

Towards a cost-competitive biorefinery: fractionation of willow with low-cost ionic liquids and synthesis of lignin-based copolymers

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Declaration of Originality

The entirety of the work described in this thesis was carried out at Imperial College London between November 2014 and December 2017. Unless otherwise stated the work is my own and has not been submitted previously for a degree at this or another university.

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Publications

The pretreatment protocol used as the basis for the experiments in this thesis has been published as a video in a peer-reviewed journal. Some of the results presented in this thesis have been published in the peer-reviewed literature.

Gschwend, F. J. V., Brandt, A., Chambon, C. L., Tu, W.-C., Weigand, L., Hallett, J.P. Pretreatment of Lignocellulosic Biomass with Low-cost Ionic Liquids. *J. Vis. Exp.* e54246, 1–6 (2016). doi:10.3791/54246.

Weigand, L., Mostame, S., Brandt-Talbot, A., Welton, T. & Hallett, J. P. Effect of pretreatment severity on the cellulose and lignin isolated from *Salix* using ionic liquid pretreatment. *Faraday Discuss.* **202**, 331–349 (2017).

Abstract

Lignocellulosic biomass is considered as a promising alternative bio-based renewable resource that can be utilized for the production of energy, fuels, chemicals and materials. In order to realise its full potential, a pretreatment step which breaks up the biomass matrix and separates the lignocellulosic biopolymers is required. Research in this field has been ongoing for several decades, however, the development of cost efficient pretreatment processes and novel materials derived from lignocellulose is still a challenging and important task.

The work described in this thesis focuses on two main aspects, namely biomass pretreatment using ionic liquids and synthesis of value added materials based on lignin, to aid the advancement of a bio-based economy. Firstly, pretreatment of the hardwood willow was undertaken using the low-cost ionic liquid solution triethylammonium hydrogensulfate $[N_{2220}][HSO_4]_{80\%}$. The effect of the pretreatment severity (described by the pretreatment temperature, residence time and ionic liquid acid/base ratio) on the pulp composition and digestibility as well as the lignin properties was studied. The pretreatment conditions significantly affected the pulp properties, lignin molecular structure and molecular weight. High pulp digestibility and glucose yields were achieved for the optimised pretreatment severity.

The hardwood willow is a potential feedstock for a biorefinery and characterized through its genomic diversity. The effects of ionic liquid pretreatment using the ionic liquid solution triethylammonium hydrogensulfate $[N_{2220}][HSO_4]_{80\%}$ on 14 willow varieties was investigated to understand which lignocellulose and pulp property influences the glucose release. The lignin content of the biomass and pulp did not significantly influence the glucose release, but pulp properties such as available surface area, pore size and cellulose degree of polymerization did play a role in pulp digestibility.

The use of lignin as a macromonomer for the synthesis of materials increases the cost efficiency of a potential biorefinery and the development of lignin based copolymers was investigated in the second part of this thesis. An ionic liquid mediated pathway for the synthesis of lignin-poly(furfuryl alcohol) copolymers was discovered in which poly(furfuryl alcohol) is grafted from the lignin polymer. The effect of different reaction conditions and lignin-to-furfuryl alcohol ratios on the properties of the synthesized copolymers was investigated. The material properties strongly depended on the reactant ratio. These copolymers display a high carbon content and thus are promising candidates for the production of carbon fibres or glassy carbon. Furthermore, protic ionic liquids were used as a combined solvent and catalyst system for the addition of aromatic compounds to the lignin polymer.

Aromatic compounds such as 6-bromo-2-naphthol and 6,6'-dibromo-1,1'-bi-2-naphthol were successfully incorporated in the lignin polymer. This introduces a bromine functionality to the lignin structure which can be used as a reactive functionality to graft polymers from lignin via cross-coupling reactions offering a novel and simple synthesis pathway for the versatile functionalization of lignin and the production of lignin based materials.

The implementation of a lignocellulosic biomass based biorefinery is still faces challenges such as high enzyme¹ and high investment costs² amongst others, thus limiting the number of the already existing biorefineries. The development of new technologies for biomass fractionation (e.g. using low-cost, recyclable ionic liquid solutions) and the utilization of lignin for the production of value added chemicals and materials are step stones to aid the realisation of a biobased economy.

¹ Klein-Marcuschamer D., Oleskiewicz-Popiel P., Simmons B.A., Blanch H.W. The challenge of enzyme cost in the production of lignocellulosic biofuels. *Biotechnol. Bioeng.* 2012;109:1083–1087.

² Valdivia M., Galan J.L., Laffarga J., Ramos J.L. Biofuels 2020: Biorefineries based on lignocellulosic materials. *Microb. Biotechnol.* 2016;9(5):585-94.

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Abbreviations

1G	First generation biorefinery
2G	Second generation biorefinery
3HT	3-hexylthiophene
5-HMF	5-(hydroxymethyl)furfural
6-b-2-n	6-bromo-2-naphthol
α	hydrogen-bond acidity of IL
β	hydrogen-bond basicity of IL
π^*	IL interactions through dipolarity and polarisability
$^{\circ}\text{C}$	degree Celsius
a/b	acid/base ratio of protic ionic liquid
BM	biomass
CMD	concerted metalation-deprotonation
DArP	Direct Arylation Polymerization
DCM	dichloromethane
DES	deep eutectic solvents
DMA	dimethylacetamide
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DOSY	Diffusion-ordered spectroscopy
DP	degree of polymerisation
EDA	electron-donor electron-acceptor complex
EOD	ease of delignification
eV	electron volt
FA	furfuryl alcohol
FWHM	full width at half maximum
g	gram
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	heteronuclear single quantum correlation NMR spectroscopy
IL(s)	ionic liquid(s)
L	litre

LA	levulinic acid
LCC	lignin carbohydrate complex
MA	maleic anhydride
mg	milligram
mL	millilitre
mmol	millimol
mol	mol
M _n	number average molecular weight
M _w	weight average molecular weight
PANI	polyaniline
PEDOT	poly(3,4-ethylenedioxythiophene)
PEG	poly(ethylene glycol)
PF	phenol-formaldehyde resin
PFA	polyfurfuryl alcohol
P3HT	poly(3-hexylthiophene)
[P(<i>o</i> -tolyl) ₃]	tri(<i>o</i> -tolyl)phosphine ligand
[PPh ₃]	triphenylphosphine ligand
[P(<i>p</i> -tolyl) ₃]	tri(<i>p</i> -tolyl)phosphine ligand
PSS	poly(styrene sulfonic acid)
PTSA	<i>p</i> -toluenesulfonic acid monohydrate
RBPD	Regional biomass processing depots
scCO ₂	super critical CO ₂
SCW	secondary cell wall
TGA	thermal gravimetric analysis
T _g	glass transition temperature
THFA	tetrahydrofurfuryl alcohol
VOC	volatile organic compounds

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Chapter 1 - Introduction

The utilization of fossil resources (e.g. coal, oil and natural gas) for the production of energy, fuels and chemical goods such as plastics, pharmaceuticals and fabrics has largely contributed to the development of the western world. However, it is now widely accepted that combustion and usage of fossil resources produces carbon dioxide which plays a significant role in global warming and causes other environmental issues such as acid rain and urban smog.¹ The consumption of liquid petroleum has tripled since the 1970s and is expected to double again by 2025.² In addition to the effects on the environment and human well-being, the increasing difficulty to extract these resources causes the need to investigate alternative carbon resources and their valorisation to valuable and more sustainable goods.

One major key player in the consumption of fossil fuels is the production of energy. The ecological footprint of the energy sector can be reduced by wide-spread implementation of the use of renewable energy in the form of nuclear, solar, hydrogen, wind, and biofuels.^{3,4} As for the production of chemicals and materials, a carbon-rich alternative resource needs to be found and processes to ensure the sustainable and efficient use of this resource need to be developed. One promising renewable resource with a high carbon content is lignocellulosic biomass. However, sustainable and cost efficient usage and processing of this resource is still not largely commercially available. One of the reasons for this is that the move towards renewable carbon feedstocks for the chemical industry needs to comply with the concepts outlined by green chemistry. The principles of green chemistry include the manufacture and application of products and processes that aim to eliminate the use or generation of environmentally hazardous chemicals.⁵ State-of-the-art biomass pulping processes often apply hazardous chemicals such as sodium sulphide and sodium hydroxide, creating a necessity to search for more environmentally friendly and sustainable biomass pulping processes.⁶

A novel pretreatment method for (lignocellulosic) biomass using ionic liquids to fractionate the biomass into a cellulose-rich pulp and a lignin fraction has been developed in recent years.⁷⁻⁹ This pretreatment method applies low-cost ionic liquids based on tertiary amines and mineral acids and is the basis of the work done and discussed in this thesis. Protic ionic liquids are a non-volatile organic solvent which are synthesized in a few steps¹⁰, easily recyclable and reusable without performance loss¹¹, thus fulfilling many requirements for environmentally friendly and sustainable solvents.

1. Ionic Liquids

1.1 Solvents as media for chemical reactions

Chemical reactions are typically carried out in solvents (organic or inorganic) to allow for thermodynamic and/or kinetic control of the reaction by influencing several parameters of the reaction system such as the rates and equilibria of the reaction, solubility of the reactants and products and their stability. Although chemical reactions can be carried out in all three states of aggregation (gaseous, liquid and solid) most reactions are performed in the liquid state to allow for more efficient heat transportation to and from endo- and exothermic chemical reactions.^{12, 13}

Various types of solvents are known to chemists and used in academia or industry and generally solvents can be categorised by their bonds. Three subgroups of solvents are differentiated: (i) molecular liquids with differing polarity such as pentane or dimethyl sulfoxide (DMSO) or methanol which are defined as molecule melts and characterised by only bearing covalent bonds, (ii) ionic liquids which are defined as molten salts with a melting point below 100 °C¹⁴ and characterised by only bearing ionic bonds and (iii) atomic liquids that consist of low-melting metals such as mercury or liquid sodium and are defined by only bearing metallic bonds.¹²

Solvents are typically used in excess to the reactants and the most commonly used type of solvents in industrial applications and research facilities are molecular liquids. Most of these solvents belong to the group of volatile organic compounds (VOC) due to convenience in product recovery (VOC can easily be removed from the reaction mixture via evaporation). The low boiling point and high volatility of VOCs and the related issues with recovery and recyclability of these solvents, environmental hazards and the production of large amounts of waste have been identified as a major disadvantage of using VOCs. This has spurred the search for alternatives and green solvents in recent years.¹⁵

A complete definition of the properties of a green solvent is still under development, but consensus has been reached in the academic community that “the idea of “green” solvents expresses the goal to minimize the environmental impact resulting from the use of solvents in chemical production.”¹⁶ The search for more sustainable solvents has led to the development of many novel classes of solvents such as ionic liquids (ILs), deep eutectic solvents (DES), liquid polymers, supercritical CO₂ (scCO₂), etc.¹⁷ Amongst those, ionic liquids are considered a promising alternative to VOCs and are utilized as solvents for chemical reactions (e.g. organic synthesis¹⁸ and catalysis¹⁹), extractions of natural products²⁰ and biopolymers from lignocellulose²¹.

The body of the work presented in this thesis focuses on applying ionic liquids as solvents for the extraction of lignin from lignocellulosic biomass as well as the synthesis of novel copolymers of lignin

in ionic liquids. For this reason, a brief history of the development of ionic liquids, their structure and chemical and physical properties, their toxicity and use as solvents for pretreatment of lignocellulosic biomass will be discussed in detail below.

1.2 A brief history of the development of ionic liquids

The first ever documented observation (mid 19th century) of an ionic liquid was the “red oil” fraction which forms during Friedel-Crafts reactions of benzene and methyl chloride. The structure of this compound remained unknown for decades until Prof Atwood revealed that the observed “red oil” is indeed an ionic liquid via structure analysis using NMR spectroscopy (Figure 1-1).

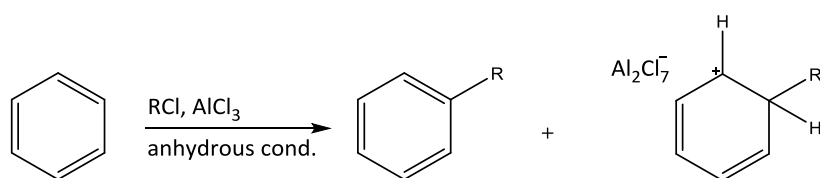


Figure 1-1. Starting materials and products of Friedel-Crafts reaction.¹⁴

This was followed by the discovery of alkylammonium nitrate salts in the early 20th century by Walden. These salts are characterized by low melting points, for example, the salt ethylammonium nitrate already melts at 12 °C. In the 1960s, Prof Yoke observed that mixtures of copper chloride and alkylammonium chloride salts are liquid at temperatures near room temperature further broadening the range of known ionic liquids.¹⁴ See Figure 1-2 for an example.

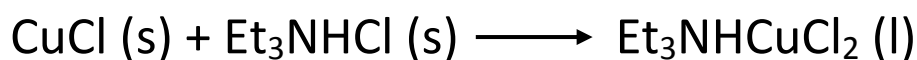


Figure 1-2. Formation of a liquid by the mixing of copper chloride and triethylammonium chloride.¹⁴

Around the same time Prof King published a study of chloroaluminate molten salts, which are mixtures of alkali chlorides and aluminium chlorides such as NaCl – AlCl₃.¹⁴

The development of liquid clathrates in the 1970s by Prof Atwood was another step towards ionic liquids as they are known today. Liquid clathrates are composed of a salt combined with an aluminium alkyl which leads to the formation of a compound with one or more aromatic molecules (Figure 1-3).²²

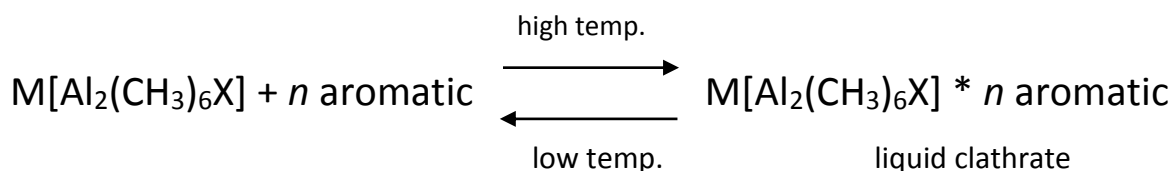


Figure 1-3. Synthesis of liquid clathrates.²²

Hurley and Wier first synthesized the ionic liquid ethylpyridinium bromide - aluminium chloride, which matches the current definition of ionic liquids (Figure 1-4).¹⁴ This ionic liquid was studied by a research group at the Air Force Academy to be used as a molten salt electrolyte.¹⁴ Koch²³ *et al.* also studied it as a medium for organic reactions in the mid-1970s.

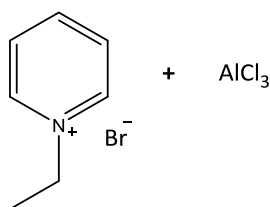


Figure 1-4. Structure of ethylpyridinium bromide - aluminium chloride, the first synthesized ionic liquid matching the current definition.¹⁴

The start of the modern era of ionic liquids was defined by Wilkes as the simultaneous discovery of the 1-butylpyridinium chloride – aluminium chloride mixture (collaborative work by groups at Colorado State University and Air Force Academy).¹⁴ Figure 1-5 shows the structure of 1-butylpyridinium chloride – aluminium chloride.

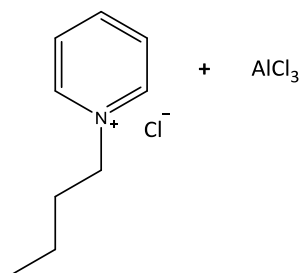


Figure 1-5. Structure of 1-butylpyridinium chloride – aluminium chloride.¹⁴

This all-chloride ionic liquid was an improvement on the mixed chloride-bromide ionic liquid synthesized by Hurley and Wier, but showed the disadvantage that the cation is easily reduced and thus not stable.¹⁴

This led to a search for a more stable cations with the goal to find a chloride salt with a low-melting point. Several cations were proposed by Hussey and Wilkes (Figure 1-6).¹⁴

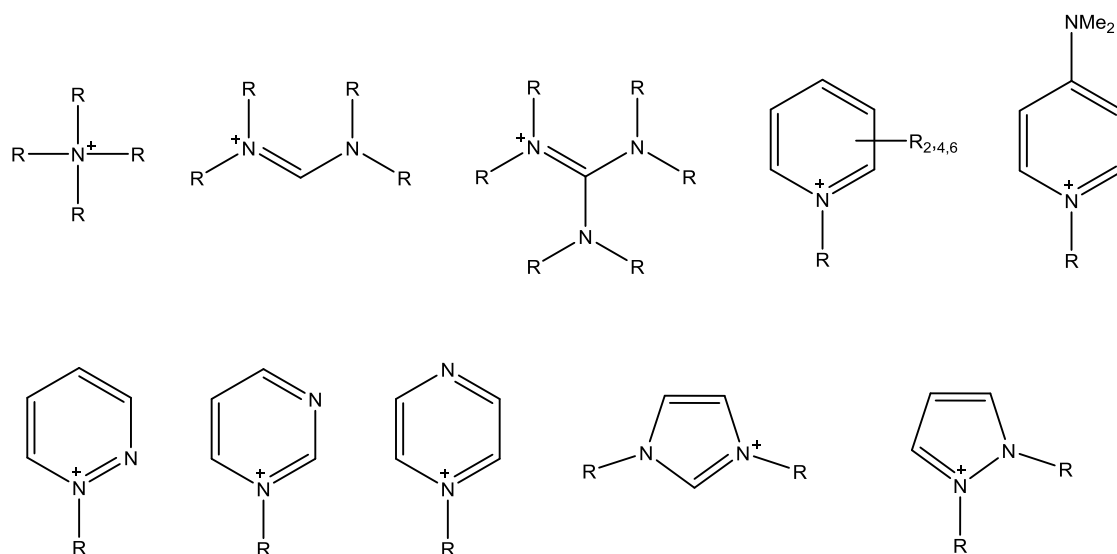


Figure 1-6. Suggested cations for the synthesis of low-melting chloroaluminate ionic liquids.¹⁴

One class of cations showed promising properties as a candidate for an ionic liquid with a wider electrochemical window (which is important for applications as electrolyte), namely the class of dialkylimidazolium cations. Thus various dialkylimidazolium chlorides were synthesized and mixtures of dialkylimidazolium chloride – aluminium chloride ionic liquids were found to have a freezing point below room temperature for specific AlCl_3 mole fractions.²⁴ However, those dialkylimidazolium – and pyridinium based chloroaluminate ionic liquids are not water stable and form hydrochloric acid in the presence of water.¹⁴

A breakthrough was the synthesis of ionic liquids based on organic cations (e.g. dialkylimidazolium) and water-stable anions (tetrafluoroborate, hexafluorophosphate, nitrate, sulphate and acetate) by Wilkes and Zaworotko in 1992.²⁵ These new ionic liquids are easily prepared and stable towards hydrolysis at room temperature. In the following years Fuller^{26,27} *et al.* expanded the amount of water-stable ionic liquids by synthesizing various ionic liquids with mono- and trialkylimidazolium cations and/or larger anions.

Nowadays, ionic liquids are defined as liquids composed only of ions with a melting point below 100 °C.^{14, 28} Modern ionic liquids typically contain an asymmetric organic cation (for example quarternized aromatic or aliphatic ammonium ions, pyridinium ions or pyrrolidinium ions but alkylated phosphonium and sulfonium ions were also reported) combined with inorganic or organic anions. The

possibilities of combinations of cations and anions are nearly infinite, resulting in the chance to tune the properties of the IL upon changing the cation or anion.²⁹ The anions are usually polyatomic and show charge distribution over more than one atom (except for halides), with fluorine substituted anions such as tetrafluoroborate being used often. Typical anions are hexafluorophosphate, trifluoromethanesulfate, dicyanamide, chloride, bromide, iodide, methyl sulphate or acetate.²¹ The great structural variety of ionic liquids allows for application in various fields. The focus of this thesis is the use of ionic liquids as fractionation media of lignocellulosic biomass, thus Figure 1-7 depicts typical examples for ionic liquids used in academia for lignocellulose pretreatment.

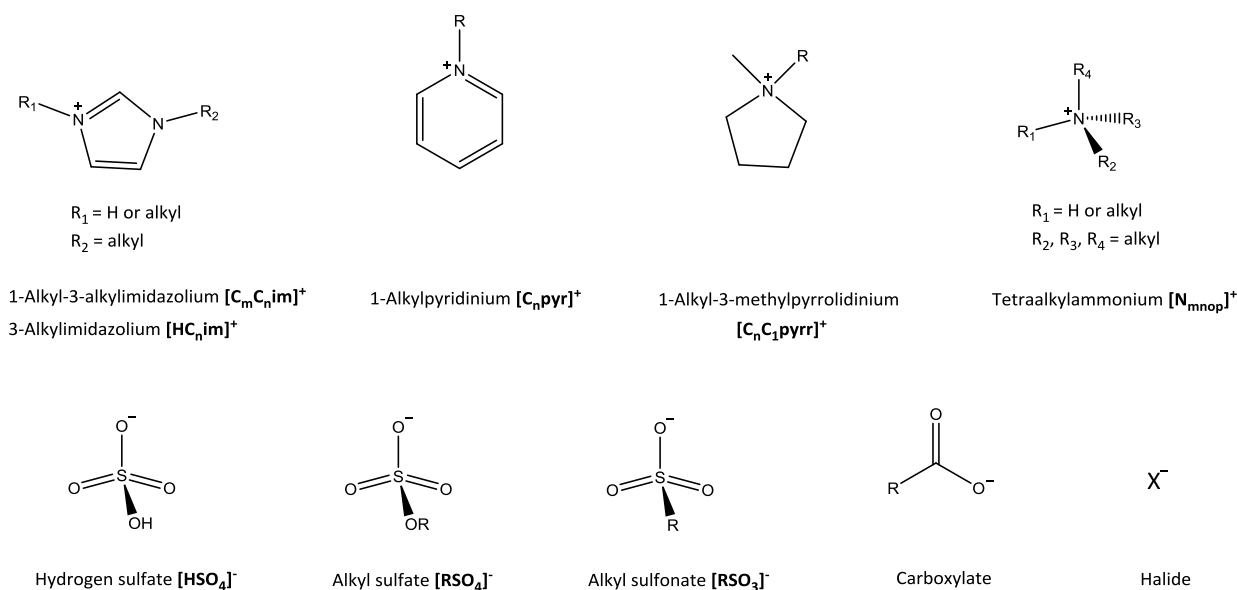


Figure 1-7. Typical cations and anions used for lignocellulose pretreatment.

1.3 Chemical and physical properties of ionic liquids

Four factors influence the melting point of an ionic liquid. In order to achieve a low melting point bulky cations and anions with a low symmetry are used which reduce the ability to form ordered structures.³⁰ Another important factor is the delocalisation of the ion charge over more than one atom which especially takes place in aromatic cations or various anions (except for halides).³¹ The substitution of hydrogen atoms with alkyl chains (linear, branched, with or without functionalities) introduces rotational degrees of

freedom at low temperature.³² Cations with more than one alkyl chain have a lower symmetry thus lowering the melting point of the ionic liquid.³⁰ The fluorination of anions not only increases the stability of the ionic liquid but also decreases its ability to form hydrogen bonds due to withdrawal of electron density by the fluorine atom. This results in a lower melting point of the ionic liquid.³³

The structure of ionic liquids is the basis of their unique properties. Ionic liquids show a large liquid range and thus a negligible vapour pressure which makes them non-volatile organic solvents.³⁴ Most ionic liquids also display a high thermal stability (typical decomposition temperatures range from 350 °C – 450 °C, if no other lower temperature decomposition pathway is accessible)³⁵, high ionic conductivity under ambient temperatures³⁶ and a high polarity whilst being non-coordinating at the same time.³⁰ Ionic liquids display highly tuneable physical and chemical properties such as hydrophobicity and polarity due to the various possible combination of the cation and anion.^{2, 30}

As mentioned above, ionic liquids are considered good solvents for many chemical applications. The unique solvent properties of ILs can be best understood by examining their polarity. The solvent group of ionic liquids is solely composed of mixtures of cations and anions, thus offering a variety of possible interactions between the solvent and solute. These interactions include dipolarity (both permanent and induced), Coulombic interactions, hydrogen bonding and electron pair donor – acceptor interactions. The currently accepted definition of polarity is that it is the sum of all possible specific and non-specific intermolecular interactions between the solvent and any potential solute. This excludes those interactions leading to chemical transformations of the solute.¹² However, the polarity can only describe the potential behaviours of the solvent in relation to the solute. This means that polarity is not an absolute property of the solvent and thus no single measure of polarity exists. Polarity can be measured using various techniques, but one has to keep in mind that different techniques will give different polarities for the same solvent and that all measured polarities are merely estimates.¹²

The Kamlet-Taft measurement of polarity has been proven to be a useful model to describe this property of ionic liquids due to the introduction of multi-parameter polarity scales by Kamlet and Taft. This scale is composed of 3 variables of the ionic liquid, namely the hydrogen bond acidity (α)³⁷, hydrogen bond basicity (β)³⁸ and dipolarity/polarizability effects (π^*)³⁹. The original Kamlet-Taft methodology has been adapted for use with ionic liquids and several studies have measured the Kamlet-Taft parameters of various ionic liquids. An overview of those was given by M. A. Ab Rani⁴⁰ *et al.*

Another important property of ionic liquids is their viscosity. Viscosity is generally defined as the internal friction of a fluid which manifests itself externally as the resistance of a fluid to flow.²⁹ Ionic liquids are generally characterised as Newtonian fluids since no data indicating Non-Newtonian behaviour in ionic liquids has been published yet. Newtonian fluids show a constant viscosity regardless of application of strain.²⁹ To measure the viscosity of ionic liquids, one of the following methods is typically applied: falling ball, capillary or rotational.

Ionic liquids are generally more viscous than regular molecular organic solvents. IL viscosity can range from values as low as 100 mPs to over 5000 mPs. The viscosity of $[\text{C}_2\text{C}_1\text{im}][\text{CH}_3\text{COO}]$ which is commonly used for biomass pretreatment was measured to be 1620 mPs.⁴¹ For comparison, the viscosity of water at room temperature is 8.9 mPs and that of ethylene glycol is 161 mPs.²⁹

The viscosity is strongly dependent on temperature and impurities present in the ionic liquids.⁴² Not surprisingly, the viscosity is also influenced by the anion of the ionic liquid. Experimental studies have established a general order of increasing viscosity with respect to the anion that is as follows: $[(\text{CF}_3\text{SO}_2)_2\text{N}]^- \leq [\text{BF}_4]^- \leq [\text{CF}_3\text{CO}_2]^- \leq [\text{CF}_3\text{SO}_3]^- < [(\text{C}_2\text{H}_5\text{SO}_2)_2\text{N}]^- < [\text{C}_3\text{F}_7\text{CO}_2]^- < [\text{CH}_3\text{CO}_2]^- \leq [\text{CH}_3\text{SO}_3]^- < [\text{C}_4\text{F}_9\text{SO}_3]^-$.²⁹ Interestingly, it can be observed that the increase in viscosity does not directly correlate with the increase in anion size. This can be explained with the effect of other anion properties on the viscosity, namely the formation of weak hydrogen bonds with the cation. However, the viscosity is not only influenced by anion properties, but in case of non-haloaluminate ILs also by the cation. Looking at ionic liquids with the same anion it was found that larger alkyl substituents on the imidazolium cation give rise to fluids with a lower viscosity. For example, ionic liquids containing the bistriflylimide anion show increasing viscosity in the following order of cations: $[\text{C}_2\text{C}_1\text{im}]^+$, $[\text{C}_2\text{C}_2\text{im}]^+$, $[\text{C}_4\text{C}_2\text{im}]^+$, $[\text{C}_4\text{C}_1\text{im}]^+$, $[\text{C}_3\text{C}_1\text{C}_1\text{im}]^+$, $[\text{C}_2\text{C}_1\text{C}_1\text{im}]^+$. Interestingly, the size of the cation seems to be not the only criterium dictating the viscosity of the ionic liquid. Impurities and experimental error between laboratories that measured the viscosity of the different ILs could explain the disparities.²⁹

The high viscosity of ionic liquids often limits possible applications of these solvents leading to the development of IL/co-solvent systems with lower viscosities. The addition of water or other organic solvents significantly lowers this ionic liquid property.⁴²

The densities of ionic liquid appeared to be the physical property least sensitive to variations in temperature and can range from 1.12 gcm^{-3} for the ionic liquid $[(n\text{-C}_8\text{H}_{17})(\text{C}_4\text{H}_9)_3\text{N}][(\text{CF}_3\text{SO}_2)_2\text{N}]$ to 2.4 gcm^{-3} for the ionic liquid $[(\text{CH}_3)_3\text{S}]\text{Br}/\text{AlBr}_3$.²⁹ Additionally, the effect of impurities on the density is far less significant than on the viscosity.²⁹ The density of non-haloaluminate ionic liquids is affected by both the cation and anion properties. For the same cation, the density of the liquid is increasing with increasing mass of the anion in the following order: $[\text{CH}_3\text{SO}_3]^- \approx [\text{BF}_4]^- < [\text{CF}_3\text{CO}_2]^- < [\text{CF}_3\text{SO}_3]^- < [\text{C}_3\text{F}_7\text{CO}_2]^- < [(\text{CF}_3\text{SO}_2)_2\text{N}]^-$. Furthermore, the size of the cation of the ionic liquid is negatively correlated with its density.²⁹

1.4 Toxicity of ionic liquids

The need to design green and sustainable processes has led research to focus on the development of ionic liquids as solvents for many applications such as electrochemistry⁴³, catalysis⁴⁴ and biomass processing.⁴⁵ However, the toxicity of the reaction medium of a process significantly determines its sustainability and “green-ness” and the toxicity of ionic liquids has only been scarcely investigated so far. Comprehensive data is still missing for the majority of ionic liquids, but a general prediction of the toxicity of an ionic liquid of the type [cation][anion] can be gained by using the known toxicity data of the salts [cation]Cl and Na[anion]. It is assumed that in the (human) body all chemical reactions take place in aqueous media resulting in the ions of the ionic liquid to be present in dissociated form. Thus, the prediction of the toxicity of an ionic liquid should be possible from a combination of the known effects of the corresponding alkali metal and chloride salts.²⁹ Some ionic liquids have been thoroughly investigated with respect of their acute toxicity. For example, the acute toxicity of 1-hexyloxymethyl-3-methylimidazolium tetrafluoroborate was assessed by Pernak⁴⁶ *et al.* using the Gadumm method. The LD₅₀ values (the amount of a toxin that is sufficient to kill 50 percent of a population of animals usually within a certain time) were determined and the authors concluded that this tetrafluoroborate ionic liquid shows low toxicity. Even though the above named ionic liquid was found to be not of high toxicity, in recent years it became clear that many imidazolium based ionic liquids are indeed (very) toxic.⁴⁷ One major concern of using ionic liquids as solvents for large scale processes was identified as the potential of ionic liquids to accumulate in nature due to their high stability and low biodegradability.⁴⁸ This led to research into development of biocompatible^{49, 50} or biodegradable^{51–53} ionic liquids to reduce environmental toxicity of these solvents. One research group has introduced additional structural motifs on alkylimidazolium cations to increase the biodegradability of those ionic liquids.^{51–53} Another focus was put on the development of ionic liquids from bio-based sources including cations or anions based on amino acids, lignin, carbohydrates or other renewable molecules.^{54,55} However, ionic liquids incorporating amino acids are generally thermally not very stable, making these ILs unsuitable for processes that require elevated temperatures.⁵⁶

Alkylammonium-based protic ionic liquids were found to be good lignin solvents and have been found to be less toxic than imidazolium based protic ionic liquids.⁵⁷ Additionally, several alkylammonium ionic liquids have been shown to be biocompatible.⁵⁸

Up to date, the majority of ionic liquids is synthesized from petroleum feedstocks which means that a process including these ILs will not entirely fulfil the criteria of a green and sustainable technology.⁵⁹ Further research on the development of environmentally friendly and sustainable ionic liquids still needs to be undertaken.

1.5 Stability of ionic liquids

One factor that increases the sustainability of an ionic liquid based process is the recyclability of said ionic liquid. In terms of biomass processing, the incubation time of the biomass in the ionic liquid is often long (up to 24 hours) and the process is carried out at elevated temperatures of over 120 °C.²¹ Thus, the thermal stability of an ionic liquid is a major factor to be considered when designing a biorefining process. As mentioned above, ionic liquids show a very broad liquid range (e.g. from – 89 °C to 450 °C for [C₄C₁im][NTf₂]).⁶⁰ Ionic liquids are known to not have a boiling point which defines the upper limit of the liquid range as the thermal degradation temperature of the IL.

In order to determine the thermal stability and the temperatures which allow for a safe application of the IL as a solvent for chemical reactions, the behaviour of ionic liquids at elevated temperatures needs to be studied. Additionally, one must also understand the degradation mechanisms of the ILs at elevated temperatures. Typically, the thermal stability of ionic liquids is measured *via* thermal gravimetric analysis (TGA). The degradation temperature measured by TGA is often termed T_{onset} which is defined as the value calculated using the baseline of zero weight loss and the tangent of weight vs. temperature upon decomposition.⁶⁰ However, it is important to note that the actual degradation of the IL already starts at lower temperatures than the calculated T_{onset} .⁶¹ One example is the ionic liquid [C₂C₁im][BF₄] which has a measured T_{onset} of 455 °C but was found to decompose at 1.37 wt% h⁻¹ at 200 °C.⁶² The reason for this discrepancy lies in the nature of the measurement technique, where very fast temperature ramping is applied.

The thermal stability of an ionic liquid is influenced by both the structure of the cation and anion. The following order of temperature resistance was observed for nitrogen containing cations: pyrrolidinium > imidazolium > pyridinium > non-cyclic tetraalkyl ammonium.^{61,63–66} Several studies have shown that increasing the alkyl chain length on nitrogen containing cations of ILs decreases their thermal stability.^{67–70} This physical parameter is also influenced by the branching and saturation of the alkyl side chain of the cation with imidazolium cations substituted with fully saturated alkyl chains showing a higher thermal stability than unsaturated ones.^{60,71} The nature of the anion also plays a significant role in the thermal stability of ILs and the following order of common anions was established: [PF₆]⁻ > [NTf₂]⁻ > [BF₄]⁻ > [Me]⁻ ~ [AsF₆]⁻ > [I]⁻, [Br]⁻, [Cl]⁻.^{63,67,72} These findings correlate well with the anion hydrophobicity, which is a measure for the H-bonding capacity of the anion and hence often the nucleophilicity.⁶⁷

In addition to the influence of the structure of the cation and anion of the IL on its thermal stability, the presence of impurities also directly influences this physical property. In general it can be noted that the presence of water alters the properties of ionic liquids significantly and drying of the ionic

liquids will improve the thermal stability. It is important to note that the choice of pan for the TGA measurement was found to influence the T_{onset} of several ionic liquids. Thermal decomposition is generally lower on aluminium than on alumina pans, but this is also dependent on the salt structure.⁶⁰

1.6 Recyclability of ionic liquids

The recycling of ionic liquids is a very important issue that needs to be addressed to guarantee an economically viable process and to find a solution for the disposal, low biodegradation and toxicity of ILs if applied as solvents in a commercial process. In terms of recycling of ILs one has to distinguish between hydrophobic ILs and hydrophilic ILs. Hydrophobic ILs are generally easily recyclable, especially if specific biphasic systems containing $[\text{PF}_6]^-$ or $[\text{NTf}_2]^-$ are applied as solvents for reactions and liquid-liquid extraction is the method of choice for recovering of the IL.^{73,74} The ease of recycling of these hydrophobic ILs is largely due to the lack of solubility in several organic solvents (e.g. diethyl ether). Products and organic impurities can thus be extracted from the IL using organic solvents and byproducts present in water-immiscible ILs can be washed out by using water with minimal loss of ILs.⁷⁵ However, the use of conventional organic solvents to recycle ILs diminishes the green aspect of using ILs as solvents. An alternative is to use distillation to recover ILs from low-boiling compounds since ILs display negligible vapour pressure. The drawback of this separation technique is the high energy consumption of distillations, especially if non-volatile compounds/IL mixtures need to be recycled.⁷⁵ The use of supercritical fluids could solve this issue since these extraction media are characterized by low costs, non-toxicity, recoverability and ease of separation from the products. The most commonly used supercritical fluid for IL recycling is supercritical CO_2 since it is soluble in ILs but ILs do not dissolve in it. This enables the extraction of ILs from IL/solvent systems without cross-contamination of the IL.^{76–78}

Recovery of the hydrophilic ILs is more difficult in comparison to hydrophobic ILs and in order to avoid cross-contamination of the IL new ways of separation need to be explored. The use of supercritical CO_2 to separate hydrophilic IL/water mixtures was studied, however, satisfactory results could not be achieved due to the solubility of CO_2 in water being very low even at high pressure.^{77,79} The addition of kosmotropic compounds to mixtures of water and hydrophilic ILs resulted in the formation of an aqueous biphasic system with an upper IL-rich phase and a lower water-rich phase. The used kosmotropic compounds were K_3PO_4 ⁸⁰ or monomeric saccharides such as sucrose⁸¹, fructose or glucose⁸². Both approaches resulted in the separation of the IL/water mixture. However, the use of K_3PO_4 has the disadvantage that introduction of inorganic ions (K^+ and PO_4^{3-}) complicates the recycling

process, since hydrophilic ILs also dissociate into ions in aqueous solutions. This issue was avoided when the monomeric saccharides were used instead.

2. Biorefining for the productions of fuels, chemicals and materials

2.1 Concept of biorefining

During the last century, various synthetic routes and processes have been developed to utilize fossil resources such as coal, natural gas and oil for the production of energy, fuels and chemical goods. However, the depletion of those resources and the role combustion of them plays in climate change causes the need to investigate alternative carbon resources. Much interest has been paid to a technology called “Integrated Biorefinery” which uses biomass as a renewable resource for the production of fuels and chemicals.^{83, 5} A biorefinery can be defined as follows: “Biorefining is the sustainable processing of biomass into a spectrum of marketable products and energy.”^a

The cell walls of woody plants such as trees (hardwood and softwood), shrubs and grasses are made of lignocellulose. Lignocellulose is an interwoven matrix of the three biopolymers cellulose, hemicellulose and lignin. Additionally, lignocellulosic biomass is the most abundant plant material on our planet, thus rendering lignocellulose as a promising alternative carbon source and renewable feedstock for biorefining.^{5, 84}

The idea to use renewable feedstocks for the production of fuels has already been commercialised in some countries, for example production of bioethanol from sugarcane in Brazil or from corn in the United States of America.⁸⁵ In Europe, production of bioethanol typically utilizes wheat or sugar beet.^b First generation (1G) biorefineries typically utilize feedstocks such as corn, wheat, cassava, barley, rye, soybean or sugarcane. The biomass is converted into sugars which are then biologically or catalytically transformed into platform molecules (e.g. lactic acid, propionic acid, 1,3-propane diol) or fuels (e.g. ethanol or butanol).⁸⁶ However, these are not the only products produced in the 1G biorefinery. Several co-products are also obtained, for example food supplies (protein rich fraction, oil, corn steep liquor, and high fructose corn syrup), animal feed (processed cake, dry distillers grains and solubles, gluten meal) and paper.⁸⁷

^a IEA (International Energy Agency) Bioenergy Task 42 on Biorefineries. Minutes of the third Task meeting, Copenhagen, Denmark, 25 and 26 March (2008).

