1 Molecular weight

Table 4.3 lists the molecular weights of lignin and hemicelluloses in the selected precipitates. The materials obtained contained both high Mw and low Mw lignin. Low Mw lignin and hemicelluloses showed similar retention times in the GPC analysis. Similar retention times of UV, IR and IV-DP responses provided evidence of LCC (Fatehi et al. 2016; Tunc et al. 2010). However, no RI and IV-DP signals were overlapped indicating that the high Mw lignin was probably carbohydrate free.

It is seen in Table 4.3 that sample 1-D did not contain carbohydrate-free lignin, while sample 3-D showed the existence of carbohydrate-free lignin with Mw of 30,000 g/mol. Based on the analysis, sample 1-D showed only a low Mw lignin peak; whereas, sample 1-E had both high Mw and low Mw lignin peaks. These changes could be related to alteration of the LCC structure caused by ethanol treatment due to the lower solubility of lignin in ethanol-water mixtures, which led to the precipitation of larger lignin fractions (Wang and Chen 2013).

Table 4.3 Molecular weight of lignin and hemicelluloses in the precipitates.

Sample ID	Molecular weight, g/mol		
	High Mw lignin	Low Mw lignin	Hemicelluloses
1-D	ND[1]	1405±130	2003±90
3-D	33320±3770	1004±55	866±110
1-E	34825±7700	3619±317	4556±246
3-A	22839±1670	2319±89	3557±800

[1]Not detected

Overall, it was shown that the molecular weight of LCC present in the precipitates was between 1,000 g/mol and 3,600 g/mol (Table 4.3). Fatehi et al. (2016) reported that the PHL of kraft-based dissolving pulp production process and the SL of NSSC process contained LCC with the Mw of 2,000 g/mol and 1,500 g/mol, respectively. It is also noted that the Mw of LCC in samples 1-E and 3-A were significantly higher than Mw of LCC in samples 1-D and 3-D. In the previous chapter, it was noted that LCC present in hydrolysate of samples 1 and 3 showed 40% and 20% decrease in Mw after ethanol and acid treatment, respectively (Tarasov et al. 2018). It was reported in the literature that the addition of ethanol could precipitate LCC (Nonaka et al. 2013; Sun et al. 2013). It is shown in Table 4.3 that the Mw of hemicelluloses of samples 1-E and 3-A were significantly

higher than that of samples 1-D and 3-D. Consequently, acidification has also likely led to the isolation of large LCC and hemicellulose fragments.

4.4.2 Thermal properties of the precipitates

The thermal stability of lignocelluloses depends on several factors, such compositions, interunit structures and functional groups (Chen et al. 2016; Obernberger and Thek, 2010). Figure 4.4 depicts the weight loss and the weight loss rate of the precipitates. Samples 1-D and 3-D showed similar behaviour with the degradation onset temperature (T_{onset}) of 200 °C and the weight loss of 37% and 40%, respectively, in the 200-300 °C temperature region. In the 300-630 °C region, the weight reduction of 30% and 27% was noted for samples 1-D and 3-D, respectively. These regions probably represented hemicelluloses and lignin decomposition (Carrier et al. 2011). Singh et al (2013) observed similar trends during pyrolysis of commercial softwood LCC. The thermal stabilities of materials obtained after ethanol or acid supplement were significantly different. Sample 1-E shows two shoulders at 230-350 °C and 350-630 °C regions with 54 wt.% and 22 wt.% weight losses, respectively. The increment in the weight loss at lower temperatures could be related to the higher sugar content of sample 1-E. Sample 3-A demonstrates one degradation region at 160-630 °C. Khazraie and coworkers (2017) reported a similar thermal behavior of precipitates obtained from hydrolysates via acidification. It was proposed that the presence of sulphuric acid in precipitates intensified the decomposition of lignin in pyrolysis (Zhou et al. 2013).

Samples 1-D and 3-D showed the peak temperature (Tp) around 230 °C with weight loss rates of 0.45 %/°C and 0.61 %/°C, respectively (Figure 3.4b). A possible reason for the higher mass loss in sample 3 can be related to the degradation of β-O-4 interlinkages and possible depolymerization of lignin structure (Chen et al. 2016; Long et al. 2014). Li et al. (2007) reported the decrease of β-O-4 substructures in lignin samples with the increment in the severity factor of steam hydrolysis from 3.2 to 4.1. It can be seen in Table 4.1 that samples 1 and 3 were generated at the S_0 of 3.53 and 3.82, respectively. As seen from Figure 4b the samples 1-E and 3-A showed T_p of 325 °C and 175 °C, respectively. The shift of T_p is most likely attributed to structures and compositions of samples 1-E and 3-A. Sample 1-E showed the degradation rate of 0.7 %/°C at T_p. Additionally, this precipitate demonstrated a shoulder in the temperature range between 275 °C and 300 °C with a 0.45 %/°C weight loss. Considering the fact that, T_p of sugar decomposition was reported to be

at 290 °C (Gasparovic et al. 2012) as well as the high sugar content in this sample (42.4 wt.%), it is possible to assume that this region is related to hemicelluloses degradation.

The reduction of T_p in case of sample 3-A could be attributed to its higher lignin content (Figure 4.2) and its smaller molecular weight (Table 4.3) (Tong et al. 2017). Another reason for a lower T_p of sample 3-A may be the enhanced decomposition of lignin due to the existence of sulfuric acid moieties as stated above (Zhou et al. 2013).

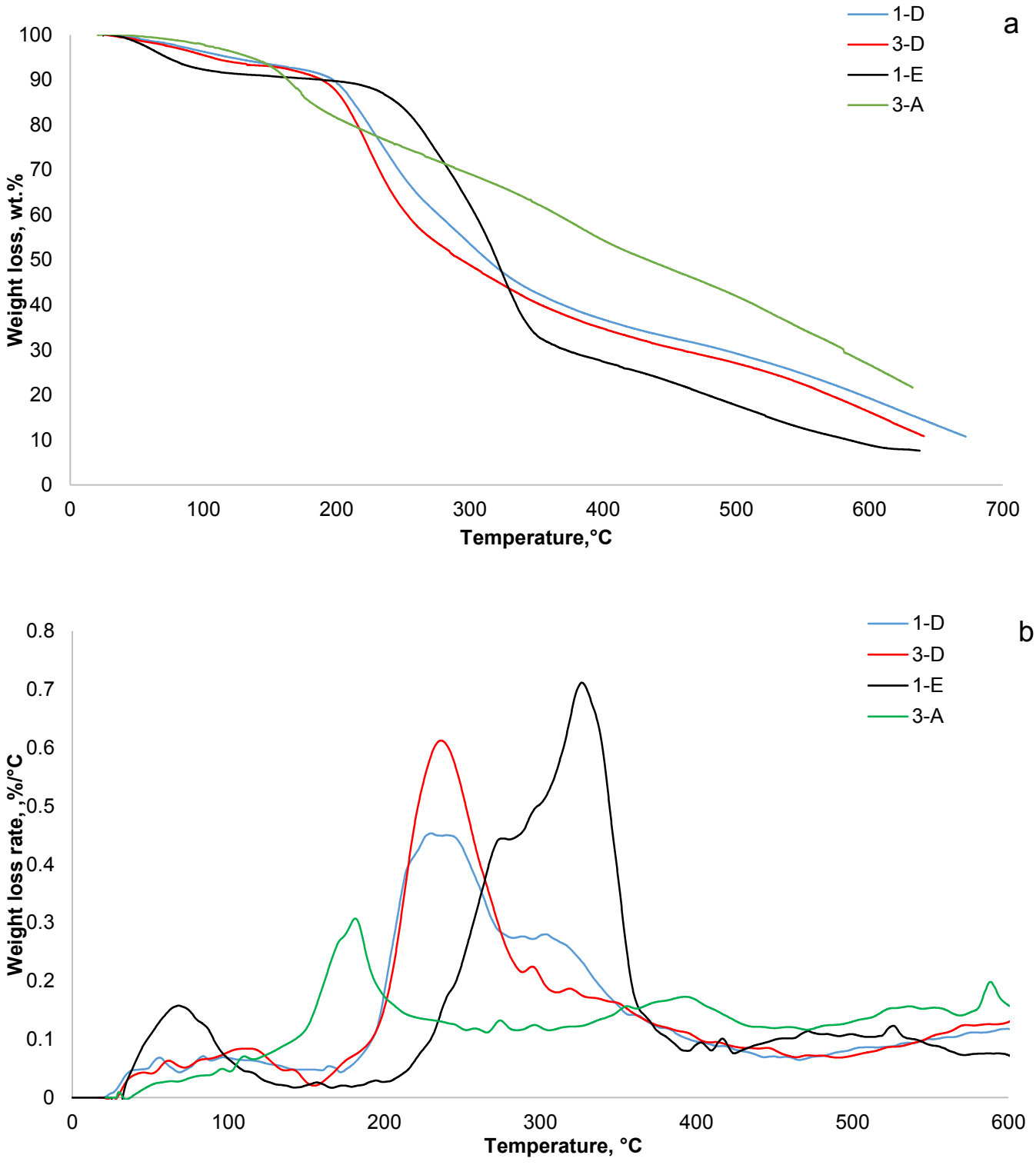

Figure 4.4 Weight loss (a) and (b) weight loss rate of the precipitates.

4.4.3 DSC analysis

Molecular weight, molecular mobility of lignin and cross-linking degree affected the glass transition temperature of lignocellulosic materials (Hatakeyama and Hatakeyama, 2010; Olsson and Salmen 1997; Guigo et al. 2009; Li and McDonald 2014). It was reported that Tg of lignin increased with Mw and cross-linking degree increased (Guigo et al. 2009; Olsson and Salmen 1997). Hydrothermal treatment led to the depolymerization of lignocelluloses and reduced their Mw (Li et al. 2007; Chua and Wayman 1979), which decreasing the Tg of lignocellulosic materials (Kong et al. 2017). Also, hydrothermal treatment caused water assimilation on the polar sites of lignin structure. This caused the modification of inter-molecular hydrogen bonds, which increases the mobility of lignin, hence decreasing Tg of lignin (Guigo et al. 2009). It was reported that Tg of hemicelluloses, lignin, and celluloses isolated from wood in aqueous conditions was 40°C, 50-100 °C and 100 °C, respectively (Kong et al. 2017; Olsson and Salmen 1997). Table 4.4 lists the Tg and the heat capacity values of the examined materials.

Table 4.4. Glass transition temperature (T_g) and heat capacity (C_p) of the precipitates.

Sample ID	T_g, °C	C_p, J/g°C
1-D	101.8	0.40
3-D	98.9	0.41
1-E	83.5	1.36
3-A	87.8	1.18

Samples 1-D and 3-D had approximately equal glass transition temperatures of 100 °C. Olsson and Salmen (1997) estimated the softening temperature of wet softwood at 90 °C, which could be considered as Tg of lignin, as the Tg of wood occurs in the same temperature range as lignin (Olsson and Salmen 1997; Kong et al. 2017). Youssefian and Ruhbar (2015) reported that the Tg of LCC extracted from bamboo was 166 °C. The lower Tg value obtained in our study could be related to lignin plasticisation by water (Irvine 1985), which resulted in higher molecular mobility of lignin units and reduction in T_g value (Guigo et al. 2009). The Tg of the precipitates obtained via acidification and ethanol treatment was slightly lower than the Tg of dried materials, whereas the Cp values of samples 1-E and 3-A were significantly higher. This could be explained by increment in lignin molecular mobility in samples 1-E and 3-A (Li and McDonald 2014) due to a higher amount of methoxyl groups (Olsson and Salmen 1997) or cross-linkage density decrease

(Hatakeyama and Hatakeyama, 2010). These assumptions were confirmed by ^{1}H-NMR spectroscopy presented later in this chapter.

4.4.4 Functional group analysis

The ^{1}H-NMR spectra of examined samples presented in Figure 4.5 generally displays, ^{1}H-NMR spectrum was partitioned into three main sections (Mainka et al. 2015). Signals in the regions of 8.00-6.00, 6.00-4.05 and 2.25-0.00 ppm are related to aromatic groups, side chain, and aliphatic groups, respectively (Nagy et al. 2010).

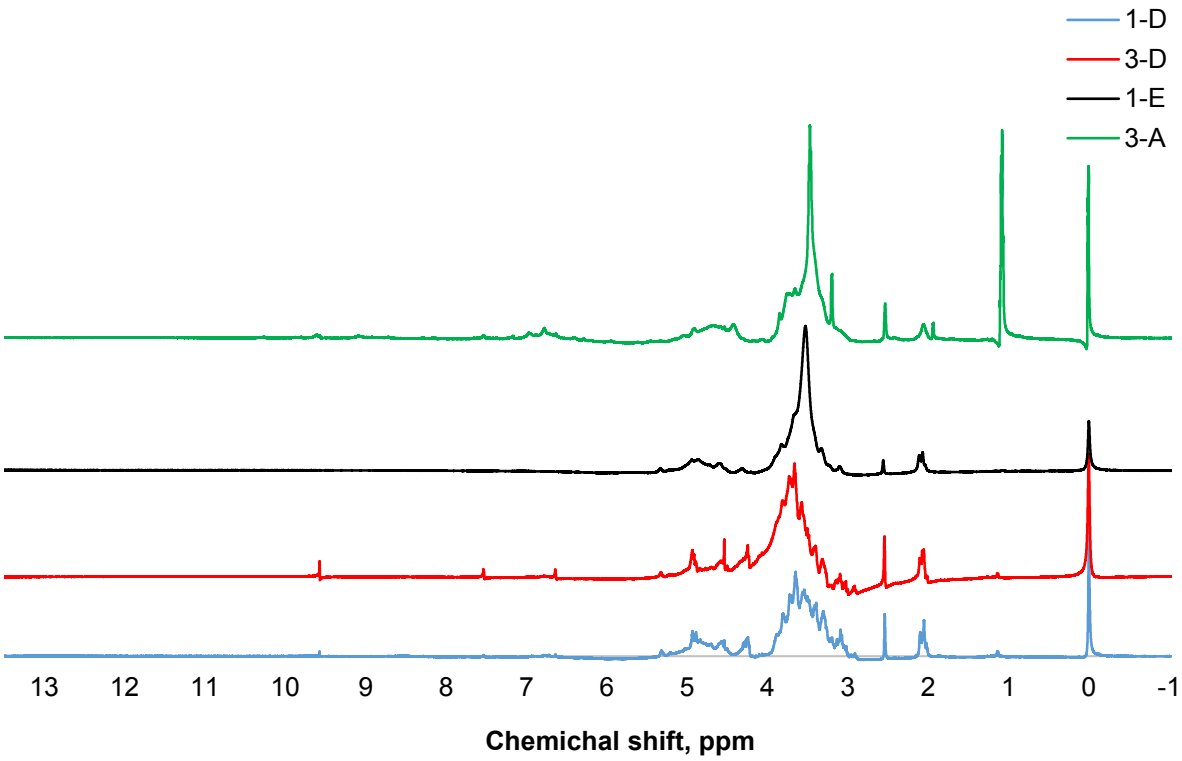

Figure 4.5 ^{1}H-NMR spectra of the precipitates.

Samples 1-D, 3-D and 3-A showed signals at 6.7 ppm, which is associated with guaiacyl units of lignin (Foston et al. 2015). The absence of this signal in the spectrum of sample 1-E could be related to its relatively low lignin content (Figure 4.2). The presence of conjugated carbonyl group was indicated by signals at 9.57 ppm (Tong et al. 2017). The higher intensity of this signal in samples 3-D and 3-A could be related to the degradation of quinoids in lignin due to the high

temperature and/or acidification, which led to the formation of carbonyl functional groups (Tong et al. 2017). The quantitative analysis of the functional groups present in the precipitates based on ^{1}H-NMR spectroscopy is shown in Table 4.5.

Table 4.5 Functional groups in the precipitates based on the ^{1}H-NMR spectroscopy.