^b From: report published by the European Renewable Ethanol association ePURE published in 2015. Data looked up on 16.11.2018 via https://epure.org/media/1215/epure_state_industry2015_web.pdf

The use of edible plant material as feedstock for 1G biorefineries soon generated concerns that this practice conflicts with the land use and supply of human food sources. Thus, the focus of research in the development of biorefineries has changed from using food plants to using non-edible lignocellulosic biomass such as agricultural residue, forest residue, municipal solid waste, industrial waste and dedicated energy crops for the production of fuels and chemicals.⁸⁸

Numerous routes for the conversion of lignocellulosic biomass to biofuels are known, which can be grouped into two main concepts of biomass valorisation. In the first known route biomass is either subjected to pyrolysis or gasification to produce synthesis gas (mixture of CO and H₂). The pyrolysis route is also called biomass-to-liquid (BTL) process and is a thermochemical route of biomass valorisation. The produced synthesis gas is the starting material to catalytically synthesize fuels via the Fischer-Tropsch reaction or by biological conversion. The second route uses microorganism to ferment cellulose or hemicellulose for the production of fuels and chemicals.⁸⁹ For a comparison of the feedstocks of the 1st and 2nd generation biorefinery and their products Figure 1-8.

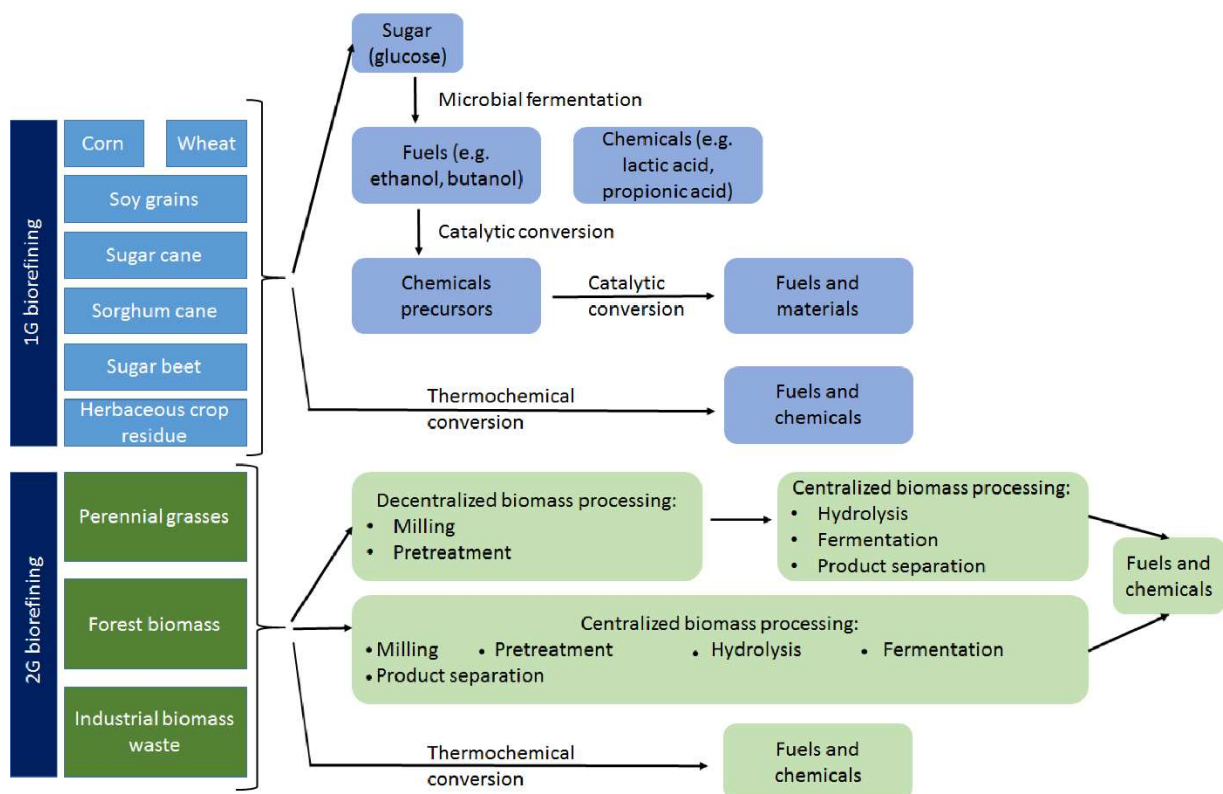


Figure 1-8. Biomass feedstocks used in the 1st and 2nd generation biorefinery, valorisation steps and their products.⁹⁰

An essential premise of a commercially competitive biorefinery is the cheap supply of biomass. Therefore, this supply chain should include several key steps: collection and storage of biomass, pre-processing such as pelleting or briquetting, transportation to the biorefinery plant and post processing at the biorefinery.^{91, 92} These steps will directly influence the cost of the feedstock delivery. There are a few major challenges for the transportation of biomass to biorefineries due to the structural nature of biomass. These challenges include texture variances of the biomass, seasonal availability, low bulk density, and distribution over a large area.⁹³ Additionally, the transportation cost is also influenced by the following factors: moisture content of the biomass, available infrastructure and the mode of transportation.⁹⁴ These factors mentioned above will influence the final cost of the bioethanol produced by a biorefinery plant, but the price of the end product will also be a subject to the size of the biorefinery plant.⁹⁵

It is widely discussed whether regional biomass processing depots (RBPDP) or processing biomass at centralised biorefineries will save more greenhouse gas emissions. It was argued that RBPDP has some advantages over the centralised approach such as a steady supply of biomass throughout the year (not depending on harvest season). This is made possible because the biomass is processed (size reduction, pretreatment and densification) near the field in a decentralised processing facility, stored there and transported when needed.^{96, 97} Life cycle analyses were prepared for the decentralised and centralised approach of biorefineries and it was reported that RBPDP saves more greenhouse gas emissions than the centralised processing of biomass.^{98, 99} However, there are some challenges for setting up an RBPDP that have to be managed. These include that small scale operations often have higher process costs than large scale ones, and that investments in new rail infrastructure or small scale biorefinery plants have to be made. In addition, further investigations in the whole biorefinery process (size reduction, pretreatment and densification) and in establishing its infrastructure have to be made.¹⁰⁰

2.2 Structure and properties of lignocellulosic biomass

As already mentioned above, lignocellulosic biomass is a composite material of three biopolymers that are assembled in a complex matrix (Figure 1-9). The individual biopolymers are composed of different monomers which gives each biopolymer a different chemical structure and different properties. The biopolymers form an interwoven matrix in native biomass that serves the purpose of stabilising the plant and proves to be highly recalcitrant to depolymerisation and decay by fungi.¹⁰¹

In general, recalcitrance of lignocellulose is associated with various properties of the biomass structure. Important factors are the degree of lignification of the plant (largely determined by the age

of the plant with younger plants generally showing less lignification), the protection of the carbohydrates by lignin and the available surface area of cellulose for enzyme degradation. The structure and composition of these three components determine the digestibility of lignocellulose and its subsequent conversion during physical, chemical and biological treatments.¹⁰²

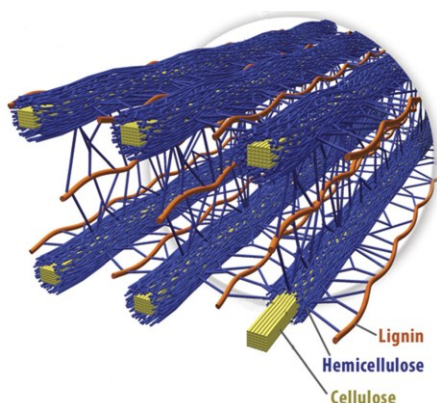


Figure 1-9. Schematic representation of arrangement of the three biopolymers in the cell walls of lignocellulosic biomass.²¹

2.2.1 Cellulose

Cellulose makes up 35 – 50 wt% of the biomass, thus being the largest single component of lignocellulose. Cellulose is a linear polymer that consists only of glucose units which are linked by 1-4- β glycosidic bonds (Figure 1-10). This results in a stretched chain conformation. The chains are linked into flat sheets via inter- and intramolecular hydrogen bonds between the chains. The linear conformation of the glucose chains enables the packing of cellulose strands which results in the formation of partly crystalline fibrils of 36 cellulose chains.²¹ Native cellulose exists in the I_α and I_β confirmation which enables three hydrogen bonds per glucosyl unit to form. Two of these are intramolecular bonds and the third one bonds intermolecularly to a neighbouring cellulose molecule in the same sheet. The interaction between sheets is thought to occur mostly via van der Waals forces which contribute significantly to the stabilisation of the cellulose fibrils.¹⁰³

Research into the ultrastructure of cellulose has shown that not only two but actually six cellulose polymorphs exist which can be interconverted by chemical or heat treatment. The polymorphs are I_α , I_β , II, III₁, III₁₁, IV₁ and IV₁₁¹⁰⁴ and differ in the way the cellulose chains are packed.¹⁰⁵ Cellulose has the highest degree of polymerisation (DP) amongst the lignocellulosic polymers, reaching a DP of 10 000 or higher.¹⁰⁶

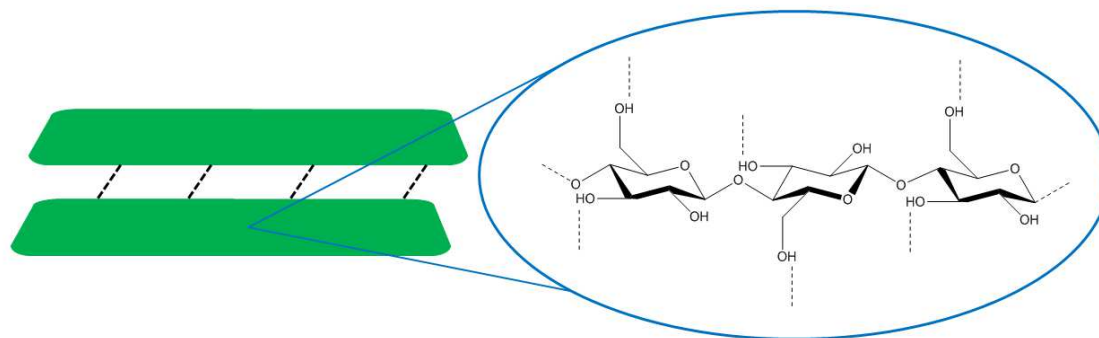


Figure 1-10. Stretched cellulose chains consisting of 1-4- β glycosidic connected glucose monomers. The chains are linked via hydrogen-bonds to form fibrils.²¹

The high degree of polymerisation and the aggregation of cellulose into fibrils is the cause of the water insolubility of cellulose (although glucose and short oligomers are water-soluble) and recalcitrance to enzymatic or chemical hydrolysis.^{107, 108} Discussions are still ongoing on the structure of cellulose with regard of the degree of crystallinity. The analytical technique of choice to answer this question is wide-angle X-Ray scattering and diffraction studies showed light and dark areas along a cellulose microfibril. These areas were attributed to crystalline and amorphous regions in the cellulose polymer.¹⁰⁴ However, it was discussed by Chanzy¹⁰⁹ that a slight curve in the cellulose microfibril brings specific domains in and out of the Bragg diffraction conditions. Thus producing the light and dark areas along the microfibril axis.

2.2.2 Hemicellulose

Hemicellulose consists of a number of polysaccharides composed of hexose sugars (glucose, mannose, galactose) and pentose sugars (xylose, arabinose), with a degree of polymerisation of around 100 – 200 (Figure 1-11).¹¹⁰

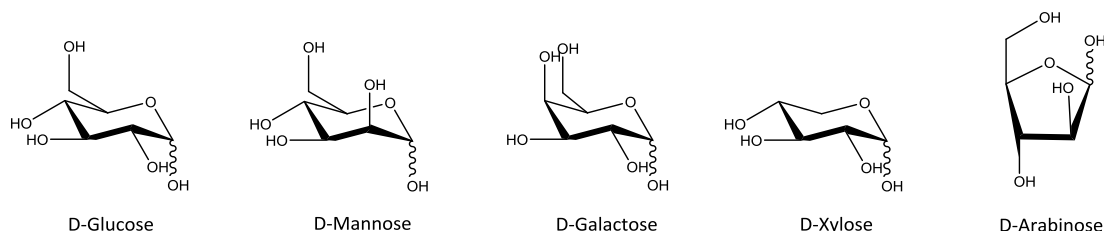


Figure 1-11. Monomers of the hemicellulose biopolymer.¹¹⁰

Around 25 wt% of biomass is built of hemicellulose.¹¹⁰ In contrast to the cellulose polymer, hemicellulose polymers can be branched and may contain various functionalities such as methyl and acetyl groups or acids (cinnamic, glucuronic and galacturonic acid). Hemicellulose acts as a matrix material which binds non-covalently to the surface of cellulose fibrils, thus holding them in place. It has been suggested by Hansen and Björkman¹¹¹ that the hydrophobic groups of hemicellulose increase the affinity of it to lignin which contributes to the cohesion between the three lignocellulosic biopolymers. The composition of hemicellulose varies depending on the lignocellulose type, with xylose being the most common monomer in grasses and hardwood, but mannose being the major hemicellulose sugar in softwood.²¹

2.2.3 Lignin

Lignin is biosynthesised from up to three monomers which are coniferyl, sinapyl and *p*-coumaryl alcohols (e.g. monolignols), and is a three dimensional, amorphous, partly aromatic, water-insoluble polymer (Figure 1-14).¹¹² The individual monomers are incorporated into the lignin structure and are then identified by the degree of substitution of methoxy groups on the aromatic ring and are called guaiacyl (G), syringyl (S) and *p*-hydroxyphenyl (H) subunits (Figure 1-12).¹¹³

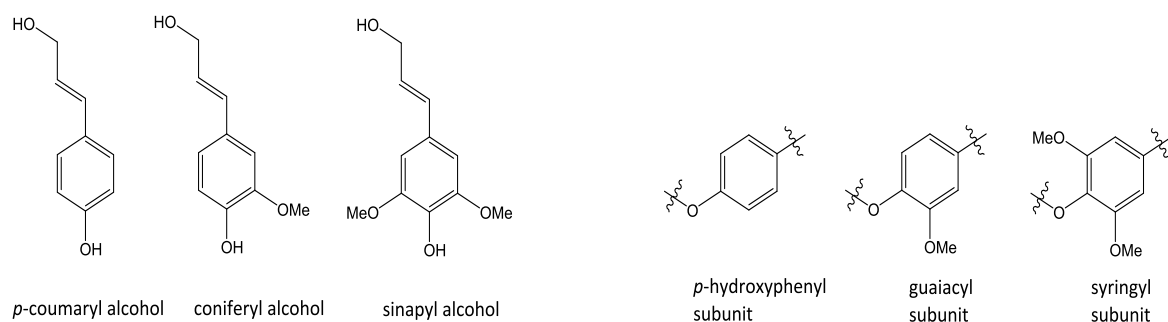


Figure 1-12. Phenolic monomers and subunits of lignin.¹¹³

The monolignols are synthesized from Phenylalanine through the general phenylpropanoid and monolignol-specific pathways. Lignin is biosynthesised in the plant cell wall via radical polymerisation of those monomers. The polymerisation consists of the following two steps: (i) oxidative radicalization of phenol subunits (dehydrogenation) and (ii) combinatorial radical coupling. The created free electron on the phenol is delocalized in the aromatic system which results in the phenolic radical being relatively stable. The coupling of two phenolic radicals forms a (dehydro)dimer and a covalent bond

between two aromatic subunits (Figure 1-13). It was found that monolignols are most reactive in the β position which results in the formation of the β -O-4, β -5 and β - β dimers. The next step in the polymerisation is again the dehydrogenation (of the dimer) to a phenolic radical followed by the radical coupling with another monomer.¹¹³ Coupling of lignin oligomers is relatively common in G-lignins and results in the formation of 5-5 interunit linkages. However, this type of coupling is rare in S/G-lignins.^{114,115} This leads to a polymer that is highly heterogeneous and random regarding the degree of polymerisation, monomer composition and branching.

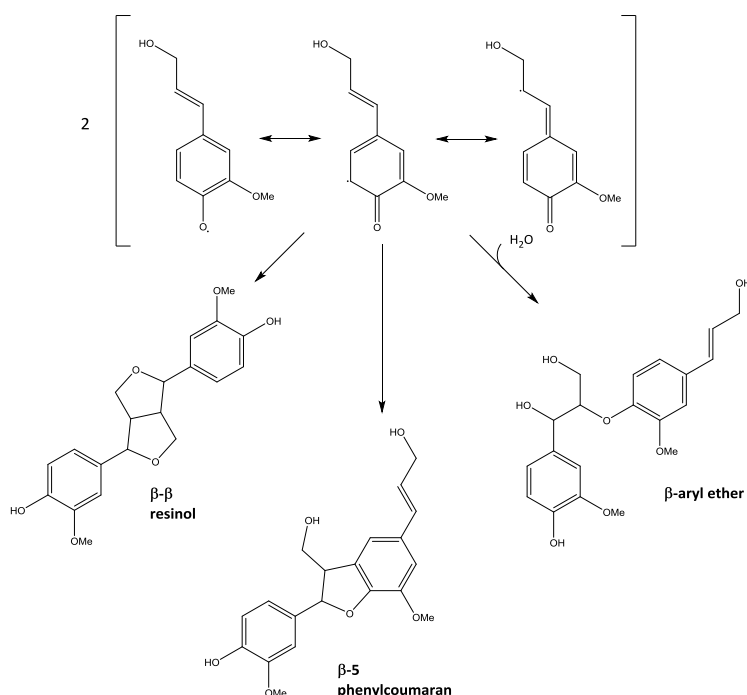


Figure 1-13. Resonance forms of dehydrogenated coniferyl alcohol and dimerization of two dehydrogenated coniferyl alcohol monomers.¹¹³

As with hemicellulose, the composition of lignin differs between grasses (mostly guaiacyl units with minor amount of p-hydroxyphenyl units), softwood (predominantly guaiacyl units) and hardwood (guaiacyl units plus a large amount of syringyl units). In addition, also the molecular weight and amount of lignin vary between the different types of lignocellulosic biomass, with softwoods having the highest lignin abundance and grasses having the lowest.¹¹² In contrast to the cellulose polymer, the structure of the lignin polymer does not show a particular sequence or order.¹¹⁷ It is important to note that the chemical structure of lignin affects the delignification chemistry and plays a major role in biomass deconstruction.¹¹⁸

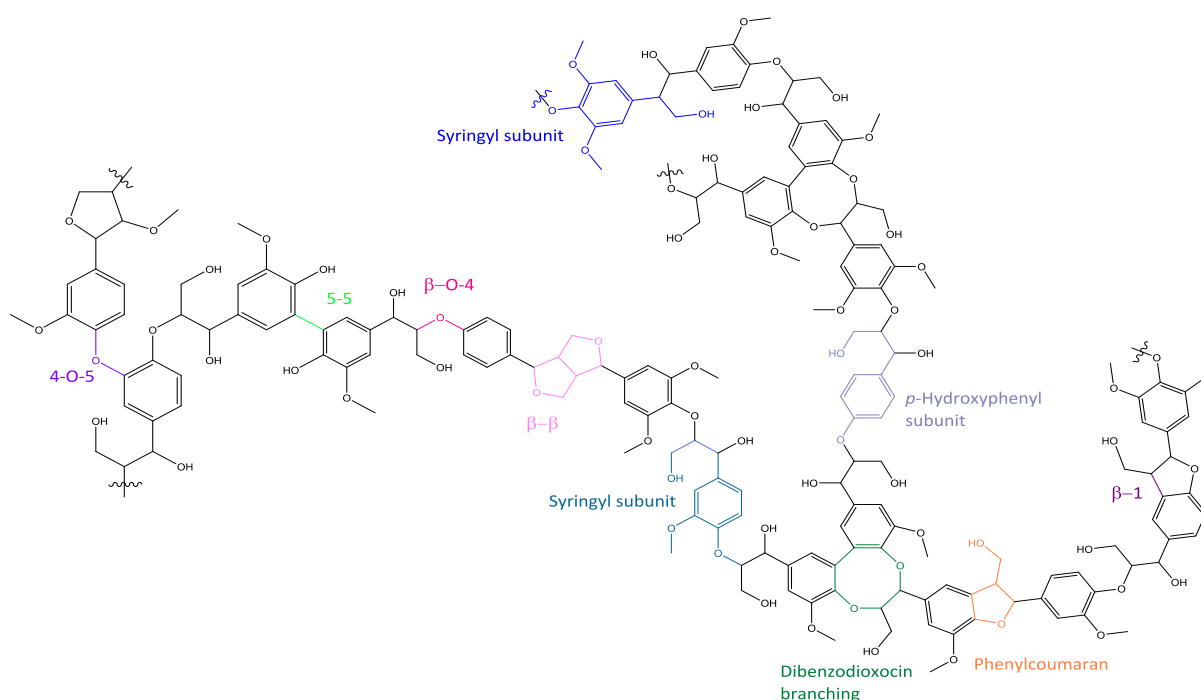
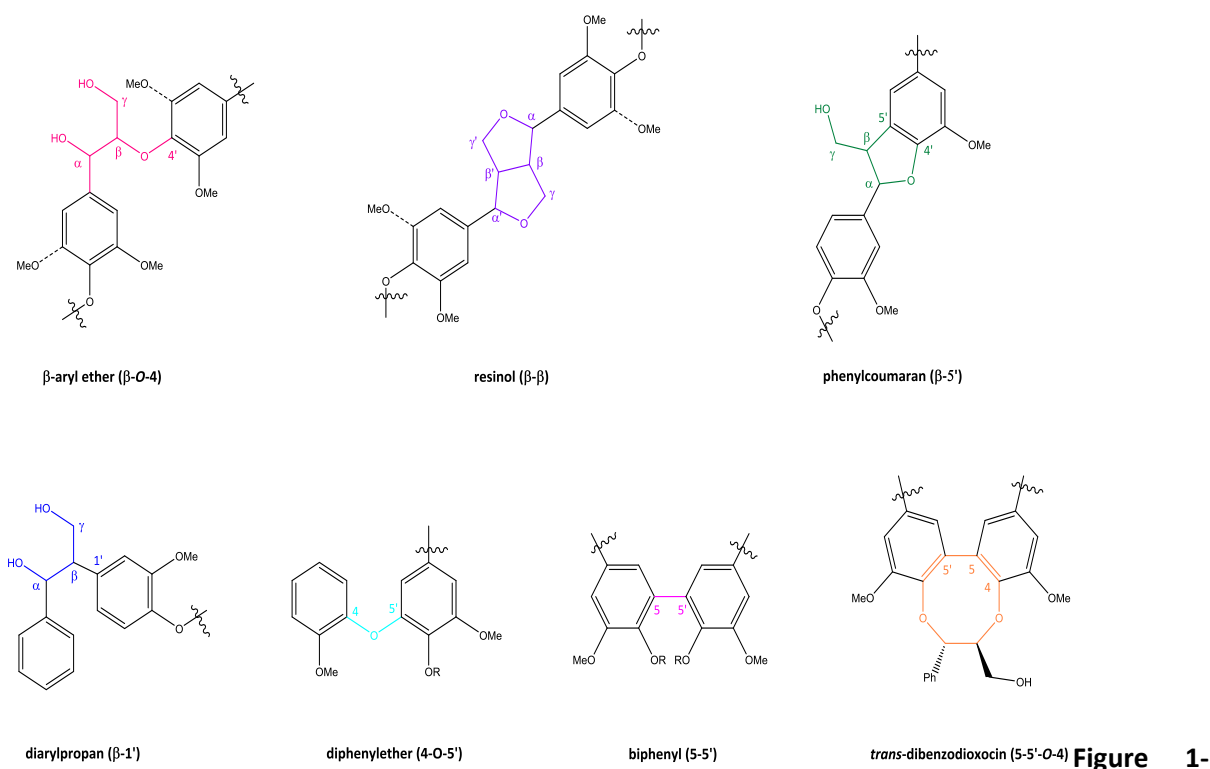


Figure 1-14. Example structure of softwood lignin.¹¹²

The lignin biopolymer contains a wide range of C-O and C-C linkages (β -O-4, 5-5, β -5, 4-O-5, β -1, dibenzodioxocin and β - β) that connect the aromatic subunits, with the β -O-4 ether linkage being the most common one (ca. 50 % of inter-subunit bonds).¹¹⁶ Figure 1-15 shows a summary of the most common interunit linkages in present lignin. The guaiacyl subunit is not methoxylated at the C5 position and is known to form C-C bonds on the C-5 position resulting in cross-linking of the aromatic ring. These C-C bonds cannot be easily hydrolysed by acid or base due to the stability of this bond, which makes delignification of softwoods more difficult than for grasses or hardwoods.¹¹⁶

Lignin becomes part of the wood composite after plant growth has ceased, providing structural stability, water-proofing and resilience.⁹¹ This function is provided because lignin is not only entangled via weak hydrogen bonds or other interactions with cellulose and hemicellulose but also forms covalent bonds and cross-links to hemicellulose. These cross-links (lignin-carbohydrate complexes or LCC) vary depending on the type of lignocellulosic biomass. In grasses, lignin and hemicellulose are linked *via* ferulic acid (Figure 1-16), which is initially bonded to hemicellulose *via* ester bonds and its aromatic ring is incorporated in the growing lignin network during lignification.^{119,120}



15. Common subunit linkages found in lignin.¹¹⁶

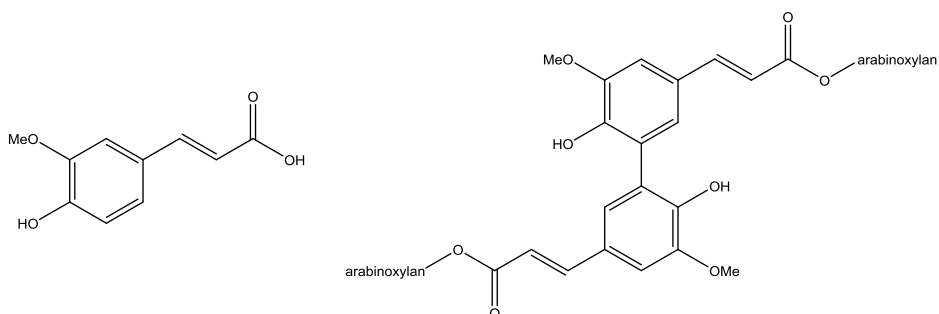


Figure 1-16. Ferulic acid and ferulic acid dimer crosslink.²¹

In contrast to that, direct complexes between lignin and carbohydrates are present in soft- and hardwood, which are also thought to form during lignification.¹²¹ It was found that an increased number of cross-links between lignin and carbohydrates provide not only a higher cell wall rigidity but also higher resistance to enzymatic digestion, leading to challenges in separation of the three biopolymers and further conversion in a biorefinery. In order to obtain an effective deconstruction process, these cross-links must be broken (e.g. by chemically hydrolysing the ester bonds between ferulic acid or hemicellulose and lignin).¹²²

In order to be able to utilize lignocellulosic biomass efficiently for the production of fuels and chemicals, the removal of lignin has proven to be a promising strategy. The presence of lignin in woody biomass guarantees its recalcitrance and decreases the efficiency of cellulose hydrolysis.¹¹⁸ This has led to the development of various pretreatment techniques over the last decades that aim to completely remove or relocate lignin from the biopolymer matrix.

2.3 Biomass pretreatment techniques

In order to be able to use lignocellulosic biomass as a renewable resource for the production of fuels and chemicals, a pretreatment step is required. The 2nd generation biorefineries utilize lignocellulosic biomass which is naturally resistant to physical and chemical attack.¹²³ One of the main products of biorefineries is the fuel bioethanol which is typically produced in a 3-step process from lignocellulose. The steps generally include pretreatment of raw lignocellulose, hydrolysis of cellulose to glucose and lastly fermentation of glucose to ethanol. In order to guarantee high yields of the cellulose hydrolysis step, the biopolymer matrix of lignocellulose needs to be disrupted to increase the access of cellulase enzymes to the substrate. This is typically accomplished in a process step that is commonly known as pretreatment (Figure 1-17).¹²⁴

Several goals of effective pretreatment have been developed over the years: it should ideally efficiently separate the three main components of lignocellulosic biomass (cellulose, hemicellulose and lignin) and provide easier access for fermentative organisms to the biopolymers. Its aim should be to remove lignin, preserve hemicellulose, reduce cellulose crystallinity as well as increase the porosity of the material.¹²⁵

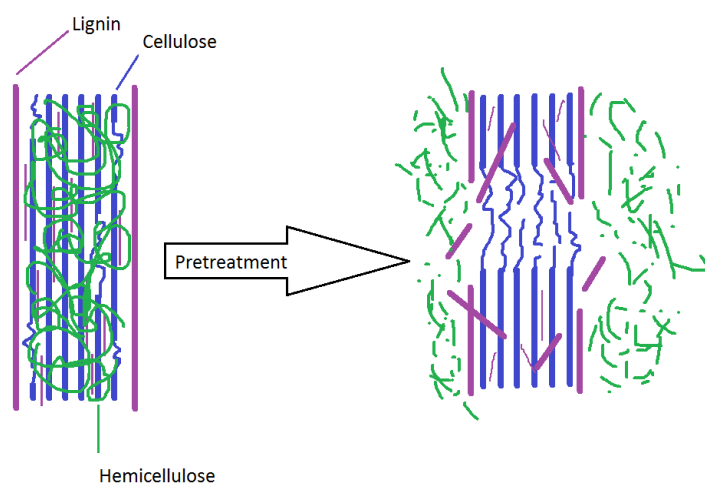


Figure 1-17. Schematic representation of the impact of pretreatment on the biopolymer matrix of lignocellulosic biomass.¹²⁶

Pretreatment processes can be divided in four different categories: physical process, physico-chemical process, chemical pretreatment and biological pretreatment.¹²⁷ Numerous pretreatment processes have been developed using various approaches. This thesis focuses on utilizing the hardwood *Salix*, which is why pretreatment methods most relevant for hardwoods are being highlighted in the following section.

Physical processes

1. Size reduction

The size reduction of biomass particles is an important step in the biorefinery process and serves the purpose of increasing the accessible surface area of the biomass material. Biomass size reduction is a two-step process, with the first step being a mechanical size reduction (chipping of wood logs) and the second step being a further size reduction from wood chips to particles (grinding or milling of wood chips).¹²⁸ Typical particle sizes of woody biomass after size reduction are 5 – 50 mm (after chipping) or 0.1 – 10 mm (after grinding and milling). However, the size reduction of biomass can be energy intensive,¹²⁹ depending on the kind of feedstock and final particle size, thus not meeting the criteria of an ideal biorefinery.

A possible solution to this problem is under investigation, a process that is called mild torrefaction. This new approach of biomass pretreatment improves the grindability of biomass fibres which reduces the energy amount needed for grinding of the biomass feedstock. Torrefaction also increases the accessibility of biomass to enzymes for hydrolysis. However, biomass pretreated *via* torrefaction only shows ethanol yields in the range of untreated biomass, which means that further research is still needed in this area.¹³⁰ However, the energy requirements also have to be considered, and a full energy balance needs to be conducted to understand the benefits or downsides of this pretreatment method.

Physico-chemical processes

1. Steam explosion

The most commonly used method for pretreatment of lignocellulosic biomass in industrial applications is steam explosion.¹³¹ This pretreatment method uses high-pressure saturated steam to treat biomass, which is followed by a sudden reduction of the pressure in the reaction vessel. This

leads to an explosive decompression of the biomass material. Reaction conditions for steam explosion typically include a temperature range from 160 – 260 °C with reaction times from several seconds to a few minutes of the high-pressure steam treatment.¹²⁶ The steam explosion process causes hemicellulose degradation and lignin transformation due to the high reaction temperature and thus increases the glucose yield during cellulose hydrolysis. However, hemicellulose is also hydrolysed to oligosaccharides and monosaccharides by acids which are released during steam explosion pretreatment.¹²⁶

The efficiency of this pretreatment method is affected by the following factors: temperature, residence time, chip size and moisture content.¹³² The steam explosion pretreatment process can be improved by adding H₂SO₄, SO₂ or CO₂ (usually 0.3 to 3 wt%) which can lead to a decrease in reaction time and temperature, as well as a decrease in production of inhibitory compounds, an increase in the yield of cellulose hydrolysis and complete removal of hemicellulose.¹³³

2. Ammonia fibre explosion (AFEX)

Ammonia fibre explosion pretreatment of lignocellulosic biomass is similar to steam explosion pretreatment. It uses high temperatures and pressure and the sudden release of this pressure to pretreat lignocellulosic biomass. However, this pretreatment process uses liquid ammonia instead of water for the treatment of biomass. The typical reaction conditions for ammonia fibre explosion are 1 – 2 kg of ammonia per 1 kg of biomass, a reaction temperature of 90 °C and a reaction time of 30 min.¹²⁶ It was found that AFEX pretreatment can be applied to many biomass types such as alfalfa, wheat straw, and wheat chaff and can also significantly improve the fermentation rates of crops and grasses.¹³⁴ AFEX pretreatment removes almost no hemicellulose or lignin from the wood matrix and only a small amount of the solid material goes into solution. During AFEX pretreatment, hemicellulose is depolymerised to oligosaccharides and deacetylated.¹³⁵ It was found that the AFEX process is not very effective for lignocellulosic biomass which contains a higher lignin content.¹³¹ One disadvantage of this pretreatment process is that it is relatively expensive as the cost of ammonia increases the overall process costs.¹³⁶

3. Carbon dioxide explosion

Both already discussed physico-chemical pretreatment processes show need for improvement of the efficiency of the process. Steam explosion faces difficulties with sugar degradation due to high reaction temperatures whereas ammonia fibre explosion is expensive and not applicable to all

biomass feedstocks. It was therefore suggested to develop a new physico-chemical pretreatment process which improves both those issues using supercritical carbon dioxide explosion. Carbon dioxide was chosen because of its comparable size to water and ammonia molecules which enables carbon dioxide to enter small pores accessible to water and ammonia.¹²⁶ It was suggested that carbon dioxide could help to hydrolyse cellulose and hemicellulose. In addition, no sugar degradation occurs under the low temperatures used in the carbon dioxide pretreatment process. The sudden release of carbon dioxide pressure disrupts the cellulose structure which increases the surface area and thus improves hydrolysis.¹²⁶

Chemical pretreatment processes

1. Ozonolysis

This pretreatment process uses ozone at room temperature under atmospheric pressure to pretreat lignocellulosic biomass. The lignin content of the biomass is reduced during pretreatment which leads to an increase in digestibility of the pretreated material. However, degradation is mainly limited to lignin with hemicellulose being slightly affected, leaving cellulose unchanged. One advantage of this process is that no toxic waste is produced and it can be applied to many different types of biomass feedstocks. Additionally, the process is environmental friendly because ozone can easily be decomposed by using either a catalytic bed or high temperatures. However, large amounts of ozone are needed in this process which increases the process costs.¹²⁶

2. Acid hydrolysis

Acid hydrolysis pretreatment can be divided in concentrated acid hydrolysis and dilute-acid hydrolysis. Concentrated acid hydrolysis uses concentrated acids like H_2SO_4 or HCl which can lead to an improvement of enzymatic cellulose hydrolysis of lignocellulosic biomass.¹²⁶ However, these chemicals are hard to handle within a process due to their corrosive nature. Special reactors which are resistant to corrosion are needed for this pretreatment process thus making it very expensive.¹³⁷

In contrast to that, the use of dilute-acid pretreatment for lignocellulosic biomass has been successfully developed. The use of dilute sulphuric acid (concentration below 4 wt%) at different temperatures has been studied intensively due to its effectiveness in the pretreatment process and its low cost.¹²⁶ Dilute sulphuric acid pretreatment hydrolyses hemicellulose to monomeric sugars such

as xylose and others. Furthermore, pretreatment of lignocellulosic biomass with dilute sulphuric acid can also improve cellulose hydrolysis.¹³⁸

It was found that the use of dilute acid removes and recovers most of the hemicellulose as monomeric, dissolved sugars. The removal of hemicellulose from the wood matrix significantly improves digestibility of the remaining cellulose leading to glucose yields of almost 100 % (for complete hemicellulose hydrolysis).¹²⁷ Although sulphuric acid is the most studied acid for use in dilute-acid pretreatment, other acids such as phosphoric acid¹³⁹, nitric acid¹²⁶ and hydrochloric acid¹³⁹ are under investigation as well. Dilute-acid pretreatment is also widely applicable to many different biomass types ranging from hardwood to grasses and agricultural residues.¹²⁶ Dilute-acid pretreatment faces some disadvantages such as high process costs, the need of pH neutralization before further downstream processes can be performed and the negative influence of the pretreatment on enzymatic hydrolysis due to lignin droplet formation on the surface of the pulp¹⁴⁰ and the formation of enzyme inhibitory by-products such as furfural, 5-(hydroxymethyl)furfural (5-HMF) and formic acid.¹⁴¹

3. Alkaline hydrolysis

Sodium, potassium, calcium, and ammonium hydroxides can be used to pretreat lignocellulosic biomass. Alkali pretreatment processes use ambient reaction conditions (lower temperature and pressure compared to other pretreatment methods) which results in longer pretreatment times (hours or days compared to minutes or seconds) but also less degradation of sugars.¹⁴²

It was found that calcium hydroxide is an effective pretreatment agent. In addition, calcium can be recovered from an aqueous reaction system by neutralising it with carbon dioxide to yield insoluble calcium carbonate. In a subsequent step calcium hydroxide can be recovered by using lime kiln technology.¹⁴²

During lime pretreatment lignocellulosic biomass material (typical particle size of 10 mm or less) is sprayed with a lime and water mixture and stored in a pile for a certain period of time (hours to weeks). It was found that an increase in reaction temperature reduces the contact time.¹²⁶ Lime pretreatment removes the amorphous biopolymers (hemicellulose and lignin) from the wood matrix which increases enzymatic digestibility of the pulp.¹⁴³ It was suggested by Kim and Holtzapple¹⁴⁴ that an effective alkali pretreatment process should remove all of the acetyl groups (of the hemicellulose and lignin) and reduce the lignin content to about 10% in the treated biomass.