Sample ID	Lignin's functional groups (ppm), mmol/g						
	Carboxylic acid (13.5-10.5)	Formyl groups (10.1-9.35)	Phenolic groups (9.35-8.0)	Aromatic and vinyl (8.0-6.0)	H_α and H_β inter-unit linkages (6.0-4.05)	Methoxyl groups (4.05-3.45)	Aliphatic groups (2.25-0.00)
1-D	ND[1]	0.05	0.22	0.71	4.95	9.43	0.21
3-D	ND	0.02	0.24	0.62	5.32	10.44	0.44
1-E	ND	ND	ND	ND	1.54	17.95	0.68
3-A	ND	0.02	0.47	0.59	0.96	12.90	2.55

[1]Not detected

It is seen in Table 4.5 that only a trace amount of formyl groups was found in the precipitates, and no carboxylic acid was present. Sample 3-A had slightly more phenolic groups compared with other precipitates. Acidification resulted in the cleavage of aryl-ether bonds, which led to the formation of phenolic groups (Cateto et al. 2008; Santos et al. 2013). The highest amount of methoxyl groups was found in sample 1-E. Samples 1E and 3-A contained higher amount of aliphatic groups comparing to samples 1-D and 3-D. This increment could be related to the liberation of α-hydroxyl groups due to lignin-carbohydrate bonds disintegration (Sun et al. 2014; Jaaskelainen et al. 2003). The decomposition of LCC linkages in sample 3-A is related to acidification (Kosikova et al. 1979; Zoia et al. 2008). It was reported that the presence of organic solvents promoted the cleavage of α-ether linkages in lignin (Azadi et al. 2013). Ether and ester linkages in LCC are generated at the α-carbon position in phenyl propane units (Saggi et al. 2016). Therefore, it is possible to assume that ethanol supplement caused partial degradation of lignin-carbohydrate linkages presented in sample 1-D, which resulted in the increment of aliphatic groups in sample 1-E. Also, the increase in the aliphatic groups in sample 1-E possibly relates to a relatively high presence of carbohydrates (Menezes et al. 2016). It is shown in Table 4.5 that the amounts of Hα and Hβ inter-units in samples 1-E and 3-A were significantly lower than that of samples 1-D and 3-D. This behaviour may be attributed to the partial decomposition of lignin inter-unit and lignin-carbohydrate linkages in sample 3-A due to the acidification (Kosikova et al 1979; Zoia et al. 2008; Monot et al. 2017; Leschinsky et al. 2008). Lower amounts of inter-units in sample 1-E could be related to its relatively low (10 wt.%) lignin content. The deficiency of

aromatic and vinyl groups in sample 1-E (Table 3.5, Figure 3.5) supports this assumption. Also, the decomposition of α-ether linkages in lignin due to the presence of ethanol (Azadi et al. 2013) could cause the reduction of Hα and Hβ inter-units content in sample 1-E.

4.4.5 Investigation of LCC linkages in precipitates

The 2D HSQC NMR technology was successfully applied for analyzing LCCs structure in the past (Du et al. 2014; Yuan et al. 2011). Lignin-carbohydrate bonds in softwood are mainly represented by benzyl ether (BE) and ester and phenyl glucoside linkages (PhyGlc) (Balakshin et al. 2011). The presence of BE, ester and PhyGlc linkages can be identified by signals in 2D HSQC spectra at the regions of δ_C/δ_H 80-81/4.5-4.7, 4-9-5.1; 62-65/4.0-4.5 and 99-104/4.8-5.2 ppm, respectively (Balakshin et al. 2011). Figure 4.6 depicts the 2D HSQC NMR spectrum of sample 1-D. The signal at δ_C/δ_H 101.23/4.89 ppm on 2D HSQC NMR spectrum revealed the presence of PhyGlc linkage in sample 1-D. Du et al. (2014) reported the existence of three types of PhyGlc linkages in enzymatically treated GM-lignin fraction. These linkages were probably generated by glycosidic bonds between phenolic hydrolxyls in lignin and three different types of carbohydrates (Du et al. 2014). The signals for PhyGlc-1, PhyGlc-2 and PhyGlc-3 were observed at δ_C/δ_H 100.2/5.03, 100.3/4.85 and 101.9/4.86 ppm, respectively (Du et al. 2014). Consequently, it is suggested that the PhyGlc bond found in sample 1-D represented the third type of PhyGlc linkage. No signals of BE and ester linkages were observed. Giummarella et al. (2016) proposed that BE linkages in softwoods mainly contained xylose, while mannose was the most abundant sugar in PhyGlc bonds. It is seen in Table 4.2 that the relative amount of mannose in sample 1-D was significantly higher than the amount of xylose. Benzyl ether linkages were not identified by 2D HSQC NMR analysis due to the relatively low content of xylose. The absence of benzyl ester bonds was also reported by other researchers (Du et al. 2014; Yuan et al. 2011). Another reason for the absence of LCC linkages could be related to a low frequency of NMR instrument used in this work. Du et al. (2013) reported that the application of 750 MHz NMR machine with a cryoplatform and that cryo-probe significantly improved the resolution of 2D HSQC NMR spectrum.

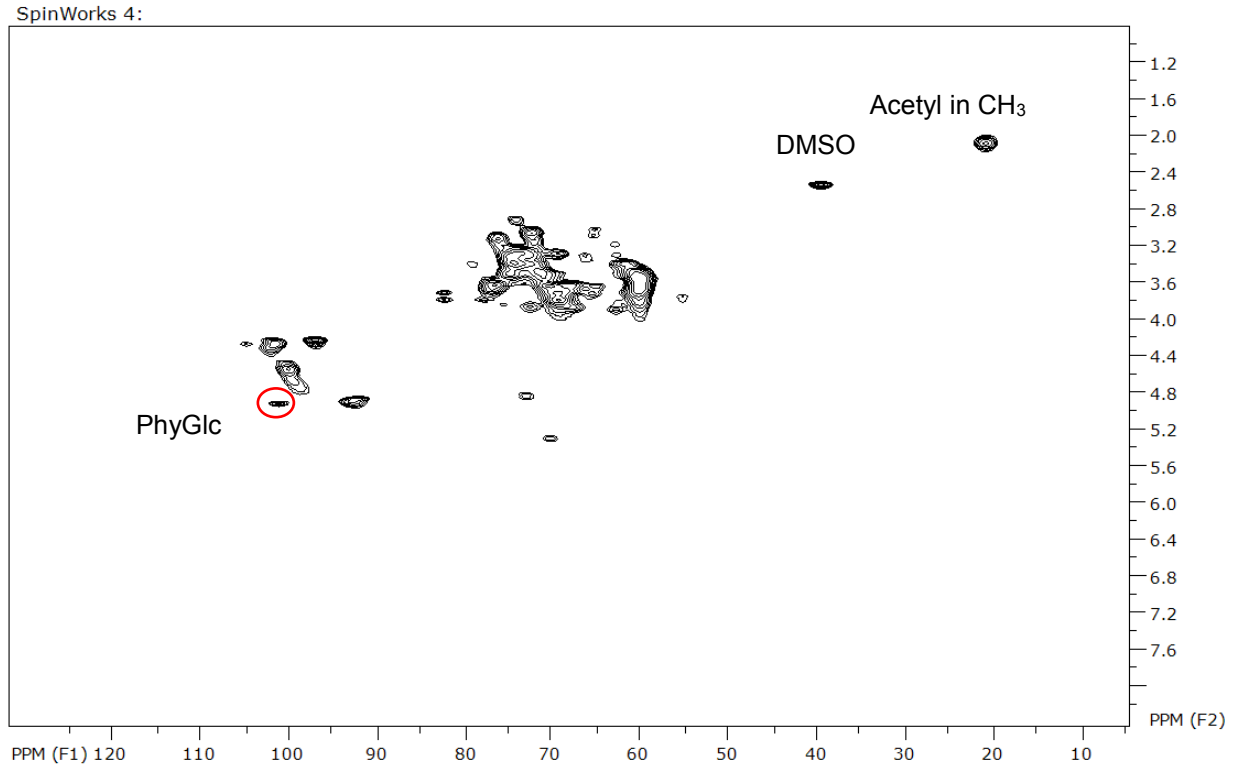

Figure 4.6 2D HSQC NMR spectra of sample 1-D.

No signals related to LCC bonds were observed in the 2-dimensional HSQC NMR spectra of samples 3-D, 3-A and 1-E (not shown). However, according to the results of GPC analysis, all tested samples contained LCCs (Table 4.3). This contradiction could be related to a low amount of LCC linkages and the poor signals of aromatic groups in the NMR analysis (Du et al. 2014; Zhang and Gellerstedt, 2007). The low content of LCC bonds in sample 3-D could be attributed to considerable decomposition of ether and glycosidic lignin-carbohydrate linkages due to severe conditions of autohydrolysis during manufacture of sample 3 (Table 4.1) (Ye et al. 2012; Fan et al. 2016). Moreover, the high acidity of sample 3-A (Figure 4.1) could lead to a significant degradation of lignin-carbohydrate bonds (Kosikova et al. 1979; Lawoko and van Heiningen 2011). The deficiency of signals assigned to LCC linkages in sample 1-E could be related to the low lignin content (Figure 3.2) and the high concentration of sugars in sample 1-E (Figure 4.3). Du et al. (2014) reported that the increment in the relative amount of lignin by reduction of carbohydrates presence in LCC fraction conduced to a stronger impulse of lignin-carbohydrate bonds on 2D HSQC NMR spectra (Du et al. 2014).

4.5 Conclusions

The compositions and properties of precipitates obtained from hydrolysates by ethanol or acid supplement were systematically analyzed in this chapter. The acidification resulted in precipitation of materials with the highest lignin content; whereas, the precipitates generated by ethanol supplement showed the highest concentration of hemicelluloses. The GPC results confirmed the existence of lignin-carbohydrate complexes in all examined samples. Acidification or ethanol supplement led to the precipitation of LCC and hemicelluloses with high molecular weights. The TG/DTG analysis suggested the partial decomposition of lignin-carbohydrate linkages in precipitates generated by acid or ethanol supplement. The ^{1}H-NMR analysis revealed that sample 3-A contained significantly higher aliphatic groups than sample 3-D, which is possibly related to the degradation of LCC linkages. The reduction of Hα and Hβ lignin inter-linkages was also noted in samples 1-E and 3-A. Acidification and the ethanol supplement increased the molecular mobility of lignin units in precipitates due to their higher content of methoxyl groups (confirmed by ^{1}H-NMR analysis). Additionally, acidification and ethanol supplement resulted in the increment of heat capacity of the precipitates from 0.41 J/g°C to 1.14 J/g°C and from 0.41 J/g°C to 1.36 J/g°C, respectively. The two dimensional HSQC spectroscopy demonstrated the presence of a third type of phenyl glycoside linkage in sample 1-D, which probably contained a high amount of mannose moieties.

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Chapter 5 Production of lignosulfonate in NSSC-based biorefinery

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5.1 Abstract

The spent liquor (SL) of a neutral sulfite semichemical (NSSC) pulping process contains a considerable amount of lignocelluloses and is treated in wastewater systems. The lignocelluloses, however, can be used for producing value-added products if they are isolated from the SL. In this paper, solvent treatment (mixing acetone, ethanol or isopropyl with SL) was used as a method for isolating lignosulfonate from SL. The maximum lignosulfonate removal was obtained via mixing isopropyl alcohol with SL at the weight ratio of 20/80, room temperature and 5.7 pH. The results also showed that the molecular weight and anionic charge density of the precipitates were in the ranges of 5,000-70,000 g/mol and 0.2-1.8 meq/g, respectively. Based on these results, a process was proposed for isolating lignosulfonate from SL and converting the NSSC process to an NSSC-based biorefinery.

5.2 Introduction

In the neutral sulfite semichemical (NSSC) pulping process, wood chips are pretreated with sodium sulfite and carbonate prior to refining the pretreated wood chips in order to produce corrugated medium papers (Biermann 1996). Generally, pretreating wood chips with these chemicals leads to the dissolution of lignocelluloses (mainly lignosulfonate) from wood chips in pulping spent liquor (SL). Currently, the SL is treated in the wastewater system of the process, and hence its lignocelluloses are not utilized (Sitter et al. 2014). As the SL is very dilute, the direct conversion of lignocelluloses in the SL to fuel or other value-added chemicals is uneconomical. This is one of the challenges in the development of an NSSC-based biorefinery (Sitter et al. 2014), however, the separation of lignocelluloses from SL will facilitate its conversion to an NSSC-based biorefinery.

Flocculation is a well-known and efficient method for isolating lignocelluloses from various pulping spent liquors. In the past, lignocelluloses were separated from SL via flocculation using polymers such as poly ethylene imine (PEI), poly diallyldimethylammnium chloride (PDADMAC) and chitosan (Sitter et al. 2014). Membrane technology was also used for separating lignocelluloses from pulping spent liquors and wastewater systems (Rojas et al. 2006). However, membrane fouling is a main issue of membrane technology, which makes it industrially unattractive (Guo et al. 2012).

Acidification is an efficient process for removing lignin from pulping spent liquors (Yang et al. 2003). In one study, lignin was separated from acidified (pH 2) pre-hydrolysis liquor of kraft-based dissolving pulp process. As acidification is a simple process, it is industrially attractive (Liu et al. 2011).

Solvent extraction was also considered as an efficient process for extracting lignocelluloses from pulping spent liquor (Ikari and Yokoyama 1973). Theoretically, lignin and hemicellulose have limited solubility in various solvents. If SL was to be mixed with solvents, the lignin and hemicelluloses could precipitate from the SL; once separated, they could be considered for the production of value-added products (Quesada-Medina et al. 2010). In one study, a combination of acidification and ethanol treatment removed about 50% of lignin and 20% of hemicelluloses from the pre-hydrolysis liquor (PHL) of the kraft-based dissolving pulp process (Lui et al. 2011a,b). Therefore, this combination of acid and solvent treatments could be an option for isolating lignosulfonate from SL. It is well known that the properties of lignin and hemicelluloses in pulping spent liquors and wastewater effluents are highly dependent on the upstream pulping process. Consequently, the results generated (and the process proposed) for treating SL of various pulping liquors are process specific, and may not be applicable to different pulping spent liquors, which implies that the results reported on the treatment of PHL may not provide useful information for the treatment of SL. In another study, hemicelluloses and lignosulfonate were separated from the SL of NSSC process by treating with different organic solvents under neutral conditions (Ikari and Yokoyama 1973). However, there is no report on the effectiveness of the combined solvent and strong acid treatment in extracting lignosulfonate from the SL of NSSC process.

Although the extraction of lignocelluloses from pulping spent liquor was studied in the past, the properties of the extracted materials have not been thoroughly investigated. It is well known that lignocelluloses with different molecular weights and charge densities have different end-use applications. For example, lignin and hemicellulose with a large molecular weight may be used as flocculants or dispersants, while the highly fragmented lignin and hemicellulose can be converted to phenols or furfural (Wang and Chen 2013; Song et al. 2012). Charge density is also an important parameter of polymers that affects their interaction with other constituents in solutions if used as a retention aid, dispersant or flocculant (Zhang et al. 2014). Moreover, it is essential to analyze the properties of separated lignocelluloses in order to identify potential end-use applications.

The main purpose of this study was to introduce a pathway to isolate lignocelluloses from the spent liquor of an NSSC pulping process, which facilitates the downstream production of value-added products from the lignocelluloses. Currently limited information is available on the application of acidification/solvent for extracting lignocelluloses from the SL of an NSSC process. The objectives of this study were 1) to investigate how solvent could facilitate the extraction of lignosulfonate from SL and 2) to examine if acidification could be used as an effective alternative for isolating lignosulfonate from SL of an NSSC process.

Furthermore, for the first time, the impact of acidification/solvent treatment on the molecular weight and charge density of precipitated lignocelluloses from NSSC process was also assessed.

5.3. Materials and Methods

5.3.1. Materials

A spent liquor sample was received from an NSSC process located in Eastern Canada. The raw material of this pulping process was mixed hardwood. The SL was initially centrifuged at 3000 rpm for 15 min in order to remove undissolved components prior to analysis. The centrifuged sample was used as the raw material in this work. Acetone (95 vol. %), ethanol (95 vol. %), isopropyl alcohol (95 vol. %) and sulfuric acid (98 wt.%) were purchased from Fisher Scientific and used as received. Polydiallyldimethyammonium chloride (PDADMAC), 20 wt.% in water, $NaNO_3$ powder and 2,2-dimethyl-2-silapentane-5-sulfonic acid (DDS), all analytical grade, were obtained from Sigma Aldrich. Potassium polyvinyl sulfate (PVSK) was obtained from Wako Pure Chemical Industries Ltd., Japan. The PDADMAC and PVSK were diluted to 0.005 M prior to use.

5.3.2 Solvent/SL mixing

Two sets of experiments were performed with or without an acidification process. In one set of experiments, the SL was mixed with acetone, ethanol or isopropyl alcohol in centrifugal tubes for the SL/solvent weight ratios of 33/66, 50/50, 25/75 and 20/80 at room temperature. The mixtures were shaken manually for 30 seconds and then kept for 10 minutes. The mixtures were then centrifuged at 3000 rpm for 15 min. This process led to the precipitation of lignocelluloses from the SL and solvent mixture. The supernatants of this centrifugation were transferred to other containers, and their lignosulfonate and hemicellulose contents were determined. The precipitates were carefully removed from the centrifugal tubes and dried in an oven at 80 °C for 24 h. After drying, the precipitates were collected for further analysis.

In another set of experiments, the SL sample was acidified to pH 1.8 with sulfuric acid in 500 ml glass flasks prior to solvent treatment at room temperature. After the treatment, the acidified samples were transferred to centrifugal tubes and centrifuged at 3000 rpm for 15 min, and the filtrates were collected for solvent treatment. The precipitates were carefully removed from the centrifugal tubes and placed into the oven at 80 °C for 24 h. The solvent treatment was conducted as stated above on the acidified SL. The precipitates of the acidification and solvent extraction were collected for FTIR, molecular weight and charge density analyses. It should be mentioned that the average of three repetitions was reported in this work.