Alkaline hydrolysis pretreatment using an aqueous solution of sodium hydroxide causes swelling in the treated biomaterial which increases the external surface area of the material and decreases the degree of polymerisation as well as the crystallinity of cellulose. It is also able to break linkages between lignin and structural carbohydrates and to disrupt the lignin structure.¹²⁶ It was reported that dilute sodium hydroxide pretreatment and pretreatment using ammonia also increase the enzymatic digestibility of lignocellulosic biomass.¹⁴⁵

4. Oxidative delignification

Oxidative delignification pretreatment uses the peroxidase enzyme in combination with H_2O_2 to biodegrade lignin.¹²⁶ It was found by Azzam¹⁴⁶ that treatment of cane bagasse with H_2O_2 removed 50 % of the lignin and almost all of the hemicellulose from the wood matrix. In addition, high efficiency of glucose production from cellulose was also achieved.

5. Organosolvation Process

The Organosolvation process (Organosolv process) uses inorganic acid catalysts (H_2SO_4 or HCl) or organic acid catalysts (e.g. oxalic, acetylsalicylic and salicylic acids) in an organic or aqueous organic solvent mixture (e.g. methanol, ethanol, acetone, ethylene glycol, triethylene glycol or tetrahydrofurfuryl alcohol) to break the internal lignin and hemicellulose bonds.^{147–150} The Organosolv pretreatment simultaneously prehydrolyses and delignifies lignocellulosic biomass which leads to a high yield of xylose if acid is added to the system.^{126,150}

The most commonly used solvent system is a 50:50 mixture of water and ethanol. This process is called lignol process. The conditions of this process vary with the type of feedstock being used but will generally be in the following ranges: reaction temperature of 180 – 195 °C, reaction time of 30 – 90 minutes, ethanol concentration of 35 – 70 wt% and a liquor-to-solids ratio ranging from 4:1 to 10:1 (wt/wt) with the pH of the liquid ranging from 2.0 to 3.8.¹²⁶ During the pretreatment process cellulose is partially hydrolysed into smaller fragments which are still insoluble in the solvent mixture, whereas hemicellulose is hydrolysed into oligosaccharides, monosaccharides and acetic acid. Under the applied reaction conditions lignin is also hydrolysed into lower-molecular-weight fragments.^{126,150}

Biological processes

Biological pretreatment processes use microorganism such as brown-, white- and soft-rot fungi which are able to degrade lignin and hemicellulose.¹⁵¹ An advantage of this process is its low energy requirement for lignin removal from lignocellulosic biomass whilst lignin is also degraded during the pretreatment.¹⁵²

The different types of microorganisms degrade different biopolymers. Brown-rot fungi mainly digest cellulose while white- and soft-rot fungi degrade cellulose as well as lignin with lignin degradation by white-rot fungi occurring through peroxidases and laccase.¹⁵³ It was reported that white-rot fungi are the most effective for biological pretreatment of biomass.¹⁵⁴ However, biological pretreatment faces the downside of long process times (typically several weeks).¹⁵⁵ Combinations of biological pretreatment with other pretreatment technologies such as the Organosolvation process¹⁵⁶ or AFEX¹⁵⁷ have also been studied and it was reported that these methods improved the yield of recovered sugars.

3. Biomass pretreatment using ionic liquids

The need for efficient and cost-effective pretreatment methods has sparked constant development and innovation in this field of research. The discovery that molten *N*-ethylpyridinium chloride in combination with nitrogen-containing bases could have the potential to dissolve cellulose by Graenacher¹⁵⁸ in 1934 marked the beginning of research into ionic liquids as solvents for biopolymers (e.g. cellulose and lignin). This laid the foundation for the development of pretreatment processes of lignocellulosic biomass utilizing ionic liquids as solvents for either the whole biopolymer matrix (dissolution process) or individual biopolymers (fractionation process).

The next section of this chapter will discuss both pretreatment methods in detail. The work carried out in this thesis used the fractionation process to selectively extract lignin and hemicellulose from the lignocellulose. With regards to that the following discussion will focus on this pretreatment method.

3.1 Dissolution process

3.1.1 Ionic liquids as solvents for cellulose

Cellulose has long been recognised as a potential starting material for the production of biofuels¹⁵⁹ and composite materials¹⁶⁰. However, the high crystallinity, high degree of polymerisation and

aggregation into fibres of the polymer introduces a great challenge for the dissolution of cellulose and only a few solvents such as *N*-Methylmorpholine-*N*-oxide (NMMO)¹⁶¹ or concentrated phosphoric acid¹⁶² are known as solvents for cellulose. The use of these solvents in an industrial process introduces several challenges such as being unstable at elevated temperatures (NMMO) or very corrosive (phosphoric acid). The need for more stable and environmentally friendly cellulose solvents has moved the focus of research towards ionic liquids.

The patent issued by Graenacher inspired R. Swatloski¹⁶³ *et al.* to study the application of ionic liquids for the dissolution of cellulose. The study involved the following ionic liquids: 1-butyl-3-methylimidazolium [C₄C₁im]⁺ based ionic liquids with various anions such as chloride Cl⁻, bromide Br⁻, thiocyanate [SCN]⁻, tetrafluoroborate [BF₄]⁻ and hexafluorophosphate [PF₆]⁻. The authors found that the ionic liquids capable of dissolving cellulose contained small, hydrogen-bond acceptor anions such as Cl⁻, Br⁻ or [SCN]⁻. They suggested that the dissolution mechanism of [C₄C₁im][Cl] worked similarly to cellulose dissolving non-derivatising solvent systems such as *N,N*-dimethylacetamide/lithium chloride (DMAc/LiCl). The dissolution is thought to occur via disruption of the hydrogen bonding of the cellulose by the interaction of the chloride anion with the cellulose hydroxyl groups. The cellulose was recovered via addition of the anti-solvent water and displayed different properties compared to the original cellulose. The dissolution of cellulose reported by Swatloski is considered to be a milestone in the field. However, the cellulose had to be mechanically treated prior to dissolution in the ionic liquid.

H. Zhang¹⁶⁴ *et al.* developed the IL 1-allyl-3-methylimidazolium chloride [C=C₂C₁im][Cl] for the dissolution of cellulose without prior treatment or activation. This novel IL successfully dissolved cellulose at elevated temperatures with a maximum concentration of cellulose in IL solution of 14.5 wt%. The cellulose was precipitated upon addition of water as anti-solvent.

The role of water as anti-solvent was studied using computational methods by K. M. Gupta¹⁶⁵ *et al.* to improve the understanding of the interactions of the biopolymer and the anti-solvent on a molecular level. The authors discovered that the addition of water to cellulose/[CH₃COO]⁻ IL mixtures leads to the formation of hydrogen bonding between the acetate anion and water molecules. The addition of water decreases the number of hydrogen bonds between the cellulose OH-groups and acetate anions and new inter- and intramolecular hydrogen bonds form between cellulose chains. This results in aggregation and finally precipitation of the cellulose from the ionic liquid.

Structural characterization of the regenerated cellulose after precipitation with an anti-solvent showed a reduced crystallinity compared to the untreated cellulose as well as a transformation from cellulose I to cellulose II. The authors concluded that this can be explained by cleavage of

intermolecular and intramolecular hydrogen bonds which results in a destruction of the crystalline form of the biopolymer.¹⁶⁶

The discovery of imidazolium based ionic liquids as solvents for cellulose has revolutionised this research field leading to numerous ionic liquids being investigated for this task.^{21, 167} Ionic liquids that are capable of dissolving cellulose typically contain cations such as alkylimidazolium, alkylpyridinium, quaternary ammonium and quaternary phosphonium cations and are shown in Figure 1-18.

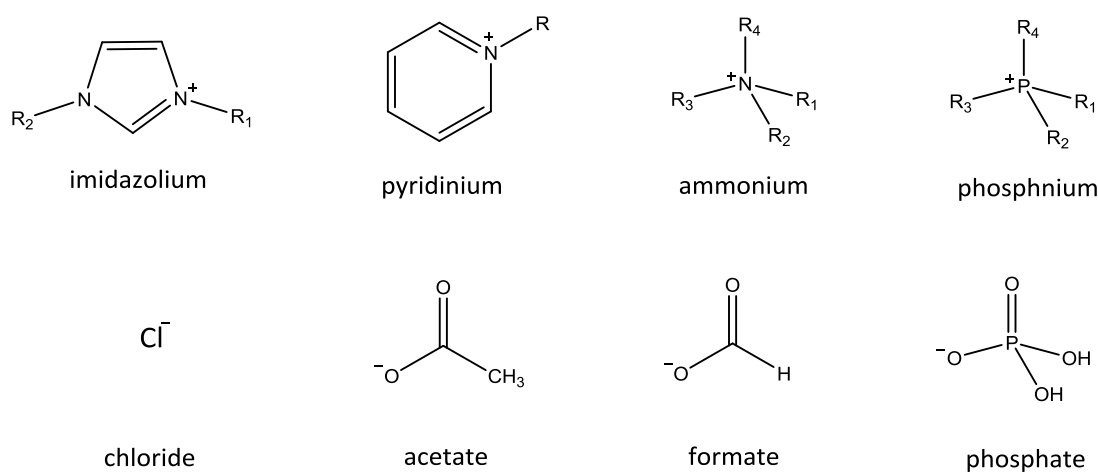


Figure 1-18. Cations and anions of ionic liquids used for cellulose dissolution.

Cellulose dissolving ILs usually contain small anions such as chloride or carboxylate or larger ones such as alkyl phosphates.²¹ Studies have been carried out to better understand which physicochemical properties of the ILs influences the capability to dissolve cellulose. One prominent method to study these properties is the Kamlet-Taft method. This method measures the ILs' hydrogen-bond basicity (β), hydrogen-bond acidity (α) and its interactions through dipolarity and polarisability (π^*). A study by Wang¹⁶⁸ *et al.* has found that all cellulose dissolving ILs are characterized by a high β value. Ionic liquids containing chloride anions have been found to dissolve the highest amount of cellulose (in terms of dissolved mass of cellulose), followed by the IL 1-ethyl-3-methylimidazolium acetate [C₂C₁im][CH₃COO].

In general, research has focused on investigating several key aspects that influence cellulose solubility in ILs. Several key factors for dissolution were identified, namely the structure of the cation^{167, 169–172} and anion^{170,173,174} of the IL or the role of organic co-solvents as additives^{175–180}. Several reviews have been published highlighting different factors of cellulose dissolution in ionic liquids as well as processing cellulose in these solvent systems.^{181–185}

Most studies focused on testing ILs for the capability of cellulose dissolution but some studies also investigated the mechanism of cellulose dissolution in ILs on a molecular level.^{186–188} A mechanism for the dissolution of cellulose in $[C_4C_1im][Cl]$ was proposed by Feng and Chen¹⁸⁹ (Figure 1-19).

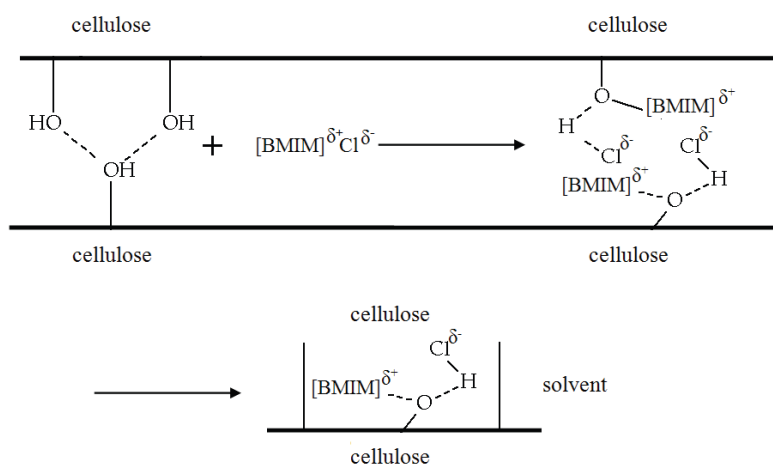


Figure 1-19. Proposed cellulose dissolution mechanism in $[C_4C_1im][Cl]$.¹⁸⁹

Upon addition of the cellulose to the ionic liquid, electron-donor electron-acceptor (EDA) complexes are formed between the solute and the solvent via the hydroxyl groups of the cellulose. The formation of these complexes results in breaking up the intra- and intermolecular hydrogen bonds of the cellulose chains which finally results in dissolution. A molecular dynamics simulation study by B. D. Rabideau¹⁹⁰ *et al.* further increased the understanding of the dissolution mechanism. The authors reported that the IL anions bind strongly to the OH groups of cellulose chains that are located on the surface of the cellulose bundle. These newly established hydrogen bonds then form negatively charged complexes, which then weakens the intermolecular H-bonding between cellulose strands. This leads to greater flexibility of the cellulose strands giving rise to the cation of the IL being able to insert between the strands which subsequently leads to dissolution.

Much of the work done to understand the dissolution mechanism and interaction between cellulose and ionic liquids has been done experimentally, but there is also a growing field that tries to tackle this problem using computational studies.¹⁶⁵

The solubility of cellulose in ILs does not only depend on the choice of cation or anion, but also significantly relies on the water content in the solvent system. Generally, the dissolution has to be undertaken under anhydrous conditions (water content in the system below 1 wt%). M. Mazza¹⁹¹ *et al.* investigated various cellulose/ionic liquid solute/solvent systems and the behaviour of these solutions upon addition of different amounts of water. Interestingly, they found that the precipitation

of cellulose from solution occurred at different amounts of water for different ILs highlighting the uniqueness of each solute/solvent system.

3.1.2 Ionic liquids as solvents for lignocellulose and lignin

Kraft pulping is the common pretreatment process applied in industry nowadays to yield cellulose. However, this process does not conform with most concepts of sustainability due to the use of environmentally hazardous chemicals such as sodium sulphide (Na_2S). In 2007, Fort¹⁹² *et al.* reported a novel method to yield pure cellulose that is considered more environmentally friendly and sustainable. This method uses ionic liquids to dissolve the whole lignocellulosic material of different hardwoods and softwoods, and a multiple-step process to separate the individual components of the dissolved lignocellulose. The cellulose was recovered upon addition of an anti-solvent (1:1 acetone-water, dichloromethane or acetonitrile). The product showed to have similar physical properties, processing characteristics and purity compared to pure cellulose samples subjected to similar treatment.

A study published by Kilpeläinen¹⁹³ *et al.* in the same year investigated the solubility of hardwoods and softwoods in different imidazolium based ILs previously applied for cellulose dissolution. The authors reported that lignocellulose is completely soluble in ionic liquids such as $[\text{C}=\text{C}_2\text{C}_1\text{im}][\text{Cl}]$ and $[\text{C}_4\text{C}_1\text{im}][\text{Cl}]$. The addition of water as anti-solvent led to recovery of the dissolved lignocellulose in form of an amorphous material.

These early studies on lignocellulose solubility can be considered a milestone in the field, although the solubility of lignocellulose in those solvent systems still remained relatively poor (8 wt% were the maximum that was achieved for sawdust in $[\text{C}_4\text{C}_1\text{im}][\text{Cl}]$ and $[\text{C}=\text{C}_2\text{C}_1\text{im}][\text{Cl}]$). Additionally, the lignocellulose was usually subjected to extensive mechanical treatment such as grinding prior to dissolution in ILs. Nevertheless, these findings lay the foundation for intensive research in this field. Many research groups studied numerous ILs to increase the overall solubility of lignocellulose. A comprehensive overview of ILs used for biomass dissolution was recently published by A. Rosatella and C. Afonso¹⁹⁴.

Another breakthrough in the field was reported again by Rogers and co-workers in 2008.¹⁹⁵ The authors reported complete dissolution of southern yellow pine or red oak at 110 °C using the IL 1-ethyl-3-methylimidazolium acetate $[\text{C}_2\text{C}_1\text{im}][\text{CH}_3\text{COO}]$. Prior to dissolution a mild grinding treatment

was performed. The chosen IL displayed a greater basicity compared to the ILs mentioned above. Rogers *et al.* suggested that this property might aid the dissolution process. The successful dissolution of biomass in $[\text{C}_2\text{C}_1\text{im}][\text{CH}_3\text{COO}]$ means that a lower energy demand for a potential process is required, since only mild grinding was needed and no additional thermo-mechanical pulping as in previous pretreatment processes. Interestingly, the dissolution of the lignocellulose in the IL was found to have a significant effect on the structure of the recovered cellulose. XRD patterns revealed that the crystallinity of the regenerated cellulose was decreased and the patterns were typical for cellulose II as compared to cellulose I which is the native form of cellulose in untreated biomass. Since then many studies investigating the effect of ionic liquid pretreatment on cellulose crystallinity have been published.^{196–200}

These early studies have significantly contributed to increase the understanding of the capability of ionic liquids to dissolve either cellulose or the whole lignocellulose matrix. However, the effects of the dissolution pretreatment on the cell wall structure of the lignocellulosic material remained unknown. To deepen the understanding of the effect of ionic liquids on the cell structure of lignocellulose, Singh²⁰¹ *et al.* studied the dynamic solubilisation mechanism of switchgrass subjected to pretreatment with $[\text{C}_2\text{C}_1\text{im}][\text{CH}_3\text{COO}]$ using the auto-fluorescence of plant cell walls. They could show that in the first minutes of lignocellulose being subjected to $[\text{C}_2\text{C}_1\text{im}][\text{CH}_3\text{COO}]$ the secondary cell walls swelled. This resulted in a complete disruption of the cell wall structure after a treatment time of 2 hours at 120 °C (Figure 1-20). Additionally, the lignin-rich parts of the plant cell walls disintegrated shortly after being contacted with the ionic liquid. Upon addition of water as anti-solvent, lignin-free fibrous structures were recovered from the ionic liquid/water mixture. Analytical investigations confirmed that these regenerated fibres are cellulose. However, the fibres displayed a significant change in cellulose morphology such as complete loss of ordered structure and crystallinity.

As discussed above, the dissolution of cellulose in ionic liquids such as $[\text{C}_2\text{C}_1\text{im}][\text{CH}_3\text{COO}]$ or $[\text{C}_4\text{C}_1\text{im}][\text{CH}_3\text{COO}]$ is significantly affected by moisture present in the system. It was found that the water content in the solvent system also impacts the solubility and degree of delignification of lignocellulose in the same ionic liquids. A study by T. V. Doherty²⁰² showed that the addition of only 5 – 10 wt% of water to the system led to a decrease in delignification of maple wood flour as well as an increase in the crystallinity index of the regenerated cellulose (compared to pretreatment under anhydrous conditions). The ability of the anion to form hydrogen bonds with water was speculated to impact its ability to disrupt the crystalline matrix of the cellulose in the biomass.

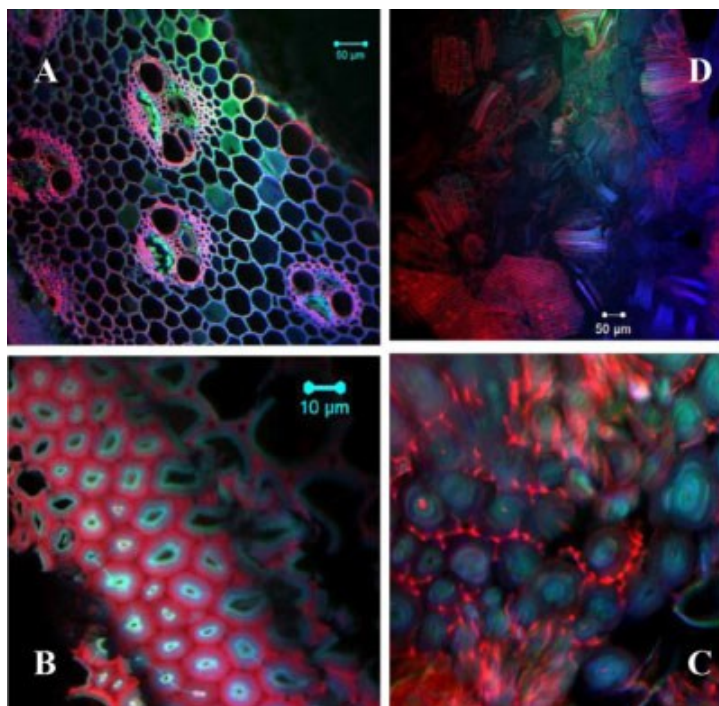


Figure 1-20. Confocal fluorescence images of switchgrass stems before pretreatment (a), and after 20 minutes (b) and 50 minutes (c) of pretreatment with $[\text{C}_2\text{C}_1\text{im}][\text{CH}_3\text{COO}]$. Complete disruption of secondary cell wall structures was observed after pretreatment for 2 h (d).²⁰¹

Residual water of the biomass is considered a disadvantage of the dissolution pretreatment approach. Freshly harvested biomass contains around 50 wt% of water²⁰³ and extensive drying of the feedstock as well as the ionic liquid is required for successful pretreatment. This introduces major cost factors for energy, storage and equipment as well as logistic challenges when scaling up the dissolution pretreatment to a commercial biorefinery scale.

A wide variety of different feedstocks was studied for dissolution in ionic liquids, with studies proving that grasses, hardwoods and softwoods can be (partially) dissolved in this novel class of solvents. Not only did the amount of different feedstocks increase quickly, but also the range of ionic liquids tested for their effectiveness in biomass dissolution. In 2009, Zavrel²⁰⁴ *et al.* introduced a high-throughput screening method which allows for a fast comparison of the dissolution capability of various ILs for either cellulose or lignocellulose dissolution.

Ionic liquids synthesised from a wide range of cations and anions have been studied empirically as possible solvents for complete dissolution of lignocellulosic biomass. However, selection criteria to determine the best solvent system were not established until 2014. Weerachanchai²⁰⁵ *et al.* suggested the use of Hildebrandt solubility parameters to define the best solvent for biomass pretreatment using

a systematical approach. The Hildebrand solubility parameter is defined as a numeric value which indicates the strength of the molecular interaction between solvent molecules. It can be used as a tool to determine promising solvents (VOC and ILs) for various applications. Weerachanchai²⁰⁵ *et al.* studied mixtures of [C₂C₁im][CH₃COO] with organic solvents such as dimethylacetamide (DMA), dimethyl sulfoxide (DMSO), dimethylformamide (DMF) and ethanolamine in a 60/40 vol% ratio. It was found that this pretreatment increased cellulose digestibility and lignin extraction. Amongst the studied solvent mixtures the highest amount of lignin extraction was reached for the solvent system of [C₂C₁im][CH₃COO]/ethanolamine.²⁰⁶

The dissolution of lignocellulose in ILs has been intensively empirically studied. However, the individual role of the cation and anion in the dissolution process of the biopolymer matrix is still not completely understood. This can be attributed to the complex nature of both the solute and its interactions with the ionic liquids. To get a better understanding of the dissolution mechanism, many studies have focused on exploring the interaction of isolated biopolymers (mainly cellulose and lignin) with different cations and anions of ILs.

Studies have shown that the solubility of lignin in ILs is mainly determined by the anion of the IL, whereas the cation seems to have a much less pronounced effect. The property of the anion that proved to be most relevant for substantial lignin dissolution was reported to be a high hydrogen bond basicity. Additionally, it was described that the planar aromatic cation (such as pyridinium or imidazolium) seemed to mainly interact with the lignin aromatic subunits.²⁰⁷ Several ILs such as [C₄C₁im][Br] or [C₄C₁im][CH₃COO] with different water contents (0 – 60 %) were tested for the dissolution of lignin. Additionally, Hansen solubility parameters were found to be able to predict which IL/water mixtures are able to dissolve lignin.²⁰⁸ An interesting study regarding the solubility of lignin in polar IL/water mixtures was published by T. Akiba²⁰⁹ *et al.* The authors reported that the solubility of lignin increased after addition of water to ILs that are defined by moderate Kamlet-Taft β -values. The added water resulted in a change of the α and β values of the studied ILs as well as a shift in polarity of the solvent system which led to improved lignin solubility. The solubility of lignin in various ILs has also been reviewed several times.^{210,211}

The cost of ionic liquids is often considered one of the major economic factors when it comes to implementing an ionic liquid based biorefinery. To lower the price of the solvent for biomass pretreatment co-solvents are added to the ionic liquid. Several studies have empirically investigated different IL/co-solvent systems such as [C₂C₁im][CH₃COO] mixed with DMSO, DMA and DMF²¹² or [C₂C₁im][CH₃COO]/glycerol²¹³ for pretreatment.

In addition, molecular dynamics simulation studies were carried out to understand the effects of co-solvents (e.g. DMSO) and anti-solvents (e.g. water) on the dissolution of cellulose in ILs.¹⁸⁰ Generally, water is used as an anti-solvent and added after pretreatment to precipitate the biopolymers, but J. Shi²¹⁴ *et al.* could show that pretreatment of biomass using [C₂C₁im][CH₃COO]/water mixtures containing 20 – 50 % water gave similar glucose yields compared to using neat ionic liquid. However, the addition of even higher amounts of water (> 50 %) into the system resulted in a significant decrease of the glucose yield after pretreatment.

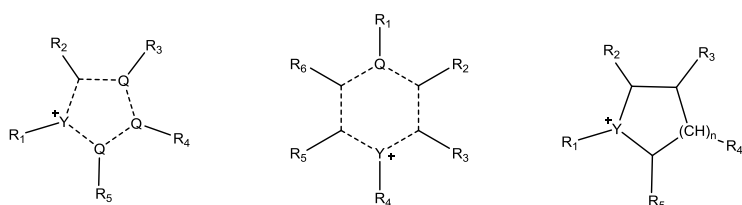
The field of research of complete dissolution of lignocellulose in ionic liquids is growing rapidly and several reviews have been published in recent years to summarize it.^{21,54,215,216} However, further discussion of this research area is beyond the scope of this thesis.

3.2 IonoSolv pretreatment

The research into ionic liquids for biomass pretreatment has led to the development of a class of ionic liquids that does not dissolve the whole lignocellulose matrix but acts as a selective hemicellulose and/or lignin solvent. This IL pretreatment method results in the fractionation of the biomass.²¹

J. Upfal²¹⁷ and co-authors were the first to report the use of ionic liquids to extract lignin from lignocellulosic biomass. The publication suggested the use of an ionic liquid-forming inorganic cation or an organic cation and a substituted or un-substituted aryl organic acid anion (Figure 1-21) to fractionate lignocellulosic material. The deconstruction of the biomass led to a lignin phase which was dissolved in the ionic liquid and a cellulose-rich pulp that was largely unaffected by the IL treatment. The lignin recovery was induced by precipitation of the polymer from the IL solution by addition of an anti-solvent or *via* change of pH. Additionally, the recovery and re-use of the IL were also mentioned in the patent

S. Varanasi²¹⁸ *et al.* issued a patent discussing the pretreatment of lignocellulosic biomass using an ionic liquid and the subsequent conversion of the resulting pulp into monomeric sugars. The developed pretreatment method did not completely dissolve the lignocellulose but extracted the lignin which resulted in an increase of the glucose and xylose yield of the enzymatic hydrolysis. The study used room temperature ionic liquids (RTILs) such as [C₂C₁im][CH₃COO], [C₄C₁im][Cl] and the novel IL 1-ethyl-3-methylimidazolium propionate [C₂C₁im][CH₃CH₂COO]. This important study could show that complete dissolution of biomass was not a necessity for high glucose yields. The results are particularly interesting, given that the used ionic liquids in this study are typically applied for pretreatment via complete dissolution of the biopolymer matrix.



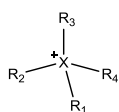
dotted lines represent single or double bonds

Q = N, CH, O or S

Y = N or P

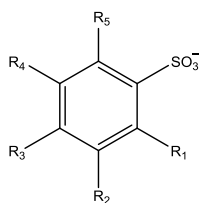
n = 1 or 2

R₁ to R₆ = H, aliphatic, aryl, aralkyl, alkylaryl, heteroaryl and haloaryl



X = N, P or S

R₁ to R₄ = aliphatic, aryl, alicyclic, aralkyl, heteroaryl and haloaryl



R₁ to R₅ = H, alkyl, aryl, alicyclic, aralkyl, alkylaryl, heteroaryl, haloaryl, halogeno and sulfonate

Figure 1-21. Cations and anions suggested by J. Upfal²¹⁷ *et al.* for the selective extraction of lignin and hemicellulose from lignocellulosic biomass.

The major difference between the two IL pretreatment methods is the biomass loading and the pretreatment atmosphere (Table 1-1).

Table 1-1. Comparison of two pretreatment methods utilizing the RTIL [C₂C₁im][CH₃COO].

Method developed by	Biomass loading	Pretreatment atmosphere	Pretreatment outcome
Swatłowski ¹⁶³ <i>et al.</i>	< 5 wt%	Under N ₂	Complete dissolution of lignocellulose
Varanasi ²¹⁸ <i>et al.</i>	33 wt%	Air	Redistribution or potential removal of the lignin on the pulp

The cost of the ionic liquid is considered one of the major factors influencing the economic viability of a biorefinery.^{219,220} The authors of the above mentioned patents showed great foresight regarding the development of biorefineries applying IL pretreatment and already studied the recyclability and reuse of the ionic liquids. Surprisingly, they found comparable results of the pretreatment outcome of fresh and recycled IL, showing that the IL can be reused.

The dissolution of hemicellulose and lignin from the biopolymer matrix was further explored by Lee²²¹ *et al.* who tested several ionic liquids for that purpose (Figure 1-22). The tested ionic liquids proved to be effective at biomass deconstruction. The authors reported that after 70 hours of pretreatment with [C₂C₁im][CH₃COO] at 90 °C, 86 % of the lignin was extracted and the pulp digestibility had increased from 46 % for raw wood flour to 96 %. Compositional analysis of the recovered pulp revealed that it mainly consists of carbohydrates (59 % cellulose and 30 % xylan) with only 3.2 % lignin.

This early study by Lee²²⁷ *et al.* was the first one to explore the effect of different ionic liquids on lignin extraction. In the same year, a study conducted by Tan²²³ *et al.* introduced a new IL as medium for lignin extraction. The IL consisted of [C₂C₁im]⁺ cations and a commercially available mixture of isomers of xylenesulfonate were used as the anions. This IL was studied for lignin extraction from bagasse and proved to be very effective with a reported high lignin recovery yield of 93%. The extracted lignin was then characterised via elemental analysis, solid state ¹³C-NMR, IR and TGA.

The effect of pretreatment severity (characterised by temperature and pretreatment time) on lignin removal and glucose release was studied by Arora²²⁴ *et al.* The authors of this study reported that the delignification of the lignocellulose increased with increasing pretreatment temperature. It is interesting to note that the authors also described a direct correlation between lignin removal and glucose yield of enzymatic hydrolysis. Furthermore, the fate of the hemicellulose was also investigated and it was suggested that this biopolymer was depolymerised into oligosaccharides. However, further depolymerisation of the hemicellulose oligomers to monomers was not observed.

The scope of ionic liquids studied for the extraction of lignin from lignocellulose grew rapidly, but the relation of their physicochemical properties to lignin extraction was still not well understood. The Kamlet-Taft-Parameters, which describe the polarity of a solvent, were measured for the 3 different imidazolium based RTILs [C₂C₁im][CH₃COO], [C₄C₁im][CH₃COO] and [C₄C₁im][MeSO₄] and related to their pretreatment performance in terms of lignin extraction.²⁰² The authors of the study showed that the hydrogen bond basicity (β) of the ionic liquid is a reliable parameter to predict the pretreatment efficiency of the solvent. A linear correlation between the hydrogen bond basicity and amount of lignin extracted was described. The pulps with a higher delignification also gave a higher sugar yield after enzymatic hydrolysis. These findings partially explains why [C₂C₁im][CH₃COO] and [C₄C₁im][CH₃COO]

(with a β value of greater than 1.0) are more effective in pretreatment than $[\text{C}_4\text{C}_1\text{im}][\text{MeSO}_4]$ ($\beta = 0.60$). A comprehensive study investigating the polarity of ionic liquids was undertaken by Ab Rani⁴⁰ *et al.* and can aid in the selection of ionic liquids for pretreatment.

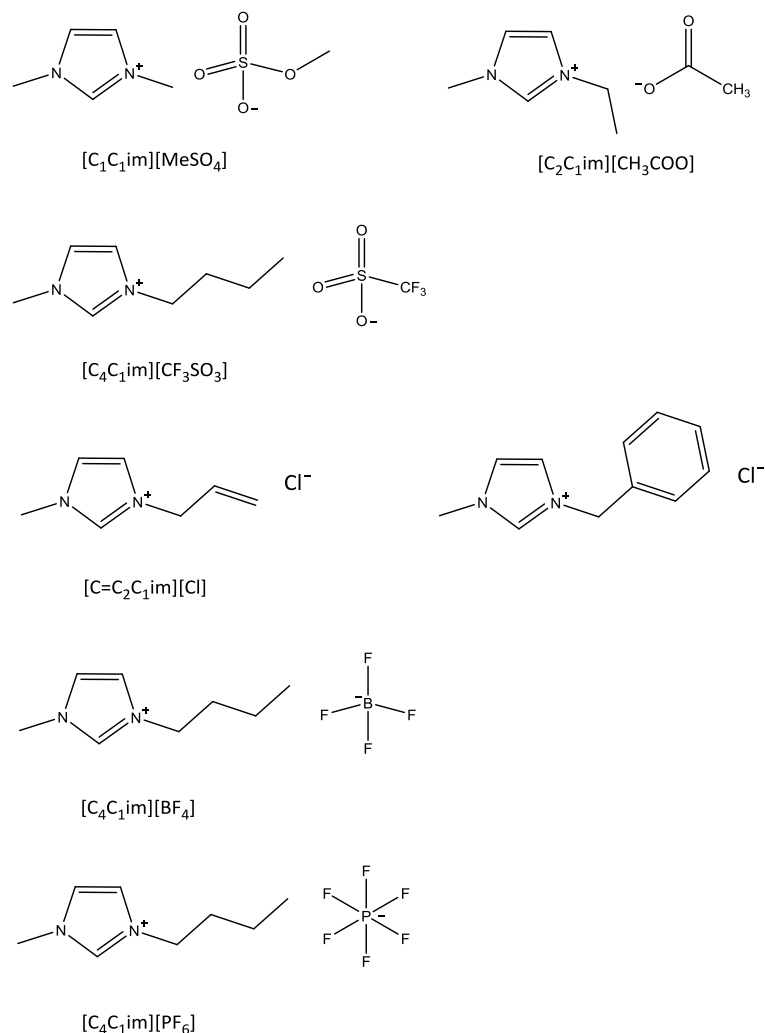


Figure 1-22. ILs explored by Lee²²¹ *et al.* for extraction of hemicellulose and lignin to produce a cellulose-rich pulp.

The field of research using ionic liquids for biomass fractionation grew rapidly and has been reviewed extensively in the recent years.^{21,225,226} The range of ionic liquids applied for pretreatment *via* deconstruction expanded quickly and several new IL solvent systems optimised for the extraction of lignin and/or hemicellulose were introduced for this purpose. Several cations such as pyridinium, 1-methylimidazolium and pyrrolidinium were combined with the acetate anion and their performance in the pretreatment of corn stover was investigated.²²⁷ The authors argued that the simple synthesis of the protic ionic liquid (PIL) involving low-cost reactants (3 amines and acetic acid) improves the economics of these ionic liquids compared to their aprotic counterparts (Figure 1-23). Unfortunately,

no absolute numbers are given by the authors due to lack of data on this subject. No reliable data could be found in other sources. However, this is a big step towards realising an economically competitive biorefinery applying ionic liquids as solvents. Protic ionic liquids are generally synthesised through a proton transfer from a Brønsted acid to a Brønsted base in dry or aqueous conditions.^{228,229}

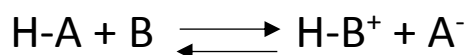


Figure 1-23. Synthesis of a protic ionic liquid *via* proton transfer from a Brønsted acid to a Brønsted base.²²⁸

After successful synthesis, protic ionic liquids are in equilibrium with their parent acid and base and have protons available for hydrogen bonding. This class of ionic liquids typically displays non-negligible vapour pressure. However, upon heating the proton transfer can be reversed and the more volatile neutral acid or base are formed which can lead to boiling rather than decomposition at elevated temperatures.²³⁰ The volatility of the ionic liquid was found to be indicated by the pK_a values of the reactants, with a large difference resulting in a more complete proton transfer and a resulting low volatility.^{231,232} Interestingly, protic ionic liquids synthesised from strong acids and strong bases behave much like aprotic ionic liquids. A less complete proton transfer results in a PIL with a higher volatility which can be evaporated under reduced pressure allowing for easier purification.²²⁹

The application of low-cost protic ionic liquid/water mixtures of triethylammonium hydrogen sulphate $[\text{N}_{2220}][\text{HSO}_4]$ or 1-butylimidazolium hydrogen sulphate $[\text{HC}_4\text{im}][\text{HSO}_4]$ was studied for the pretreatment of various feedstocks such as grasses, hardwoods and softwoods.^{7, 8, 233} The addition of water to acidic ILs decreases the viscosity of the solvent resulting in easier handling and does not appear to impact the performance of the ILs. The reuse and recyclability of the IL $[\text{N}_{2220}][\text{HSO}_4]$ was investigated and a repeatedly high lignin extraction and glucose yield of enzymatic hydrolysis was shown.²³⁴ Additionally, the price of these ionic liquids at a large scale was calculated to be 1.24 \$ per kg²³⁵ for $[\text{N}_{2220}][\text{HSO}_4]$, and their pretreatment performance was compared to $[\text{C}_2\text{C}_1\text{im}][\text{CH}_3\text{COO}]$.²¹⁹

This new generation of protic ILs proved to simplify the pretreatment method due to the high tolerance of the solvent system to moisture originated both from residual moisture in biomass or from addition of water to the IL. The addition of water to the ionic liquid resulted in a lower viscosity and a reduced amount of IL used during pretreatment. The mixture of 20 wt% water and 80 wt% IL (such as

[C₄C₁im][CH₃SO₄], [C₄C₁im][HSO₄], [HC₄im][HSO₄] or [N₂₂₂₀][HSO₄]) proved to yield high delignification and hemicellulose removal from the lignocellulose providing successful biomass fractionation.^{7, 8, 234} The authors suggested that the water plays a role in several chemical reactions occurring during the pretreatment such as hydrolysis of glycosidic hemicellulose linkages as well as lignin aryl ether linkages.

Another class of ionic liquids for pretreatment combined imidazolium cations such as [C₄C₁im]⁺ or [C₂C₁im]⁺ with acesulfamate anions (Figure 1-24) to successfully extract lignin from *Pinus radiata* and *Eucalyptus nitens*.²³⁶ However, it was found that the IL anion reacted with the lignin polymer during pretreatment resulting in IL loss and thus reducing the recyclability of this pretreatment solvent.

The search for an optimized protic ionic liquid for lignin extraction led to the synthesis of ILs containing unique cations or anions. P. Yan²³⁷ *et al.* tested the combination of tetraethylammonium [N₂₂₂₂]⁺ or choline [Ch]⁺ cations (Figure 1-24) with amine-sulfonate functionalized anions for the pretreatment of eucalyptus bark and N. Muhammad²³⁸ *et al.* introduced a nitrile functionality to imidazolium based cations and synthesised chloride ILs containing these cations for the pretreatment of bamboo biomass.