5.3.3 Lignosulfonate, hemicellulose, furfural and acetic acid analyses of SL

The lignosulfonate content of the SL before and after any treatment was determined according to TAPPI UM 250 using a UV spectrophotometry at 205 nm (GENESYS 10S UV-Vis, Thermo Scientific) (Sitter et al. 2014). The hemicelluloses content of SL before and after any treatment was measured according to an established method by Dashtban and his co-workers (2011). To determine the hemicelluloses' concentration of SL, samples (5 g) were treated with 4 wt.% sulfuric acid and then placed in an oil bath for 1 h at 121 °C. This process converted oligomeric sugars to monomeric sugars. The monomeric sugar content of the SLs was then determined via treatment with 3.5-dintrosalicylic acid (DNS) (Sitter et al. 2014). A 60 µL of the sample was mixed with 120 µL of DNS and 120 µL of deionized water, and the solution was then boiled for 5 min. The concentration of monomeric sugars was determined using a UV spectrophotometer at 540 nm. The concentrations corresponding to the measured absorbance were calculated using a calibration curve. The total monomeric sugars determined by this method were considered as hemicelluloses in the SL, in this work. An average of three repetitions was reported in this work. The furfural and acetic acid of SL were determined using a Varian Unity Inova 500 MHz Nuclear Magnetic Resonance (NMR) with DDS as an internal reference (Saeed et al. 2012).

5.3.4 Molecular weight analysis

About 50 mg of air dried samples were dissolved in 10 ml of 0.1 mol/L $NaNO_3$ solution, stirring at 300 rpm for 12 h and then the samples were filtered with a 0.2 µm nylon filter (13 mm diameter). The filtered solutions were used for molecular weight analysis, which was carried out using a Gel Permeation Chromatography system, Malvern GPCmax VE2001 Module + Viscotek TDA305 with multi-detectors (UV, RI, viscometer, low angle and right angle laser detectors). The columns

of PolyAnalytic PAA206 and PAA203 were used, and 0.1 mol/L $NaNO_3$ solution was used as a solvent and eluent. The flow rate was set at 0.70 ml/min, while the column temperature was 35 °C and poly (ethylene oxide) was used as a reactive standard for this aqueous system.

5.3.5 Charge density analysis

The charge density of all precipitates was determined via using a Mütek PCD04 charge detector. The measurements were conducted according to the method described by Saeed et al. (2011). Approximately, 0.2 g of each precipitate sample was dissolved in deionized water at 1wt.% concentration. Subsequently, the samples were placed in the water bath shaker for 8 h at 100 rpm and 30 °C. The standard solutions of PVSK and PDADMAC (0.005N concentration) were used as titrants.

5.3.6 FTIR analysis

In this set of experiments, the dried precipitates generated under the conditions of SL/solvent 20/80 weight ratio were selected for Fourier-transform infrared spectroscopy (FTIR) analysis. About 0.5 g of the precipitates were ground and then analyzed by Bruker Tensor 37 FTIR spectrophotometer. The FTIR of the samples was conducted at room temperature using a Bruker Tensor 37 FTIR spectrophotometer. Each spectrum was recorded using 32 scans in the frequency range of 4000-600 cm^{-1} with a resolution of 4 cm^{-1}.

5.4 Results and discussion

5.4.1 SL properties

The hemicelluloses and lignosulfonate contents of SL are listed in Table 5.1. The SL contained approximately 13 g/L hemicelluloses and 53 g/L lignosulfonate. The results in Table 5.1 also show that the lignosulfonate and hemicelluloses contents of SL dropped through acidification to 51 g/L and 4.8 g/L, respectively, implying that the acidification was inefficient in lignosulfonate removal. However, there are contradictory reports on the impact of acidification in removing lignosulfonate from various spent liquors. In one report, it was stated that decreasing pH to 3.5-4 resulted in a significant lignin removal from black liquor (Yang et al. 2003). In contrast, acidification resulted in insignificant lignosulfonate isolation from sulfite-based spent liquor in another study (Ringena et al. 2005). The limited removal of lignosulfonate via acidification in the current work is attributed to the fact that lignin is sulfonated (i.e. water soluble) in the SL of NSSC process. It was stated in

the literature that sodium sulfite treatment of wood chips produces lignosulfonate that was soluble in acidic pH (Ringena et al. 2005). Since SL contains a significant amount of lignosulfonate, the main focus of this study was to investigate the impact of an acidification/solvent extraction process on the properties of the isolated lignosulfonate. Furthermore, it can be seen in Table 5.1 that SL comprises acetic acid and furfural. Although the furfural content was very low in original SL, its content was marginally increased in the SL after acidification. Acetic acid was not detected after acidification in the SL, and the removal of acetic acid via acidification/solvent extraction was out of the scope of this study.

Table 5.1 Spent liquor properties

pH	Hemicelluloses (g/L)	Lignosulfonate (g/L)	Acetic acid (g/L)	Furfural (g/L)
5.7	12.9	52.7	26	0.5
1.8	4.8	50.9	ND[1]	0.9

[1]not detected

5.4.2 Lignosulfonate and hemicellulose removals

Figure 5.1 shows lignosulfonate and hemicellulose removals from untreated SL as a function of SL/solvent weight ratio. As shown, the results depict that by decreasing the ratio of SL/solvent (i.e. increasing the amount of solvent), the lignosulfonate removal from SL increased. In the literature, it was stated that the solubility of lignosulfonate decreased with the addition of acetone to a spent liquor of organosolv pulping that contained lignosulfonate (Quesada-Medina et al. 2010). The current work results also confirmed that by adding more acetone to SL, more lignosulfonate precipitated. Furthermore, isopropyl treatment seemed to be more effective than acetone and ethanol treatments in removing lignosulfonate from untreated SL. The highest lignosulfonate removal in acetone, ethanol and isopropyl treatments were 59.0%, 28.3% and 59.1%, respectively. The polarity of solvents may also be an influencing factor in the precipitation of lignosulfonate. The polarity indices of isopropyl, acetone and ethanol are 3.9, 5.1 and 5.2, respectively. As lignosulfonate was in its sodium form in the SL at the original pH (5.7) of SL (i.e. it is sodium lignosulfonate salt), it could be isolated from the solution via mixing the solution with a solvent that had a low polarity. The lower polarity of the isopropyl can be the reason for more precipitation of lignosulfonate in SL/isopropyl alcohol mixture. Melms and Muhlberg (1969) also reported a higher isolation of lignosulfonate from spent sulfite liquors via mixing them with a less

polar solvent (e.g., methanol), which supports our hypothesis on the impact of solvent polarity on the isolation efficiency of lignosulfonate from SL.

The results in Figure 5.1b also show that, the maximum hemicelluloses removal from SL was achieved at SL/solvent weight ratio of 50/50 and a further decrease in the ratio reduced the hemicellulose removal from SL, regardless of the solvent type used.

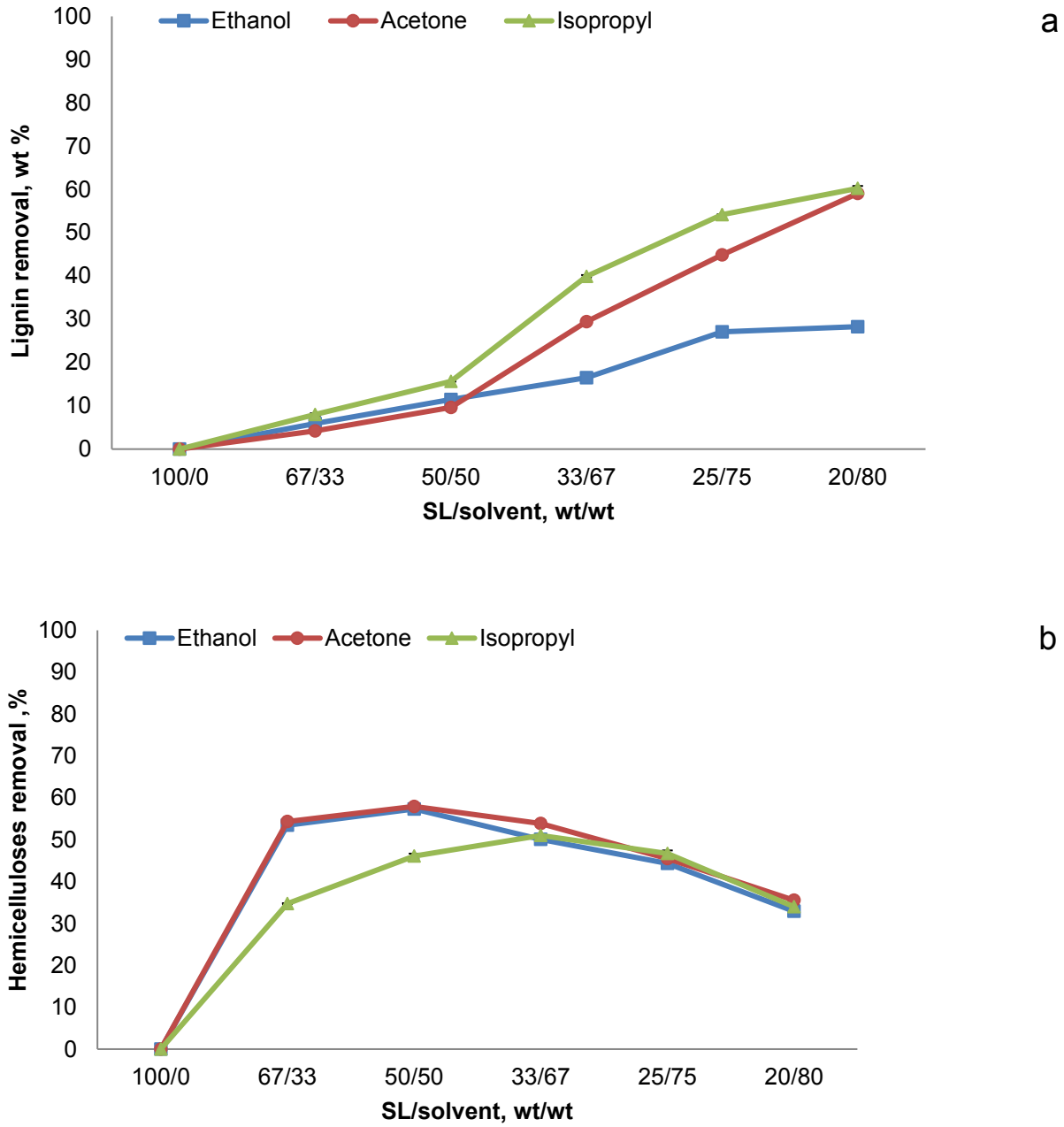

Figure 5.1 Lignosulfonate (a) and hemicellulose (b) removals from untreated SL via treating SL with different solvents.

Figure 5.2 shows the lignosulfonate and hemicelluloses removals from the acidified SL as a function of SL/solvent weight ratio. It is apparent that lignosulfonate removal increased with an increase in the amount of solvent used in the mixtures, which is similar to the trends observed in Figure 5.1. However, the lignosulfonate removal from acidified SL was less than that from untreated SL. The maximum lignosulfonate removal from acidified SL was 22.7%, 21.7% and 44.1% via ethanol, acetone and isopropyl treatments, respectively. Isopropyl alcohol was more efficient than ethanol and acetone in removing lignosulfonate from acidified SL. The results in Figures 5.1 and 5.2 reveal that by decreasing the ratio of SL/solvent, the removal of lignosulfonate was improved, but that of hemicellulose had a peak at a 50/50 SL/solvent ratio.

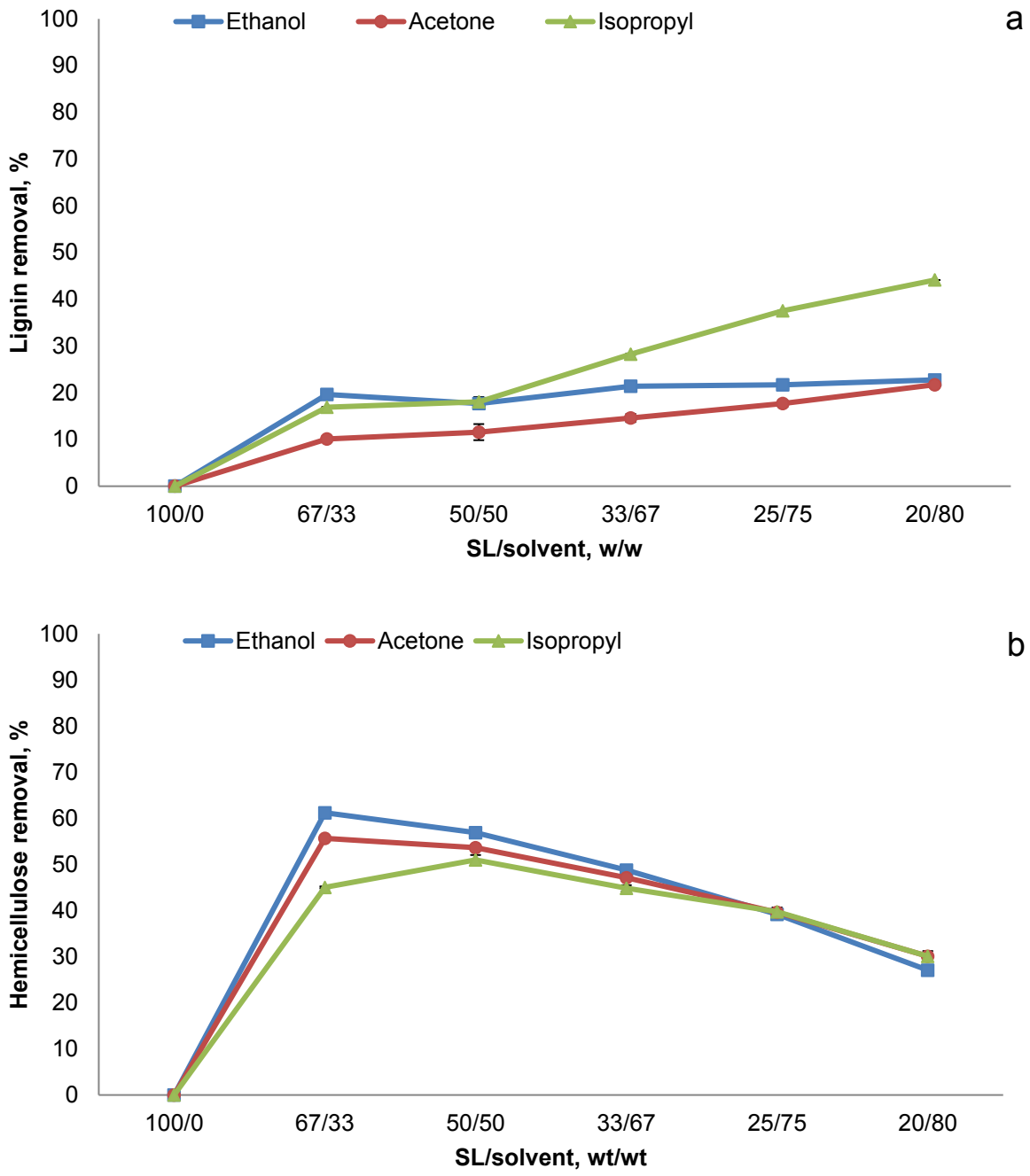

Figure 5.2 Lignosulfonate (a) and hemicellulose (b) removals from acidified SL via treating with different solvents.

Based on the original concentrations of lignosulfonate and hemicelluloses in the SL (Table 5.1) and the results reported in Figures 5.1 and 5.2, it can be claimed that the overall lignosulfonate removal was higher than hemicellulose removal. Therefore, the precipitates were mainly composed of lignosulfonates (lignosulfonate/hemicellulose weight ratio ranging between 4/1 and

52/1). The precipitates that were formed via treating untreated SL with 20/80 SL/solvent ratio were selected as the precipitates with the highest lignosulfonate content.

5.4.3 Molecular weight analysis of precipitates

The solubility of lignin and hemicellulose in SL depends on their molecular weight and charge density. Molecular weight (MW) is an important parameter of polymers that influences their end-use applications. For example, large polymers can be used in the production of retention aids, strength additives and flocculants; while, small ones can be used in the production of chemicals such as furfural and phenols. Molecular weight distribution of lignosulfonate isolated from SL heavily depends on the extraction method of lignosulfonate from the SL (Hatakeyama and Hatakeyama 2005). The molecular weight of kraft lignin in black liquor and lignosulfonate of spent liquor is in the range of 1000 g/mol to 100,000 g/mol (Kim, 1987). It is important to investigate how the solvent extraction process used in this work affected the MW of lignosulfonate and hemicellulose separated from the SLs.

Figure 5.3 presents the molecular weights of precipitates separated from untreated and acidified SL via solvent treatment. By adding small amount of solvent to SL (e.g. SL/solvent ratio of 66/33), the lignosulfonate and hemicellulose with large molecular weights were removed, but the overall removal mass of lignosulfonate and hemicellulose was limited at this ratio, as seen in Figures 5.1 and 5.2. Generally, by increasing the molecular weight of polymers, their solubility in solutions is reduced (Miller-Chou and Koeniq 2003). Therefore, one way to isolate large polymers from solutions is to mix the solutions with solvents that have less polarity. Wang and Chen (2013) also reported that by increasing the molecular weight of lignin, the solubility of lignin in the ethanol–water mixture decreased, and hence larger lignin segments were isolated from the solutions. By reducing the overall polarity of the mixture via adding more solvents to solutions (e.g., a lower SL/solvent ratio), more of small polymers could also be precipitated along with large polymers. By increasing the precipitation of small polymers, the overall average molecular weight of precipitated polymers is reduced, which supports the decrease in molecular weight of precipitates shown in Figure 5.3. Furthermore, large lignin-carbohydrate (i.e. lignin-hemicellulose) complexes might be affected by the addition of solvent to SL, and thus these large complexes would precipitate from the SL (Nonaka et al. 2013; Sun et al. 2013).

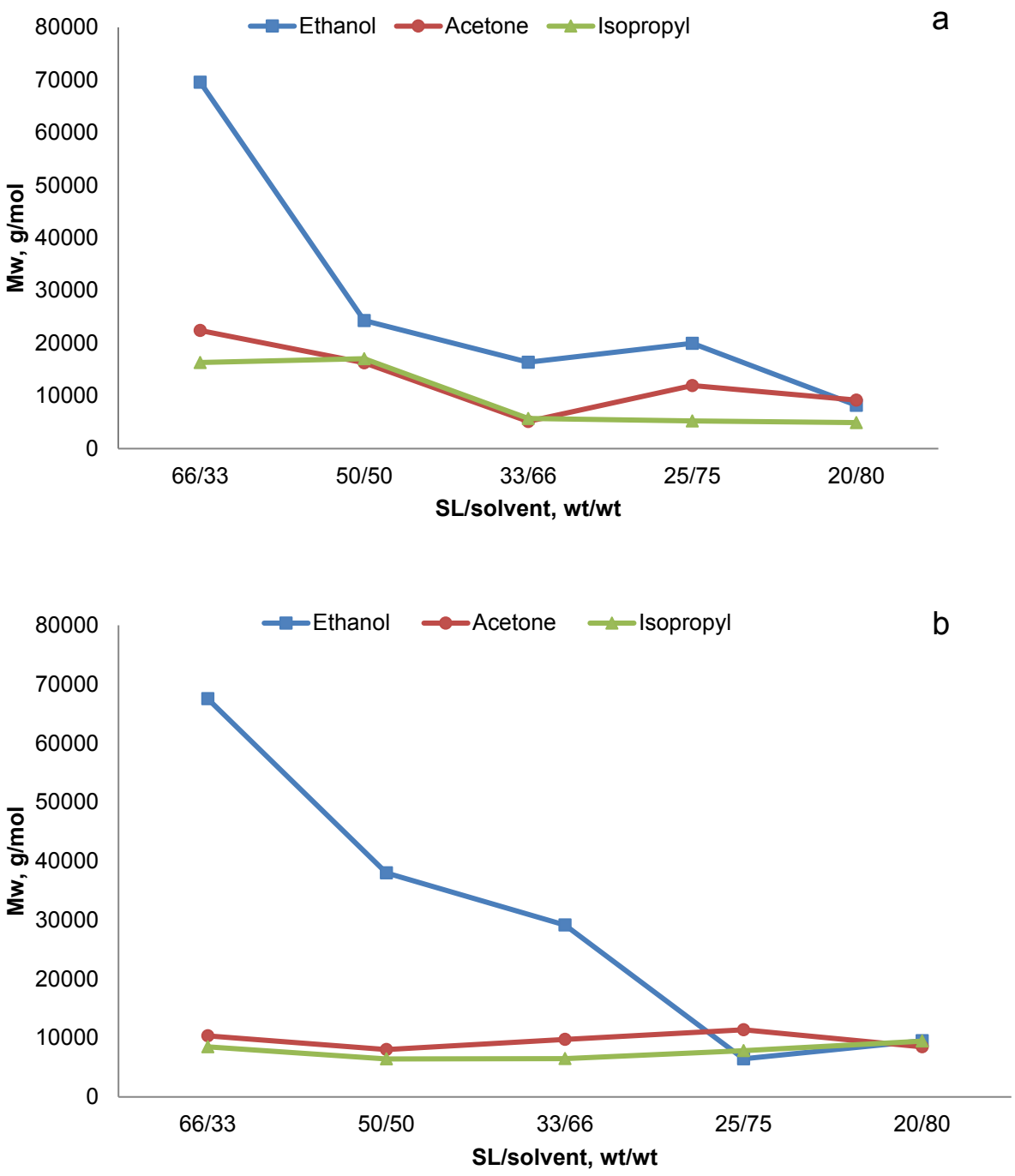

Figure 5.3 Molecular weights of the precipitates isolated from (a) untreated SL and (b) acidified SL.

As seen in Figures 5.1 and 5.2, by reducing the SL/solvent ratio, more lignosulfonate was removed from the SL. As explained previously, the overall removal of hemicellulose was less than that of lignosulfonate. Therefore, the removal of hemicellulose from SL had a less significant impact on the molecular weight of the precipitates. The results shown in Figures 5.1, 5.2 and 5.3 suggest that the removal of more lignosulfonate with a broader molecular weight distribution was the reason for a decrease in the overall molecular weight of precipitates at a low SL/solvent ratio.

The results also imply that the molecular weight of precipitates collected via ethanol treatment was higher than those of other solvent treatment. This is in agreement with the fact that higher removal of lignosulfonate with a broad molecular weight distribution leads to precipitates with a lower overall molecular weight, and that less lignosulfonate removal leads to a higher overall molecular weight. Although black liquor of kraft pulping process and the spent liquor of NSSC process have different properties, a study on the black liquor showed that the molecular weight of the isolated lignin was reduced with increasing the mass of precipitated lignin (via decreasing pH) (Zhu and Theliander 2015), which supports the phenomenon observed in Figures 5.1, 5.2 and 5.3.

5.4.4 Charge density analysis of precipitates

Figure 5.4 presents the charge density of the precipitates extracted from untreated and acidified SL/solvent mixtures. The results show that the charge density of precipitates generally increased by reducing SL/solvent ratio, because the charged lignocelluloses were more soluble than uncharged ones in solutions, and more solvent was required (i.e. lower SL/solvent ratio) to remove the charged lignosulfonate and hemicelluloses. Consequently, it can be concluded that by decreasing the ratio of SL/solvent, more of the charged lignosulfonate and hemicelluloses were removed. The highest extraction rate of lignosulfonate was obtained when the amount of solvent was significantly high. In some cases (e.g. SL/ethanol with a low ratio), however, the solubility of low charged low molecular weight lignosulfonate and hemicelluloses was also reduced, which resulted in their precipitation, and this was the reason for the overall low charge density of precipitates made of small SL/solvent ratio (e.g., 20/80).

It is also apparent in figure 5.4 that the charge density of precipitates made of SL/ethanol treatment was higher than that of the other precipitates. In this case, the precipitates isolated via ethanol treatment probably contained a higher amount of sulfonate and carboxylate groups that contributed to the charge density. Hu et al. (2013) postulated that lignin extracted from the black liquor via acetone treatment contained less aromatic and hydroxyl groups than lignin precipitated via

benzene-ethanol treatment. It can be inferred from that study that the type (and polarity) of solvent plays a role in removing various lignin segments from SL. This phenomenon is also seen in Figure 5.4 in that mixing SL with different solvents led to precipitates with different charge densities. Considering the results in Figures 5.1 to 5.4, it can be concluded that ethanol treatment of SL led to less removal of lignosulfonate, but the removed lignosulfonate had a relatively higher MW and charge density than other precipitated samples.

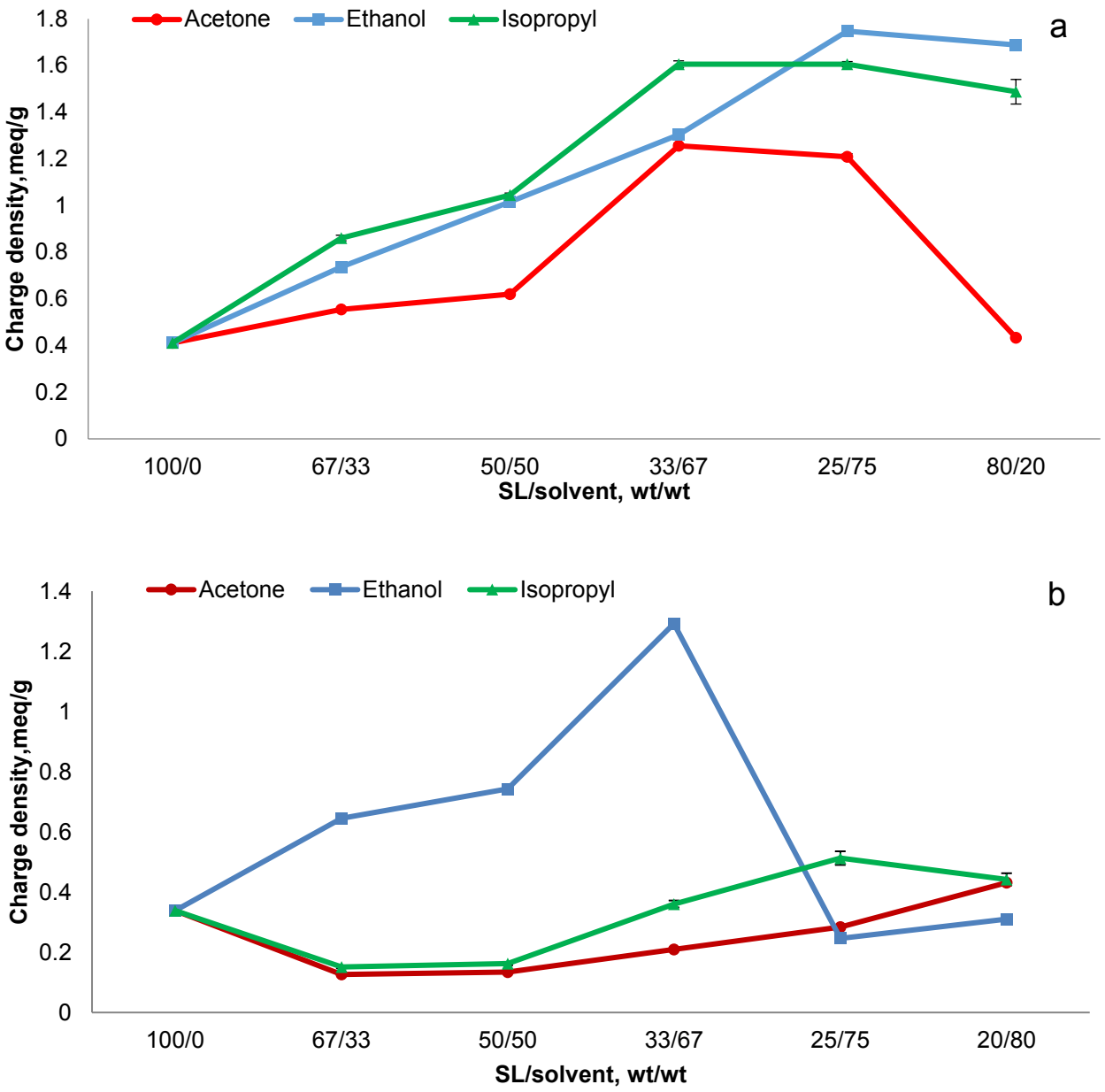

Figure 5.4 Charge density of precipitates generated from untreated (a) and acidified (b) SL/solvents mixtures.

5.4.5 Fourier transformed infrared (FTIR) spectroscopy

Figure 5.5 shows the FTIR spectra of precipitates made of SL/solvent weight ratio of 20/80 g/g. The samples were chosen for further analysis as these precipitates had the highest lignosulfonate content. All spectra displayed a broad band at 3600-3200 cm^{-1}, which corresponded to O-H stretch and that at 2934 cm^{-1} was due to the C-H band (Liu et al. 2011c). The band at 1730 cm^{-1} was assigned to non-conjugated C=O stretch in hemicelluloses (Xu et al. 2013). The band at 1595cm^{-1} was attributed to aromatic vibration in lignin (Xu et al. 2013). The band at 897 cm^{-1} referred to β-anomers, meaning that β-glycosidic bonds were dominant between the sugar units (Postma et al. 2014), which was observed in all the precipitates. However, the peak at 1184 cm^{-1} was assigned to C-O stretching vibrations of lactones derived from xylan (Dworzanski et al. 1991). The absorbance at 1035 cm^{-1} could be related to C-C, C=O and C-C-O stretching (Xu et al. 2013). Additionally, this peak could be assigned to cellulose, hemicelluloses or lignin (Postma et al. 2014). Faix (1991) stated that this peak could be related to G-type lignin predomination, but Postma noted that this peak reflected the presence of xylan in analysis (Postma et al. 2014). In the current work, the studied precipitates contain the highest amount of lignosulfonate and the lowest amount of hemicelluloses. Consequently, the peak was most probably attributed to the lignosulfonate and the presence of G-type lignin in the precipitates.

It was noted that SL/ethanol and SL/isopropyl footprints were identical. However, ethanol spectra displayed a peak at 1167 cm^{-1}, which was assigned to C-O stretching of ester group (Dworzanski et al. 1991). The spectrum of the sample made of SL/acetone treatment displayed some divergence. It displayed a peak at 1111 cm^{-1}, which was assigned to syringyl lignin (Kline et al. 2010). Also, the peak at 1406 cm^{-1} on the precipitates of SL/acetone treatment reflected CH_2 scissoring (Kline et al. 2010). This precipitate also displayed absorption at 1327 cm^{-1}, which was assigned to C-O stretching of S -type lignin (Faix 1991; Kline et al. 2010). These results may imply that acetone treatment might lead to fractionation of the S and G types of lignosulfonate (peaks at 1111 and 1327 cm^{-1}, respectively), whereas ethanol or isopropyl treatment might result in S type lignosulfonate separation (peak at 1111 cm^{-1}). However, further analyses are required to prove this hypothesis.

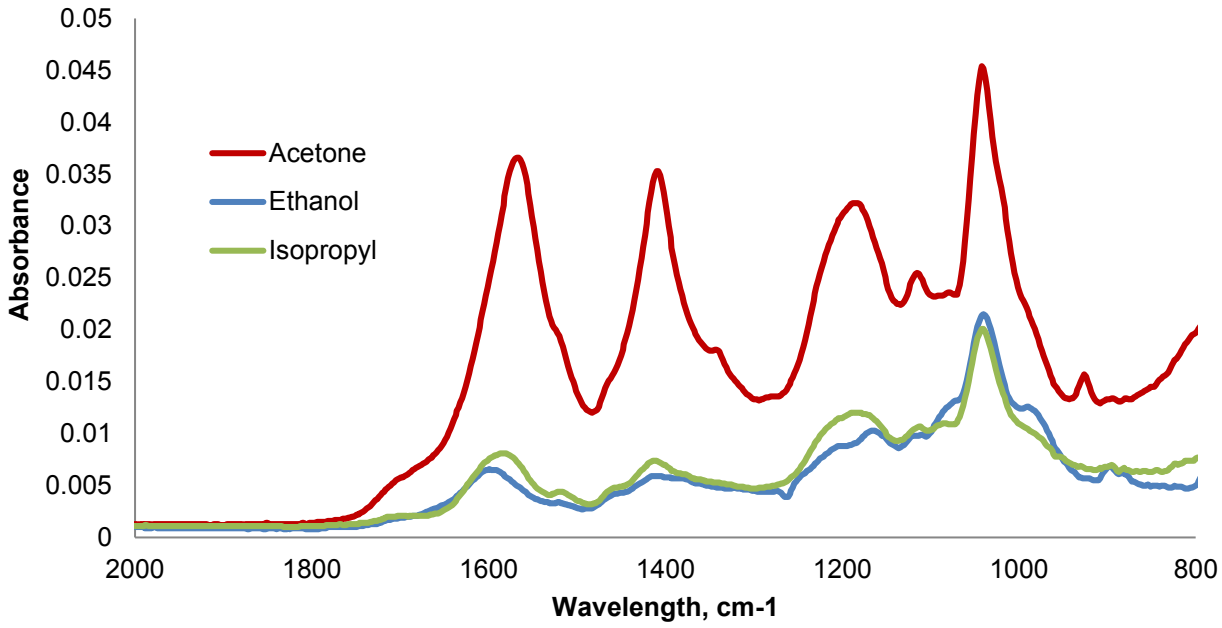

Figure 5.5 FTIR spectra of precipitates made of SL/acetone, SL/ethanol and SL/isopropyl mixtures with 20/80 SL/solvent weight ratio.