Growing concerns regarding the toxicity of ILs²³⁹ have led to the search for alternative ionic liquids with lower toxicity such as choline or amino acid based ILs (Figure 1-24). These 2nd generation ionic liquids were shown to successfully extract lignin from biomass.^{240–244} It was also described that choline based ionic liquids can tolerate substantial amounts of water without compromising effectiveness of pretreatment of rice straw. High lignin extraction of 58.2 % was reported when using a 50/50 mixture of the ionic liquid choline lysine [Ch][Lys] compared to 60.2 % lignin extraction when the neat IL was used.²⁴⁵

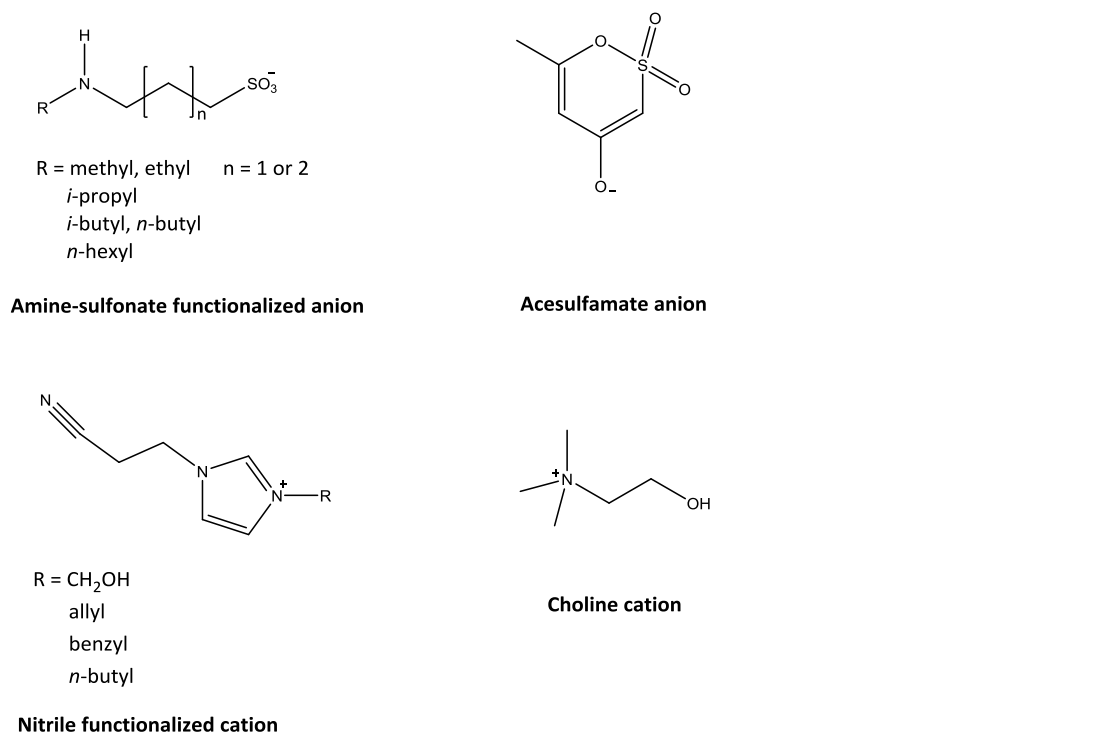


Figure 1-24. Examples of cations and anions used in 2nd generation ionic liquids for biomass pretreatment.

The 3rd generation of ionic liquids designed for lignin extraction from lignocellulose are switchable ionic liquids (SIL). These ILs are typically composed of inexpensive starting materials such as glycerol or monoethanolamine (MEA) and 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU). The formation of the ionic species is induced by addition of CO₂, SO₂ or CS₂ (Figure 1-25).^{246–248}

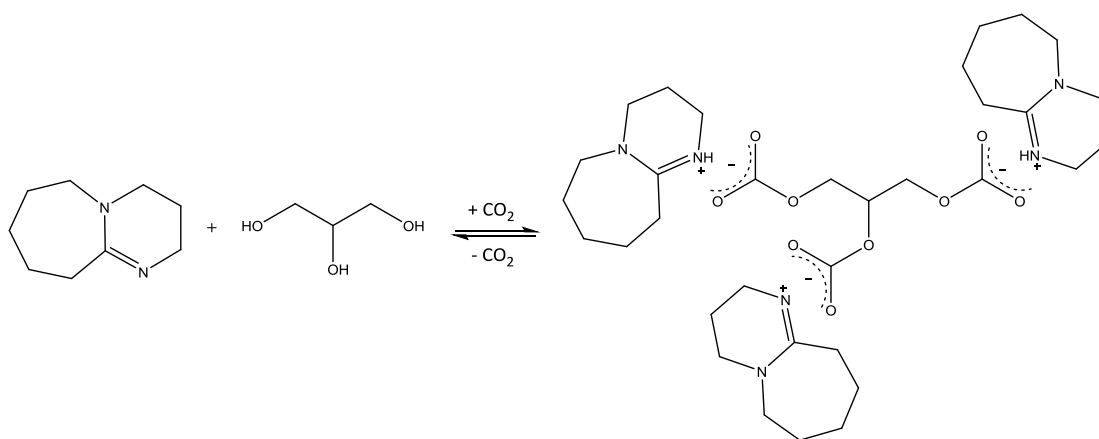


Figure 1-25. Example of a switchable ionic liquid synthesised from glycerol and DBU using CO₂ as trigger.²⁴⁷

The extraction mechanism of both lignin and hemicellulose from the biopolymer matrix on a molecular level using ILs is still not well understood. The literature on the fractionation of biomass with ILs generally focuses on optimization of the delignification of lignocellulose to achieve high glucose yields. Only very few studies give a hypothesis for the mechanism of lignin and hemicellulose extraction. E. Achiniu²²⁷ *et al.* showed that dissolution of hemicellulose is necessary for successful and high lignin extraction with protic ionic liquids. The authors argued that the dissolution of hemicelluloses leads to fibre disruption of the lignocellulose enabling the protic IL to extract lignin more efficiently. Furthermore, studies on pretreatment using $[\text{HSO}_4]^-$ containing protic ILs were able to show that hemicellulose gets dissolved into the IL solution and is subsequently depolymerised into monomeric sugars and partially further degraded to furfural or HMF.²⁴⁹

The extraction of lignin with protic ionic liquids is thought to alter the structure of the polymer. The structural properties of the isolated lignin (especially molecular weight and amount of aryl ether linkages present) significantly depend on the pretreatment severity. Increasing the pretreatment time and/or temperature will first lead to depolymerisation of the lignin chains *via* cleavage of β -O-4 bonds. Harsher pretreatment conditions will then result in lignin condensation, which leads to an increase of molecular weight (Figure 1-26).^{233,250}

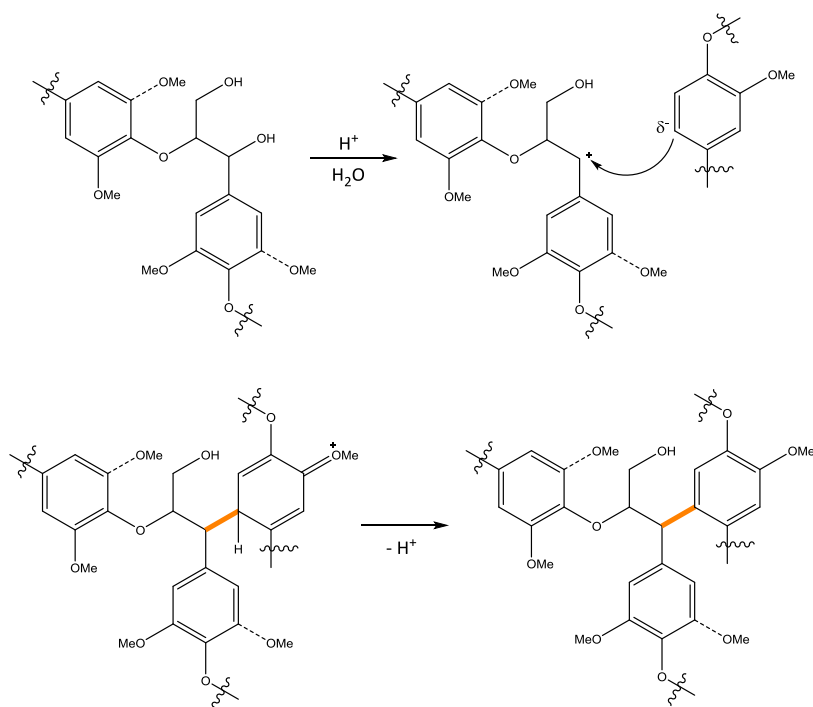


Figure 1-26. Formation of lignin carbocation via dehydration and subsequent condensation reaction at another aromatic subunit of lignin.²⁵⁰

Although much research has been undertaken in the field of lignocellulose pretreatment with ionic liquids, several obstacles still need to be overcome for the successful implementation of an IL based biorefinery. As mentioned above, the economic feasibility of this technology is crucial for the commercialisation of pretreatment using ionic liquids. Life cycle analysis was carried out to better understand the challenges this pretreatment technique is currently still facing.^{220, 251} The life cycle analysis identified the cost of the ionic liquid and the IL loading as key cost parameters that need to be reduced. Additionally, recycling and reuse of the IL needs to be implemented into the process. Furthermore, using lignin as a starting material for the production of valuable chemicals and materials will aid in making an IL based biorefinery economically competitive.

4. Products of biorefineries

Two differing approaches were proposed for the implementation of a biorefinery based chemical industry. In the first scenario, the value chain approach, value added compounds present in the biomass are isolated in different processing and (bio)conversion steps. The remaining biomass is then transformed into a universal substrate which is used as a feedstock for the synthesis of various chemical products. This approach is based on the idea that it is technologically and economically more beneficial to extract valuable chemicals and polymers from biomass compared to synthesising these compounds from universal building blocks in several subsequent steps. However, this approach faces technological challenges in the area of biomass refining, separation technology and bioconversion technology. Additionally, a realization of the value chain approach requires a far reaching integration of food, feed and chemical industries as well as a major investment in infrastructure.²⁵² The second approach, the integrated process chain approach, is largely based on the conversion of crude oil to chemical products in the petrochemical industry. It focuses on the transformation of a universal substrate into platform chemicals, based on which chemical products are needed. In this approach it is believed that it is economically and technologically more feasible to synthesize chemicals in highly integrated production facilities. The main technological challenges for this approach lay in the high-efficiency transformation of lignocellulose into platform chemicals commonly known from the petrochemical industry.²⁵³

The bio-resource biomass does not only consist of the carbohydrate polymers cellulose and hemicellulose and the partially aromatic polymer lignin but also contains other heterogeneous fractions such as oils and (depending on the feedstock) starch and proteins. This variety in components

allows for the production of non-food and food products, intermediate agro-industrial products and materials *via* fractionation and functionalization (Table 1-2).²⁵⁴

This requires the development of several specific technologies to guarantee efficient transformation of each fraction into value added products. Depending on the nature of the recovered fractions they can be either used directly as chemicals or need to be converted by several routes such as chemical, enzymatic and/or microbial processes. The conversion of these fractions will be beneficial in several ways, as it will reduce the cost of biofuels, increase the revenue of the biorefinery, minimize waste production and reduce the dependence of humankind on petroleum-based products.²⁵²

Bioethanol is the most widely known example of a biorefinery product, however, many other high-volume commodity products were identified that can be produced from lignocellulosic biomass.²⁵⁵ These products can be divided into the following generic categories: naturally occurring carbohydrate polymers, chemical products of carbohydrate containing materials, fermentation products of carbohydrate containing sources, fats and oils from plants and terpene based materials. Purified cellulose is a very versatile starting material and a large subgroup of products is derived from this polymer *via* processing or modification. Much research has been undertaken to develop cellulose based products. Derivatization of cellulose produces cellulose acetate which is a biodegradable polymer used to make photographic films, acetate rayon, various thermoplastic products and lacquers.²⁵² In addition, cellulose can be converted to lactic acid *via* fermentation which serves as a platform chemical to produce various other products such as methyl lactate, lactide and polymer polylactic acid (Figure 1-27). Polylactic acid is already commercially available and is a fully biodegradable replacement for polyethylene terephthalates.²⁵⁶ Furthermore, efficient processes to biologically convert lactic acid into methacrylic and acrylic acid (starting compounds for polymeric materials) are currently being developed.²⁵²

Much research has lately been directed towards the development of products from the low cost compound glycerol resulting in the use of glycerol as a building block for the synthesis of propylene glycol. Additionally, the use of glycerol for the production of epichlorohydrin is currently investigated. Epichlorohydrin is a starting material of epoxy resins and elastomers.²⁵⁷ The aromatic compound 5-(hydroxymethyl)furfural is another important platform chemical derived from cellulose.²⁵⁸ 5-HMF is an intermediate in the production of products such as 1,6-hexanediol, adipic acid, levulinic acid 2,5-dimethylfuran, 2-methylfuran, caprolactone, 2,5-dimethyltetrahydrofuran.

Table 1-2. Potential platform chemicals and value added products produced from the lignocellulosic biopolymers cellulose, hemicellulose and lignin.²⁵²

Biopolymer	Platform chemical	Product
Cellulose	HMF	1,6-hexanediol, adipic acid, levulinic acid 2,5-dimethylfuran, 2-methylfuran, caprolactone, 2,5-dimethyltetrahydrofuran
	Levulinic acid	Succinic acid, THF, MTHF, 1,4-butanediol, NMP, lactones
	Ethanol	
	Lactic acid	Acrylic acid, Acetaldehyde, 2,3-pentanedione, Pyruvic acid
	3-hydroxy-propanioc acid	
	Itaconic acid	3-MTHF, 3-methyl pyrrolidone, 2-methyl-1,4-butane, Itaconic diamide
	Glutamic acid	
	Glucuronic acid	
	Succinic acid	2-pyrrolidones, 1,4-butanediol, THF
Hemicellulose	Xylitol	
	Ethanol	
	Butanol	
	2,3-butandiol	
	Ferulic acid	Vanillin, Vanillic acid, Protocatechuic acid
	Lactic acid	
	Furfural	
	Chitosan	
	Xylo-oligosaccharides	
Lignin	Syngas	
	Syngas products	Methanol, Dimethyl ether, Ethanol, Mixed liquid fuels
	Hydrocarbons	Cyclohexanes, higher alkylates
	Phenols	Cresol, Eugenol, Coniferols, Syringols
	Oxidized products	Vanillin, Vanillic acid, DMSO, Aldehydes, Quinones, aromatic and aliphatic acids
	Macromolecules	Carbon fibres, Activated Carbon, Polymer alloys, Polyelectrolytes, substituted lignins, thermosets, composites, wood preservatives, adhesives, resins

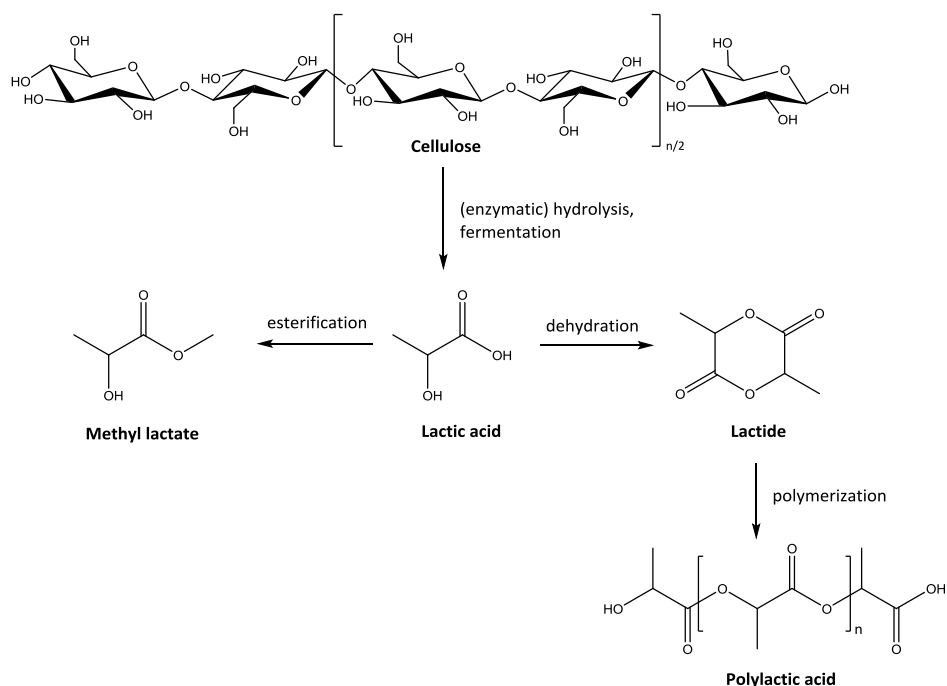


Figure 1-27. Transformation of cellulose into value added chemicals such as lactic acid and polylactic acid.²⁵²

The hydrolysis of hemicelluloses yields mixed C₆ and C₅ platform chemicals which can theoretically be converted into the same products as derived from cellulose *via* fermentation. However, more research needs to be undertaken to overcome technical, biological and economic barriers of this product route. Potential chemicals produced from hemicellulose *via* selective dehydration, hydrogenation and oxidation are sorbitol, furfural, glucaric acid, 5-(hydroxymethyl)furfural and levulinic acid. Sorbitol is used as an ingredient in food and personal care products such as toothpaste.²⁵⁹

In contrast to the (relatively) structurally homogeneous carbohydrate polymers, lignin is built of a heterogeneous structure and consists of alkyl linkages and aromatic subunits. The presence of aromatic subunits have led to the idea to use lignin as a source for aromatic chemicals. However, to realise this goal depolymerisation of the lignin polymer is required.²⁶⁰ In practice, the valorisation of lignin to aromatic chemicals to substitute petroaromatics remains a challenge to this day.²⁶¹ The one aromatic chemical that is currently produced from lignin on an industrial scale is vanillin.²⁶⁰

Several techniques have been developed to yield aromatic monomers from lignin, namely pyrolysis of the isolated lignin, catalytic hydrogenolysis, alkaline hydrolysis, supercritical water and solvent depolymerisation.²⁶¹ Figure 1-28 depicts possible products of the depolymerisation of lignin.

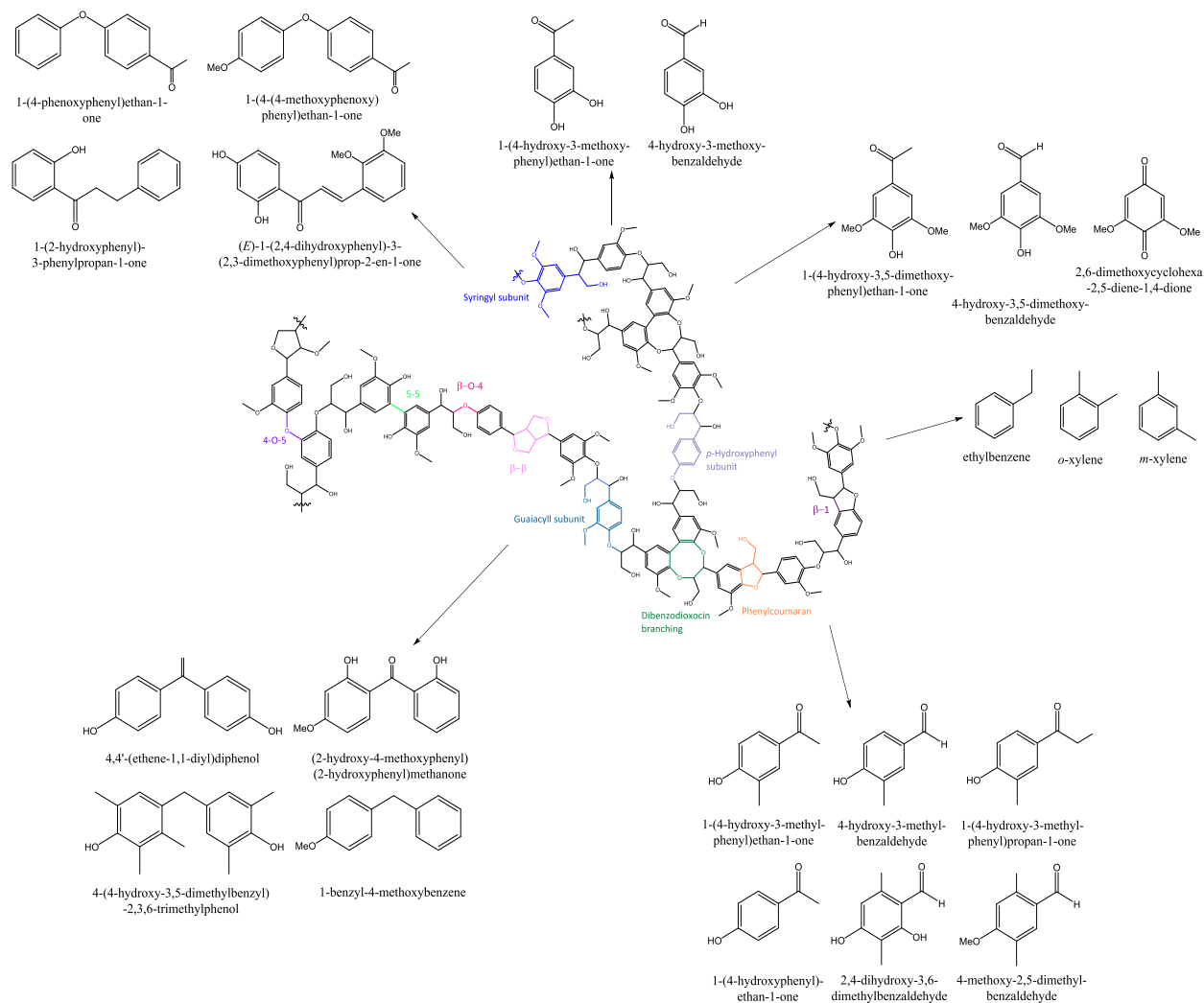


Figure 1-28. Possible reaction pathways of lignin depolymerisation into aromatic monomers and dimers.²⁶²

Pyrolysis of lignin occurs over a wide range of temperature (160 °C to 900 °C) which can be explained by the structure of the polymer itself.²⁶³ The two main products of lignin fast pyrolysis are lignin bio-oil and char. The bio-oil typically contains a wide product mixture of aromatic and non-aromatic compounds with a high polydispersity.²⁶¹ Generally, lignin from various sources such as alkali^{263, 264}, Organosolv^{265, 266}, soda^{267, 268}, acid hydrolysis^{268, 269}, milled wood lignin^{270, 271}, steam explosion²⁷⁰ and enzymatic²⁶⁶ isolation were studied for pyrolysis.

Catalytic hydrogenolysis of lignin is the generic term for the transformation of lignin into a liquid product mixture with the use of a suitable catalyst and hydrogen. Hydrogenolysis is generally carried out at lower temperatures than pyrolysis and different terminologies have been used in literature to describe this technology such as hydrotreating, hydrocracking, hydrodeoxygenation, hydrotreating,

and hydrogenolysis. A wide range of chemical reactions occurs simultaneously during hydrogenolysis. These reactions include the cleavage of the interunit linkages, ring hydrogenation, deoxygenation and removal of the alkyl and methoxyl moieties, resulting in a complex oil mixture resembling pyrolysis bio-oil. However, the oxygen content of hydrogenolysis oil is lower compared to pyrolysis bio-oils which renders these oils more chemically stable.²⁶¹

Processing of lignin in sub- and supercritical water can produce smaller lignin fragments through cleavage of the (ether) linkages. However, cross linking between the reactive fragments resulting in high molecular weight compounds has been often described as an issue of the technology.^{272, 273, 274} The reactions primarily occurring during the processing of lignin in a hydrothermal medium are dealkylation and demethoxylation with higher molecular weight residues still present in the product mixture. However, the amounts of higher molecular weight residue can be minimized by simultaneous optimization of process parameters such as the reaction temperature, the residence time and the water density.²⁶¹ Another technology that applies supercritical conditions is the depolymerisation of lignin with solvents like ethanol²⁷⁵, methanol²⁷⁶, CO₂/acetone/water²⁷⁷ or butanol²⁷⁸. The depolymerisation of lignin in organic solvents is usually carried out in the temperature range of 200 °C – 350 °C under high pressures and soluble lignin fragments in high yields are recovered after depolymerisation. Lignin solvolysis processes can be divided into two general groups: base-catalyzed depolymerization and hydrogenolysis.²⁶¹

In general, lignin depolymerisation can be performed in the liquid or solid phase. However, independent of the depolymerisation technology applied, the process always yields a complex product mixture with the individual fractions of each compound barely exceeding a few percent. Additionally, the conversion of the polymer into monomers is restricted by the amount of stable C-C linkages present in lignin that cannot be hydrolysed. This results in an overall low yield of aromatic monomers and a product mixture that consists of unreacted lignin, oligomers and monomers which introduced great challenges in product separation due to the similar molecular weight and polarity of the desired monomeric product fractions.²⁶¹

Depolymerisation of lignin is not the only strategy to create value-added products from this biopolymer. Even though the structure of lignin strongly depends on the biomass source and the extraction process it always contains reactive aromatic and aliphatic hydroxyl groups. These groups can be used as reactive sites to create lignin-based polymers. This strategy has been applied to synthesize polymers such as polyurethanes, polyesters, phenolic resins or epoxy resins. Lignin can either be used as a macromonomer (without prior depolymerisation) or as monolignols (after depolymerisation).²⁷⁹ In this thesis, the focus was on using lignin without further modification in order

to keep the cost of the production of lignin materials low, and thus only examples of lignin being used as a macromonomer for the production of polymers will be discussed in detail here.

Although lignin holds great potential for multiple industrial applications, these are limited by the immiscibility of lignin with many thermoplastics and thus only small amounts of lignin can be blended into plastics. This led to the research focusing on the development of modified lignin or lignin-based polymers. The complex structure of the polymer provides several reactive functionalities such as hydroxyl, carbonyl and carboxyl groups. These can be used to directly synthesize polymers from lignin. A second approach of using lignin as a macromonomer is to introduce new chemically active sites to the polymer (Figure 1-29).²⁸⁰

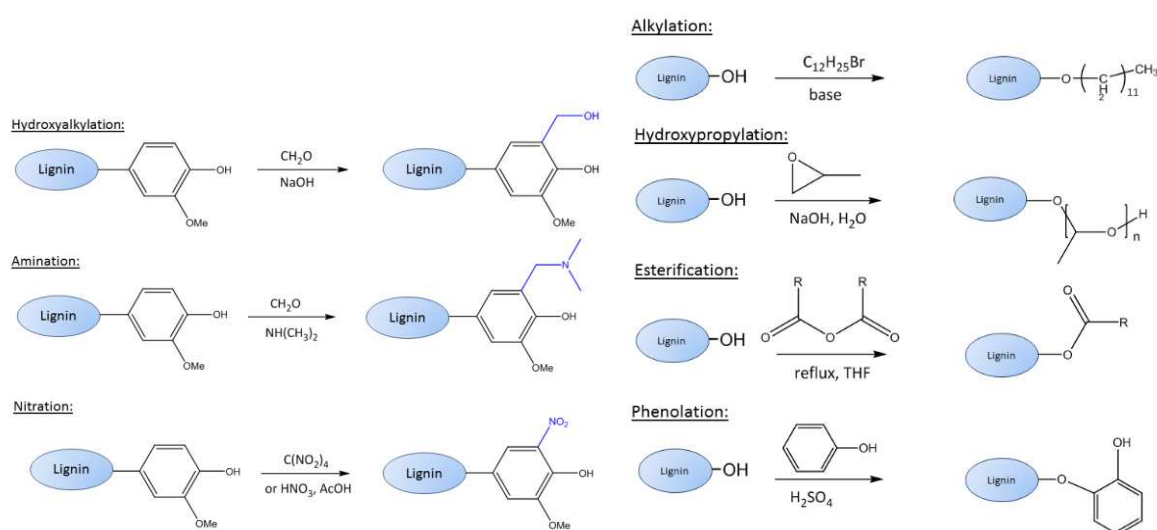


Figure 1-29. Chemical modification techniques of lignin to synthesize lignin-based polymeric materials.²⁸⁰

The phenolic hydroxyl group in lignin is the most reactive functionality on the biopolymer and is thus often used for modifications. One of the easiest ways to change the structure of lignin is to esterify the hydroxyl groups. Different esterifying agents, including acidic compounds, acid anhydrides and chloride acids, have been utilized for this purpose.^{281–283} Another way of using the hydroxyl groups as reactive centres is the phenolation of lignin. This synthesis route introduces more phenolic hydroxyl groups into the lignin structure *via* the condensation of phenol with the lignin aromatic subunits and side chains in acidic conditions. The chemical reactivity of the biopolymer is thus improved.²⁸⁰ The most investigated etherification method of lignin is the oxypropylation using propylene oxide and an alkaline catalyst (e.g. KOH).²⁸⁴ This reaction, which involves the lignin hydroxyl groups, creates lignin-based macropolyols.²⁸⁰

The addition of functional groups on lignin to increase its reactivity was also studied. Using basic reaction conditions lignin can be alkylated or alkoxyated. These reactions produce lignin ethers or hydroxyalkyl lignin which can then be used as macromonomers for the synthesis of lignin-based copolymers.^{285–287} The introduction of a tertiary amine group to lignin occurs *via* the Mannich reaction which utilizes a formaldehyde and an amine. The synthesized products are used as fillers in polymer/lignin composites or for the preparation of a cationic surfactant.^{288–290} Nitration is another approach to modify lignin. This is performed using nitration agents or nitric acid in acidic conditions. The resulting product was used to synthesize lignin-based polyurethane.^{291–293}

These examples shows the broad application opportunity of lignin in polymeric products. However, a gap between research and commercialization of lignin product is evident²⁹⁴ which might be due to the still not optimum properties of the developed lignin products and shows that further research in this field is required.

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Chapter 2 – Pretreatment of *Salix* with [N₂₂₂₀][HSO₄] with different acid/base ratios

2.1 Introduction

The successful implementation of biorefineries depends amongst other things on the cost of the lignocellulose used for production of fuels, chemicals and materials. The feedstock should typically fulfil the following requirements: fast growth and high biomass yield, beneficial carbohydrate and lignin content, low requirement of nutrients and water as well as insusceptibility to diseases and pests.¹ Several feedstocks conform to these criteria such as the perennial grass *Miscanthus giganteus* or the hardwood *Salix*. The work conducted in this thesis focussed on using *Salix*, hence the introduction will mainly discuss this feedstock.

The hardwood *Salix* has been used by humankind for many centuries and has actively been cultivated due to its many useful properties such as high strength, low weight and flexibility. It was traditionally used as the raw material of basketry, fencing and hurdle making. During the 1970s and in light of the occurring oil crisis new interest arose with regard to using willow as a source of fuels. Nowadays, willow is also grown commercially as a raw material for the production of bioenergy.²

The species willow consists of around 400 varieties and some are cultivated as short-rotation coppice (Figure 2-1) for the production of biomass for biorefineries. This practice is applied for commercially grown bioenergy plants to maximise biomass yield and accelerate growth rates of the plants.³ Another advantage of cultivating willow as a bioenergy and biofuels crop is the low requirement for nitrogen containing fertilizers. Short rotation coppice willow has been ranked first place amongst crops cultivated for bioenergy and biofuel production with a nitrogen intensity of 90 kg per 1 000 GJ⁴ energy produced. The production and use of fertilizers causes both financial and greenhouse gas emission costs that should be kept to a minimum in the implementation of biorefineries.²

Second generation biorefineries use lignocellulosic biomass which is not in competition with human food supply, however, one has to keep in mind that the available arable land is limited as well. This introduces the need to grow bioenergy crops on subprime land which is not suitable for food production. Willow has been proven to be an excellent candidate for this as many species are already adapted to grow on more marginal lands.²



Figure 2-1. Short rotation coppice willow plantation at Rothamsted Research Centre. (Picture downloaded from <https://www.bbsrc.ac.uk/research/institutes/bsbec/images-bsbec/> on 01.02.2018)

Even though willow is a promising feedstock for use in biorefining, up to now only limited research has been undertaken to investigate its potential as a crop for this purpose. Typically research into lignocellulose pretreatment utilizes perennial grasses (e.g. *Miscanthus giganteus*), agricultural residues (e.g. wheat straw, rice straw and rice husk) or poplar. One reason for this is that willow typically grows in more temperate regions of the world and is mainly cultivated in only three countries, namely Sweden, the United Kingdom and the United States of America.²

As discussed in the background chapter of this thesis, many pretreatment methods have been developed in the last decades with the ionoSolv approach being a relatively novel one. Several studies have been published which investigate various acidic ionic liquid solutions for the fractionation of lignocellulosic biomass.^{5, 6, 7} The ionoSolv pretreatment utilizes acidic ionic liquid solutions such as triethylammonium hydrogensulfate [N₂₂₂₀][HSO₄] or 1-butylimidazolium hydrogensulfate [HC₄im][HSO₄] to selectively extract lignin and hemicellulose from the biomass matrix.

The deconstruction of the lignocellulosic biomass is influenced by several factors such as residence time of the biomass in the IL solution, pretreatment temperature and acid/base ratio of the ionic liquid solution. Verdia *et al.*⁶ showed that [HC₄im][HSO₄] solutions prepared with an excess of acid and a resulting acid/base ratio of greater than 1 were more effective at delignification of the lignocellulose compared to IL solutions with an acid/base ratio below 1. However, a substantial excess of acid in the ionic liquid solution led to poor fractionation of the biopolymer matrix as well as major degradation

of the cellulose in the pulp. These findings are a crucial part of better understanding the effects of ionic liquid pretreatment on the digestibility of the recovered cellulose-rich pulp. However, the study lacks investigation into the effect of the ionic liquids acid/base ratio on the chemical structure and molecular weight of the isolated lignin fractions. Given that lignin is subjected to changes such as hydrolysis of aryl ether bonds during acidic pretreatment methods⁸, it can be speculated that a difference in acidity of the ionic liquid solution also greatly impacts the structural properties of the lignin polymer. The structural characteristics (e.g. content of inter-unit linkages, aromatic subunit composition, molecular weight and linearity) and macromolecular properties of the isolated lignin (e.g. stiffness, density, strength and toughness) will determine the later application of the biopolymer.

Changing the acidity of the ionic liquid solution not only impacts the effectiveness of the fractionation of the lignocellulosic feedstock, but also the enzymatic hydrolysis of the pulp. It is well known that acidic pretreatment methods favour the formation of sugar degradation products such as furfural, 5-hydroxymethyl furfural (5-HMF) and formic acid which are considered to be inhibitory for enzymatic hydrolysis (Figure 2-2).

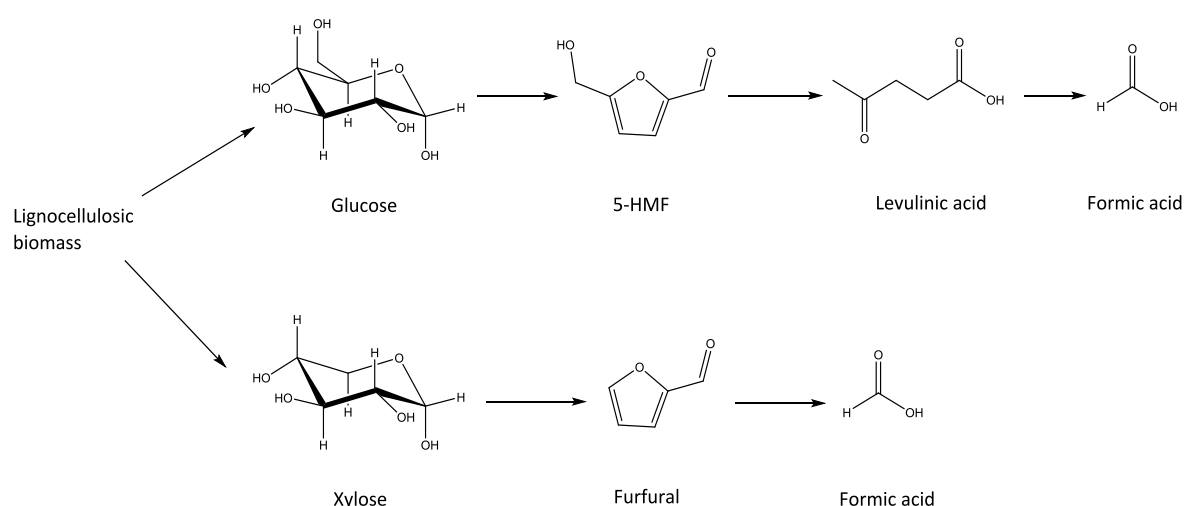


Figure 2-2. Proposed conversion pathway of lignocellulosic biomass to sugar degradation products furfural, 5-HMF and formic acid.⁸

Being able to control the acidity of the pretreatment medium should also allow for minimizing the production of sugar degradation products. Verdia *et al.*⁶ could show that the acidity of the ionic liquid solution greatly influenced the glucose yield of the saccharification. Pulps pretreated with IL solutions with an acid/base ratio below 1 generally gave higher glucose yields most likely due to less cellulose degradation during pretreatment.

Preserving high amounts of cellulose in the pulp is not only important in respect of subsequent enzymatic hydrolysis and fermentation of glucose to ethanol but also in light of cellulose being an interesting starting material for various other applications including composite materials⁹ and hydrogels¹⁰.

The study presented in this chapter investigates the hardwood willow (variety Endurance) as a potential feedstock for the IonoSolv pretreatment. So far, it was reported that pretreatment of herbaceous lignocellulose (*Miscanthus giganteus*) and softwood (*pinus*) with protic ionic liquids such as triethylammonium hydrogensulfate [N2220][HSO₄] and 1-butylimidazolium hydrogensulfate [HC4im][HSO₄] resulted in successful delignification and a significant increase in glucose yield of enzymatic hydrolysis of the recovered pulp.¹¹ However, the IonoSolv process has so far never been applied for the delignification of hardwood.

The lignocellulosic biomass was pretreated using the low-cost (estimated to be 1.24 \$/kg¹²) ionic liquid/water solution [N2220][HSO₄]_{80%} with different acid/base ratios at three temperatures for different pretreatment times to investigate the effect of pretreatment severity on the lignin structure, pulp composition and glucose yield of enzymatic saccharification.