5.4.6 NSSC based biorefinery

It is inferred from the results of Figures 5.1 and 5.2 that solvent treatment of untreated SL generated more promising results than that of acidified SL. Based on these results, a process is proposed for treating the SL to convert NSSC process to an NSSC-based biorefinery as shown in Figure 5.6. In this process, the SL is treated with isopropyl or acetone in a mixer, which leads to mainly lignosulfonate precipitation. The precipitates can then be collected from the system using a filter, such as a drum filter. The SL/solvent mixture will be sent to a distillation tower, in which the solvent will be separated from the SL, and is recycled in the process. As the boiling points of acetone and isopropyl alcohol are low (56 °C and 82.6 °C under atmospheric conditions, respectively), the energy required for the distillation (separation of acetone or isopropyl from water) may not be significant. Also, the steam that is generated in mechanical refining of the NSSC process can partly supply the heat demand of the distillation tower. As lignosulfonate (mainly) and hemicelluloses contents of SL are substantially reduced in this process, the load to wastewater treatment system is reduced, which can financially help the process as well.

Furthermore, it can be concluded from the results in Figures 5.1 to 5.4 that precipitates with a molecular weight in the range of 5,000 to 70,000 g/mol and charge density in the range of 0.4 and 1.2 meq/g can be obtained in this process. The precipitates with a higher MW and anionic charge density, generated via SL/solvent weight ratio of 50/50, can be used as flocculants in tailing waste management (Vedoy and Soares 2014). The precipitates that contained a higher concentration of low MW lignosulfonate, which were generated from the SL/solvent weight ratio of 20/80, can be used in phenol production (Song et al. 2012). By changing the ratio of SL/solvent, it is possible to produce different lignosulfonate products that can be further processed to value-added products in an NSSC-based biorefinery process.

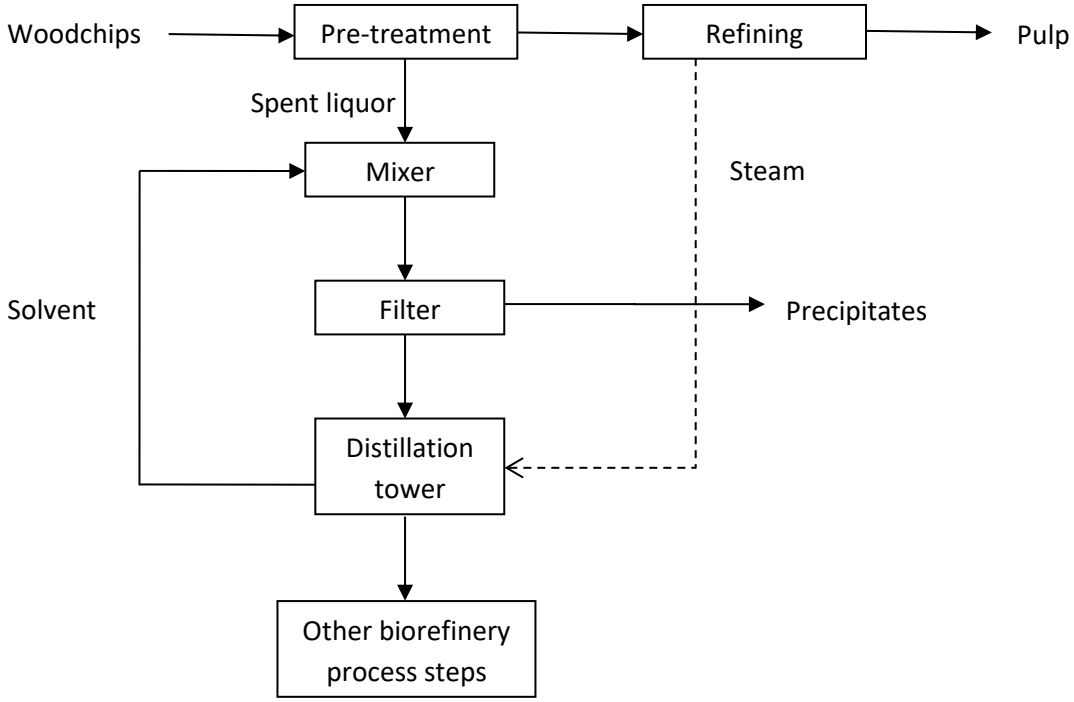

Figure 5.6 The proposed process for extracting lignosulfonate from SL of an NSSC pulping process.

5.5 Conclusions

A new process for isolating lignosulfonate from the SL of NSSC process was introduced and it could generate lignosulfonate as a potential raw material for a variety of applications. The solvent

treatment was less effective on acidified SL than untreated SL. The experimental results showed that isopropyl and acetone led to more effective lignosulfonate removal than did ethanol. However, ethanol generated the precipitates with higher MW and charge density. The most efficient ratio of SL/solvent was 20/80 wt/wt, which caused 59 % lignosulfonate removal from SL via isopropyl or acetone treatment. The molecular weight and charge density analyses confirmed that the mixing ratio played an important role in the properties of precipitates. Both acetone and isopropyl alcohol have a comparable price and low boiling points. Therefore, the energy required for the separation of acetone or isopropyl from water may not be significant in the proposed separation process.

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Chapter 6 Thermal properties of lignocellulosic precipitates from neutral sulfite semichemical pulping process

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6.1 Abstract

In a neutral sulfite semichemical (NSSC) pulping process, wood chips are pretreated with sodium sulfite and sodium carbonate solution. This pretreatment dissolves a part of hemicellulose and lignin from wood chips. The spent liquor (SL) that is produced in the pretreatment process contains a considerable amount of lignosulfonate and hemicelluloses, but SL is generally treated in the wastewater effluent system of the mills (i.e. lignocelluloses are wasted). In this paper, these lignocelluloses were separated from SL with organic solvents, and their thermal properties were determined. The results showed that the precipitates isolated from SL/acetone, SL/ethanol or SL/isopropyl mixtures with the weight ratio of 67/33 had the highest heating values of 18.61, 17.59 and 17.05 MJ/kg, respectively. The precipitates made from mixing acidified SL and solvents had lower heating values than those made from mixing untreated SL and solvents, which is likely due to the relatively high ash content of the precipitates made from mixing acidified SL and solvent. The theoretical and experimental heating values of the precipitates were compared in this work. The precipitates displayed lower ignition temperatures compared with other biomass-based solid fuels, implying that the combustion of precipitates would require lower activation energies.

6.2 Introduction

Fossil fuels are the primary energy resources in the world (Maity 2015). The uncertainties in the price of oil and environmental challenges associated with the use of oil-based materials are great concerns these days (Dashtban et al. 2014). It is crucial to decrease the fossil fuel use by replacing it with biomass-based fuel for sustainable energy production.

Recently, the consumption of some paper products has declined very significantly, which severely impacted the pulping industry. Pulping processes are seeking for alternatives to increase their overall revenues. Forest biorefining has been proposed as a means to revisit pulping industry. Pulping processes can be converted to forest biorefining processes if their under-utilized biomass, e.g. hemicellulose or lignin, is effectively converted into value-added products (Moshkelani et al. 2013). In forest biorefining processes, hemicelluloses can be converted to ethanol or furfural (Moshkelani et al. 2013; Rafione et al. 2014), while lignin can be converted to phenol or used as energy source for instance (Marinova et al. 2009; Mao et al. 2008). Song et al. (2012) reported a method for lignosulfonate conversion into phenol via heterogeneous nickel catalysts. In this process, the aryl-alkyl C-O-C linkages of lignin are cleaved in heterogeneous Ni-based catalytic

systems in the temperature range of 473–513 K. It was noted that the use of diatomic and triatomic alcohols in this process resulted in a high lignosulfonate conversion. The conversion of hemicelluloses and lignin to other value-added products is possible if they are in pure form and/or highly concentrated in solutions.

In the neutral sulfite semi-chemical (NSSC) pulping process, the majority of cellulose and hemicellulose of wood chips are collected and then converted to corrugated medium papers, while lignin (in lignosulfonate form) and the rest of hemicelluloses are wasted in the pulping spent liquors (Dashtban et al. 2014; Oveissi and Fatehi 2014; Sitter et al. 2014). To convert NSSC pulping process to an NSSC based forest biorefinery, the wasted lignocelluloses in the spent liquor (SL) should be converted to value-added products or energy (Sitter et al. 2014). As is, the concentrations of lignosulfonate and hemicelluloses in the SL are very low, which hampers the direct use of SL in producing value-added chemicals or fuel (Oveissi and Fatehi 2014). It is necessary to extract lignosulfonate and hemicelluloses from SL for further conversion of lignocelluloses to value-added chemicals or fuel.

In previous studies, it was reported that flocculation was a useful technique for isolating lignocelluloses from various spent liquors (Sitter et al. 2014, Liu et al. 2011, Liu et al. 2013; Shi et al. 2011). In one study, polyethylene imine (PEI) and poly diallyl dimethyl ammonium chloride (PDADMAC) were used for isolating lignocelluloses from SL (Sitter et al. 2014). However, polymers might be expensive, and flocculation processes may be ineffective in some systems. Acidification was claimed as a feasible method to isolate lignocelluloses from spent liquors (Liu et al. 2011; Yang et al. 2003). More interestingly, the combination of polyethylene oxide treatment and acidification led to sufficient lignin isolation from pre-hydrolysis liquor (PHL) in one study (Shi et al. 2011). In the previous chapter, we reported that lignocelluloses of SL could be isolated from SL with the help of solvents (Tarasov et al. 2015). The combination of acidification and ethanol treatment was effective in hemicellulose extraction in another report (Liu et al. 2011). In the past, different products were proposed to be produced from lignocelluloses extracted from pulping spent liquors (Quesada-Medina et al. 2010; Sing et al. 2012; Wang and Chen 2013; Van Loo and Koppejan 2008). Solvents were reported to be efficient in extracting lignocelluloses from pulping liquors, and thus can be used in forest biorefining to produce value-added products (Liu et al. 2011; Tarasov et al. 2015). However, a solvent recovery unit may be necessary to have a cost

effective biorefining process. The type of solvents will significantly impact the solvent recovery yield, the complexity of recovery process, and thus the overall cost of producing value-added products in forest biorefining. In the past, the use of ethanol and acetone for this purpose was reported to be promising due to their low boiling points and thus relatively efficient and less complicated recovery process. Results presented in the previous chapter showed that adding solvents (e.g. ethanol and acetone) to the spent liquor of NSSC process at the ratio of 20/80 wt./wt. would result in a significant isolation of lignocelluloses from the spent liquor (Tarasov et al. 2015). However, the potential application of extracted lignocelluloses was not clear, and the application would significantly impact the overall practicality and economic feasibility of the proposed process. To investigate the end-use application of the extracted lignocelluloses, it is essential to investigate the properties of the extracted lignocellulose. The first objective of this work was to study the thermal properties of precipitates obtained via solvent treatment of SL.

The elemental components of lignocelluloses impact their heating values, which is an important property of fuels (Van Loo and Koppejan 2008). It was reported that the increase in carbon, hydrogen and sulfur contents of lignocelluloses improved their heating values; while, oxygen, nitrogen and ash contents negatively impacted the heating values (Gaur and Reed 1998). It was also reported that the use of biomass-based fuel with low Cl and Si, but with a high Ca content led to equipment corrosion and slagging problems (Vassilev et al. 2013). Furthermore, a high K and Na contents resulted in a low melting temperature for ash, which caused ash slagging and carbon deposition in the combustion unit of a boiler (Obernberger and Thek 2010). Another objective of this study was to determine and relate the elemental components of the extracted lignocelluloses with their thermal characteristics.

The work presented herein focused on analyzing the properties of lignocelluloses isolated from an NSSC spent liquor. The properties of precipitates that were isolated from SL via mixing SL with ethanol, acetone, and isopropyl were experimentally evaluated. The main novelty of this work was the thermal analysis of precipitates that were produced via solvent extraction from spent liquors. For the first time, the heating values and elemental compositions of the precipitates were experimentally and theoretically determined and compared. The paper also reported the heating values and ignition temperatures of the precipitates.

6.3 Materials and methods

6.3.1 Materials

The SL sample was supplied by an NSSC process located in Eastern Canada. During this process, mixed hardwood chips were treated with sodium sulfite and caustic alkali for 15–18 min at 180 °C (Oveissi and Fatehi 2014). At first, the SL was centrifuged at 3000 rpm for 15 min for removing undissolved materials. After centrifuging, the filtrate was separated and considered as the raw material for this study. Acetone (95 vol. %), ethanol (95 vol. %), isopropyl alcohol (95 vol. %) and sulfuric acid (98 wt.%) were procured from Fisher Scientific and used without further purification.

6.3.2 Sample preparation and mixing

In one set of experiments, the SL was mixed with ethanol, acetone and isopropyl alcohol with the weight ratios of 67/33, 50/50, 25/75 and 20/80, and kept at room temperature for 10 min. Afterward, the samples were centrifuged at 3000 rpm for 15 min, which isolated lignocelluloses from SL. The isolated precipitates were separated from the supernatants and dried in an oven at 80 °C for 24 h. Alternatively, the pH of the SL sample was decreased to 1.8 via adding sulfuric acid to the SL. After acidification, the samples were centrifuged at 3000 rpm for 15 min. The precipitate was then collected (Sample 4). The supernatants were mixed with solvents with various ratios as explained above. The resulting precipitates were separated by centrifugation and dried at 80 °C for 24 h. The dried samples were used in subsequent tests.

6.3.3 Chemical compositions of precipitates and SL

In one set of experiments, 0.3-0.5 g dried precipitates made from acidifying SL (Sample 4) and from mixing solvents with untreated or acidified SL with the weight ratio of SL/solvent 67/33 were dissolved in 30-50 g of deionised water. The mixtures were placed in a water bath shaker (Boekel Scientific) and shaken at 50 rpm and 30 °C for 24 h. After mixing, the samples were filtrated using Whatmann filter paper, Cat No#1001-070. The treated filter papers were placed in the oven at 60 °C for 48 h and the amounts of undissolved lignocelluloses remained on filter papers were determined. Alternatively, the compositions of dissolved lignocelluloses in SL and produced solutions were determined. The lignosulfonate content of the mixtures was examined in accordance with the TAPPI UM 250 standard via UV spectrophotometry at 205 nm (Genesys 10S UV–Vis, Thermo Scientific) (Area et al. 2000). The hemicelluloses content of the solutions was determined using an ion chromatography (IC) unit (Dionex ICS-5000^{+} DP. Thermo Scientific)

equipped with Dionex CarboPac SA10 column, Thermo Scientific and pulsed amperometric detector (Thermo Scientific Electrochemical Detector). KOH was used as an eluent with 1mM concentration and flow rate 1mL/min. In this set of experiments, 1 g of the solutions was mixed with 4% sulfuric acid (5g) and placed in the thermal bath for 1 h at 121 °C. The total amount of monomeric sugars determined in this analysis was considered as hemicelluloses content of the precipitates (Tarasov et al. 2015). Acetic acid and furfural concentration of the dissolved samples were determined using Varian Unity Inova 500 MHz Nuclear Magnetic Resonance (NMR) (Saeed et al. 2011).

6.3.4 Ash content of precipitates

The ash content of the precipitates was quantified in accordance with ASTM D-1102-84 standard described by Rowell et al. (2013). At first, crucibles were placed in the muffle furnace (ThermoLyne 1400, ThermoFisher Scientific) at 575 °C for contamination removal. Then, the crucibles were weighted and filled with samples. The crucibles with samples were placed in the furnace at 575 °C, and ash content was determined based on the weight difference of empty and filled crucibles after the heating treatment.

6.3.5 Heating value analysis

In calorimetric analysis, the low heating value or net heating value of biomass is defined as energy generated via combusting biomass and contains moisture in vapor form in the combusted products at 0.1 MPa (Obernberger and Thek 2010). A 0.3-0.4 g of samples was combusted according to ASTM E711-87 using a Parr 6200 oxygen bomb calorimeter, which was attached to a Parr 6510 water handling system.

6.3.6 Elemental analysis

The elemental analysis of the precipitates was conducted using a Vario EL Cube Instrument (Germany) according to the procedure illustrated in the literature (Fadeeva et al. 2008). In this method, 5-10 mg of the samples was loaded in an integrated carousel of the instrument. First, the samples were flushed with a carrier gas (He). Subsequently, the samples were combusted, which was followed by the reduction of the burned gasses in order to determine the mass of elements in the samples (Fadeeva et al. 2008).

6.3.7 Thermogravimetric analysis

The thermogravimetric analysis (TGA) of the precipitates was performed by a TGA analyzer (Instrument Specialist, i1000) in order to characterize the thermal behavior of the precipitates. The samples were heated from room temperature to 700 °C at the heating rate of 20 °C/min in nitrogen or oxygen gas at the flow rate of 100 mL/min.

6.3.8 Ignition temperature determination

The ignition temperature of biomass was determined using TGA analysis in the past (Wang et al. 2009). This method was used as a technique for estimating coal's ignition temperature (Qing et al. 2011). Biomass and coal exhibited similar thermal performance implying that this method could be used for identifying the ignition temperature of biomass (Wang et al. 2009). A lower ignition temperature led to easier burning of biomass (Qing et al. 2011). Also, Sun and Kozinski (2000) stated that ignition had an impact on the flame stability, pollutants, and emissions configuration. In this method, the temperatures that the weight loss of biomass started and the temperature that the maximum weight loss rate of biomass occurred were taken into account. The lines tangent to these two temperatures intercepted at an ignition temperature (Wang et al. 2009). The ignition temperature analysis was performed with Microsoft Excel 2013 considering TGA results.

6.3.9 DSC Analysis

The precipitates displayed complex compositions due to the presence of lignosulfonate and hemicelluloses, which impacted the glass transition of the precipitates. The glass transition temperatures (T_g) of the precipitates were determined using a differential scanning calorimeter, TA Instruments Q2000. The analysis was conducted according to the procedure described by Sammons et al. (2013) and Persson et al. (2012). In this set of experiments, 2-5 mg of the oven dried precipitates was sealed in DSC pans. The research was conducted in the temperature range of 0 to 200 °C with a 3 °C/min rate (Persson et al. 2012). After heating to 200 °C, the precipitates were cooled to 0 °C and reheated to 200 °C. The heat capacity of precipitates was determined using the reheating data generated by the instrument (Sammons et al. 2013).

6.4 Results and discussion

6.4.1 Heating values of precipitates made from untreated SL with solvents

It was described in the previous chapter that by adding solvent to SL, lignosulfonate and hemicelluloses could be removed from SL. Figure 6.1 shows the heating values of precipitates

made from untreated SL mixed with solvent as a function of the SL/solvent weight ratio. Generally, a heating value of 14-18 MJ/kg was achieved for these precipitates.

The heating values of hemicelluloses and lignin were reported to be 13.6 MJ/kg and 27.0 MJ/kg, respectively (Lee et al. 2003; Lyytikainen et al. 2011). The heating value of activated sludge was 22.96 MJ/kg in one study (Eckenfelder and O'Connor 2013). The heating values of black liquor and wood pellets varied from 13 to 15.5 MJ/kg and from 17.63 to 20.8 MJ/kg, respectively (Gavrilescu 2008; Telmo and Lousada 2011). The results in Figure 6.1 suggest that the precipitates could be used as fuel. Furthermore, by reducing the SL/solvent ratio, the heating value reduced, in particular for that made from mixing untreated SL and acetone. These results were possibly attributed to the fact that the precipitates isolated from SL/acetone mixtures with the ratio of 25/75 and 20/80 wt./wt. had a higher ash content. The ash content of the precipitates with SL/acetone ratio of 33/66, 25/75 and 20/80 were 43.3%, 50.5% and 72.2%, respectively.

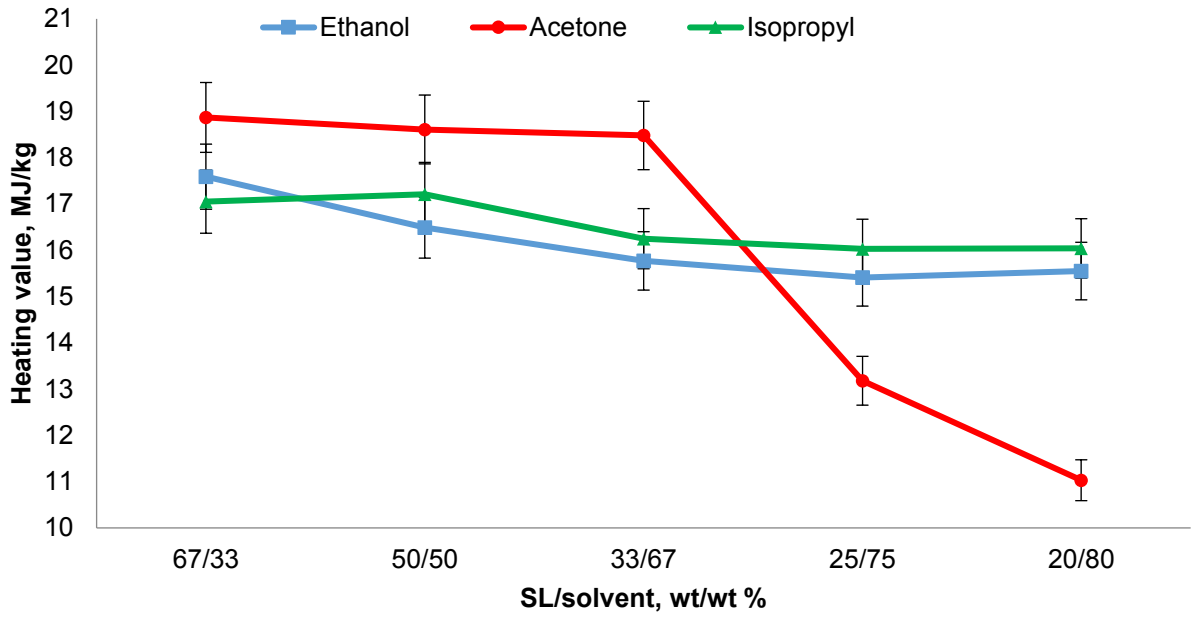

Figure 6.1 Heating values of the precipitates made from mixing untreated SL with solvents at different ratios.

6.4.2 Heating values of precipitates made from acidified SL with solvents

Figure 6.2 shows the heating values of precipitates made from acidified SL mixed with solvents as a function of the acidified SL/solvent weight ratio. The results showed that smaller heating values were generally obtained for precipitates made from mixing acidified SL with solvents and that by reducing the SL/solvent ratio, the heating value decreased when the ratio was larger than 50/50 wt./wt. (Figure 6.2). When the ratio was smaller than 50/50 wt./wt., the heating value increased for the precipitates made from mixing acidified SL and ethanol or isopropyl. As these heating values are almost half of that reported in Figure 6.2 the use of these precipitates as a fuel source and, more importantly, the use of combined acidification and solvent treatment for isolating lignocelluloses from the SL of NSSC process may be uneconomical.

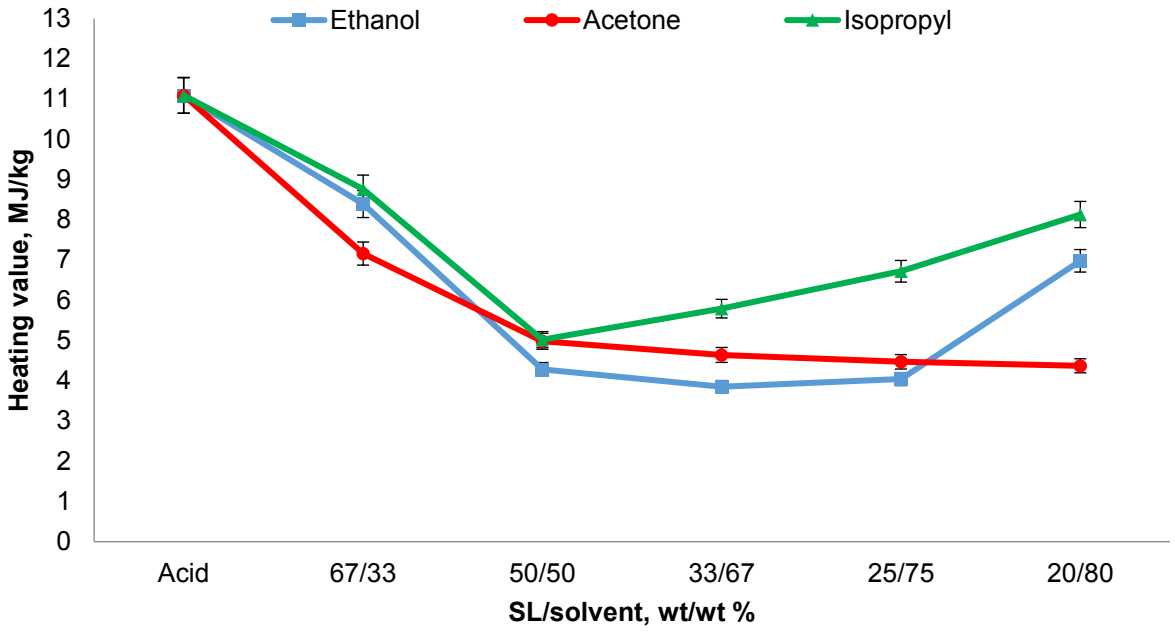

Figure 6.2 Heating values of the precipitates made from mixing acidified SL with solvents at different ratios.

6.4.3 Chemical compositions of precipitates with the highest heating values

The chemical compositions of the precipitates made from acidification of SL are listed in Table 6.1. As seen, the acidification of SL generated the precipitates containing a significant amount of soluble lignosulfonate, some hemicelluloses and insoluble materials. The untreated SL contained

52.7 g/L of lignosulfonate and 13.18 g/L of hemicelluloses. After acidification, the lignosulfonate and hemicellulose contents of SL dropped to 50.9 g/L and 4.8 g/L, respectively, implying more hemicellulose removal than lignosulfonate. The low ash content of the precipitates suggests that the insoluble and soluble materials were more organic. These results may imply that the acidification of SL led to insoluble precipitates that contained more hemicellulosic compounds as they were significantly removed from SL, but they were not present in the soluble part of the precipitates (i.e. hard to dissolve after extraction from SL).

Table 6.1 also lists the chemical compositions of the precipitates with the highest heating values depicted in Figures 6.1 and 6.2 (SL/solvent weight ratio of 67/33). As seen, more lignosulfonate and hemicelluloses were present in the precipitates made from mixing untreated SL and solvent (Samples 1 to 3) than those made from mixing acidified SL and solvents (Samples 5 to 7). However, the precipitates of acidified SL/solvent contained more ash, indicating that they contained more inorganic materials. It can be inferred from these results that the solvent treatment of the acidified SL resulted in more precipitation of organic materials than did that of untreated SL. However, it is not clear why treating SL with different solvents led to altered organic or inorganic precipitation. The amounts of furfural and acetic acid were marginal in the precipitates.

Table 6.1 Chemical compositions of the precipitates with the highest heating values reported in Figures 6.1 and 6.2 (SL/solvent 67/33 wt./wt.).

	Untreated SL/solvent			Acidified SL	Acidified SL/solvent		
Components	Ethanol Sample 1	Acetone Sample 2	Isopropyl Sample 3	Sample 4	Ethanol Sample 5	Acetone Sample 6	Isopropyl Sample 7
Lignosulfonate, wt.%	57.08	63.22	74.06	69.18	38.87	27.93	25.02
Hemicelluloses, wt.%	20.62	17.65	18.94	6.28	9.22	12.19	14.46
Insoluble materials, wt.%	N/A	N/A	N/A	29.93	N/A	0.14	N/A
Acetic acid,wt.%	0.21	0.13	N/A	0.07	N/A	N/A	N/A
Furfural, wt.%	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Ash, wt.%	22.9	13.21	16.91	19.52	63.47	50.63	53.01

6.4.4 Elemental compositions

Table 6.2 lists the elemental components of the precipitates isolated from mixing untreated or acidified SL with solvents at the weight ratio of 67/33 as the highest heating values obtained at this ratio.

Generally, the carbon and hydrogen contents of the precipitates made from mixing untreated SL and solvents were similar but higher than those of precipitates made from acidified SL and solvents. However, oxygen, sulfur and sodium contents of precipitates obtained from untreated SL and solvents were lower than those of acidified SL and solvents. The higher inorganic elements of the precipitates, made from acidified SL and solvents were in agreement with their higher ash content showed in Table 6.1. Jenkins et al (1998) postulated that the increase in the carbon content of biomass by 1% led to the heating value rise by 0.39 MJ/kg. A similar phenomenon was reported by Shafizadeh (1981) in combusting wood and wood products. Jenkins et al. (1988) also reported that a 1% increase in the ash content of biomass led to 0.2 MJ/kg decrease in its heating value. Therefore, the low ash content (Table 6.1) and high carbon and hydrogen contents (Table 6.2) of the precipitates made from mixing untreated SL and solvents were the reasons for their higher heating values than those made from mixing acidified SL and solvents (comparison of Figures 6.1 and 6.2). Table 6.2 showed that all samples contained a trace of nitrogen, calcium, potassium, and magnesium. The nitrogen content of precipitates originates from wood (Kymalainen et al. 1999). It was reported that nitrogen was in the form of amino acid in the black liquor of hardwood species (Veverka et al. 1993). In the literature, calcium, potassium, and magnesium elements were claimed as the main components contributing to the ash of wood (Meier et al. 2013). Baxter et al. (1998) postulated that potassium, calcium, and sulfur were also present in the ash of herbaceous fuels. The results also showed that the precipitates had more sodium and sulfur than other elements; both were originated from sodium compounds used in the NSSC pulping process. Comparing the precipitates made from untreated SL/solvent with those made from acidified SL/solvents, it is inferred that the acidification pretreatment of SL increased the sodium and sulfur compounds of the precipitates.

Table 6.2 Elemental analysis of the precipitates with highest heating values reported in Figures 6.1 and 6.2 (SL/solvent ratio of 67/33 wt./wt.)

	Untreated SL/solvent			Acidified SL	Acidified SL/solvent		
Solvent	Ethanol	Acetone	Isopropyl		Ethanol	Acetone	Isopropyl
Sample/ Element, %	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
N	0.06	0.04	0.06	0.19	0.04	0	0
C	38.4	39.82	40.86	28.04	19.53	17.08	15.82
H	5.21	5.64	5.4	3.34	3.41	2.59	2.21
S	1.49	1.89	3.94	8.94	10.57	10.79	13.34
O	45.30	43.25	45.53	41.1	46.12	47.2	47.25
Ca	0.08	0.11	0.08	0.10	0.15	0.80	1.10
K	0.17	0.19	0.18	0.28	0.18	0.47	0.48
Mg	0.02	0.04	0.03	0.04	0.03	0.16	0.14
Mn	0.02	0.02	0.02	0.02	0.02	0.07	0.06
Na	5.30	6.22	5.43	7.49	4.65	17.36	11.84

6.4.5 Theoretical heating value determination

The heating values of the precipitates can be evaluated by considering their carbon and hydrogen contents in Table 6.2. According to the literature, a close correlation was identified between the carbon and hydrogen contents and the heating value of biomass (Jablonsky et al. 2013; Hu et al. 2010); whereas, an inverse correlation was obtained between oxygen content and lignin's heating value (Hu et al. 2010). Jablonsky et al. (2013) predicted the heating value of lignin via using equation 6.1:

$$Heating\ value = 0.40659 \times C \tag{6.1}$$

where C is the carbon content of lignin (wt.%).

The heating value of biomass (MJ/kg) was related to its elements according to equation 5.2 (Gaur and Reed 1998):

$$Heating\ value = 0.3491C + 1.1783H - 0.1034O - 0.0211A + 0.1005S - 0.0151N \tag{6.2}$$

where C, H, O, A, S and N are carbon, hydrogen, oxygen, ash content, sulfur and nitrogen contents (wt.%) of biomass, respectively. Figure 6.3 presents the predicted heating values of the precipitates generated from 67/33 wt./wt. untreated SL/solvent or acidified SL/solvent mixtures, respectively.