2.2 Results and discussion

2.2.1 Synthesis of [N2220][HSO₄]_{80%} and determination of acid/base ratio

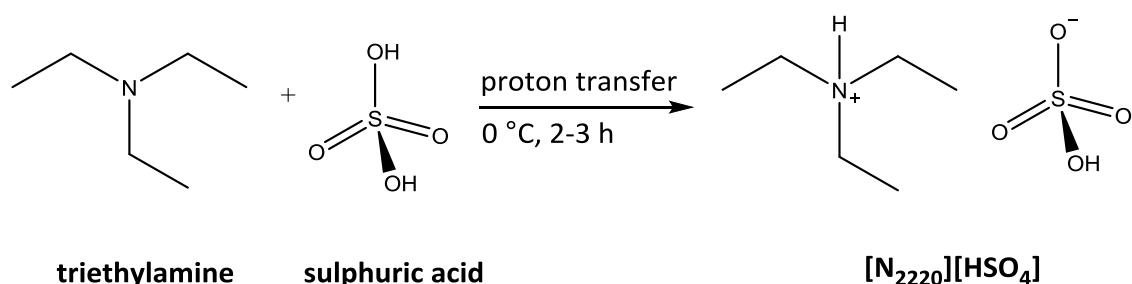
Protic ionic liquids are a subgroup of ionic liquids with unique properties. Typically, ionic liquids containing the 1,3-dialkylimidazolium cation and anions such as [PF₆]⁻, [BF₄]⁻, [CH₃COO]⁻ and others are synthesised via an anion metathesis reaction. The anion metathesis synthesis pathway involves the treatment of the halide salt with either silver, potassium or sodium salts of the desired anion or with the corresponding free acid of the appropriate anion. Table 2-1 for examples of common ionic liquids. The cation is synthesised either via quaternization reactions of the amine with a haloalkane under elevated temperatures or by protonation of the amine with an acid. The synthesis of these ionic liquids contains several steps and expensive reactants which is reflected in the high price of the product.¹³

As discussed in the introduction part of this thesis, an industrial biorefinery has to be cost competitive with an oil refinery. This calls for the need for low-cost ionic liquids for the pretreatment step of lignocellulosic biomass. The development of protic ionic liquids such as [N2220][HSO₄] is considered one step into further realising this goal.

Table 2-1. A few examples of the anion source and final product of ionic liquids.¹³

Salt	Anion source
[Cation][PF ₆]	HPF ₆
[Cation][BF ₄]	HBF ₄ , NH ₄ BF ₄ , NaBF ₄
[Cation][CH ₃ COO]	Ag[CH ₃ COO]

Protic ionic liquids are synthesised *via* a simple acid base reaction between an amine and a mineral acid. The ionic liquid used in this study [N₂₂₂₀][HSO₄] has an estimated price of *ca.* 1.24 \$kg⁻¹ if produced at an industrial scale and thus lies in the range of the cost of many other organic solvents.¹⁴ The prize of ILs typically used for IL pretreatment of biomass was estimated to be around 50 \$kg⁻¹¹², showing that the use of protic ionic liquids containing sulphate ions significantly lowers the process cost for this type of biorefinery. Figure 2-1 displays the synthesis pathway of the ionic liquid [N₂₂₂₀][HSO₄].

**Figure 2-3.** Acid base reaction that produces the protic ionic liquids [N₂₂₂₀][HSO₄].

This ionic liquid is usually synthesised with equal molar ratios of amine and acid to yield an ionic liquid with an acid/base ratio (a/b) of 1:1 and has been shown to be an effective pretreatment medium for lignocellulose fractionation into a cellulose-rich pulp and a lignin-rich fraction.¹¹ However, a study performed by P. Verdia *et al.* showed that the a/b ratio of protic ionic liquids impacts the outcome of the pretreatment of *Miscanthus giganteus* with regards of pulp and lignin yield, pulp composition and saccharification yield.⁶

The severity of ionic liquid pretreatment can be adjusted *via* the following parameters: residence time of the lignocellulose in the ionic liquid solution, pretreatment temperature and the acid/base ratio of the ionic liquid. The last process parameter (a/b of IL) is easily tuneable and the pretreatment of *Salix* was investigated under various levels of severity (Table 2-3).

Firstly, the synthesis and characterisation of the different ionic liquid solutions will be discussed below.

The desired acid/base ratios for this study were aimed to be 1.02 (meaning 2 % excess acid) and 0.98 (relating to 2 % excess amine). To synthesise these two ionic liquids, 1.250 mol of triethylamine was mixed with 1.275 mol or 1.225 mol of 5M sulphuric acid for the IL with $a/b = 1.02$ and $a/b = 0.98$, respectively.

Previous studies in our group have shown that an IL/water mixture containing 20 wt% water gave the best pretreatment performance compared to the neat IL or other IL/water ratios.^{6, 11} Thus, the water content of the synthesised IL [N₂₂₂₀][HSO₄] was adjusted to 20 wt%.

Table 2-2 shows exemplary pH and density values obtained for both synthesised [N₂₂₂₀][HSO₄]_{80%} solutions. The ionic liquid solutions clearly display a difference in pH and density thus confirming the success of the synthesis of ionic liquid solutions with a different acid/base ratio. The more acidic ionic liquid solution has a measured density of 1.1924 g mL⁻¹ and a pH of 1.52 compared to 1.1889 g mL⁻¹ and a pH of 1.65 for the less acidic one.

Table 2-2. Examples of measured pH and density values for the synthesised [N₂₂₂₀][HSO₄]_{80%} with two different a/b .

Entry	pH	density [g/mL]	acid/base ratio
1	1.52 ± 0.01	1.1924 ± 0.0001	1.02
2	1.65 ± 0.01	1.1889 ± 0.0002	0.98

To verify the acid/base ratio, the synthesized ionic liquid solutions were characterized using two common methods for this task, namely pH and density measurements. The a/b of both the synthesized ionic liquid solutions was calculated according to the method developed by M. S. Y. Jennings¹⁵, using the following equation:

$$y = 0.0772x + 1.1135 \quad (1)$$

where y is the density of the ionic liquid solution (g cm⁻³) and x is the acid/base ratio.

2.2.2 Influence of acid/base ratio on the pulp and lignin yield after pretreatment

In this study, the hardwood willow is used to expand the feedstock range that can be pretreated using the IonoSolv approach. Additionally, the effect of the pretreatment severity with special focus on the influence on the lignin structure is investigated. Lignin has been put in the focus of sustainable chemistry research in the last years due to it being a potential starting material for aromatic chemicals¹⁶ or can be used as a macro-monomer for polymeric applications¹⁷.

The main feedstocks studied previously for the IonoSolv pretreatment method are grasses (*Miscanthus giganteus*)¹¹ and agricultural residues (rice straw, rice husk and bagasse)¹⁸. However, the recalcitrance of hardwood cell walls is higher compared to the cell walls of grasses, which calls for more severe pretreatment conditions in order to guarantee a successful lignocellulose fractionation.

The ionic liquid solution [N₂₂₂₀][HSO₄]_{80%} synthesised with an excess of 2 % of sulphuric acid (a/b = 1.02) was first used to pretreat *Salix*. The more acidic IL solution was chosen in order to overcome the higher recalcitrance of the hardwood cell structure to increase the effectiveness of the pretreatment. The excess amount of acid can potentially facilitate undesirable acid catalysed cellulose hydrolysis to glucose¹⁹ which in turn raises the concern that this will lead to cellulose degradation in the pulp. One desired outcome of a successful pretreatment is to recover (near) quantitative amount of the cellulose in the pulp to maximise glucose yields of the enzymatic hydrolysis and subsequently the yield of ethanol during the fermentation step.²⁰ Cellulose degradation during pretreatment will decrease the glucose yield after enzymatic hydrolysis leading to an undesired pretreatment result. To prevent this issue, a second IL solution with a/b = 0.98 was also used for pretreatment of *Salix*.

The feedstock *Salix* Endurance was pretreated using the following conditions which are listed in Table 2-3.

Table 2-3. Conditions used for pretreatment of *Salix* Endurance in this study.

Pretreatment temperature [°C]	Pretreatment time [h]	IL solution
120	0.5, 1, 2, 4, 8	a/b = 1.02 and a/b = 0.98
150	0.5, 1, 2, 4	a/b = 1.02 and a/b = 0.98
170	0.3, 0.5, 0.7, 1	a/b = 1.02 and a/b = 0.98

Two fractions were recovered after pretreatment of *Salix* Endurance with the IL solution, one pulp fraction and the other one was speculated to be a lignin-rich fraction, similar to pretreatment results using the same IL solution reported earlier by Gschwend *et al.*⁵ Figure 2-4 shows the physical appearance of the isolated pulp after pretreatment with the IL solution $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 1.02$. It is clearly visible that the colour and general appearance of the pulp changed significantly with the severity of the pretreatment. It can be seen that a higher pretreatment severity resulted in a darker pulp and an almost char like product for very severe treatments such as 4 hours at 150 °C and 1 hour at 170 °C. It is hypothesized that the formation of the dark colour is caused by the formation of pseudo-lignin which precipitated on the pulp. The char like appearance of some pulps is most likely due to charring of the biomass induced by the very severe pretreatment conditions used. This gave a first indication of the success of the pretreatment.



Figure 2-4. Examples of the physical appearance of raw *Salix* before pretreatment and pulp after pretreatment at 120 °C (top), 150 °C (bottom left) and 170 °C (bottom right) for different amount of time.

The pulp and lignin recovery after pretreatment were calculated and can be used as a second parameter on the effectiveness of the fractionation of the lignocellulose. Figure 2-5 gives an overview of the pulp and lignin recovery after pretreatment at various levels of severity.

Figure 2-5 shows that using $[N_{2220}][HSO_4]_{80\%}$ decreased the amount of pulp recovered after pretreatment compared to the starting material independent of the pretreatment conditions applied. In general, the conditions of the pretreatment (time, temperature and acid/base ratio) dictated the amount of pulp and lignin that was recovered.

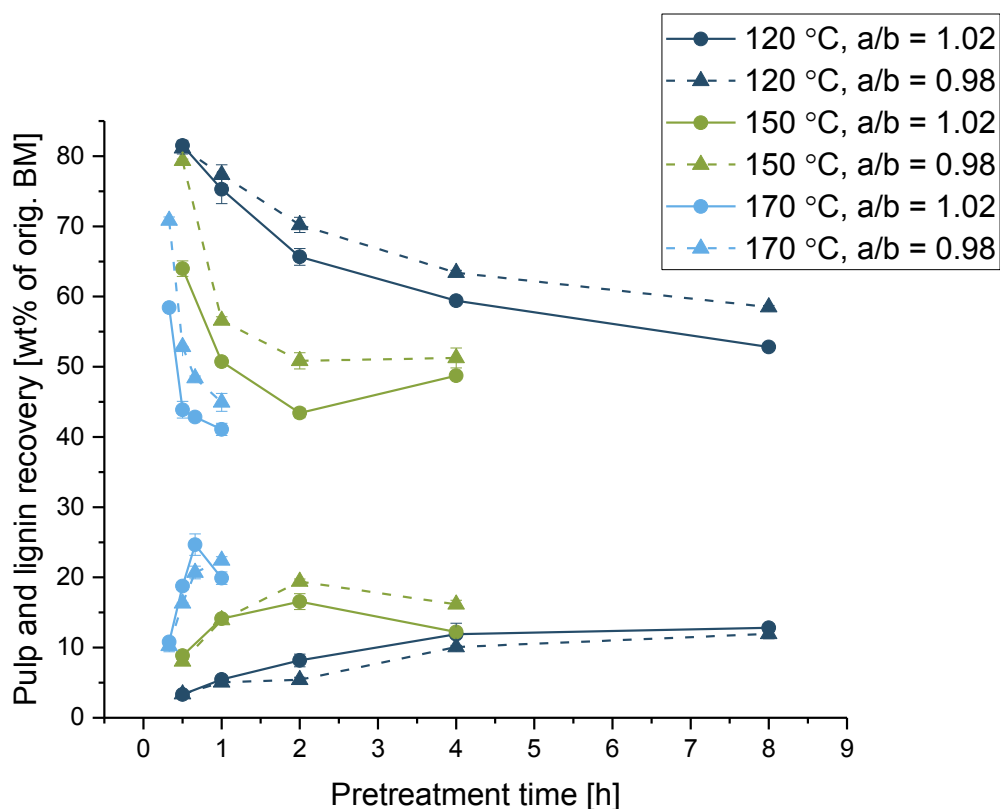


Figure 2-5. Pulp (top part of the graph) and lignin (bottom part of the graph) recovery yields after pretreatment of *Salix* with the ionic liquid solution [N₂₂₂₀][HSO₄]_{80%} with acid/base ratios of a/b = 1.02 (solid line) and 0.98 (dashed line).

. An increase in pretreatment severity resulted in a lower pulp yield (i.e. removal of more material from the biomass) and a higher lignin yield upon precipitation. The pretreatment conditions were selected to understand the point at which overtreatment occurred (reflected by an increase in pulp yield compared to milder pretreatment conditions) and once this point was reached, no further increase in pretreatment severity was studied.

The effect of an increase in pretreatment time at constant temperature was studied and it was found that at 120°C an increase in residence time steadily improved the removal of material from the pulp and resulted in a higher lignin recovery. It was found that using the more acidic IL solution resulted in a lower pulp yield and higher lignin yield as compared to the ionic liquid solution with a/b = 0.98 (Figure 2-5). The lowest pulp yield of 52.8 wt% and highest lignin yield of 12.8 wt% (of the original biomass) were found for pretreatment of 8 hours.

Applying higher pretreatment temperatures to speed up the fractionation of lignocellulose was already performed successfully with the IL solution [N₂₂₂₀][HSO₄]_{80%}.¹¹ Additionally, it was reported that conducting pretreatment at temperatures above the glass transition temperature of lignin ($T_g = 130 - 150\text{ }^\circ\text{C}$ depending on the source of lignin) might aid the delignification of biomass.^{21, 22} For more severe pretreatments of *Salix* Endurance at $150\text{ }^\circ\text{C}$ and $170\text{ }^\circ\text{C}$ an increase in pretreatment effectiveness was discovered which was indicated by a greater amount of material being removed from the biomass in less time compared to pretreatment at $120\text{ }^\circ\text{C}$.

The most effective pretreatment conditions in terms of lignocellulose fractionation were found to be 1 hour at $170\text{ }^\circ\text{C}$ using the IL solution [N₂₂₂₀][HSO₄]_{80%} with $a/b = 1.02$. The pulp recovery at these conditions was 41.1 wt%. However, the highest lignin yield was 24.7 wt% for pretreatment of *Salix* with ([N₂₂₂₀][HSO₄]_{80%} with $a/b = 1.02$ at 40 min at $170\text{ }^\circ\text{C}$. The discrepancy in pretreatment conditions that resulted in the lowest pulp yield and highest lignin yield indicates that lignin gets partially depolymerised resulting in dissolution of the lignin fragments in the IL solution. These fragments are then not recovered after precipitation of the lignin fraction with water.

The trend observed for the time course at $120\text{ }^\circ\text{C}$ could not be observed when higher pretreatment temperatures were applied. Initially, an increase of material removal from the biomass was found similar to pretreatment at $120\text{ }^\circ\text{C}$, but for longer pretreatment times at elevated temperatures (e.g. 4 hours at $150\text{ }^\circ\text{C}$ and 1 hour at $170\text{ }^\circ\text{C}$) over-treatment of the pulp was discovered. Signs of over-treatment include the increase in pulp recovery and decrease in lignin recovery compared to the previous time point at the same temperature. This phenomenon indicates the occurrence of lignin re-deposition on the pulp as also observed previously by Gschwend *et al.*⁵ and Chambon¹⁸ for other feedstocks. The increase in pulp yield can be explained by two different events: (i) re-deposition of lignin on the pulp occurring because of condensation of lignin molecules and the resulting formation of lignin fragments *via* intermolecular condensation reactions²³ with too high molecular weight to be soluble in the IL solution and (ii) formation of pseudo-lignin during pretreatment under acidic conditions²⁴. Pseudo-lignin is considered to consist of the polymerisation product of sugar degradation products that form during pretreatment under acidic conditions and lignin fragments²⁴. This hypothesis is supported by findings discussed in the compositional analysis section of this chapter. Over-treatment of the pulp was more prominent when the more acidic IL solution was used for pretreatment at elevated temperatures.

The severity of the pretreatment in terms of acidity seemed to have an interesting effect on the lignin yield. Pretreatments under milder conditions (such as all pretreatments at $120\text{ }^\circ\text{C}$ and pretreatments with short exposure times at $150\text{ }^\circ\text{C}$ and $170\text{ }^\circ\text{C}$) using the IL solution [N₂₂₂₀][HSO₄]_{80%} with $a/b = 1.02$

consistently gave higher lignin recovery compared to the less acidic IL solution. However, after pretreatment of 2 hours and 4 hours at 150 °C the lignin yield was found to be lower compared to [N₂₂₂₀][HSO₄]_{80%} with a/b = 0.98. This suggests that the lignin in the more acidic IL solution was more prone to condensation and re-deposition onto the pulp rather than precipitating during the lignin isolation step. A second explanation for the lower lignin yield could be that the lignin fragments were more water-soluble and not able to precipitate. To verify either hypothesis, compositional analysis of the pulp was performed and the isolated lignin was thoroughly analysed, which will be discussed later on in this chapter.

2.2.3 Effect of [N₂₂₂₀][HSO₄] treatment on pulp composition

Compositional analysis is an analysis technique that enables a better understanding of the changes in biomass composition that occur during pretreatment. These changes include delignification, hemicellulose removal and glucan degradation. The glucan, hemicellulose and lignin content of untreated *Salix* and pretreated pulps are shown in Figure 2-6. The composition of native *Salix* Endurance was determined as follows: 42.8 % glucan, 18.5 % hemicellulose and 27.3 % lignin (plus an additional 10.1 % of extractives).

It was found that pretreatment of *Salix* with both IL solutions [N₂₂₂₀][HSO₄]_{80%} resulted in a fractionation of the lignocellulose via removal of lignin and hemicellulose from the biomass matrix, leaving a cellulose-rich pulp. The effectiveness of the pretreatment as demonstrated by the composition of the pulp was strongly dependent on the acid/base ratio of the ionic liquid. In general, using [N₂₂₂₀][HSO₄]_{80%} with a/b = 1.02 gave a higher delignification and hemicellulose removal (Figure 2-6). However, the composition of the pulp was also greatly influenced by the pretreatment temperature and time.

Pretreatment at 120 °C only partially extracted the lignin from the biomass resulting in a pulp that still contains significant amounts of lignin. At this pretreatment temperature, the highest delignification and hemicellulose removal were found for the 8 h residence time, which led to 44.9 % and 56.6 % lignin removal and 54.0 % and 72.3 % hemicellulose removal, using ionic liquid solutions with a/b = 0.98 and a/b = 1.02, respectively. These findings suggest a concerted lignin and hemicellulose extraction under the applied pretreatment conditions, which is followed by (partial) hydrolysis and dissolution of the hemicellulose into the ionic liquid. Heteronuclear single quantum correlation (HSQC) spectra of the isolated lignin show no significant carbohydrate cross-peaks, proving that hemicellulose remains in the ionic liquid liquor after pretreatment (see appendix for HSQC spectra). In general, using

the more acidic IL solution was found to be more successful at delignification and hemicellulose removal and thus produced a pulp with a higher cellulose content.

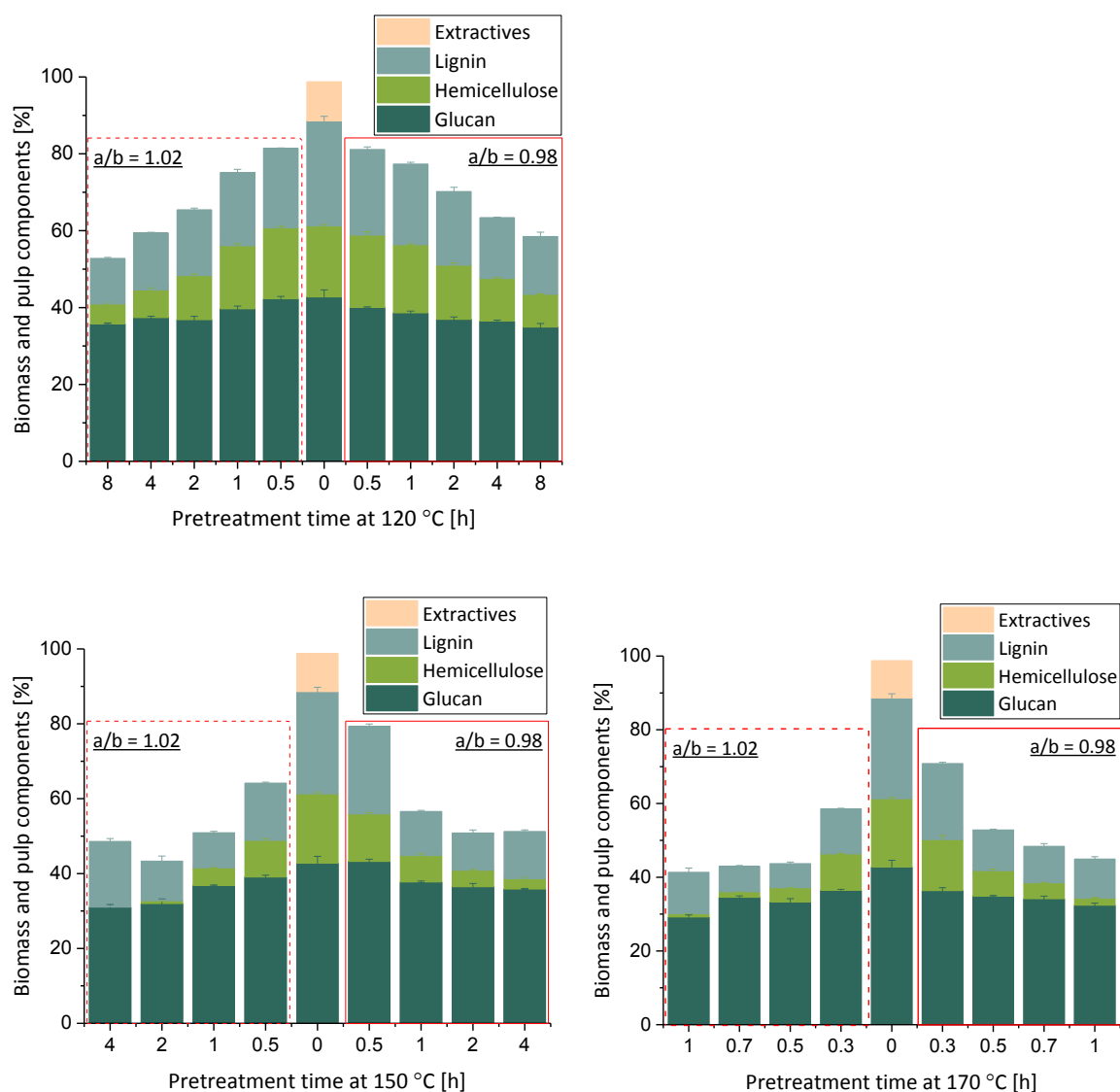


Figure 2-6. Glucan, hemicellulose and lignin content of raw *Salix* and pretreated pulp with the IL solution $[N_{2220}][HSO_4]_{80\%}$ with two different acid/base ratios at 120 °C (top), 150 °C (bottom left) and 170 °C (bottom right).

Even though using the mild pretreatment temperature of 120 °C proved not to be very effective in terms of delignification and hemicellulose removal it was found to be beneficial for preserving a high percentage of the glucan in the pulp. More than 80 % of the glucan remained in the pulp after 8 hours of pretreatment compared to the untreated biomass, for both ionic liquid solutions used. It was also found that for mild pretreatment conditions (0.5 h and 1 h at 120 °C, 0.5 h at 150 °C and 0.3 h at 170

°C) around 10 % of the original glucan was already extracted from the biomass most likely due to hydrolysis of amorphous cellulose and/or hemicellulose during the pretreatment.

Applying a more severe pretreatment by increasing the temperature to 150 °C significantly changed the composition of the recovered pulp and the influence of the acid/base ratio of the ionic liquid solution on the content of the pulp became more prominent. After only 30 min of treatment, 47.2 % and 31.6 % of hemicellulose were removed as well as 44.0 % and 14.3 % of the lignin, for [N₂₂₂₀][HSO₄]_{80%} with a/b = 1.02 and a/b = 0.98, respectively. This showed that the more acidic IL solution is much more effective at hydrolysing the lignin-carbohydrate ether bonds and depolymerising the lignin. Interestingly, this significant difference in delignification of the pulp was not reflected in the saccharification yields. Enzymatic hydrolysis of both pulps yielded 55.9 % and 44.1 % glucose for [N₂₂₂₀][HSO₄]_{80%} with a/b = 1.02 and a/b = 0.98, respectively. This discrepancy could be explained by the higher glucan degradation that occurred during pretreatment with the more acidic IL solution. Only 91.4 % of the glucan were preserved in the pulp compared to almost quantitative 99.2 % for the less acidic ionic liquid solution (based on the original amount of glucan in untreated *Salix*).

Increasing the pretreatment time and thus the severity of the pretreatment at 150 °C from 30 minutes up to 2 hours showed that the more acidic ionic liquid was better at delignifying the biomass as well as removing the hemicellulose. After treatment of 2 hours, 96.0 % of hemicellulose and 57.8 % of the lignin were removed from the pulp when [N₂₂₂₀][HSO₄]_{80%} with a/b of 1.02 was used. Compared to that treatment with [N₂₂₂₀][HSO₄]_{80%} with a/b of 0.98 extracted only 76.2 % of the hemicellulose but 63.6 % of the lignin. These findings can be explained as follows: the excess amount of sulphuric acid in the ionic liquid solution not only accelerated the lignin and hemicellulose extraction, but also sped up the formation of pseudo-lignin that then re-deposited on the pulp. Compositional analysis is a useful technique to analyse pulp composition, however, it faces the disadvantage that it cannot distinguish between actual lignin present in the cell walls of the pulp and pseudo-lignin that formed and deposited onto the pulp during pretreatment under acidic conditions. If the amount of lignin on the pulp is found to be higher than the lignin content of the untreated lignocellulose it is safe to assume that pseudo-lignin was formed during pretreatment. The difference in pretreatment severity introduced by the difference in acidity of the ionic liquid solution was especially obvious for pretreatment for 2 hours at 150°C, where the formation of pseudo-lignin was observed for pulp pretreated with the more acidic ionic liquid, whereas no formation of pseudo-lignin on the pulp was found for the ionic liquid with a lower acid/base ratio.

Comparing the delignification for the pretreatment of 2 hours to the pretreatment of 1 hour (at 150 °C), it can be seen that the delignification decreased when [N₂₂₂₀][HSO₄]_{80%} with a/b = 1.02 was used

but increased when [N₂₂₂₀][HSO₄]_{80%} with a/b = 0.98 was used. This indicates that lignin re-deposition occurred under more acidic pretreatment conditions. However, glucan degradation took place for pretreatment under these conditions, with the glucan content in the pulp decreasing to levels as low as 73.1 % of the original glucan in the biomass when using the more acidic ionic liquid solution. Glucan degradation was found to be less prominent when the less acidic ionic liquid solution was used and 83.9 % of the original glucan was contained in the pulp after pretreatment.

The pulp isolated after pretreatment for 4 hours at 150 °C showed signs of heavy over-treatment and formation of pseudo-lignin. The lignin content of the pretreated pulp was found to be higher compared to the lignin content in untreated biomass or pulp pretreated at milder conditions. These effects were more pronounced for the pulp pretreated with the more acidic ionic liquid solution, where hemicellulose was removed nearly quantitatively in addition to 27.4 % of the glucan. The delignification of the pulp decreased even further from 57.8 % (150 °C, 2 h) to 35.8 %. The less acidic ionic liquid caused less glucan degradation (only 16.8 % of the glucan were removed) and pseudo-lignin formation (a decrease of delignification from 63.6 % (150 °C, 2 h) to 54.0 %).

Pretreatment at 170 °C again accelerated the lignin and hemicellulose extraction and yielded a pulp high in cellulose after only 30 minutes when [N₂₂₂₀][HSO₄]_{80%} with a/b = 1.02 was used. This pretreatment condition exhibited the highest delignification success of all the studied pretreatment severities, with only 23.9 % of the original lignin left in the pulp. Interestingly, this did not correlate with the highest amount of hemicellulose removal which was found to be 96.0 % after pretreatment for 2 hours at 150 °C (a/b = 1.02). Pretreatment at 170 °C for 30 min only dissolved 79.1 % of the hemicelluloses in the ionic liquid solution.

Lengthening the pretreatment time at 170 °C from 30 min up to 1 hour affected the composition of the pulp significantly. At a residence time of the biomass in the ionic liquid solution of 1 hour the recovered pulp appeared to consist only of glucan, lignin/pseudo-lignin and a negligible amount of hemicellulose. On first sight this seems to be close to the idea of an ideal pulp recovered after pretreatment, however, glucan degradation was very prominent under these conditions with a glucan loss of 25% into the ionic liquid solution. Losing a significant amount of the glucan available will negatively impact the glucose yield of enzymatic hydrolysis. This hypothesis will be discussed further in the next section of this chapter.

2.2.4 Enzymatic hydrolysis of the pulp and untreated biomass

Enzymatic hydrolysis of the pulp was performed in order to assess the success of the pretreatment which can be defined by an increase in glucose yield compared to the untreated lignocellulose. Also, enzymatic hydrolysis was used to be able to determine the best pretreatment conditions for willow with [N₂₂₂₀][HSO₄]_{80%} which are shown by the highest glucose yield. It is generally believed that the lignin in the biomass and pretreated pulp hinders the cellulase enzymes from accessing the cellulose via formation of a protective layer of lignin around the cellulose fibrils or non-productive binding of the cellulase to the lignin.²⁵ Given the negative impact of lignin on enzymatic hydrolysis high delignification of the lignocellulose is considered a key aspect of a successful fractionation pretreatment.²⁶ The glucose yields after 7 days of enzymatic hydrolysis of the pulp are shown in Figure 2-7, demonstrating the influence of the acidity of the ionic liquid solution and the residence time on glucose release.

The enzymatic hydrolysis of *Salix* Endurance resulted in high glucose release of almost 40 % even without a pretreatment step. Untreated lignocellulosic biomass usually yields glucose yield in the range of 1 - 20 %, depending on the type of biomass used.²⁷ This unlikely high glucose yield of untreated lignocellulose can be explained by the origin of the biomass. The *Salix* used in all the studies in this thesis was provided by Rothamsted Research Centre that specialises in breeding willow varieties for use in biorefineries. The biomass received is the product of years of research in optimisation in glucose release of enzymatic hydrolysis.

Nevertheless, the ionic liquid pretreatment of *Salix* further improved enzymatic digestibility for certain conditions compared to the untreated biomass (Figure 2-7). However, this was not universally so. It was found that the glucose release was highly dependent on the applied pretreatment severity.

For very mild pretreatment conditions such as 0.5 hour, 1 hour and 2 hours at 120 °C (for both a/b ratios), 0.5 hours at 150 °C (for a/b = 0.98) and 0.3 hours at 170 °C (for a/b = 0.98), the glucose yield was found to be lower than the yield from the raw biomass. The same result was also previously observed for mild pretreatment of *Miscanthus giganteus*.¹¹

This might be due to acid catalysed hydrolysis of parts of the amorphous cellulose followed by dissolution of the sugar monomers or oligomers into the ionic liquid solution. The amorphous part of cellulose is generally hydrolysed faster under acidic conditions than the crystalline part²⁸ thus

decreasing glucan recovery for pulp treated even under mild conditions. The compositional analysis of the pulp revealed a glucan loss of around 10 % even for very mild pretreatment conditions, supporting the above statement.

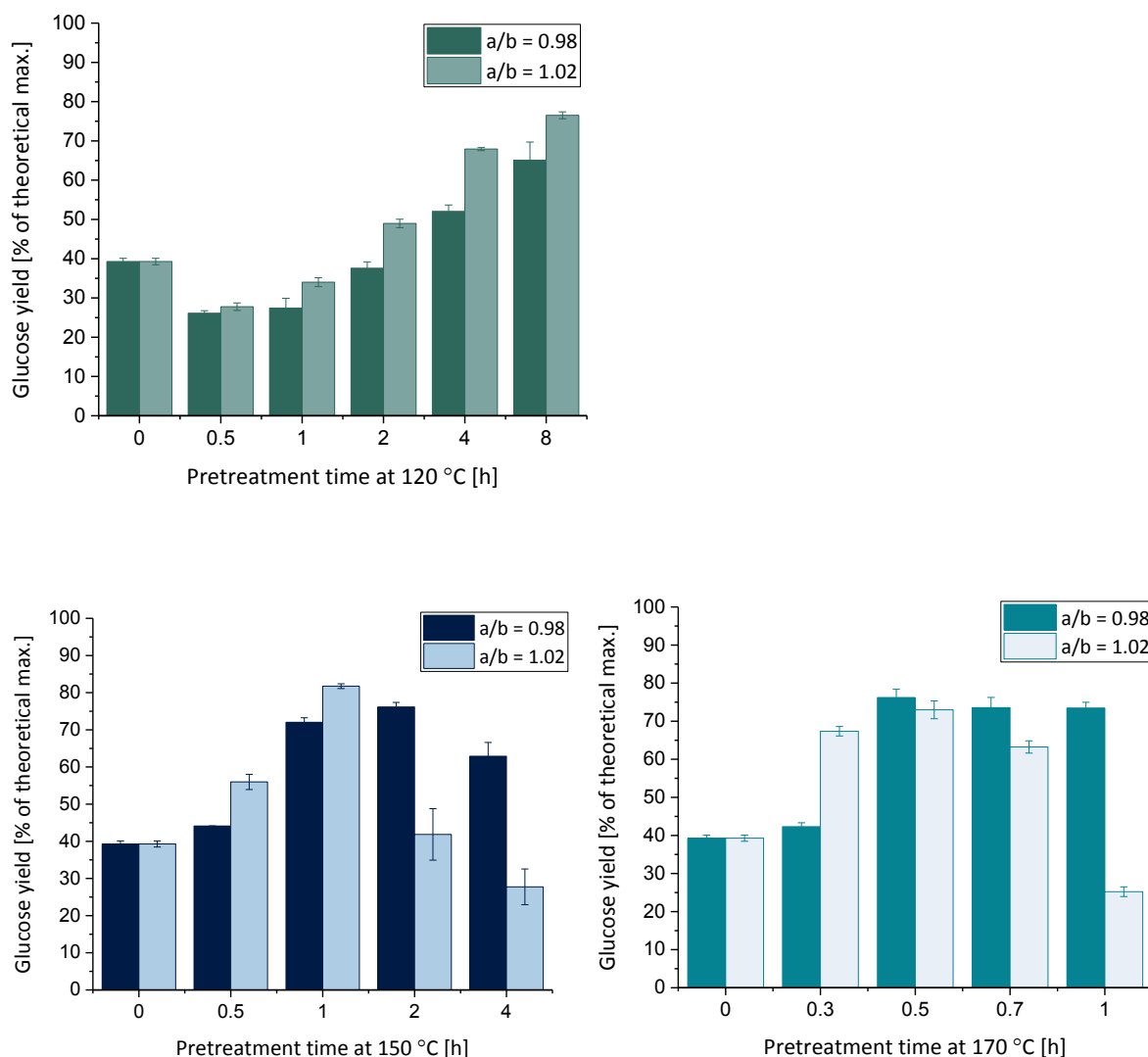


Figure 2-7. Glucose yield after enzymatic hydrolysis of raw *Salix* and pulps after pretreatment using [N₂₂₂₀][HSO₄]_{80%} with two acid/base ratios at 120 °C (top), 150 °C (bottom left) and 170 °C (bottom right). BM = biomass.

It was found that the glucose yield after enzymatic hydrolysis of the pulp pretreated with the more acidic ionic liquid solution at 120 °C was always higher compared to treatment with [N₂₂₂₀][HSO₄]_{80%} with a/b of 0.98. The highest yield was found to be 76.5 % for pretreatment of 8 hours at 120 °C. These results are in contrast to earlier findings reported by P. Verdía *et al.*⁶ for pretreatment of *Miscanthus giganteus* at 120 °C with the ionic liquid 1-butyl-3-methylimidazolium hydrogensulfate [C₄C₁im][HSO₄].

The authors of the Verdía study reported that the less acidic ionic liquid solution gave slightly higher glucose yields (90 % and 84 % for $a/b = 0.99$ and 1.01 , respectively) after 96 hours of enzymatic hydrolysis. The contrary findings support the hypothesis that hardwoods are more recalcitrant towards pretreatment and harsher conditions are required to successfully break-up the biopolymer matrix and create access to the cellulose fibrils. The higher glucose yields for *Salix* pretreated with [N₂₂₂₀][HSO₄]_{80%} ($a/b = 1.02$) can be found in the compositional analysis results of this pulp. The ionic liquid solution with an excess of sulphuric acid yields a purer pulp by extracting more lignin and hemicellulose from the pulp which results in an increase in accessibility of the cellulose substrate to the enzymes.^{29, 30} Additionally, lignin acts as a non-productive binding site for cellulases, which decreases the amount of available enzymes that productively bind to the substrate. This lowers the efficiency of the enzymatic hydrolysis and explains why a pulp with higher delignification gave higher glucose yields.²⁵

The highest glucose yield was found for pretreatment of 1 hour at 150 °C with [N₂₂₂₀][HSO₄]_{80%} with an a/b of 1.02. The pulp recovered after these pretreatment conditions released 81.8 % of the glucose that was contained in the untreated *Salix*. This proves that the ionic liquid solution [N₂₂₂₀][HSO₄]_{80%} can be used to successfully pretreat the hardwood *Salix* to improve glucose yields.

However, using very severe pretreatment conditions such as long pretreatment times at 150 °C and 170 °C led to over-treatment of the pulp which resulted in a dramatically decreased enzymatic digestibility. Using [N₂₂₂₀][HSO₄]_{80%} with an a/b of 0.98 produced high glucose yields even at harsh pretreatment conditions such as 2 hours and 4 hours at 150 °C as well as 1 hour at 170 °C compared to the more acidic ionic liquid solution. For example, at 150 °C and 2 hours, a glucose yield of 76.2 % ($a/b = 0.98$) vs. 41.9 % ($a/b = 1.02$) was achieved and at 150 °C and 4 hours, the glucose yield was found to be 62.9 % ($a/b = 0.98$) vs. 27.8 % ($a/b = 1.02$). The most significant effect of the acidity of the ionic liquid solution was observed for pretreatment for 1 hour at 170 °C. Here the glucose yield was 73.5 % (for $a/b = 0.98$) compared to 25.3 % (for $a/b = 1.02$). These findings are in good agreement with the reduced glucan content of the pulp after severe pretreatments, showing that the less acidic ionic liquid reduces the propensity for severe glucan degradation of the pulp.

2.2.5 Lignin linkages in the aliphatic region

The extraction of lignin from lignocellulosic biomass in biorefineries ideally serves two purposes: (i) increasing accessibility of the cellulose substrate to cellulases, which leads to an increase in glucose

yield compared to the untreated biomass and (ii) providing the starting material for either aromatic chemicals or polymeric materials.

However, the structure of lignin changes depending on the isolation method applied and strongly influences the processing possibilities of the extracted lignin.³¹ It has been previously reported that isolating lignin from the biopolymer matrix using acidic conditions (such as in the dilute acid pretreatment⁸ or IonoSolv pretreatment²³) results in the cleavage of aryl ether C-O bonds and in a decrease of the molecular weight of lignin. The formation of high molecular weight lignin condensation products under severe conditions was also described.³² Previous work investigating changes of the lignin structure of *Miscanthus giganteus* during IonoSolv pretreatment showed that the final structure of the isolated lignin is majorly impacted by the applied pretreatment conditions.³²

The heterogeneous nature of lignin introduces challenges regarding the characterisation and analysis of its structure. However, much progress in this field was made in recent years because of the development of powerful analytical techniques such as heteronuclear single quantum correlation (HSQC) NMR spectroscopy. HSQC NMR analysis is a two-dimensional NMR technique that resolves signals that otherwise overlap in one-dimensional spectra. The technique is semi-quantitative and is often applied for analysis of lignin linkages and subunit composition. The resulting NMR spectra can be divided into two sub-sections, namely the aromatic region that shows cross-signals of the aromatic sub-units (H, G and S as well as *p*-coumarates and ferulic acid) and the region that shows cross-signals for the linkages between the aromatic subgroups.

The HSQC pulse sequence employed in this study is optimised for both spectral resolution and signal strength. It is important to note that this method is not a quantitative one, as signal relaxation following each pulse will not be complete for some correlations, especially for end groups such as coumarates, which relax more slowly than the bulk.³³

A selection of common linkages and subunits of hardwoods is shown in Figure 2-8.

The lignin isolated from *Salix* Endurance using various levels of severity was analysed using the previously described HSQC NMR technique. Several structural units of the complex lignin polymer can be detected by this technique and many have been previously assigned by comparison with cell wall model compounds and can be found in an NMR database.³⁴

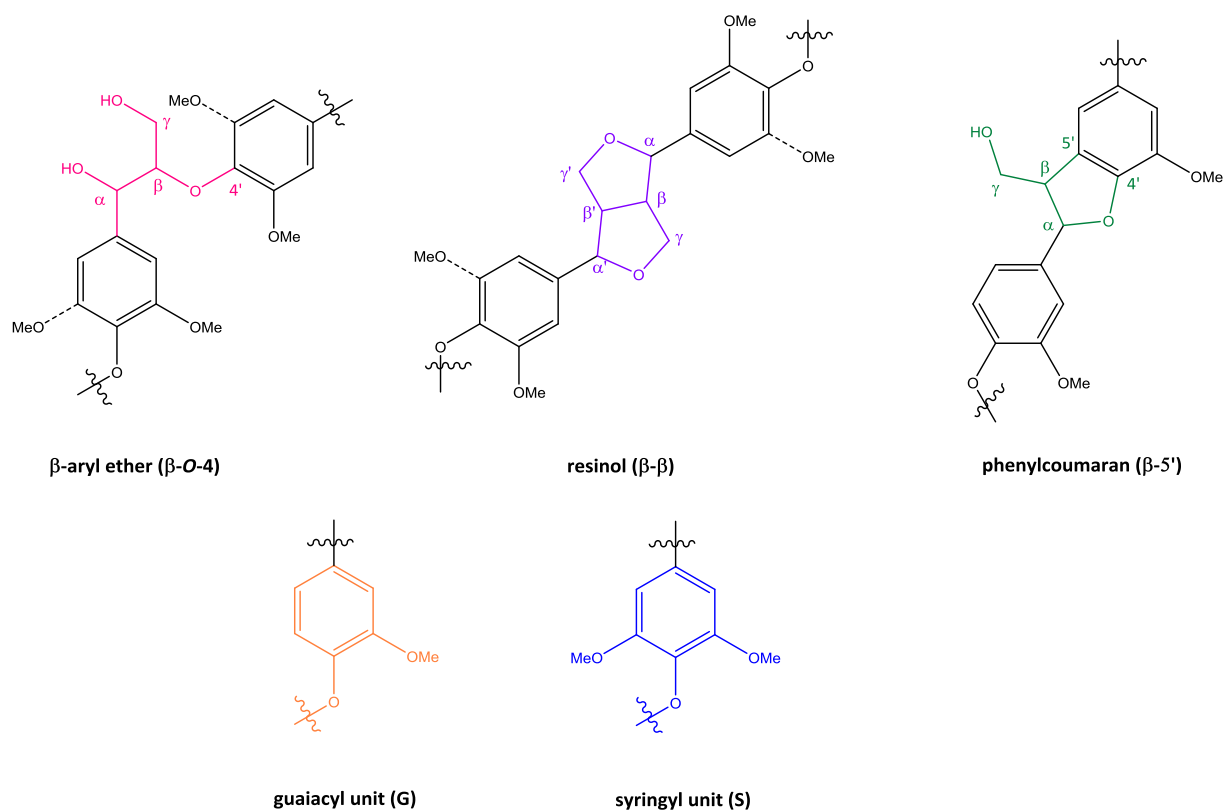
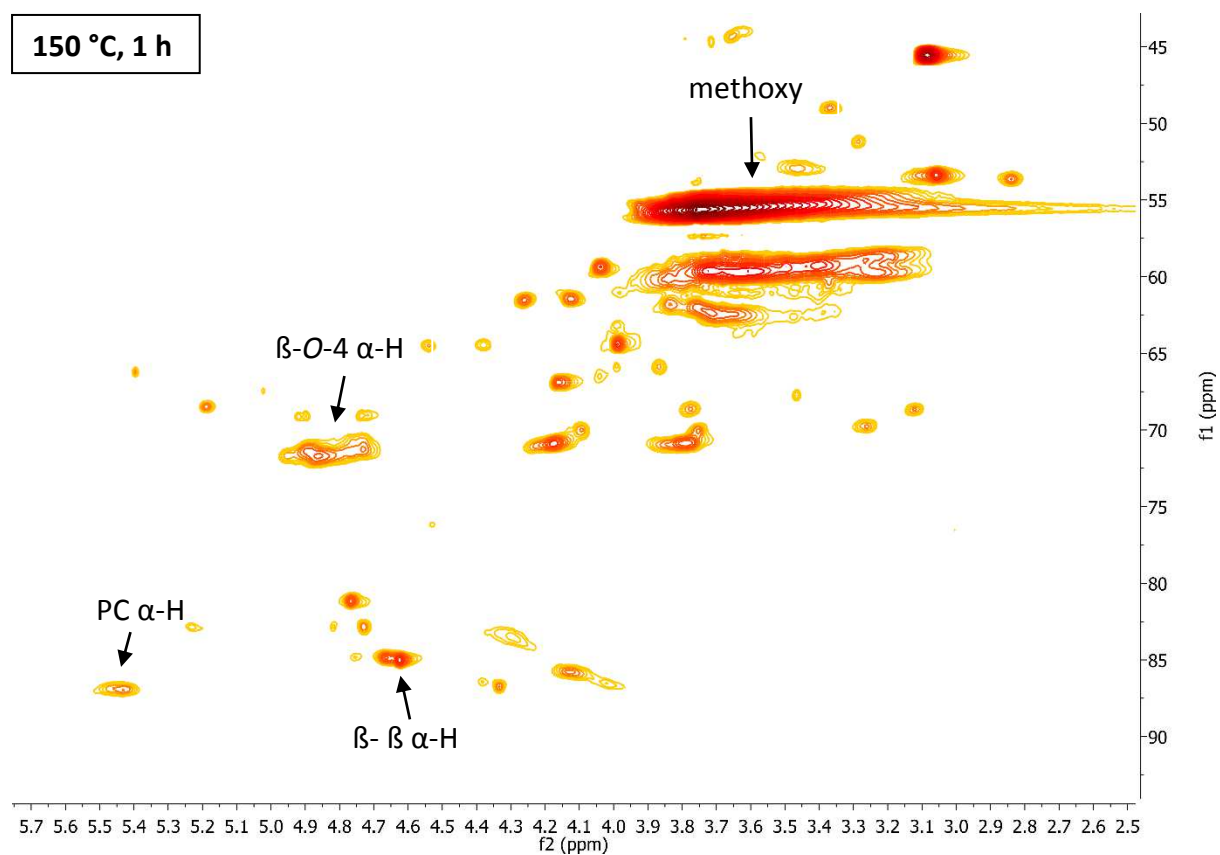
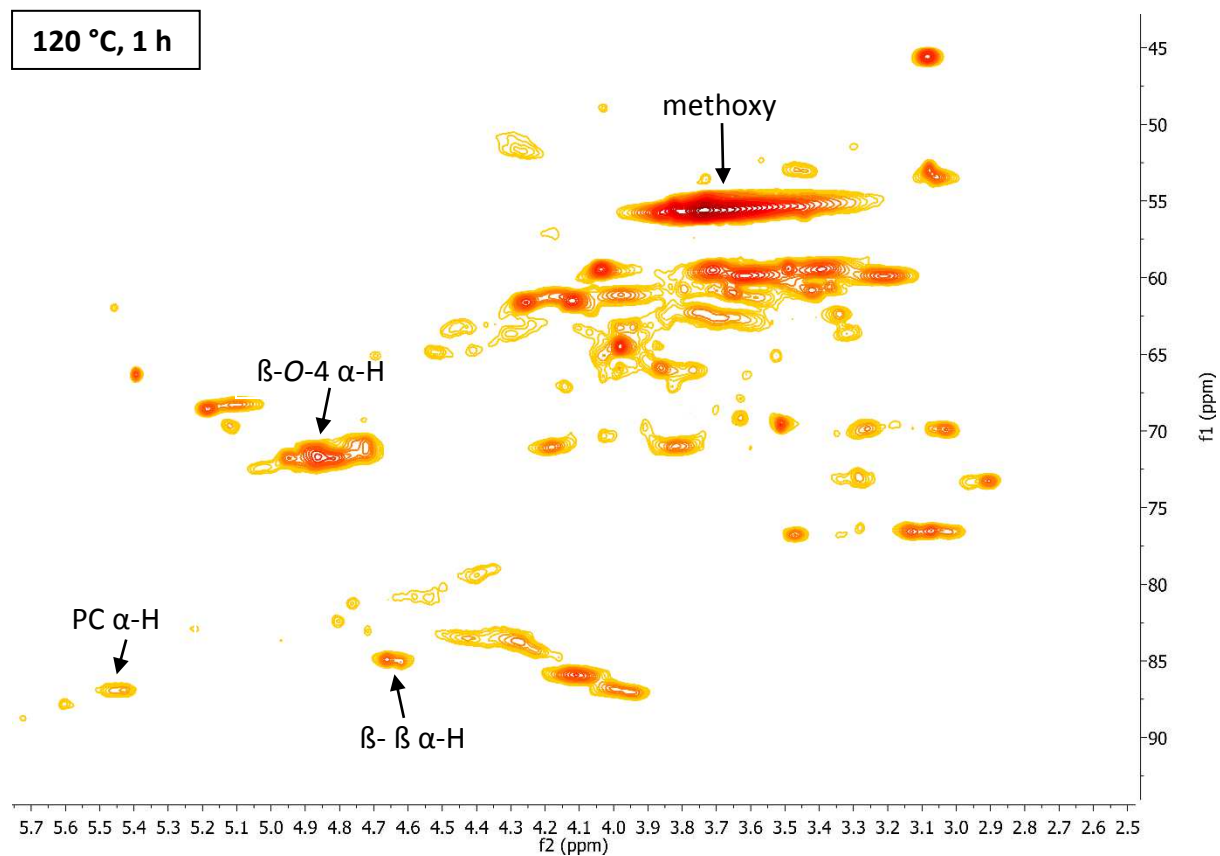


Figure 2-8. Some typical structural motifs found in hardwood lignins: β -O-4 alkyl-aryl ether (A), β - β (resinol) (B), phenylcoumaran (C), guaiacyl unit (G) and syringyl unit (S).

Examples of common subgroups and linkages that are resolved by HSQC NMR are β -ether (β -O-4, A_α at 4.87/71.8 ppm), β - β (resinol, B_α at 4.62/85.0 ppm) and phenylcoumaran (β -5, C_α at 5.45/86.9 ppm). The aromatic rings of the G (G_2 at 6.92/110.2 ppm, G_5 at 6.78/114.9 ppm and G_6 at 6.75/118.4 ppm) and S units ($S_{2,6}$ at 6.64/103.6 ppm) are also resolved. For calculation of the amount of inter-unit linkages, the α protons of β -O-4, β - β and phenylcoumaran were used. Three exemplary spectra of the inter-unit linkage region of lignin isolated from *Salix* Endurance after pretreatment with [N₂₂₂₀][HSO₄]_{80%} ($a/b = 1.02$) under different pretreatment conditions are shown in Figure 2-9.



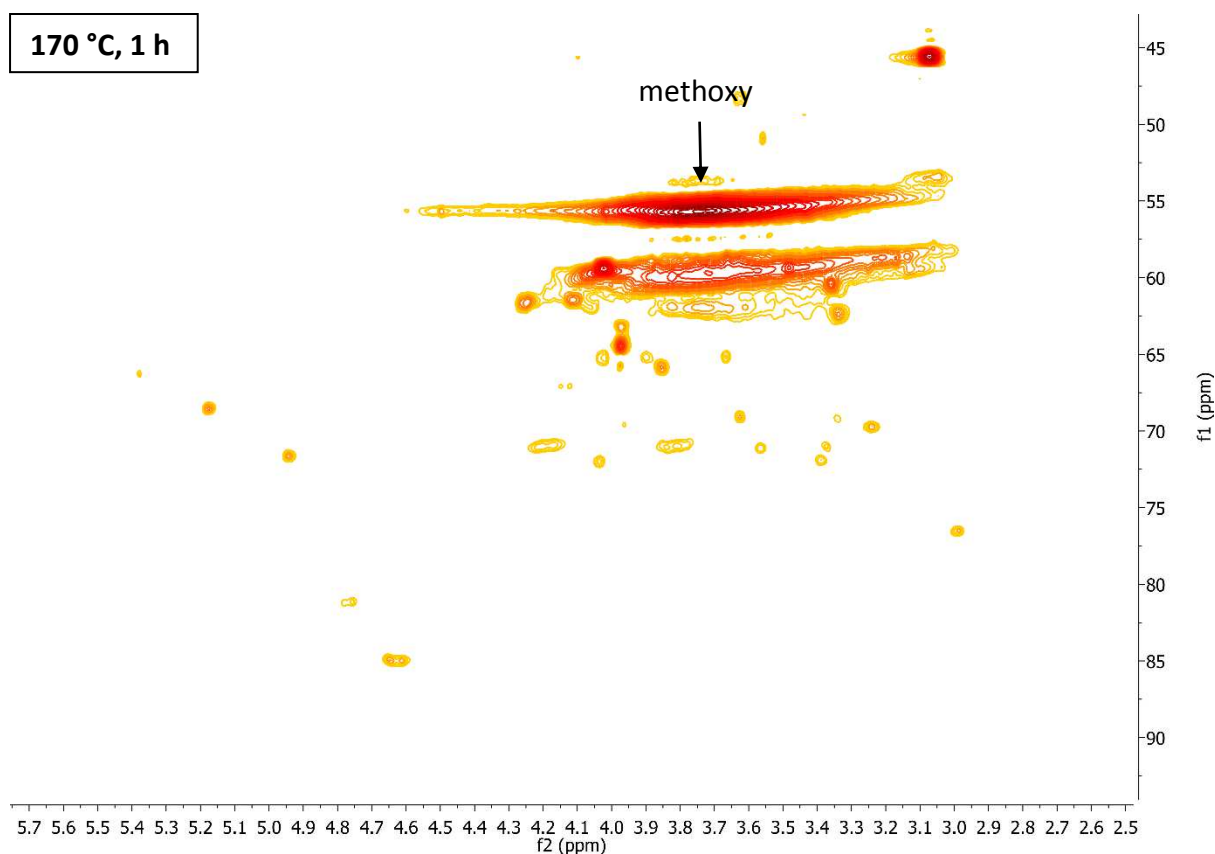


Figure 2-9. Example HSQC NMR spectra of lignin in DMSO-*d*₆ recovered after pretreatment of *Salix* Endurance with [N₂₂₂₀][HSO₄]_{80%} with *a/b* = 1.02 with the following conditions: 1 hour at 120 °C (top), 1 hour at 150 °C (middle) and 1 hour at 170 °C (bottom).

HSQC NMR analysis of the lignin isolated from *Salix* using [N₂₂₂₀][HSO₄]_{80%} showed that the inter-unit linkage composition of the lignin was surprisingly unaffected by the different acidities of the ionic liquid solutions at mild (all time points at 120 °C and 30 min at 150 °C) or very harsh (1 hour at 170 °C) pretreatment conditions. The acidity of the ionic liquid solution seemed to have little impact on the amount of linkages present in the recovered lignin (Figure 2-10). Three linkages were mainly present in the extracted lignin, namely the β-O-4 aryl ether linkage, the β-β linkage and the phenylcoumaran linkage.

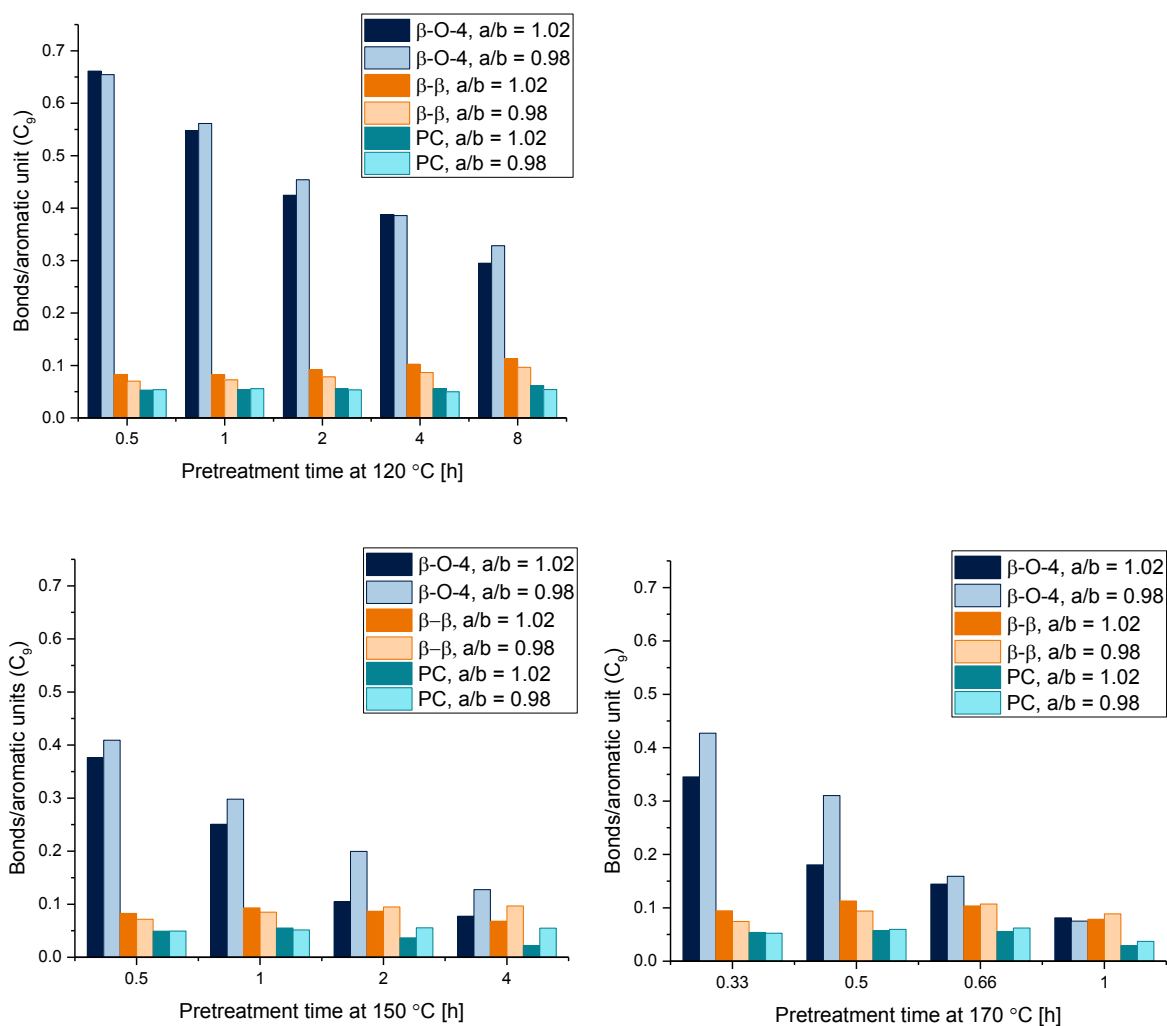


Figure 2-10. Typical lignin linkages (β -O-4 ether linkage, β - β (resinol) linkage and phenylcoumaran (PC) linkage) present in lignin isolated after pretreatment at different temperatures with [N₂₂₂₀][HSO₄]_{80%} with different acid/base ratios (a/b=1.02 and 0.98). Top left: 120 °C, bottom left: 150 °C and bottom right: 170 °C.

The most abundant linkage in lignin isolated at 120 °C with both IL solutions was the β -O-4 aryl ether linkage as also described earlier by Prado *et al.*³⁵ It was found that the amount of the β -O-4 linkage in the isolated lignin is strongly influenced by the applied severity of the pretreatment. The highest amount of β -O-4 bonds of 0.66 bonds per C_9 unit was found for pretreatment at 120 °C for 30 min for both ionic liquid solutions. This very mild pretreatment condition most likely did not alter the lignin structure too much and this lignin still contains the majority of the aryl ether linkages found in native lignin. This means that this lignin could be a promising feedstock for depolymerisation reactions leading to the production of small aromatic compounds such as syringyl aldehyde, *o*-methoxy catechol, vanillin, guaiacol or syringol¹⁶ (Figure 2-11).

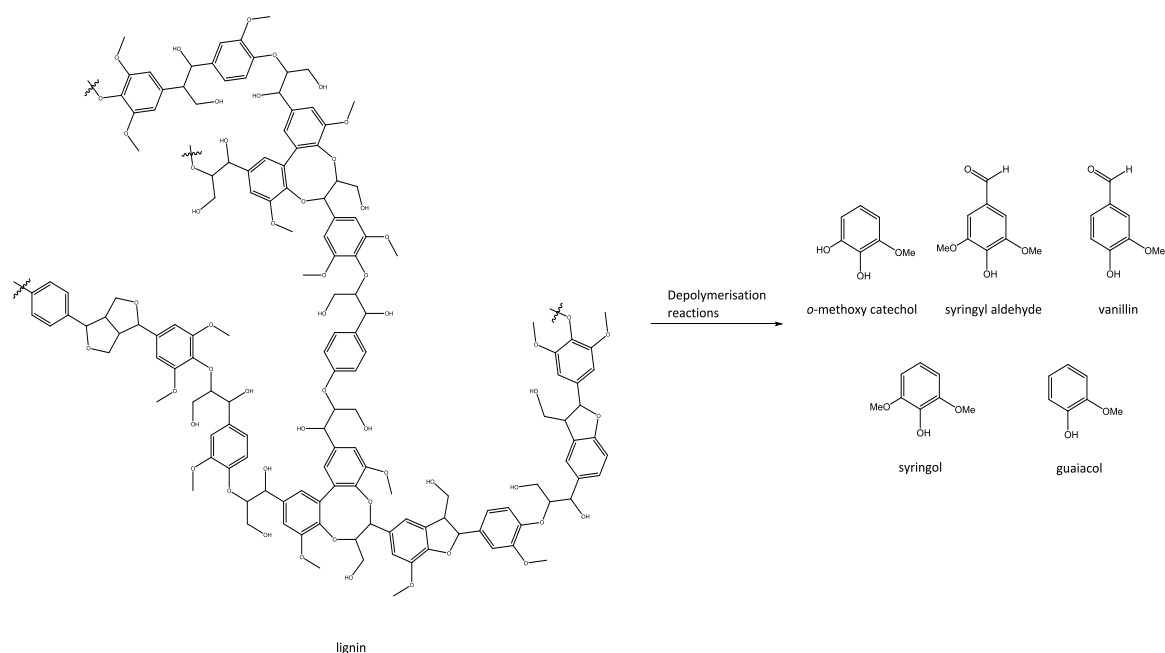


Figure 2-11. Possible lignin depolymerisation products.

Longer pretreatment times resulted in the cleaving of more aryl ether linkages and the amount of β -O-4 bonds dropped to 0.30 and 0.39 bonds ($a/b = 1.02$ vs. $a/b = 0.98$) per C_9 subunit after 8 hours at 120 °C. Figure 2-12 for a schematic representation of the reaction mechanism that leads to the cleavage of the aryl ether bond in lignin using acidic ionic liquids during pretreatment. First, the hydroxyl group at the α carbon of the aryl ether bond is protonated, followed by the elimination of a water molecule and formation of a double bond between the α and β carbon. This is followed by the formation and subsequent hydrolysis of a keto-ether structure, which then undergoes a 1,3 proton shift. This finally leads to the cleavage of the hydrolysed intermediate and the formation of an alcohol and the Hibbert ketone as products of the hydrolysis reaction.³⁶

The lignin isolated at elevated temperatures contained a lower amount of β -O-4 linkages compared to lignin isolated at 120 °C. A comparison of lignin extracted for 30 min at the three different investigated pretreatment temperatures is listed in Table 2-4. It can clearly be seen that with increasing severity of the pretreatment more aryl ether bonds were hydrolysed. Additionally, the acidity of the ionic liquid solution played a significant role in the structural changes of the isolated lignin with using the less acidic IL solution resulting in lignin with more preserved aryl ether bonds.

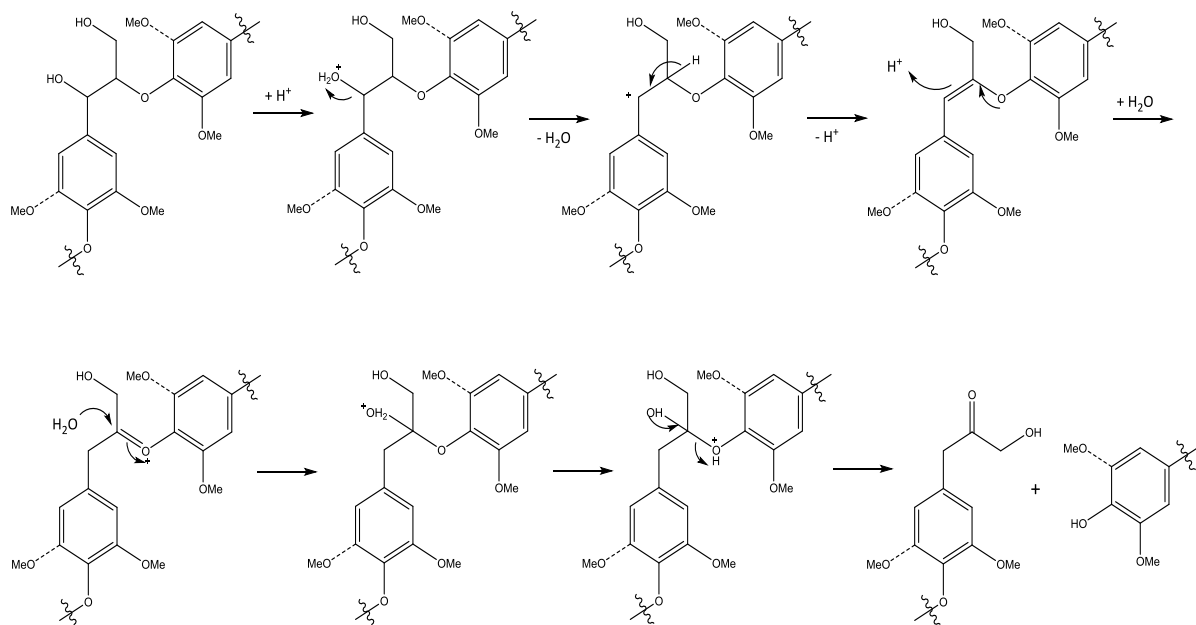


Figure 2-12. Cleavage of β -O-4 bond of lignin during pretreatment with acidic ionic liquids. X^- represents the anion of the ionic liquid.³⁶

Table 2-4. Amount of β -O-4 linkages in lignin isolated under different pretreatment conditions.

Pretreatment temperature [°C]	a/b = 1.02	a/b = 0.98
	β -O-4 bonds [per C ₉ unit]	β -O-4 bonds [per C ₉ unit]
120	0.66	0.65
150	0.38	0.41
170	0.18	0.31

However, the same structural changes of the β -O-4 bond cleavage were found for lignin isolated at elevated pretreatment temperatures. Interestingly, the more acidic ionic liquid was more effective at breaking the ether bond than the less acidic solution. This phenomenon was more pronounced for pretreatments with higher severity at temperatures of 150 °C and 170 °C.

Lignin recovered after pretreatment at 150 °C for 2 hours showed a significant difference in its structure with twice as many β -O-4 bonds per C₉ (0.20 vs. 0.10) when the less acidic ionic liquid was used. A similar ratio was found for lignin isolated after 30 minutes at 170 °C, where 0.18 β -O-4 bonds per C₉ (a/b = 1.02) are still preserved compared to 0.31 β -O-4 bonds per C₉ (for a/b = 0.98).

The structure of lignin isolated under very severe pretreatment conditions (e.g. 4 hours at 150 °C and 1 hour at 170 °C) is hypothesised to be highly condensed and rich in aromatic subunits, whereas most

inter-unit linkages have already been cleaved or altered. This hypothesis can be verified via molecular weight analysis of the samples and will be discussed in more detail later on in this chapter.

The abundance of β - β and phenylcoumaran linkages was less than 0.10 per C₉ unit for all the lignins, and the number of these linkages was largely unaffected by pretreatment severity. A minor decrease of the amount of β - β and phenylcoumaran linkages was observed for very severe pretreatment conditions. These linkages contain stable C-C bonds that are not easily hydrolysed under acidic reaction conditions.

2.2.6 Lignin subunits in aromatic region

Changes in lignin structure during acidic ionic liquid pretreatment do not only occur at the interunit linkages but also in the aromatic region of the polymer, as shown previously for *Miscanthus giganteus* lignin.³²

However, as discussed above, HSQC NMR analysis is not a flawless technique and one limitation of integrating HSQC NMR spectra in the aromatic region is that condensation reactions can occur on the aromatic rings³² which replace aromatic C-H bonds with C-C bonds, particularly at the C₆ position at the guaiacyl subunit. It must be noted that these C-C bonded aromatic positions do not produce cross correlations in HSQC spectra, meaning that the calculated subunit composition may be misrepresented when examining condensed lignins. With these restrictions in mind, volume integration was attempted for estimating the subunit composition. It should be noted that the C₅-H₅ and C₆-H₆ peak of the guaiacyl ring cannot be relied on for quantification of the guaiacyl content because of overlap with the PCA_{3,5} correlations (C₅-H₅) and participation in cross-condensation reactions (C₆-H₆). Due to these reasons, the C₂-H₂ resonance as well as the S_{2,6}-H_{2,6} signal were selected to calculate the S/G ratio.

The native S/G ratio of lignin in the cell walls cannot be determined easily due to modifications in the structure of lignin that occur with any extraction technique applied.³¹ However, the S/G ratio of lignocellulose has been reported to be linked to the ease of delignification (EOD) of hardwoods³⁷ as well as the ease of enzymatic hydrolysis of cellulose and hemicellulose^{38, 39}.

Additionally, the S/G ratio also gives information about the degree of condensation of the isolated lignin since G subunits are more prone to condensation reactions on the ring than S subunits. The condensation was observed to appear in acidic solvents via the formation of a carbocation on the α carbon of the β -O-4 linkage and a subsequent nucleophilic attack of the G subunit (Figure 2-13).³² The

characterisation of the S/G ratio of isolated lignin is an important tool to deepen the understanding of the structural composition of the polymer.

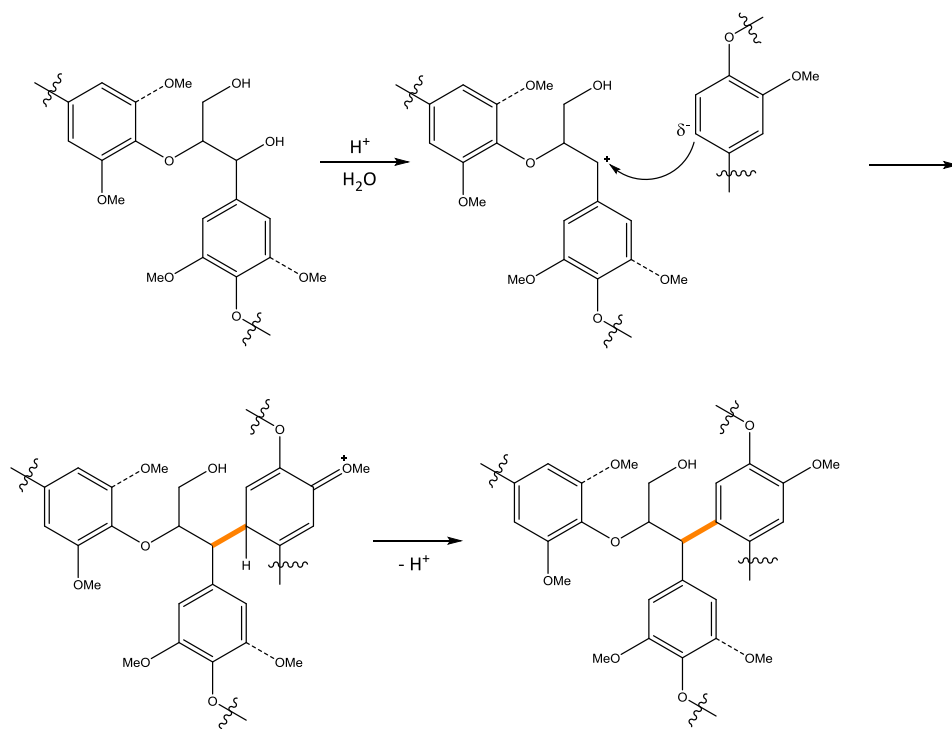
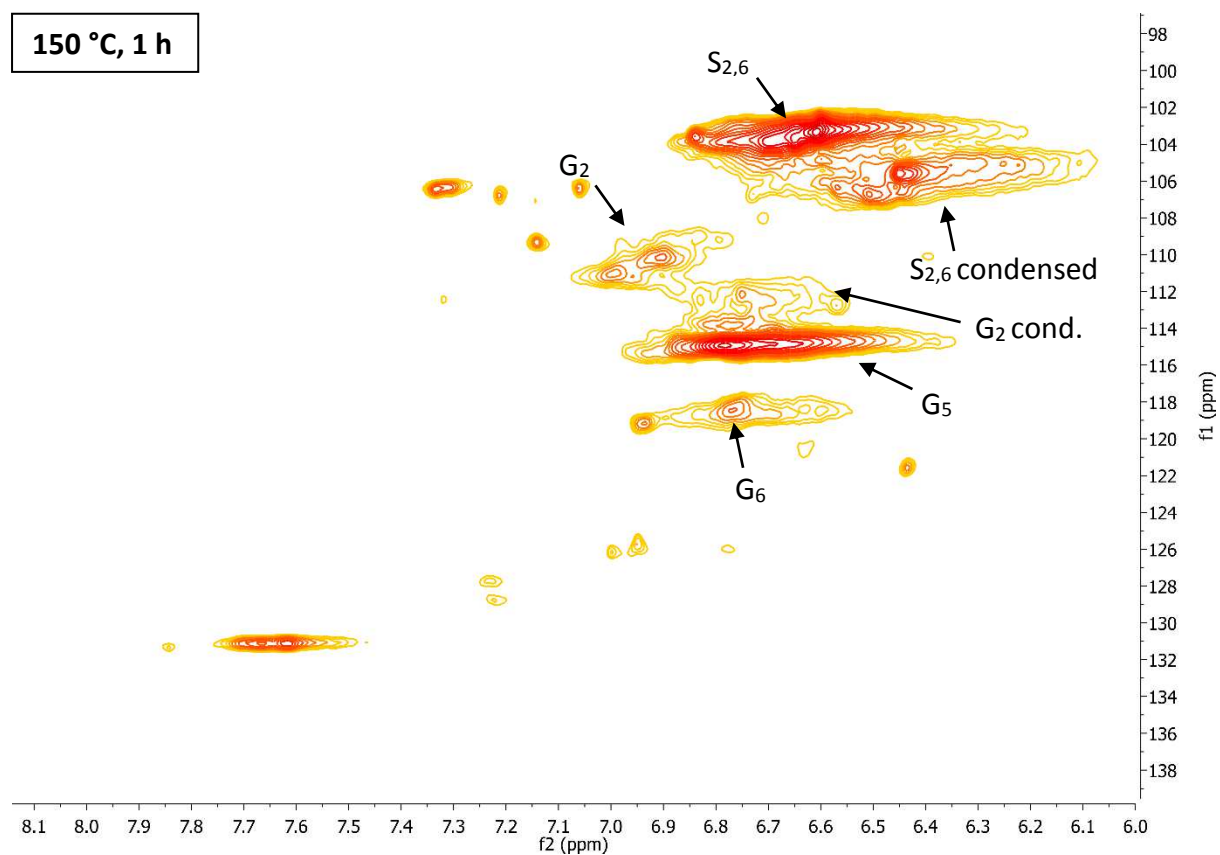
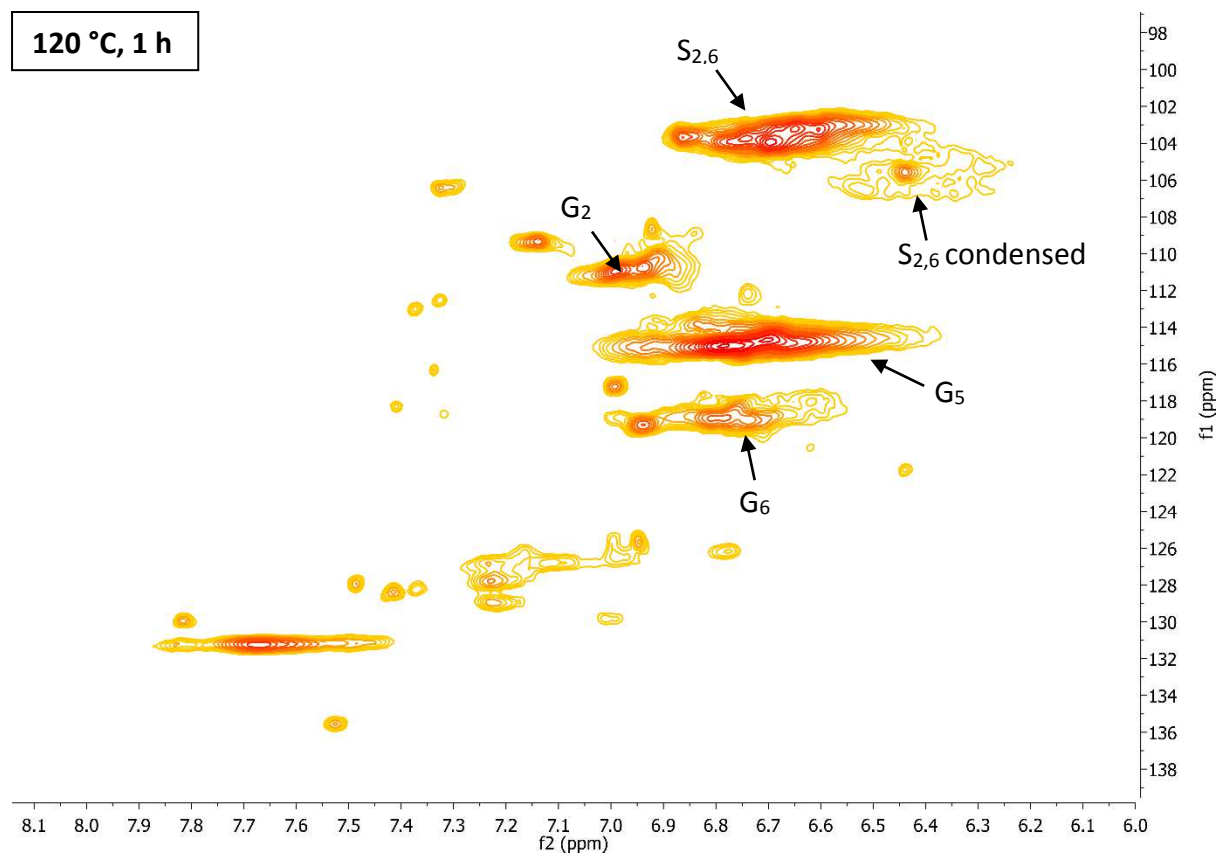


Figure 2-13. Formation of condensed lignin structures at the C₆ position at the G unit in acidic media.