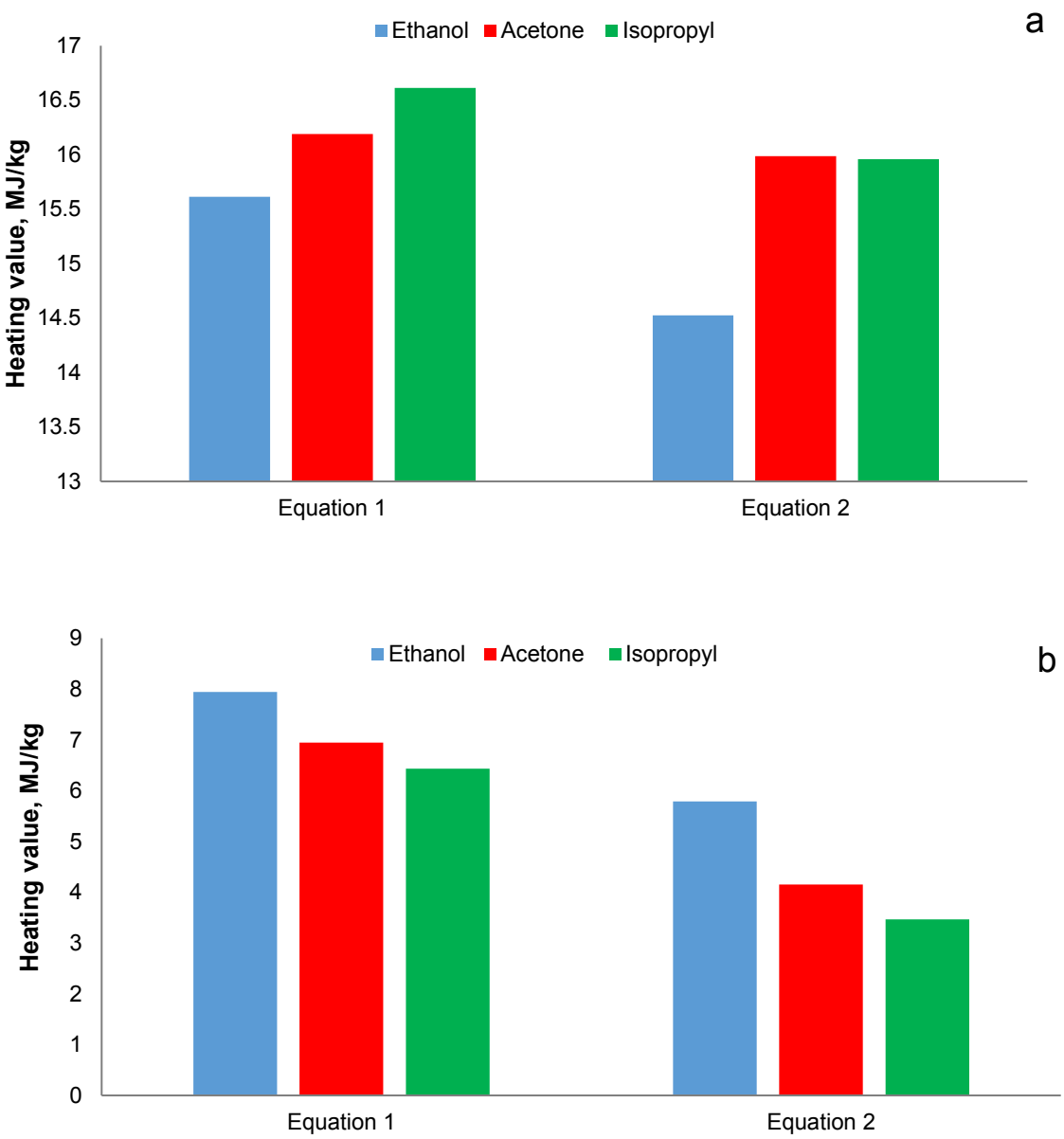

Figure 6.3 The theoretical heating values determined using equation 1 and 2 for precipitates made from mixing (a) untreated SL or (b) acidified SL with solvents.

Generally, the heating values estimated theoretically were close to the experimental data reported in Figures 6.1 and 6.2. Interestingly, Equation 6.1 predicted the experimental results better than did Equation 6.2. Equation 6.1 is designed for the lignin of black liquor, and the SL samples studied in this work contained mainly lignosulfonates, which are derivative products of lignin. Equation

6.2 is designed for heating value analysis of biomass. As the experimental and theoretical values based on equation 6.1 were close, it is implied that other components in the precipitates affected the heating value of the precipitates to a less extent.

6.4.6 DSC analysis

It was stated that the T_g of lignin and hemicelluloses was in the range of 180 °C and 205 °C, respectively (Stokke et al. 2013). The solvent treatment of polymers may affect their plasticization and thus their T_g (Wypych et al. 2011). Table 6.3 lists the T_g and heat capacity of the precipitates. The T_g value of acidified SL precipitate (Sample 4) was similar to the T_g value reported by Hatakeyama and Hatakeyama (2009) for lignosulfonate. The precipitates isolated from untreated SL/solvent mixtures displayed slightly lower T_g than samples precipitated from acidified mixtures. It was noted that low T_g corresponded to high C_p (Table 6.3). A correlation was obtained by Li and McDonald (2014) in that a high Cp revealed a high molecular mobility index, which led to a Tg decrease. Glass transition temperature depends on several factors such as molecular weight, thermal history, cross-linking and pressure (Hatakeyama 1992). As shown in Table 6.2, the precipitates made from mixing untreated SL and solvents had higher organic but lower inorganic compounds. The Tg and Cp of precipitates made from mixing SL and ethanol or isopropyl are similar but lower than those made from mixing SL and acetone, regardless of acidification. The precipitates made from mixing SL and acetone had higher inorganic components. Therefore, it can be claimed that the inorganic compounds of precipitates were directly related to the thermal properties of the precipitates, and increased the Tg, but reduced the Cp of precipitates.

Table 6.3 Glass transition temperature (T_g), heat capacity (C_p) and ignition temperature (T_i) of the precipitates with the highest heating values reported in Figures 6.1 and 6.2.

Sample ID	Tg,°C	Cp, J/g°C	Ti, °C
1	147.15	0.2549	202.2
2	169.32	0.1110	202.9
3	149.37	0.2587	207.3
4	128.6	0.1239	157.4
5	153.46	0.2190	187.6
6	170.84	0.1228	202.3
7	158.69	0.1694	192.0
Rice husk*			258
Poplar*			248
Miscanthus*			233

* (Kok and Ozgur, 2013)

6.4.7 TGA/DTGA analysis of precipitates made from mixing untreated SL with solvents

Figure 6.4 presents the weight loss and weight loss rate of the precipitates made from mixing untreated SL with solvent. The weight loss rate results of the sample displayed a maximum weight loss in the range of 200-300 °C and a minimal weight loss in the temperature range of 300-500 °C. These two regions are most likely related to hemicelluloses and lignin degradation (Carrier et al. 2011). Lignin degradation started at 230 °C and xylan degradation occurred in the range of 200-315 °C (Bonini and D'Auria 2006; Pasangulapati 2010). Gasparovic et al. (2012) reported that the thermal decomposition of hemicelluloses mainly occurred in the range of 190-380 °C with the maximum peak at 290 °C. It was also reported that the degradation of lignin occurred mainly in the temperature range of 190-500 °C (Gasparovic et al. 2012). Mansaray and Ghaly (1998) reported that biomass degradation occurring in the temperature range of 184-380 °C was due to active pyrolysis and the release of light volatile compounds originating from the decomposition of hemicelluloses. The second decomposition temperature range (300-500 °C) was related to the generation of heavy volatile compounds and to lignin decomposition (Mansaray and Ghaly 1998).

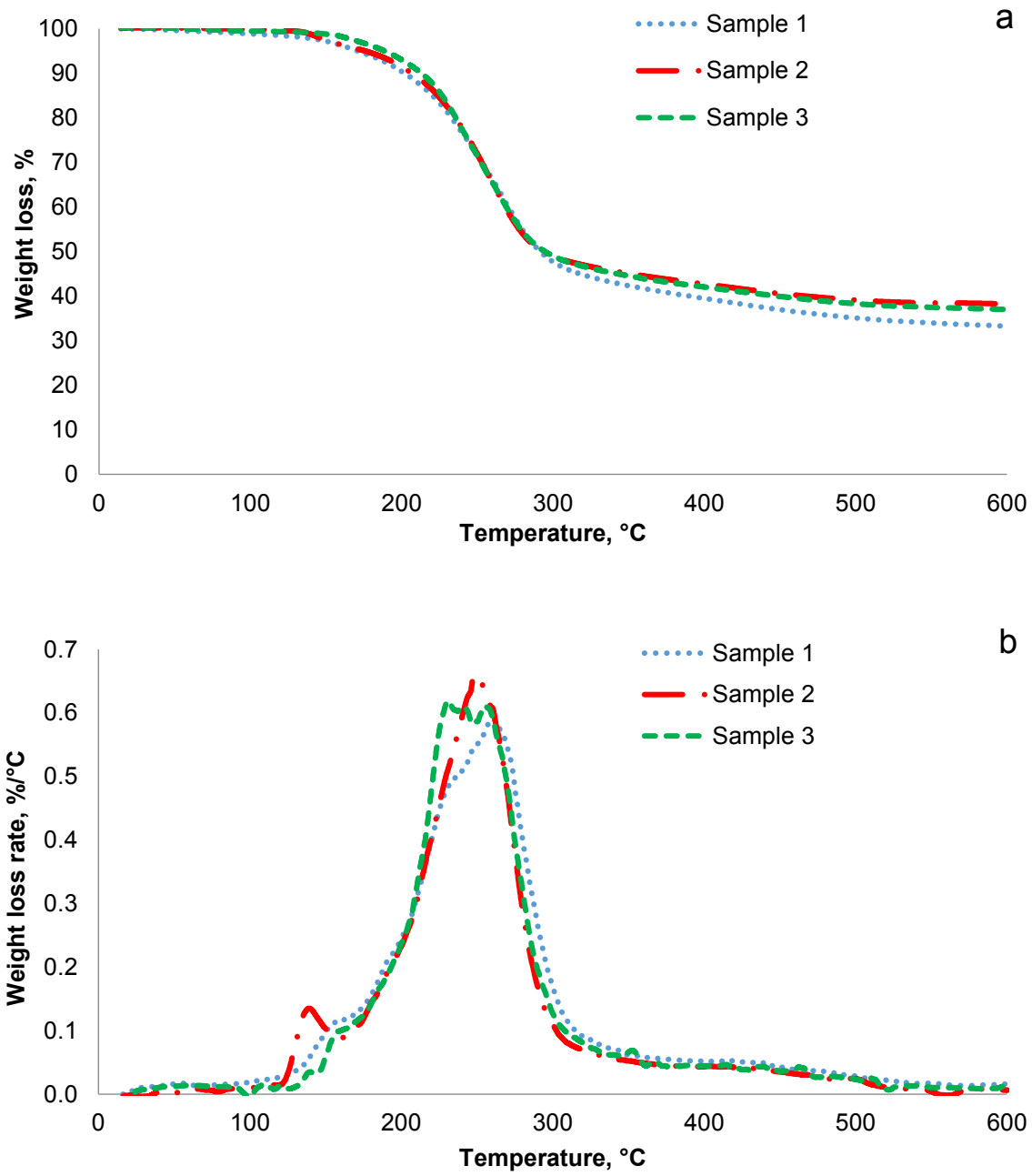

Figure 6.4 Weight loss (a) and weight loss rate (b) of precipitates made from mixing untreated SL with solvents.

6.4.8 TGA/DTGA analysis of precipitates made from mixing acidified SL with solvents

Figure 6.5 presents the weight loss and weight loss rate of the precipitates made from mixing acidified SL with solvents. The precipitates had a broad temperature decomposition range of 200-

300 °C, which is due to the wide range of lignin and extractive decompositions (Gomes et al. 2007). Samples 1, 2 and 3 had higher weight lost rates compared to Samples 5, 6 and 7, which was due to the higher ash content of the latter.

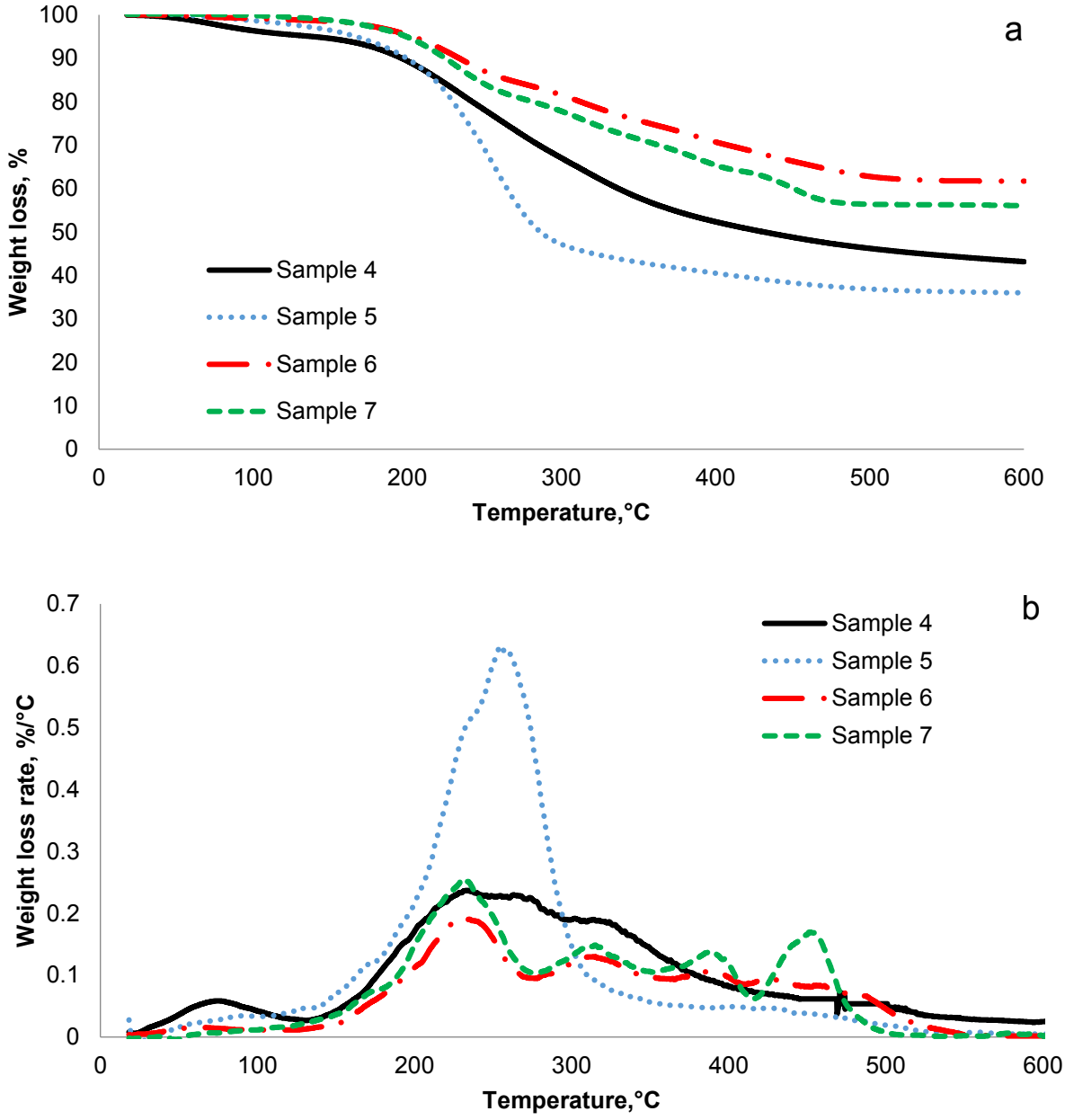

Figure 6.5 Weight loss (a) and weight loss rate (b) of precipitates made from mixing acidified SL with solvent.

6.4.9 Ignition temperature analysis

Ignition temperature is the turning point from pyrolysis to combustion (Gomez et al. 2007). Ignition temperature is an important fuel property as it impacts flame stability, pollutants formation and flame extinction (Sun and Kozinski 2000). Grotkjær et al. (2003) reported that ignition temperature depended on the sample mass, the heating rate, and gas atmosphere. Ignition temperatures of the precipitates are also listed in Table 6.3. Generally, a low ignition temperature was preferred as the material could be flamed in a lower temperature (Qing et al. 2011).

The results in Table 6.3 depict that the samples obtained via solvent treatments generally had higher ignition temperatures compared to acidified SL (Sample 4), but they had lower ignition temperatures than commercial lignosulfonate, wood, and rice. Kok and Ozgur (2013) reported that the ignition temperature is directly related to its heating rate. Furimsky (1988) postulated that the ignition temperature of coals had an inverse correlation with its H/C ratio. Grotkjær et al. (2003) found the same correlation between the ignition temperature of wood and straw samples and their H/C ratios. As shown in Table 6.2, the precipitates made from mixing untreated SL with solvent had a slightly lower H/C ratio than those made from acidified SL with solvents, and thus had higher ignition temperatures (Table 5.3).

6.4.10 Application

Lumadue et al. (2012) reported that the lignin isolated from kraft black liquor was used as a binder in the anthracite briquettes. It was found that the briquettes containing 3-8 % lignin possessed a similar combustion rate, but 38 % higher energy content than did coke (Lumadue et al. 2012). In another report, lignin was used as a binder for coal briquettes production (Malhotra 2010). Tabil et al. (1997) reported an increase in the durability of low quality alfalfa pellets from 65.1 to 85.8 % by adding 1.25 wt.% of lignosulfonate without a noticeable change in energy consumption of the system. Since the precipitates reported in the present work were mainly lignosulfonates (Tarasov et al. 2015), thus could be used in briquette production.