A more condensed and cross-linked lignin contains more stable C-C bonds which are more resistant to cleavage, thus rendering these lignins unfeasible for the production of aromatic chemicals. However, these lignins might be considered interesting starting materials for polymeric applications such as resins⁴⁰ or carbon fibres⁴¹ due to their more condensed structure and higher aromaticity. Figure 2-14 for exemplary spectra of the aromatic region of lignin isolated after pretreatment with [N₂₂₂₀][HSO₄]_{80%} with a/b = 1.02 for 1 hour at 120 °C, 150 °C and 170 °C.



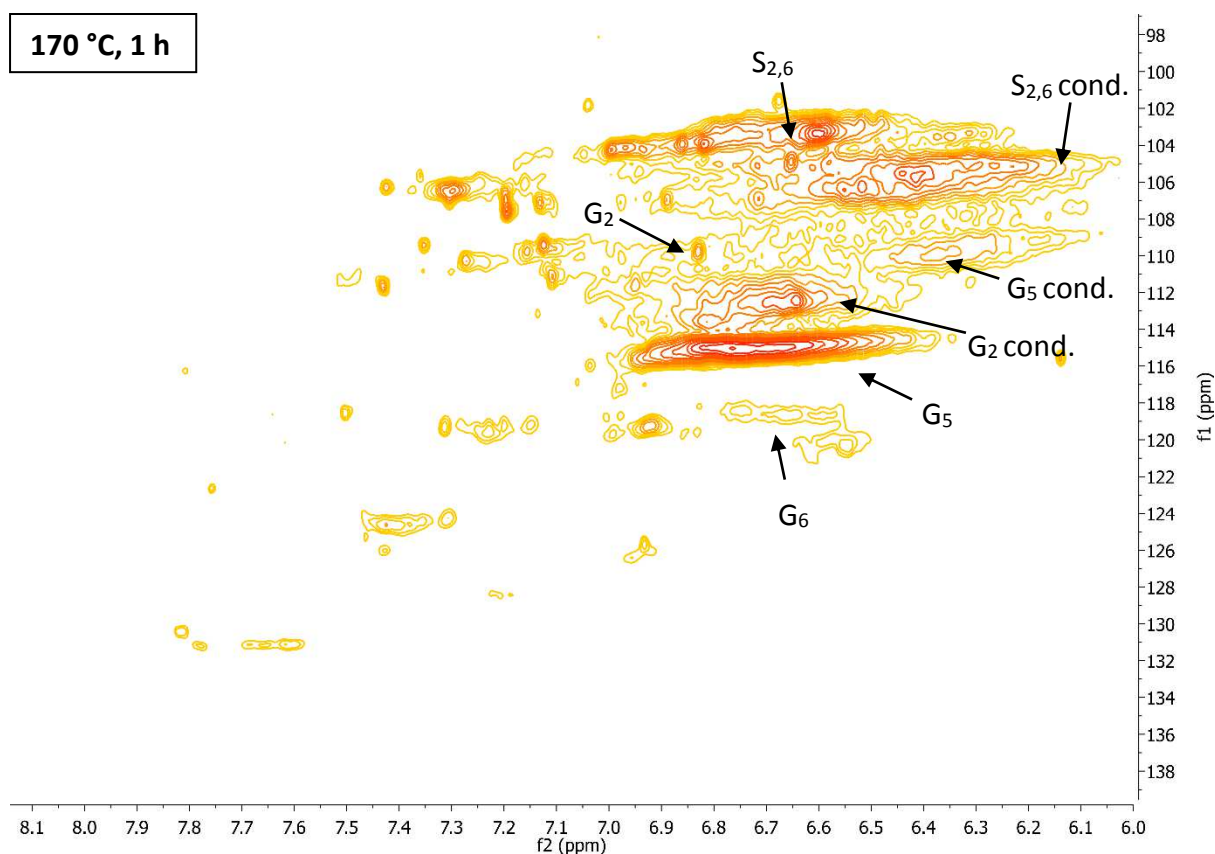


Figure 2-14. Example spectra of lignin recovered after pretreatment of *Salix* Endurance with [N₂₂₂₀][HSO₄]_{80%} with a/b = 1.02 with the following conditions: 1 hour at 120 °C (top), 1 hour at 150 °C (middle) and 1 hour at 170 °C (bottom).

It was found that the S/G ratio of lignin extracted during pretreatment at 120 °C steadily increased from 2.3 to 5.4 (for a/b = 1.02) and from 2.1 to 4.2 (for a/b = 0.98) with increasing pretreatment times. The S/G ratios determined here are in agreement with S/G ratios found for another hardwood (poplar) after hydrothermal pretreatment with different severity.²⁵ As discussed above, the acidity of the ionic liquid solutions did not significantly affect the linkage composition at this temperature, but it did affect the S/G ratio. Lignin isolated with the more acidic ionic liquid solution consistently showed a slightly higher S/G ratio, meaning that a greater amount of S subunits was extracted from the cell wall as compared to the lignin isolated with [N₂₂₂₀][HSO₄]_{80%} with a/b = 0.98 (Figure 2-15). Increasing the pretreatment time led to an increase in the S/G ratio up to 5.4 (for a/b = 1.02) and 4.2 (for a/b = 0.98) meaning that the lignins extracted under these conditions are around 5 times as rich in S subunits as in G subunits.

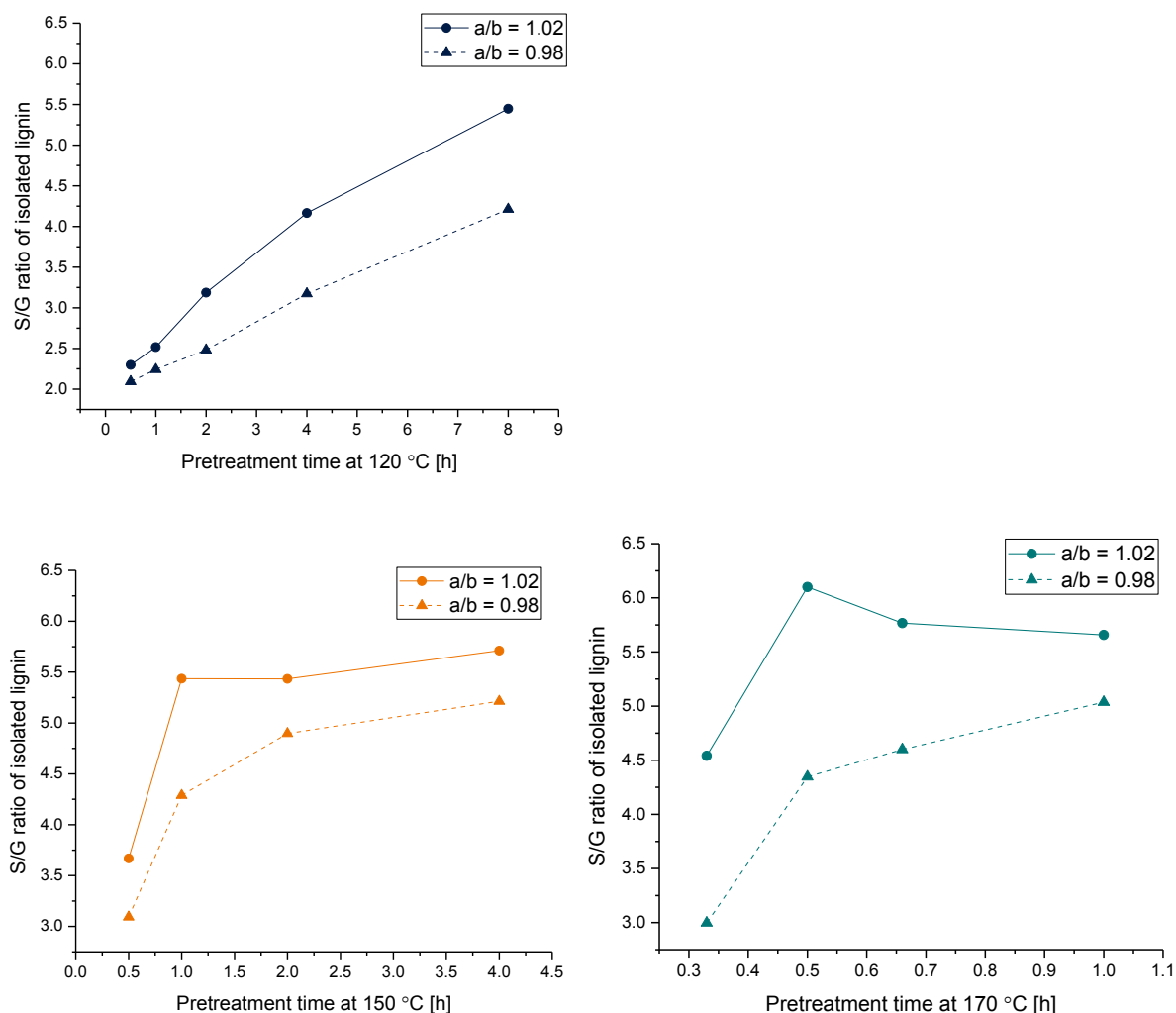


Figure 2-15. S/G ratio of lignin isolated after pretreatment at different temperatures with [N₂₂₂₀][HSO₄]_{80%} with different acid/base ratios (a/b=1.02 and 0.98) at 120 °C (top left), 150 °C (bottom left) and 170 °C (bottom right).

The acidity of the ionic liquid solution strongly influenced the S/G ratio for the lignin extracted for pretreatment at 150 °C. The aromatic subunit ratio for lignin isolated with the IL solution [N₂₂₂₀][HSO₄]_{80%} a/b = 1.02 initially significantly increased from 3.7 to 5.4 (for the pretreatment of 0.5 h and 1 h, respectively) and then reached a plateau around 5.5 for longer pretreatment times. This indicates that the pretreatment for 1 hour at 150 °C (with a/b = 1.02) extracted all the available S-lignin from the cell walls and longer pretreatment times did not influence the subunit composition. Interestingly, a different trend was observed for pretreatment with the less acidic ionic liquid. Here, the S/G ratio gradually increased from 3.1 to 5.2 with increasing residence time of the biomass in the

ionic liquid solution. This suggests that the lower acidity of this ionic liquid solution affected the kinetics of the extraction of S-units.

Increasing the pretreatment severity further by increasing the pretreatment temperature to 170 °C showed the same trend for the S/G ratio as observed for milder pretreatment conditions of 150 °C and 120 °C (when using the less acidic ionic liquid). The trend of the subunit composition of S and G units differs greatly when the more acidic ionic liquid solution was used for lignin extraction. The lignin extracted after pretreatment of 20 minutes was already enriched in S subunits. Increasing the pretreatment time further led to an increase in S/G ratio for the isolated lignin from 4.5 to 6.1, reaching the highest of S/G ratio observed for the lignins isolated for all the conditions studied. Interestingly, the very harsh pretreatment conditions (high temperature and high acidity of the ionic liquid solution) led to a decrease in S/G ratio from 6.1 (for 30 min) to 5.8 (for 40 min) and 5.7 (for 1 h) of the isolated lignin. This implies that the extracted lignin got enriched in G-units over time. The data presented here might indicate that S-rich lignin may be extracted under milder reaction conditions, due to it being less cross-condensed and thus more easily extracted.⁴² Very harsh pretreatment conditions, such as 40 minutes and 1 hour at 170 °C, also extracted the G-rich part of the lignin and thus led to a decrease in S/G ratio.

The S/G ratio of isolated lignin can also be attributed to properties of the polymer, such as degree of branching and stiffness. Lignins with a high S/G ratio are generally less rigid and more linear compared to lignins with a low S/G ratio.⁴³ Applying this principle to the lignins isolated in this study, it can be hypothesised that lignin isolated under mild pretreatment conditions would make a good macromonomer for applications where a stiffer material is needed. However, the properties of the polymer are not only influenced by its chemical structure but also by its chain length (i.e. molecular weight) and polydispersity.

2.2.7 Molecular weight of isolated lignin

The extracted lignin was investigated via GPC analysis in order to understand the effect of the pretreatment severity on the molecular weight (weight average M_w and number average M_n) of the polymer. However, it is important to note that the measured molecular weight of different lignin samples strongly depends on several factors including the nature of the biomass (grass, hardwood or softwood), the extraction method as well as the solvent and instrument used for analysis. This makes comparison with lignin isolated via other pretreatment techniques challenging.

A summary of the weight average molecular weight (M_w) of lignin using different pretreatment conditions is shown in Figure 2-16. A summary of both the weight average molecular weight (M_w), the number average molecular weight (M_n) and polydispersity of the extracted lignins can be found in Table A-1 in the appendix.

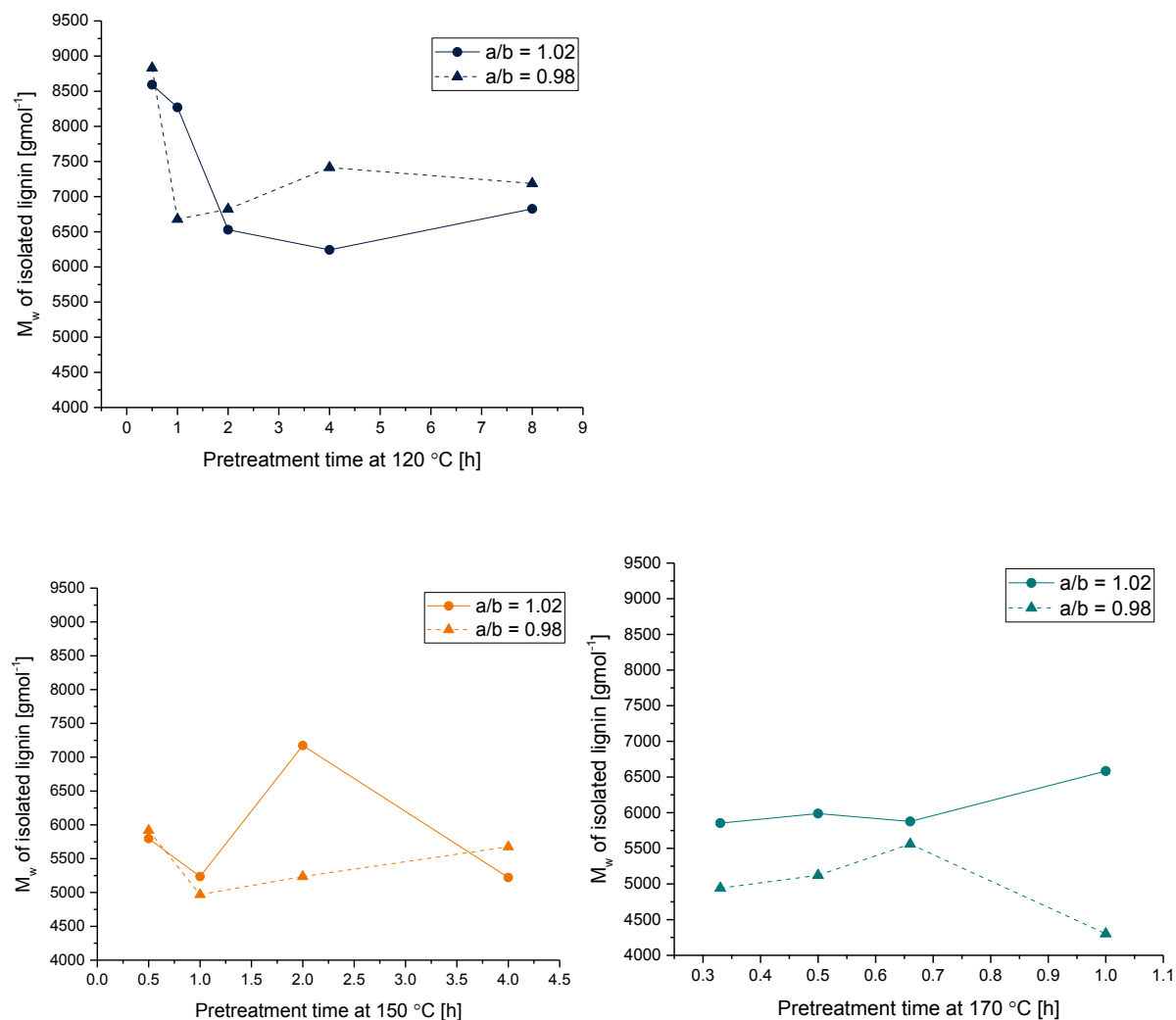


Figure 2-16. Weight average molecular weight ($\bar{M}_w$) of lignin isolated after pretreatment with [N₂₂₂₀][HSO₄]_{80%} with different acid/base ratios at 120 °C (top left), 150 °C (bottom left) and 170 °C (bottom right).

The weight average molecular weight of the lignin changed significantly with the applied pretreatment conditions. The molecular weight of the polymer was influenced by the residence time, pretreatment temperature and acid/base ratio of the ionic liquid solution.

The M_w of lignin isolated after pretreatment at 120 °C decreased dramatically with increasing pretreatment time from ca. 9 000 gmol^{-1} for pretreatment of 30 min to ca. 6 500 gmol^{-1} for pretreatment after 2 hours. This was caused by lignin depolymerisation through cleavage of β -O-4 bonds as discussed in the previous section, which is likely catalysed by the acidic conditions in the ionic liquid solution.^{18, 21} The acidity of the IL solution played a significant role regarding the chain length of the isolated lignin. Using the less acidic IL solution seemed to favour condensation reactions of the polymer over hydrolysis of the aryl ether bonds compared to the more acidic IL solution. The molecular weight of the lignin isolated with [N₂₂₂₀][HSO₄]_{80%} started to increase after only 1 hour of pretreatment, whereas depolymerisation still dominantly occurred until 4 hours of pretreatment in the reaction mixture using the more acidic IL solution. However, increasing the pretreatment time to 8 hours finally also led to condensation of the lignin in the more acidic IL solution.

For pretreatment at higher temperatures, a different trend for the molecular weight was observed. At 150 °C and using the less acidic ionic liquid solution, the molecular weight first decreased from ca. 6 000 gmol^{-1} after 30 min to ca. 5 000 gmol^{-1} for lignin isolated after 1 hour. This clearly shows the occurrence of depolymerisation of the lignin polymer. However, a steady increase in molecular weight to ca. 5 700 gmol^{-1} was observed for longer residence times (2 and 4 hours) for pretreatments with the IL with $a/b = 0.98$. This indicates that lignin condensation reactions were favoured, as suggested earlier by Brandt *et al.*²³ Interestingly, the acidity of the ionic liquid solution greatly impacted the molecular weight of the lignin isolated at this temperature. The initial depolymerisation step observed for lignin isolated with the more acidic IL was followed by a significant increase of M_w to ca. 7 200 gmol^{-1} (for pretreatment of 2 hours), showing that lignin cross-condensation had occurred as well. However, the polymeric product recovered using these pretreatment conditions showed a significantly larger chain length compared to using the less acidic IL solution. This indicates that the greater acidity of this ionic liquid solution supported the cross-condensation of lignin.

Interestingly, the conditions applied for the pretreatment of 4 hours led to a decrease in lignin molecular weight to ca. 5 000 gmol^{-1} , which might indicate that under these conditions cleavage reactions of several lignin linkages were favoured over cross-condensation due to the high severity of this treatment. This hypothesis is supported by the HSQC NMR data discussed previously showing that the amount of β - β and phenylcoumaran bonds decreased for lignin isolated under these conditions compared to shorter pretreatment times at the same temperature.

The molecular weight of lignin was significantly influenced by the pretreatment severity and pretreatment at very high temperatures of 170 °C resulted in a trend that differed from the ones observed for lower temperatures. Using [N₂₂₂₀][H₂SO₄]_{80%} with an a/b of 1.02 the M_w of the isolated

lignin was found to slightly increase with increasing pretreatment time from ca. 6000 g mol⁻¹ (20 minutes) to ca. 6500 g mol⁻¹ (1 hour) indicating that at these high temperatures condensation reactions of the lignin polymer are favoured over cleavage reactions. Using the IL solution triethylammonium hydrogensulfate with a lower acid to base ratio of 0.98 an overall lower molecular weight of the extracted lignin was found supporting the hypothesis that condensation reactions are occurring more frequently than depolymerisation reactions when using the more acidic IL solution. Interestingly, the observed trend for the M_w of lignin isolated with [N₂₂₂₀][H₂SO₄]_{80%} with a/b did not follow the one found for pretreatment with the more acidic IL solution. The weight average molecular weight first increased from ca. 5000 g mol⁻¹ (20 minutes) to ca. 5500 g mol⁻¹ (40 minutes) and then significantly decreased to ca. 4200 g mol⁻¹ (1 hour). This suggests that under these reaction conditions lignin depolymerisation takes place most likely induced by the very severe pretreatment conditions and the less acidic ionic liquid solution.

2.3 Summary and Future work

The conditions such as residence time, pretreatment temperature and acidity of the ionic liquid solution significantly impact the outcome of the pretreatment in terms of lignin and pulp yield and the composition of the pulp and properties of the lignin. Generally, using the more acidic ionic liquid solution resulted in a more effective pretreatment and higher glucose yields but only if the severity of the pretreatment was well controlled through the residence time and pretreatment temperature. The composition of the recovered pulp strongly depended on the acid/base ratio of the ionic liquid solution used and compositional analysis revealed that pretreatment with the more acidic IL solutions led to the formation of pseudo-lignin on the pulp for severe pretreatment conditions. This negatively impacted the glucose release of the pulp and high saccharification yields were observed to be preferable for pretreatments of medium severity. Using the less acidic ionic liquid solution resulted in a higher degree of glucan preservation and high saccharification yields even at very severe pretreatment conditions.

The structure and the properties of the isolated lignin were also significantly impacted by the severity of the pretreatment. Lignin extracted under mild pretreatment conditions showed to have a relatively high molecular weight and a high amount of intact β-O-4 bonds which would make this lignin a promising candidate for the production of aromatic chemicals from lignin *via* depolymerisation. However, more severe pretreatment conditions altered the structure of the lignin and caused hydrolysis of the aryl ether bonds and cross-condensation reactions of the lignin resulting in a polymer

that has a high aromaticity and increased amount of C-C bonds. This type of polymer could be used in applications where a condensed aromatic structure is required such as carbon fibres or resins.

As mentioned above, the genus *Salix* consists of around 400 varieties with only limited knowledge of how the different varieties perform as feedstocks for the ionic liquid pretreatment and which property of the pulp affects its digestibility the most. Additionally, little is known about the differences in the structure of lignin of the different varieties and how that would affect the use in possible applications. The next chapter of this thesis will thus discuss these questions.

2.4 References

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Chapter 3 – Towards understanding the factors influencing enzymatic hydrolysis of *Salix* varieties

3.1 Introduction

The hardwood willow (*Salix*) is considered a promising feedstock for the production of liquid biofuels in biorefineries. Around 400 different species of the genus *Salix* are known and the genotypes differ widely in many biomass properties, amongst them size and leaf shape. Willows are generally known as tall (weeping) trees, but the size spectrum also includes shrubs and bushes as well as dwarf and rockery plants (Figure 3-1).¹



Figure 3-1. Examples of diversity in leaf form (A–F), stem shape (G) and size (H–I) in willow genotypes.¹

Some varieties are grown commercially in short rotation coppice as feedstock for the production of biofuels and bioenergy. For this purpose, shrubby willow varieties have been identified to be the most promising candidates due to high biomass yield and desirable biomass properties.¹ The breeding of various willow varieties has been undertaken in Europe for several decades and some species have been cultivated predominantly for biorefining purposes. These include mainly *S. viminalis*, but also *S. dasyclados*, *S. schwerinii*, *S. trianda* and *S. caprea*. Hybrid cultivars with improved biomass yield and pest resistance resulted from these breeding programs. New varieties derived from *S. viminalis* (such as Orm, Rapp, Ulv, Jorr and Jorrun) were reported to have 15 – 20 % higher biomass yields compared to non-bred willow clones. Additional progress in terms of biomass yield and resistance to the fungal disease rust (derived from *S. schwerinii*) of the specifically bred varieties Bjorn and Tora were

reported. Later on, promising hybrid varieties such as Resolution and Terra Nova have emanated from a collaboration of the UK and Swedish breeding programme.²

However, the achievable biomass yield, chemical composition of the stems and energy value not only strongly depend on the willow variety but also on the harvest cycle of the plantation.³ An overview of the influence of the length of the growth cycle on the biomass yield of six willow varieties is demonstrated in Figure 3-2. This demonstrates the difference in achievable biomass yield of willow genotypes, which is under genetic control and presents one issue in the search for the best willow variety for biorefining.

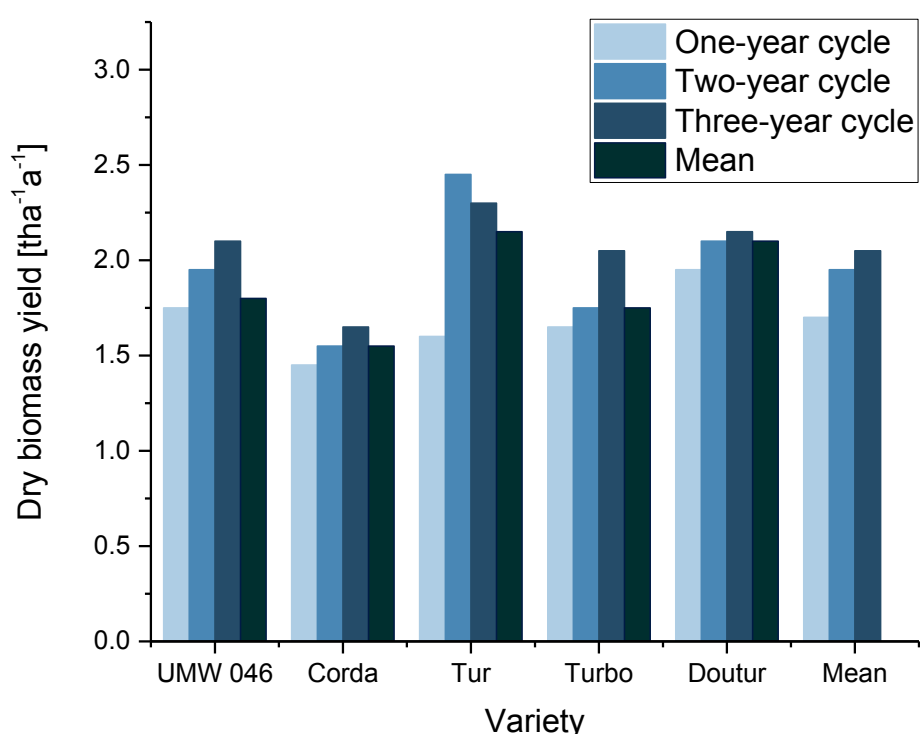


Figure 3-2. The relationship of dry biomass yield of six willow varieties and the harvest cycle.³

Typically, willow biomass contains around 40 wt% cellulose, 20 wt% hemicellulose and 25 wt% lignin. However, a study conducted by Ray *et al.*⁴ showed significant variation in cellulose, hemicellulose and lignin content of the 35 willow varieties studied.

Additionally, the individual willow varieties also differ in the chemical structure of the biopolymers hemicellulose and lignin. Particular focus has been placed on the structure of hemicellulose due to its role of acting as a bridge between the cellulose microfibrils and lignin polymer. Xylose is the most

abundant monomer in the hemicellulose polymer forming the backbone *via* β -(1,4)-linked D-xylose units (Figure 3-3). The polymer contains *O*-2-linked glucuronic acid or methyl-glucuronic acid moieties and can be highly acetylated.⁵ Studies investigating the role of genes of different varieties have shown that those are responsible for variations in the backbone synthesis of the polymer⁶, addition of glucuronic acid⁷, xylan chain length⁸ and xylan *O*-acetylation⁹. The difference in hemicellulose structure has been suspected to be a key parameter in the ease of enzymatic hydrolysis of different genotypes.

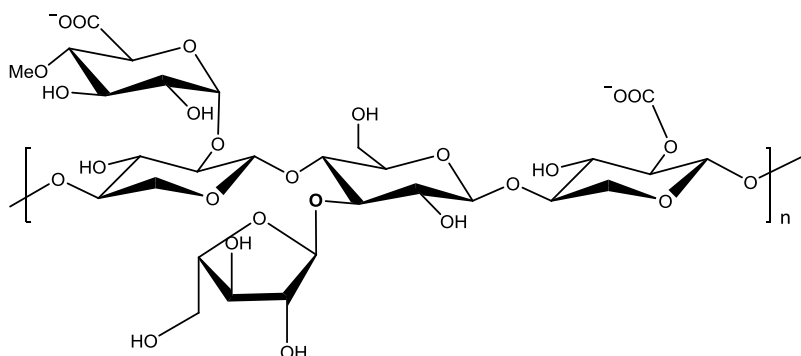


Figure 3-3. Generic structure of β -(1,4)-linked d-xylose units that form the backbone of the xylan polymer.¹⁰

In order to utilize willow for the production of bioethanol and in light of the genetic variety of the different genotypes, a deeper understanding of the response to pretreatment of the varieties needs to be achieved. The information derived from this can aid in selection of the most suitable willow variety for biorefining.

A first attempt in relating specific biomass properties of 35 willow varieties to the suitability of those for the production of liquid fuels was undertaken by M. Ray *et al.*⁴ The authors investigated four traits of the different varieties, namely lignocellulose composition, response to pretreatment, yield of enzymatic saccharification of raw and dilute acid pretreated varieties and projected ethanol yields. The study concluded that the optimum properties (e.g. biomass composition, pulp digestibility and glucose yield) of willow as a dedicated feedstock for biofuel production are genotype specific. Surprisingly, the authors also found no correlation between lignin content of the untreated lignocellulose and glucose yield of the pretreated pulps. This suggests that varieties with a high lignin content might be the superior feedstock compared to low lignin genotypes if lignin can be successfully turned into value-added products that have a higher market price than pulp or monomeric sugar.

This result is particularly interesting, since much research is conducted to reduce the lignin content of bioenergy crops to guarantee higher glucose yields. However, lowering the lignin content of a plant

(either by breeding or genetic modification) also comes along with severe disadvantages to the health and recalcitrance of the plant as well as a decrease in biomass yield.¹¹

A second study investigating the potential of willow genotypes as feedstock for biorefineries was conducted by M. J. Serapiglia¹² where 10 genetically diverse genotypes were pretreated *via* hot water pretreatment. The authors also found genotype specific differences of the glucose yield of enzymatic hydrolysis and hypothesized that this might also be subject to lignin properties such as amount of phenolic groups and S/G ratio of the lignin.

The above mentioned studies gave interesting insight into the potential use of willow genotypes as biorefinery feedstocks, but are lacking a detailed analysis of the biomass properties that actually influence the glucose yield of enzymatic hydrolysis. The results discussed in this chapter aim to deepen the understanding of the factors controlling the digestibility of the untreated lignocellulose and pulp.

The first chapter of this thesis discussed the outcome of the ionic liquid pretreatment of the willow variety Endurance regarding biopolymer recovery, yield of enzymatic hydrolysis and lignin properties. The focus of this chapter lies on understanding changes of the physical and chemical properties of the pulp of 14 willow genotypes induced by pretreatment with the ionic liquid solution [N₂₂₂₀][HSO₄]_{80%} and how these influence the cellulosic glucose release from the untreated biomass and pretreated pulp.

The varieties selected were chosen based on a broad range of glucan and lignin content as reported previously by Ray *et al.*⁴ The different willow varieties investigated in this study and information regarding their parents are listed below in Table 3-1.

Some of the varieties investigated in this study are related to each other, for example, the variety Tora is descendent from Orm as well as the mother of Nimrod. This guarantees genetic similarities and might play a role in regard to response of ionic liquid pretreatment.

Table 3-1. 14 willow genotypes used for ionic liquid pretreatment with [N₂₂₂₀][HSO₄]_{80%}.

Willow variety	Abbreviation	Species
Asgerd	A	<i>S. viminalis</i> x <i>S. schwerinii</i>
Baldwin	B	<i>S. triandra</i>
Bowles Hybrid	BO	<i>S. viminalis</i>
Corail	C	<i>S. triandra</i> x <i>S. viminalis</i>
Discovery	D	<i>S. schwerinii</i> 'K3 Hilliers' x (<i>S. vim.</i> x <i>S. schwerinii</i>) 'Bjorn'
Endurance	E	<i>S. rehderiana</i> x <i>S. dasyclados</i> '77056'
Jorr	J	<i>S. viminalis</i> x <i>S. viminalis</i>
Nimrod	N	(<i>S. schwerinii</i> 'L79069' x (<i>S. viminalis</i> 'L78195' x <i>S. viminalis</i> 'L78101') 'Orm') 'Tora' x <i>S. miyabeana</i> 'Shrubby'
Orm	O	<i>S. viminalis</i> x <i>S. viminalis</i>
Resolution	R	((<i>S. viminalis</i> x <i>viminalis</i> 'Jorunn') x (<i>S. schwerinii</i> x <i>viminalis</i> 'Bjorn') 'SW930812') x ((<i>S. viminalis</i> 'Pavainen') x (<i>S. schwerinii</i> x <i>viminalis</i> 'Bjorn') 'Quest')
Shrubby Willow	SW	<i>S. miyabeana</i>
Stott10	St10	<i>S. viminalis</i> x <i>S. dasyclados</i> 'Korso'
Terra Nova	TN	(<i>S. viminalis</i> 'Bowles Hybrid' x <i>S. triandra</i> 'Brunette Noire') 'LA940140' x <i>S. miyabeana</i> 'Shrubby'
Tora	T	<i>S. schwerinii</i> 'L79069' x (<i>S. viminalis</i> 'L78195' x <i>S. viminalis</i> 'L78101') 'Orm'

3.2 Results and discussion

3.2.1 Composition of untreated willow genotypes

Compositional analysis was performed on the willow varieties prior to ionic liquid pretreatment to enable comparisons between the untreated biomass and pretreated pulp as well as to draw conclusions of the effect the pretreatment has on the relative amounts of the main biopolymers and other components.

Most biorefinery scenarios envision bioethanol to be one of the main products of the refinery leading to the search for sufficient feedstocks with high cellulose content.¹³ However, high cellulose content is not the only factor that influences the glucose yield obtained by enzymatic hydrolysis. It is assumed that the lignin content in the native biomass significantly affects the cellulose digestibility. For this reason, biologists and plant geneticists focus on breeding plants with a low lignin content.¹¹ The resulting loss in biomass yield and recalcitrance is considered a disadvantage of this approach of improving the pulp digestibility. Further insight into the relationship of lignin content, delignification

and ease of enzymatic hydrolysis of lignocellulose could answer the question if genetic modification of the lignin content of a plant is a road worth pursuing.

The varieties were first analyzed regarding their biomass composition and it was found that the 14 genotypes showed significant differences in glucan, hemicellulose and lignin content as well as amount of extractives (Table 3-2 and Figure 3-3).

Table 3-2. Glucan, hemicellulose and lignin content and extractives of the 14 willow genotypes. The ash content of all the samples was negligible. The standard deviation is displayed in brackets.

Variety	Glucan	Xylan	Galactan	Arabinan	Mannan	ASL	AIL	Extractives	Mass closure
A	42.28 (0.45)	13.04 (0.05)	2.66 (0.09)	0.58 (0.11)	3.76 (0.21)	2.22 (0.02)	23.27 (0.27)	9.62 (0.36)	97.42
B	39.75 (0.07)	15.59 (0.22)	2.41 (0.03)	0.93 (0.09)	2.60 (0.01)	2.17 (0.00)	23.15 (0.25)	7.59 (0.10)	94.19
BO	40.79 (0.07)	13.19 (0.13)	2.64 (0.05)	0.74 (0.03)	3.65 (0.01)	2.20 (0.00)	24.05 (0.31)	8.88 (0.41)	96.13
C	44.45 (0.53)	16.30 (0.07)	2.22 (0.08)	0.61 (0.02)	2.60 (0.01)	2.16 (0.01)	20.88 (0.13)	8.18 (0.09)	97.39
D	41.16 (0.12)	13.03 (0.13)	2.22 (0.27)	0.20 (0.15)	2.98 (0.26)	1.93 (0.01)	25.52 (0.02)	8.99 (0.61)	96.03
E	41.29 (0.08)	13.61 (0.07)	2.52 (0.09)	0.80 (0.03)	3.27 (0.00)	2.27 (0.02)	23.42 (0.28)	11.59 (0.06)	98.76
J	40.65 (0.07)	13.70 (0.06)	2.32 (0.08)	0.88 (0.02)	2.98 (0.04)	2.20 (0.02)	23.41 (0.06)	9.29 (0.04)	99.48
N	41.49 (0.54)	13.52 (0.27)	5.50 (0.06)	1.01 (0.03)	3.31 (0.07)	2.28 (0.02)	23.96 (0.09)	8.47 (0.34)	99.53
O	41.08 (0.60)	14.98 (0.12)	2.28 (0.00)	0.39 (0.00)	3.34 (0.04)	2.15 (0.01)	20.92 (0.20)	7.43 (0.49)	92.74
R	43.05 (0.02)	14.21 (0.05)	5.67 (0.07)	0.79 (0.04)	3.88 (0.06)	2.13 (0.00)	22.41 (0.25)	8.50 (0.30)	100.64
SW	36.96 (0.58)	13.20 (0.33)	5.65 (0.04)	1.46 (0.07)	3.23 (0.01)	2.28 (0.02)	25.34 (0.47)	11.63 (0.85)	99.75
St10	47.11 (0.41)	14.19 (0.00)	1.98 (0.02)	0.24 (0.03)	3.52 (0.03)	2.33 (0.02)	21.01 (0.12)	5.43 (0.10)	95.81
TN	46.58 (0.13)	12.79 (0.13)	2.19 (0.07)	0.44 (0.07)	3.55 (0.01)	2.30 (0.03)	21.83 (0.04)	7.50 (0.10)	96.57
T	40.29 (0.00)	14.76 (0.19)	2.19 (0.08)	0.56 (0.09)	3.62 (0.08)	2.34 (0.03)	24.20 (0.19)	8.04 (0.49)	96.01

The lowest glucan content of 36.96 % was found for Shrubby Willow and the highest glucan content of 47.11 % was detected for Stott 10. The varieties Terra Nova and Corail also showed high glucan content of the untreated biomass. Simply speaking, the glucan content of lignocellulose should

correlate with enzymatic glucose release, making these three varieties promising candidates for high saccharification yields.