The precipitates isolated from untreated SL/solvent mixtures could also be considered as raw materials for biofuel production, which could be an alternative for lignite coal. In North America, the heating value of lignite A is 14-19.3 MJ/kg with 7.97 wt.% ash and average moisture of 18 % (Zavodska and Lesny 2006). To the best of our knowledge, lignite may release radioactive materials during combustion, and it needs expensive drying technology (Zavodska and Lesny

2006). The precipitates isolated from SL/solvent mixtures with lowest SL/solvent had a similar heating value to that of lignite and thus they can potentially replace lignite. The use of limited amount of solvent for a precipitate generation will be beneficial for the NSSC process as it potentially reduces the cost of solvent recovery and thus the production costs of precipitates. However, further investigation is needed to determine the feasibility of this replacement.

It should be noted that, the high ash (35-40 wt.%) and sulfur (1.8-2.1 wt.%) contents of precipitates may make barriers for their use. It was reported that *Thiobacillus ferroxidans* bacteria was used for coal desulfunization (Tillet and Myersen 1987). A similar approach may be considered for the removal of sulfur from precipitates, which would broaden the industrial applications of the precipitates. Vishtal and Kraslawsky (2011) stated that lignin's ash content could be decreased by water flushing. Lowering ash content will allow using the precipitates as a fuel source for industrial applications, e.g. for wood pellet production.

6.5 Conclusions

The results showed that the precipitates isolated from untreated SL generally had higher heating values and ignition temperatures, but a lower ash content and H/C ratio, compared to other acidified SL. The amount and type of the solvent used impacted the heating value of the precipitates. Generally, the precipitates made from mixing untreated SL and solvent had higher heating values compared with those made from acidified SL/solvent mixtures. These results are due to the higher inorganic content and lower carbon contents of the precipitates made from untreated SL and solvents. The highest heating values were obtained via mixing SL and solvent with the weight ratio of 67/33, regardless of SL pretreatment. The highest heating values of 18.87, 17.59 and 17.05 MJ/kg were obtained for precipitates generated via mixing untreated SL with acetone, ethanol, and isopropyl alcohol, respectively. The highest heating values of 8.39, 7.16 and 8.76 MJ/kg were obtained for mixing acidified SL with ethanol, acetone, and isopropyl, respectively. The heating value of the precipitates obtained from untreated SL is similar to that of wood pellets (17.64–18.72 MJ/kg) (Vassiliev et al. 2013). However, the ash content of the precipitates is significantly higher than that of other biomass-based fuels (Van Loo and Koppejan 2008). The reduction in ash content of precipitates should be studied to widen their potential application. Currently, it may be possible to mix the precipitates with fuel pellets or briquettes (Tabil et al. 1997). Although the precipitates showed promising results for use as fuel, a pilot scale

study of this process is essential in evaluating the feasibility of this process. The feasibility study should include a comparison between the solvent extraction process and multi-effect evaporation.

The precipitates made from untreated SL/solvent mixtures had an approximately similar ignition temperature, between 202 and 207 °C. The ignition temperatures for precipitates made from acidified SL/solvent mixtures were 187, 202 and 192 °C for ethanol, acetone, and isopropyl alcohol, respectively. The experimental and theoretical heating values were close, implying that the heating value of the precipitates can be predicted based on their compositions. The TGA analysis showed that precipitates made from untreated SL or acidified SL lost 50 % or 57 % of their weights, respectively, when the temperature was increased to 700 °C. The DSC analysis showed that glass transition temperatures of solvent treated precipitates were higher than those obtained without solvent treatment.

6.7 References

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Chapter 7 CONCLUSIONS AND FUTURE DIRECTIONS

7.1 Summary of conclusions

The study presented in this dissertation focused on the investigation of the properties, structure, and compositions of lignocellulosic materials extracted from biomass during flow-through autohydrolysis and NSSC pulping processes. The information presented in the literature on the properties, methods of extraction and analysis, and possible applications of lignin-carbohydrate complexes (LCC) were systematically analyzed. The impact of autohydrolysis pretreatment parameters on the efficiency of lignocelluloses removal from softwood was fundamentally studied. The effect of hydrothermal pretreatment conditions on the structure and properties of LCC present in the produced hydrolysates was thoroughly analyzed. The impact of acidification and organic solvent treatment on the composition and properties of lignin, hemicelluloses and LCC in materials precipitated from hydrolysates was investigated in detail. The efficiency of application of various organic solvents for lignosulfonate and carbohydrates removal from non-treated and acidified SL of NSSC pulping process was assessed. The thermophysical properties and elemental compositions of lignocellulosic materials generated via treatment of acidified and not-acidified SL of NSSC process were comprehensively studied.

The results of flow-through autohydrolysis pretreatment of softwood chips confirmed that the process temperature made the most critical effect on lignin removal. The hydrolysate obtained at the slowest liquid flow rate applied in this study showed a higher sugar concentration than the samples produced at a higher liquid flow velocity. Molecular weights of hemicelluloses slightly increased with the liquid velocity rate increment, whereas lignin Mw was stable regardless of the conditions applied. GPC analysis revealed LCC existence in the produced hydrolysates. The results of this work suggested that the hydrolysate produced at the L/S ratio of 10/1 wt./wt. contained 19% of lignin moieties in LCC form, which were involved in the lignocelluloses with larger molecular weights.

Lignocellulosic materials were isolated from hydrolysates by ethanol or acid supplements. Acidification was found to be the most effective for lignin removal, while ethanol supplement led to the highest hemicelluloses extraction rate. It was found that the application of ethanol supplement led to the precipitation of large molecular weight hemicelluloses. The heat capacity of the precipitates obtained due to ethanol or acid treatment was higher than the C_p values of the

initial solid material present in hydrolysates, which could be attributed to structural modifications of precipitates caused by the reagents applied. This hypothesis was supported by TGA and ^{1}H NMR analysis. Two-dimensional HSQC NMR spectroscopy showed the presence of LCC linkages only in the initial solid material of hydrolysate produced at autohydrolysis pretreatment with the lowest intensity of autohydrolysis applied in this study.

The experimental results revealed that mixing organic solvents with the untreated SL of NSSC process led to a higher lignosulfonates removal than that with acidified SL. It has been found that the application of acetone or isopropyl facilitated the extraction of 60% of lignosulphonate from SL. The highest removal of hemicelluloses was achieved via the mixture of acidified SL and ethanol in 67/33 wt./wt. ratio. The charge density of the obtained precipitates generally increased with the SL/solvent ratio decrease. The precipitates obtained from SL/solvent mixtures with 50/50 wt./wt. ratio could be applied as flocculants due to its high molecular weight and anionic charge density. The materials with low Mw produced via SL/solvent mixture with 20/80 wt./wt. ratio could be used for phenols production.

The materials produced from mixing organic solvents with untreated SL showed higher heating values and ignition temperatures than the precipitates obtained from the acidified SL/solvent mixtures. The highest heating values were observed for the precipitates produced from SL/solvent mixture with 67/33 wt./wt. ratio. The materials obtained from solvents mixture with acidified SL displayed significantly lower heating values due to a lower content of carbon and hydrogen and a higher amount of ash. The ignition temperatures of the precipitates made from untreated and acidified SL/solvent mixtures ranged from 202 to 207 °C and from 187 to 202 °C, respectively. The glass transition temperature was found to be directly correlated with inorganic compounds content. Based on these findings, the application of precipitates obtained from untreated SL/solvent mixtures as an additive to fuel pellets and briquettes was proposed.

7.2 Recommendation for future work

In this dissertation, the effects of flow-through autohydrolysis parameters on the proportions of extracted lignocelluloses were comprehensively studied. It was found that an autohydrolysis treatment led to a significant isolation of lignocellulosic materials from biomass. It is possible to assume that the removal of the lignocellulosic material in the hydrothermal treatment could result in considerable modifications of the structure and properties of the biomass material. Significant

isolation of hemicelluloses during autohydrolysis increased the relative concentration of lignin and improved the hydrophobic properties of the treated wood, which can be used as a raw material in producing advanced wood pellets.

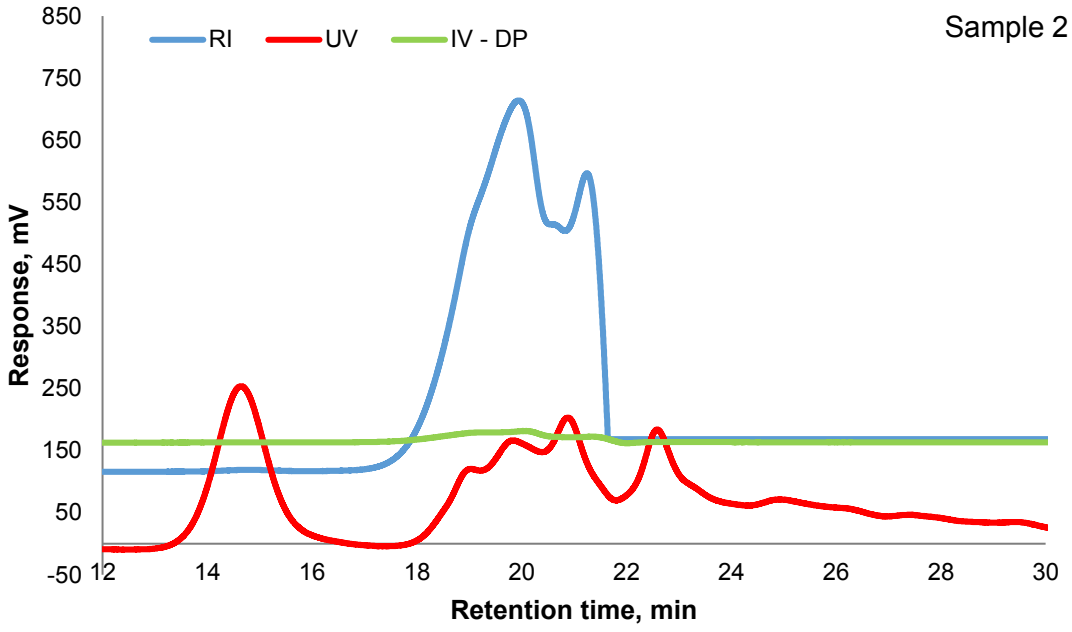
This PhD study did not focus on the pretreated wood chips after hydrolysis, but the treated wood chips have significant potential to be used in value-added production. Therefore, a detailed analysis of the compositional, viscoelastic, and physicochemical parameters of softwood chips after hydrothermal pretreatment should be conducted. Also, post-treated wood chips could be fractionated in lignin-carbohydrate complexes, which allows for more detailed analysis on the impact of hydrolysis on wood structure and LCC formation and use.

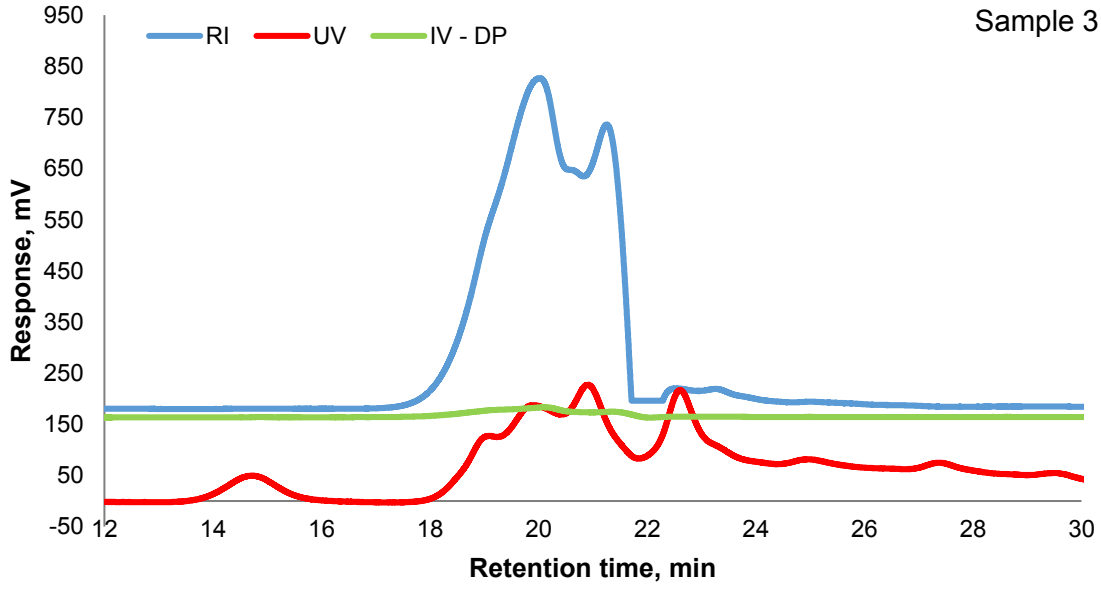
In the current study, the isolation and characterization of lignocellulosic material from biomass and spent liquors were systematically investigated. As it is discussed in the review chapter of this dissertation, the presence of LCC in SL of NSSC was reported in the literature. However, the structure and compositions of LCC in the materials obtained from SL mixture with organic solvents are unclear and should be investigated for a better understanding of LCC behavior. The materials extracted from SL of NSSC process showed a relatively high heating value and a low heating capacity. The use of these materials as additives for fuel pellets and briquettes is suggested. On the other hand, a high proportion of ash content will lead to stove deficiency, and make these materials less industrially attractive. Therefore, methods for decreasing the ash content should be investigated. Also, the hydrophobic and binding properties of the obtained lignocellulosic materials after extraction should be studied systematically for potential application development.

PUBLICATIONS LIST

1. Tarasov D, Leitch M, Fatehi P (2018) Flow through autohydrolysis of spruce wood chips and lignin carbohydrate complex formation. Cellulose 25(2): 1377-1393.
2. Tarasov D, Leitch M, Fatehi P (2017) Thermal properties of lignocellulosic precipitates from neutral sulfite semichemical pulping process. Fuel Process Technology 158: 146-153.
3. Tarasov D, Leitch M, Fatehi P (2015) Production of lignosulfonate in NSSC-based biorefinery. Biotechnology Progress Journal 2015:1508-1514.
4. Khazraie T, Zhang Y, Tarasov D, Gao W, Price J, DeMartini N, Hupa L, Fatehi P (2017) A process for producing lignin and volatile compounds from hydrolysis liquor. Biotechnol Biofuels 10(1): 47.

APPENDIX


Sample 2
RI
UV
IV - DP
Response, mV
850
750
650
550
450
350
250
150
50
-50
12
14
16
18
20
22
24
26
28
30
Retention time, min


Sample 3
RI
UV
IV - DP
Response, mV
950
850
750
650
550
450
350
250
150
50
-50
12
14
16
18
20
22
24
26
28
30
Retention time, min

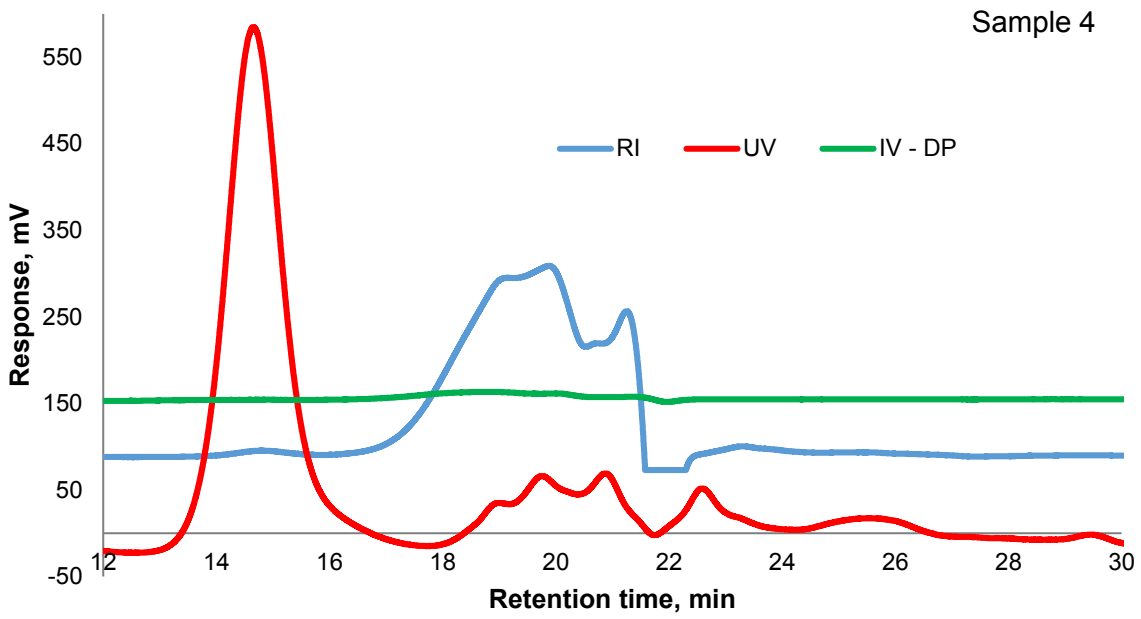


Figure 1a RI, UV and IV-DP detectors data related to retention time data in case of different autohydrolysis liquor samples.