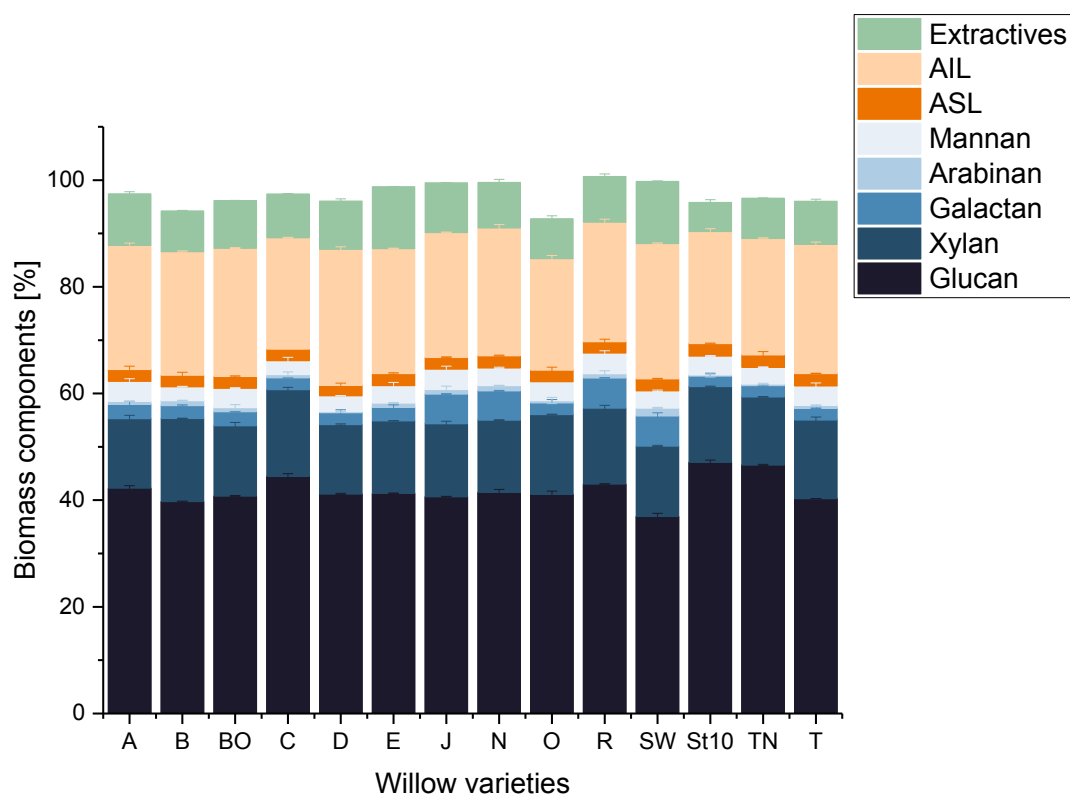


Figure 3-4. Composition of the 14 native willow varieties investigated in this study.

The hemicellulose fraction of the examined willows mainly consists of xylan (ranging from 12.79 % for Terra Nova to 16.30 % for Corail), with low amounts of galactan and mannan and traces of arabinan, which is in good agreement with previous findings reporting *O*-acetyl-4-*O*-methyl glucuronoxylan being the dominant hemicellulose in secondary cell walls (SCW) in hardwoods.¹⁴ The chain length and degree of acetylation of hemicellulose amongst the willow varieties is believed to be under genetic control, leading to a variation in those properties throughout the genotypes.⁵ Hemicellulose fulfills a structural role in the SCW, acting as the bridging interface between the lignin and the cellulose microfibrils and is thus assumed to play a key role in biomass properties such as accessibility of cellulose for enzymatic hydrolysis.⁵

Applying logical thinking, it would be expected that the willow varieties with a high glucan content would have a correspondingly low lignin content and vice versa. But interestingly, no correlation of glucan and lignin content were observed, suggesting that there is no compensatory relationship between these two major cell wall polymers.

The willow genotypes do not only differ in glucan and hemicellulose content but also significantly in lignin content ranging from a low amount of 20.88 % (Corail) to a high one of 25.52 % (Discovery). The access of cellulose enzymes to cellulose is restricted by lignin¹⁵, suggesting that varieties with a high lignin content will result in low pulp digestibility. Theoretically, a higher lignin content should result in higher recalcitrance of the lignocellulose and lower degree of delignification after pretreatment. The following section will discuss this hypothesis in more detail.

3.2.2 Influence of ionic liquid pretreatment on pulp and lignin recovery

A previous study conducted by Weigand *et al.*¹⁶ showed that the ionic liquid pretreatment of willow (genotype Endurance) with triethylammonium hydrogen sulfate solution [N₂₂₂₀][HSO₄]_{80%} increased the glucose yield of the biomass. However, selecting the correct pretreatment conditions was found to be vital for the success of the pretreatment. It was shown that very mild conditions (e.g. 120 °C for 30 min, 1 hour and 2 hours) led to a decrease in enzymatic glucose yield compared to the untreated biomass due to acid catalyzed hydrolysis of the amorphous part of the cellulose and simultaneous poor delignification. Pretreatment applying very severe conditions was found to cause over-treatment of the pulp which again decreased the glucose yield.

Based on these findings, mild conditions of the ionic liquid pretreatment were chosen for this study to deliberately undertreat the 14 genotypes which should highlight the differences in biomass cell structure and the response of the willow varieties towards the ionic liquid pretreatment. Applying a too severe pretreatment might lead to some varieties wrongly seeming to perform worse due to over-treatment. The conditions of this study were chosen to be 120 °C for 4 hours using the ionic liquid solution [N₂₂₂₀][HSO₄]_{80%}.

Ionic liquid pretreatment of the different genotypes resulted in a deconstruction of the biomass and a pulp and lignin fraction were recovered (Figure 3-5). The ionic liquid proved to be very effective in breaking up the biopolymer matrix and removed nearly 50 % of the lignocellulose material independent of the genotype. The pulp yield of willow Endurance in this study is lower compared to the pulp yield of the same variety observed after pretreatment with [N₂₂₂₀][HSO₄]_{80%} in chapter 1 of this thesis. A different batch of IL solution was used in this study and the a/b ratio most likely differed slightly, resulting in a slightly different pretreatment outcome. However, the pretreatment of the 14 willow varieties used in this chapter was undertaken with one batch of IL to ensure comparability of the results. The pulp and lignin recovery of the willow varieties after IL pretreatment varied between

50.1 % (Shrubby Willow) to 54.9 % (Discovery) and 8.2 % (Stott10) to 11.9 % (Jorr) of the original dry biomass, respectively. These findings suggest that the recalcitrance towards ionic liquid pretreatment is dependent on the genotype.

Interestingly, a direct correlation between pulp recovery and lignin recovery was not observed, meaning that a lower pulp yield did not correspond with a higher lignin yield for the same genotype. This suggests that the recovery of the pretreatment fractions is influenced by several factors. Firstly, the complexity of the starting material (in terms of diversity of biopolymer content, biopolymer structure and amount of linkages between lignin and carbohydrates) plays a role in the fractionation process. Secondly, the pretreatment process itself is thought to be a cascade of individual reactions^{17, 18} such as cleavage of lignin-carbohydrate complexes and lignin aryl ether bonds, lignin condensation reactions as well as lignin re-deposition on the pulp that the different genotypes are more or less structurally inclined to undergo.

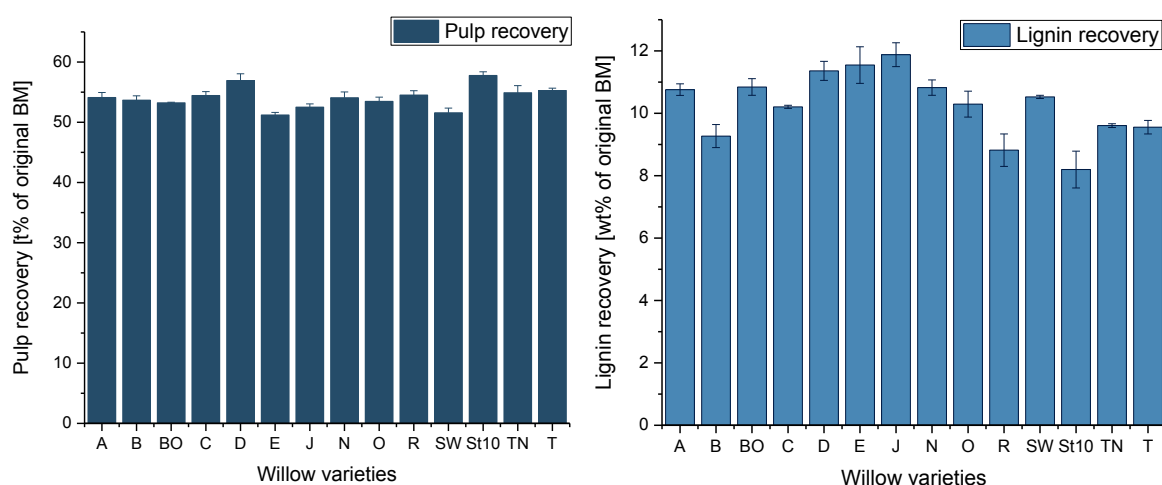


Figure 3-5. Pulp yield (left) and lignin yield (right) recovered from 14 willow varieties after pretreatment with $[N_{2220}][HSO_4]_{80\%}$ at 120 °C for 4 hours.

The untreated willow genotypes are characterized by a wide range of lignin content, but interestingly no correlation between lignin content in untreated biomass and lignin recovery after pretreatment was found. This suggests that the lignin content of the genotypes is not the only factor influencing lignin extraction during ionic liquid pretreatment. It is well known that lignin depolymerisation occurs in acidic media¹⁹ and it is assumed that the lignin was partially depolymerized into low molecular weight compounds during the extraction with the acidic IL. Those short lignin oligomers remained dissolved in the pretreatment solvent $[N_{2220}][HSO_4]_{80\%}$ after the addition of the anti-solvent and only parts of the extracted lignin were recovered. It seems that the lignin of the different genotypes is more

or less prone to depolymerisation under the applied pretreatment conditions, most likely due to a different content of easily hydrolyzed aryl ether bonds in the lignin polymer.

Another factor influencing lignin recovery is re-deposition of lignin on the pulp induced by condensation reactions of lignin under acidic conditions.^{20, 21} Previous unpublished research undertaken in our group indicates that the solubility of the lignin in the ionic liquid solution is determined by its molecular weight. High molecular weight polymers formed under severe pretreatment conditions are not soluble in the ionic liquid solution. This leads to re-deposition of lignin on the pulp and an observed increase in the pulp yield compared to pulps recovered from lower severity pretreatment. This effect has previously been observed for the pretreatment of willow (variety Endurance) with $[N_{2220}][HSO_4]_{80\%}$ for harsh pretreatment conditions.¹⁶ However, mild pretreatment conditions were applied in this study and no evidence for lignin re-deposition on the pulp was found for the varieties investigated here.

The ionic liquids utilized in the ionicSolv pretreatment are not capable of disrupting the H-bonding network of the cellulose fibrils, resulting in a cellulose-rich pulp after pretreatment. Interestingly, the pulp recovery was found to mostly correlate with the glucan content in the untreated willow genotypes (see Figure 3-6). This result supports the hypothesis that no *pseudo*-lignin was formed on the pulp. Furthermore, cellulose degradation seemed to be kept minimal under the applied pretreatment conditions.

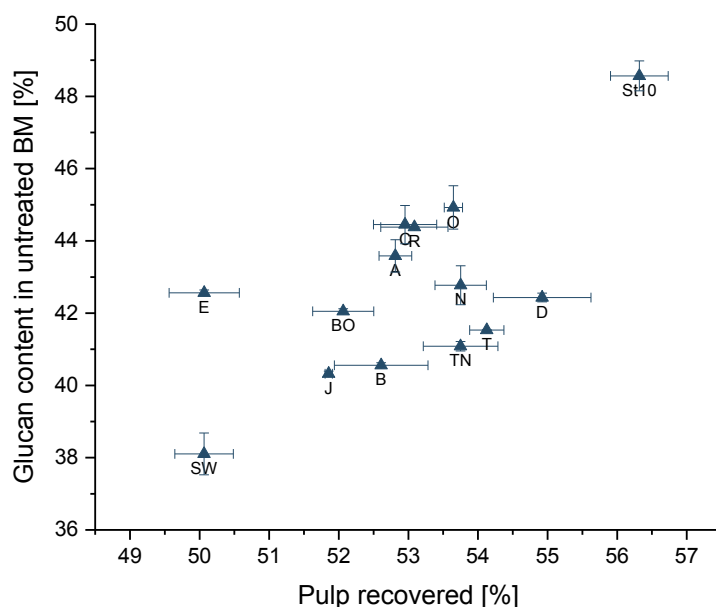


Figure 3-6. Relationship between glucan content in raw biomass and pulp recovery.

3.2.3 Enzymatic hydrolysis of raw lignocellulose and pulp

Enzymatic saccharification using Cellic CTec3 was performed to allow for evaluation of the effectiveness of the pretreatment on the 14 genotypes. Research over recent years has shown that several factors influence the glucose yield of pretreated pulp. These include but are not limited to: (i) glucan content of the pulp, (ii) delignification and hemicellulose removal, (iii) formation of *pseudo*-lignin, (v) cellulose crystallinity and (vi) exposed cellulose surface area.²² However, to the best of my knowledge no study investigated the influence of structural properties of the untreated and pretreated biomass of willow varieties on the yield of enzymatic saccharification.

The willow varieties used in this study were specifically bred for use as bioenergy crops and thus show a very high glucose release even for the untreated lignocellulose. Typically, glucose yields using the same enzyme mixture in our laboratory were found to be less than 20 % of the theoretical maximum for feedstocks such as *Miscanthus giganteus* or pine.²³

The glucose release of the untreated willow varieties varied significantly after saccharification with yields ranging from 35.8 % for Resolution to 63.3 % for Endurance (see Figure 3-7).

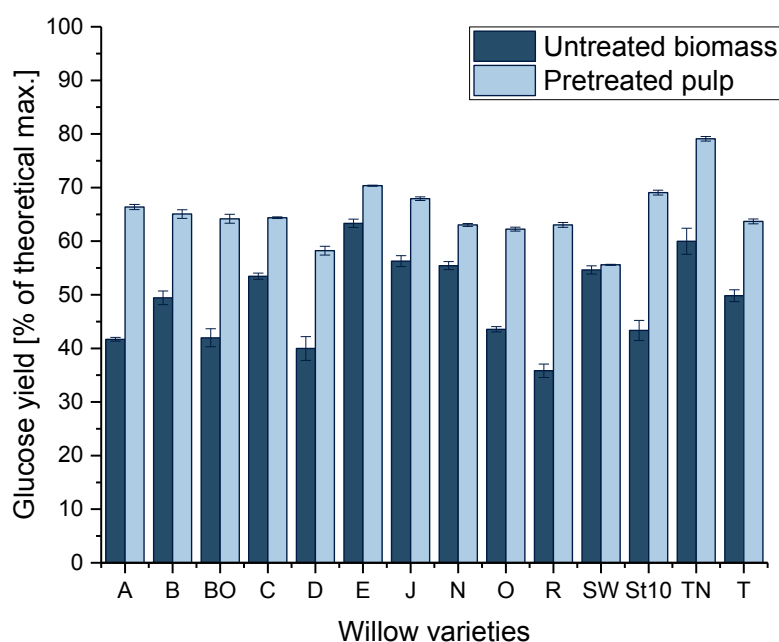


Figure 3-7. Glucose yields as percent of theoretical maximum for untreated willow genotypes and genotypes after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

Even though high pulp digestibility was already realized for untreated biomass, ionic liquid pretreatment led to an increase in glucose yield for all genotypes. Interestingly, the increase in glucose yield compared to the untreated samples was observed to not be the same for all the varieties. The

glucose release in untreated biomass. The variety Shrubby Willow (with a high lignin content of 25.3 %) displayed good digestibility with a relatively high glucose yield of 54.6 %, whereas Stott 10 (lignin content of 21.0 %) only showed a relatively low glucose release of 43.3 %. This is in agreement with a study by M. J. Ray *et al.*⁴ where the authors also describe that no correlation between glucose yield and initial lignin content of the untreated biomass.

These findings indicate that the sugar release of different willow genotypes is not solely controlled by the biomass composition, but other properties of the lignocellulosic material also play a significant role in biomass digestibility.

After ionic liquid pretreatment with [N₂₂₂₀][HSO₄]_{80%} the correlation relationship between glucan or lignin content in the pulp and saccharification yield changed completely. Interestingly, a correlation was now observed for glucose release and pulp composition (see Figure 3-8, right), meaning that a higher glucan content and a lower lignin content of the pulp resulted in higher glucose yields after enzymatic hydrolysis. This clearly shows that the removal of lignin from the biopolymer matrix positively influences the pulp digestibility.

The following sections of this chapter will discuss the influence of structural and chemical properties of the lignocellulose (before and after pretreatment) on the yield of enzymatic hydrolysis.

3.2.4 Composition of pretreated biomass

Compositional analysis of the pulp was performed to study the effect of pretreatment on the 14 genotypes regarding ease of delignification and cellulose degradation.

The pretreatment of lignocellulose with protic ionic liquid solutions fractionates the biopolymer matrix and extracts hemicellulose and lignin by cleaving the lignin-carbohydrate complexes and hydrolyzing the hemicellulose as well as breaking (some of) the β -O-4 bonds of the lignin.^{17, 20}

The pulp of the willow genotypes showed a significantly different composition compared to the raw lignocellulose (see Figure 3-9) where around 50 % of the biomass material had been removed and an enrichment of cellulose content was observed. However, the amount of material dissolved in the ionic liquid solution varied, demonstrating that the different genotypes do indeed respond differently to pretreatment.

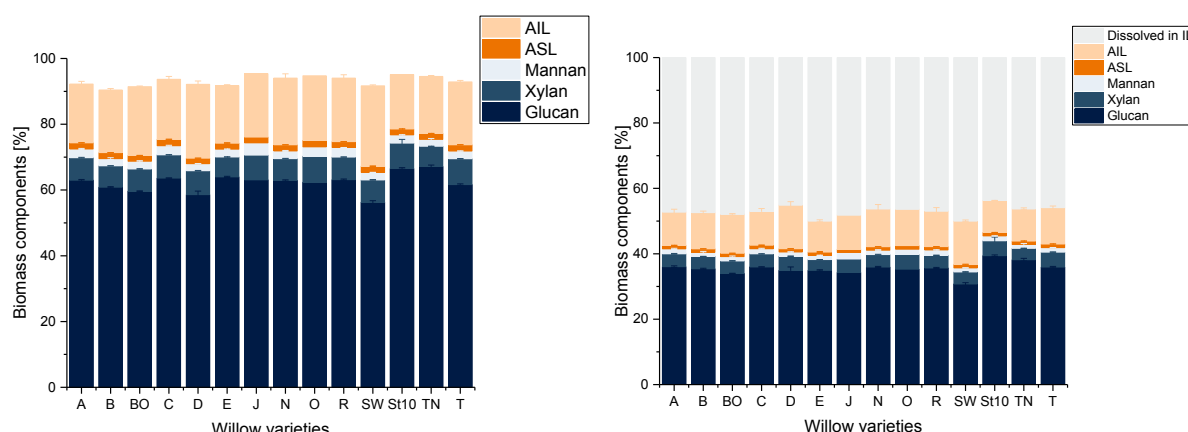


Figure 3-9. Pulp composition of 14 willow genotypes after pretreatment with [N₂₂₂₀][HSO₄]_{80%} (left) and pulp composition after pretreatment normalized to composition of untreated willow samples (right).

The pretreatment extracted a vast amount of the hemicelluloses, reducing the xylan content by 67 – 76 % for all the genotypes and the mannan content by 40 – 64 % as well as completely extracting the galactan and arabinan fractions of the hemicellulose. In general, a higher percentage of xylan was extracted from the biomass compared to mannan, indicating that the majority of the LCCs are formed between lignin and xylose, and to a lesser extent between lignin and mannose as also described elsewhere.²⁴ The significant difference observed for the hemicellulose extraction for the 14 genotypes suggests that the structure of the hemicellulose as well as the linkages between the polysaccharides and lignin are under genetic control.

One key factor for high glucose yields of enzymatic hydrolysis is a high glucan content in the pulp, which can be achieved by extraction of hemicellulose and lignin. Pretreatment with protic ionic liquids increases the relative glucan proportion in the pulp. However, when using acidic ionic liquids, glucan degradation becomes an issue that decreases the saccharification yield, especially for severe pretreatment conditions.²⁰ The glucan removal observed for pretreatments applying acidic ionic liquids is believed to be caused by partial hydrolysis of the amorphous part of cellulose as well as hydrolysis of the glucomannan fraction of the hemicellulose.^{25, 26} It was found that the willow varieties investigated in this study show a significant difference in glucan extraction after pretreatment with [N₂₂₂₀][HSO₄]_{80%} solution ranging from 10.80 % for Tora to 19.06 % for Corail. Given that the genotypes were subjected to the same pretreatment severity, this suggests that raw biomass might differ in the amorphous and crystalline parts of the cellulose. Interestingly, the glucose yield of both varieties after pretreatment was very similar (around 65 %), indicating that the decrease in glucan content observed

here was mainly due to hemicellulose extraction. Considering the mild pretreatment conditions applied in this study, attack of the cellulose (amorphous or crystalline) seems very unlikely.

The pulp isolated after ionic liquid fractionation showed an increase in glucan content for all genotypes. Generally, the glucan content increased around 50 % due to removal of lignin and particularly hemicellulose. The pulp of the varieties Endurance and Jorr was found to have a glucan increase of 55 %, whereas the pulp of Stott10 only showed an increase of 41 %. This difference is also reflected in the glucose yield after enzymatic hydrolysis. The genotype Endurance was found to have the second highest saccharification yield, but interestingly, the variety Stott10 also gave high glucan yields. This indicates that the digestibility of the pulp is not only controlled by the glucan content of the samples.

The IonoSolv pretreatment uses protic ionic liquids to delignify lignocellulosic biomass by cleaving the lignin β -O-4 bonds and lignin carbohydrate complexes which results in an increase in pulp digestibility.^{17, 20} A previous study investigating the pretreatment efficiency of the IL [N₂₂₂₀][HSO₄]_{80%} for the hardwood willow (variety Endurance) showed that high delignification (76.1 %) of this feedstock can be achieved under optimized pretreatment conditions.²⁰ However, applying a mild pretreatment in this study, a low lignin removal was expected. Surprisingly, it was observed that relatively high delignification of the genotypes was achieved after pretreatment, with 11 out of the 14 varieties showing lignin removal of more than 50 % based on the lignin content in untreated biomass. The genotype least recalcitrant to IL pretreatment was found to be Endurance, where 59% of the lignin had been extracted. Interestingly, Endurance was also found to give the second highest glucose yield after pretreatment. The lowest amount of lignin was removed for the genotypes Orm (47 %) and Discovery (48 %) which both gave low to medium glucose release. This implies that the lignin properties such as content of aryl ether bonds, S/G ratio and chain length of the different genotypes differ, altering the reactivity of the polymer and play a significant role in the recalcitrance of the individual varieties. However, further studies on the lignin structure need to be conducted to support this hypothesis.

Interestingly, a study conducted to explore the potential of 35 willow genotypes for biofuel production *via* acid hydrolysis reported the formation of *pseudo*-lignin on the pulp.⁴ While an exact definition of *pseudo*-lignin is still not agreed upon it is considered to be formed by sugar degradation products and lignin fragments and is known to negatively impact pulp digestibility. Typically, the formation of *pseudo*-lignin is observed under acidic pretreatment conditions (see also chapter 1 of this thesis) but none was detected for the 14 willow varieties studied here. This is most likely due to the chosen mild pretreatment conditions in this study.

3.2.5 Effect of ionic liquid pretreatment on pore volume and surface area

Ionic liquid pretreatment not only alters the chemical composition of the lignocellulose but also induces physical changes in the pulp. Lignocellulosic biomass typically contains pores that serve as water and nutrient conducting vessels and that allow cellulases to partially access the cellulose substrate in native biomass.²⁷ SEM pictures of untreated willow (Shrubby Willow) clearly show small pores in the biomass structure (see Figure 3-10).

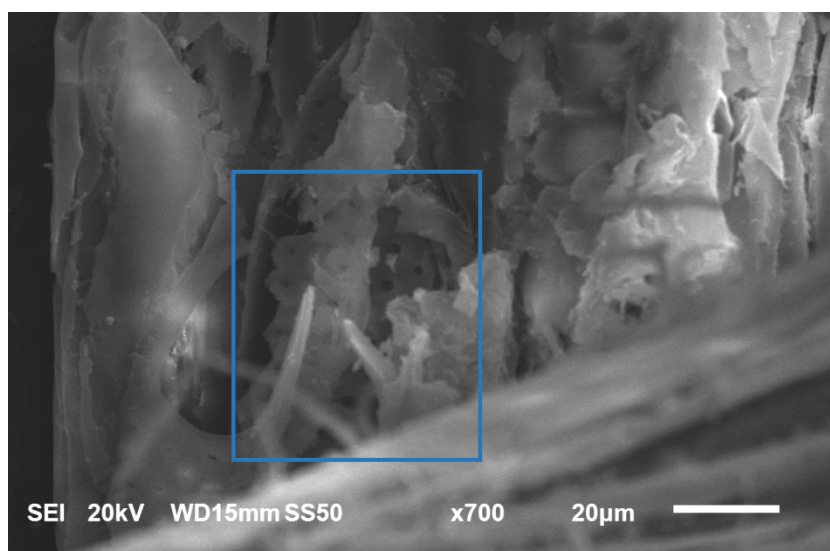


Figure 3-10. SEM image of untreated willow genotype Shrubby Willow showing small pores (blue box) in the wood structure.

However, the cellulose is embedded in a matrix of hemicellulose and lignin which decreases accessibility of the enzymes. Several studies have reported that pretreatment is able to increase the surface area and pore size of lignocellulosic biomass *via* extraction of hemicellulose and lignin as well as disruption of the fibrous structure.^{28,29} All of these studies reported that a higher surface area resulted in an increase in glucose yield of enzymatic hydrolysis.

The complete mechanism of cellulose hydrolysis is a complex multi-step reaction but it is well-known that the close contact between the substrate and the enzymes is a requirement for hydrolysis to occur. In order for the enzymes to come to close proximity with the cellulose fibrils the diffusion of the enzyme is strongly controlled by the pore size of the lignocellulose and larger pores allow for the complete enzyme to diffuse to the substrate whereas smaller pores only allow for parts of the enzyme to reach the cellulose which in turn deactivates the enzyme.³⁰

In this study, the pore volume and surface area are investigated using the Brunauer-Emmett-Teller (BET) theory of adsorption. Interestingly, the measurements revealed the pore volume of the 14

genotypes differs significantly for untreated samples, ranging from $0.0061 \text{ m}^3\text{g}^{-1}$ (Resolution) to $0.0119 \text{ m}^3\text{g}^{-1}$ (Baldwin), respectively (see Figure 3-11). Not surprisingly, the variety Baldwin which was found to have the largest pore volume also had a high glucose yield of 49.4 % of the untreated biomass.

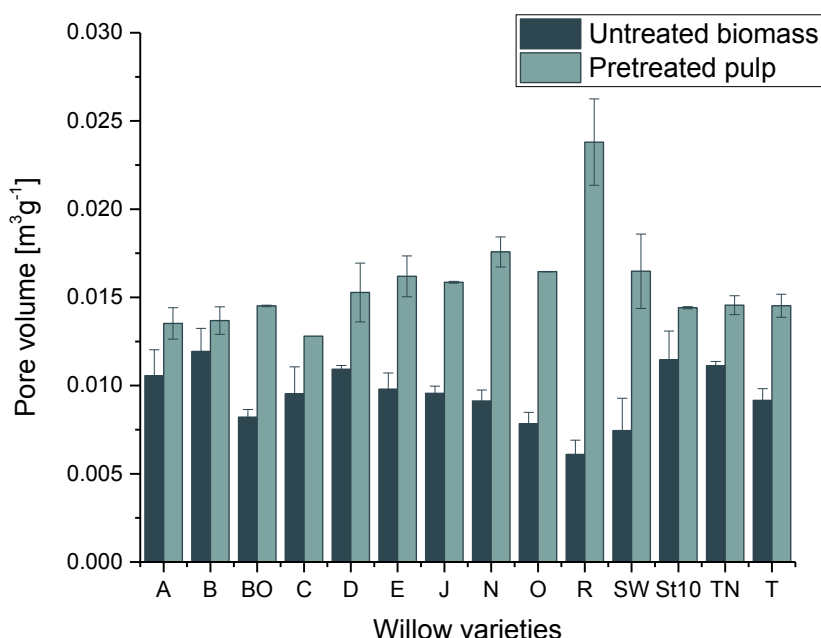


Figure 3-11. Pore volume of 14 willow genotypes before and after pretreatment with $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$ measured via N_2 adsorption according to the BET theory.

However, the majority of the samples showed a pore volume around 0.009 to 0.010 m^3 which is large enough to allow cellulases access to the cellulose substrate. Typically, cellulose enzyme mixtures contain enzymes that are $2 - 7 \text{ nm}$.³¹

After pretreatment, the pore volume of all 14 genotypes had increased, however, not by the same amount. This might suggest that the varieties differ in lignin and hemicellulose distribution in the cell walls in addition to some being more recalcitrant to pretreatment than others. The largest pore volume after pretreatment was measured for the variety Resolution ($0.0238 \text{ m}^3\text{g}^{-1}$) with this also being the largest increase in pore volume. It is important to note that Resolution did not only show the highest increase in pore volume amongst all the varieties but also the highest increase in saccharification yield compared to the untreated biomass, strengthening the hypothesis that accessibility of the cellulose substrate plays a crucial role in enzymatic hydrolysis reactions. The pretreatment of some varieties, like Asgerd and Baldwin resulted in only a small increase of pore volume from $0.0106 \text{ m}^3\text{g}^{-1}$ to $0.0135 \text{ m}^3\text{g}^{-1}$ and $0.0119 \text{ m}^3\text{g}^{-1}$ to $0.0137 \text{ m}^3\text{g}^{-1}$, even though a medium

increase of saccharification yield was observed for these varieties. However, the yield of enzymatic hydrolysis is not solely dependent on the pore volume or increase thereof, as seen for the variety Shrubby Willow which showed a large pore volume after pretreatment but almost no increase in glucose yield.

An exemplary BET isotherm for untreated lignocellulose and pretreated pulp of the genotype Shrubby Willow can be found in Figure 3-12.

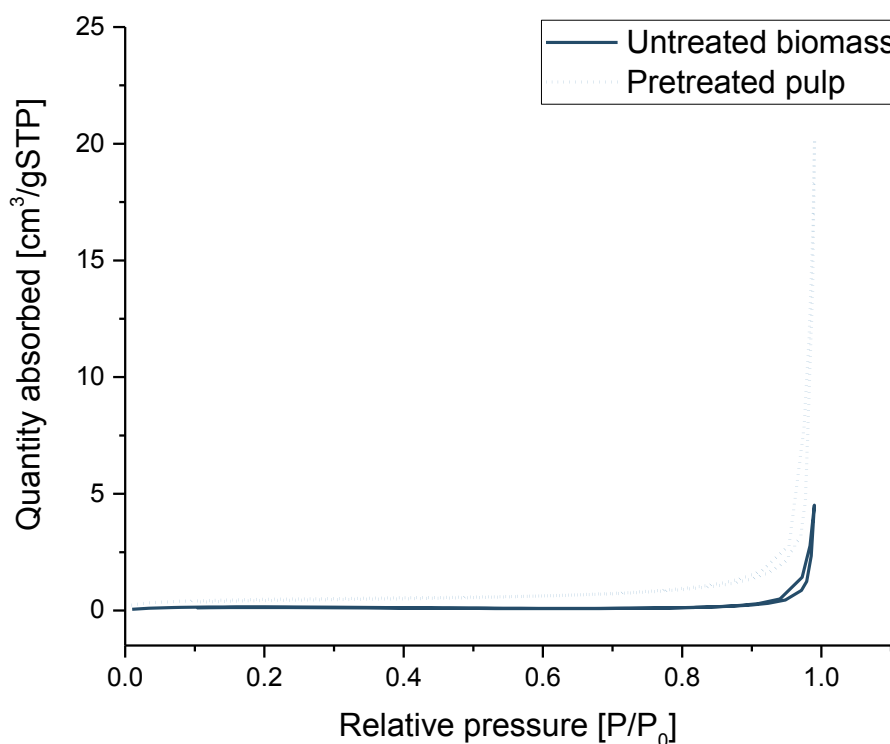


Figure 3-12. BET isotherms of the willow genotype Shrubby Willow before and after pretreatment with the ionic liquid solution $[N_{2220}][HSO_4]_{80\%}$.

The BET isotherms for all varieties show a classical shape that characterizes mesoporous materials.³² Previous research in the field of BET theory has led to a wide acceptance of a correlation between pore geometry of a mesoporous material and the shape of the adsorption/desorption isotherm. Analysis based on the IUPAC classification of the hysteresis loops obtained from untreated and pretreated willow varieties has shown that the samples investigated in this study contain pores with a well-defined cylindrical-like shape or agglomerates of approximately uniform spheres.³²

The accessible surface area of the cellulose is considered another important factor in the digestibility of the pulp. Similar to the pore volume, the surface area (of both untreated and pretreated samples) differs amongst the genotypes. The largest surface area after ionic liquid deconstruction of $2.3 \text{ m}^2\text{g}^{-1}$

was found for the variety Shrubby Willow and the highest increase of 262 % of this pulp property was detected for the genotype Orm. Surprisingly, the significantly larger surface area for the genotype Shrubby Willow did not result in a notable increase in glucose yield which was found to be only 1.7%.

Interestingly though, the variety Resolution with the smallest pore size is not the one with the smallest surface area. This indicates that the specific cellulose surface area is not only accessible *via* (internal) pores but also *via* the surface layer of the biomass particles. Most untreated varieties displayed a surface area of around $0.7 - 0.8 \text{ m}^2\text{g}^{-1}$ with Asgerd and Baldwin having the largest ones of $1.2 \text{ m}^2\text{g}^{-1}$ (see Figure 3-13).

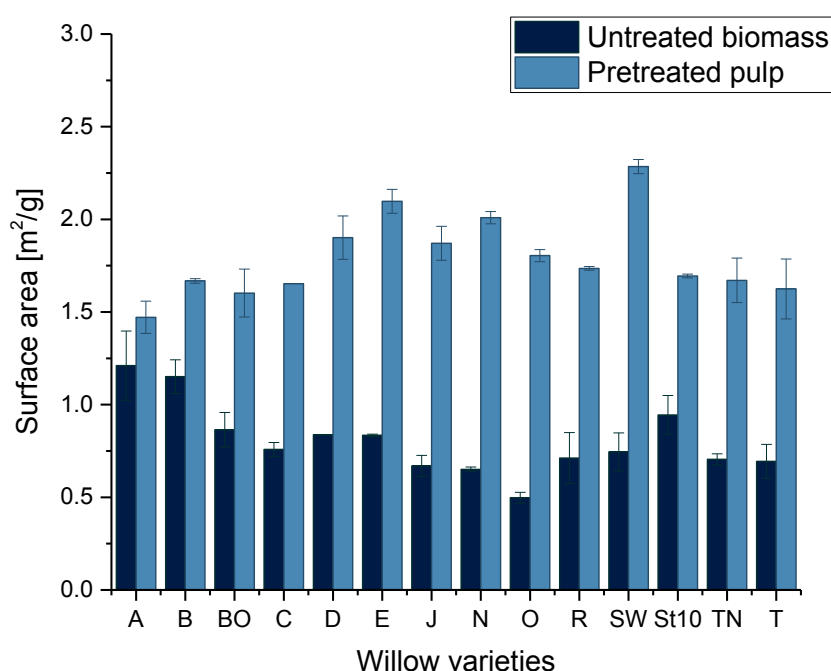


Figure 3-13. Surface area of 14 willow genotypes before and after ionic liquid pretreatment.

The increase in surface area after pretreatment for those two varieties was the lowest, as already observed for the increase in pore volume. Interestingly, the hemicellulose and lignin removal was found to be high, indicating that other factors such as wood density and location of lignin in the lignocellulose matrix also play a role in the disruption of the biopolymer matrix.

The difference in increase of pore volume and surface area after pretreatment clearly shows that the genotypes do respond differently to ionic liquid fractionation. The effect of this behaviour on the enzymatic hydrolysis is clearly given, although one has to keep in mind that the structure of the biomass is very complex and many wood properties influence the final glucan hydrolysis.

3.2.6 Chemical changes of pulp induced by ionic liquid pretreatment

Degree of polymerization of cellulose

Even though the hydrolysis of cellulose by cellulases is still not completely understood, the degree of polymerization of the cellulose biopolymer was identified as another factor that influences the digestibility of lignocellulosic material. Typically, enzymes such as endoglucanases, exoglucanases and β -glucosidases are all required to successfully depolymerize cellulose.^{33, 34} The hydrolysis of the substrate is undertaken by a cascade of reactions that involves several individual steps. These steps include (i) diffusion of the enzymes to the surface of the substrate, (ii) adsorption of the enzyme with subsequent formation of enzyme-substrate complexes, (iii) hydrolysis of the cellulose polymer, (iv) diffusion of the oligomeric and monomeric hydrolysis products from the substrate surface to the bulk aqueous phase, and (v) further hydrolysis of the carbohydrate oligomers into monomeric sugar in the aqueous phase.³⁵ The three types of enzymes play different roles in the breakdown of the cellulose polymer. The endoglucanases were found to cleave the internal hydrogen bonds of the polymer, whereas the exoglucanases depolymerize the chain ends resulting in carbohydrate oligomers. The β -glucosidases then hydrolyses the oligomers into monomers.³⁶

In this study, the cellulose chain length of the willow genotypes is analyzed to better understand the effect of cellulose structure on the enzymatic hydrolysis. All the pulp samples have been treated to completely remove the lignin prior the GPC analysis in order to solely measure the degree of polymerization of the carbohydrate polymers. Interestingly, the pulp of all 14 genotypes gave bimodal GPC profiles showing the presence of both hemicellulose and cellulose as can be seen in an exemplary chromatogram in Figure 3-14.

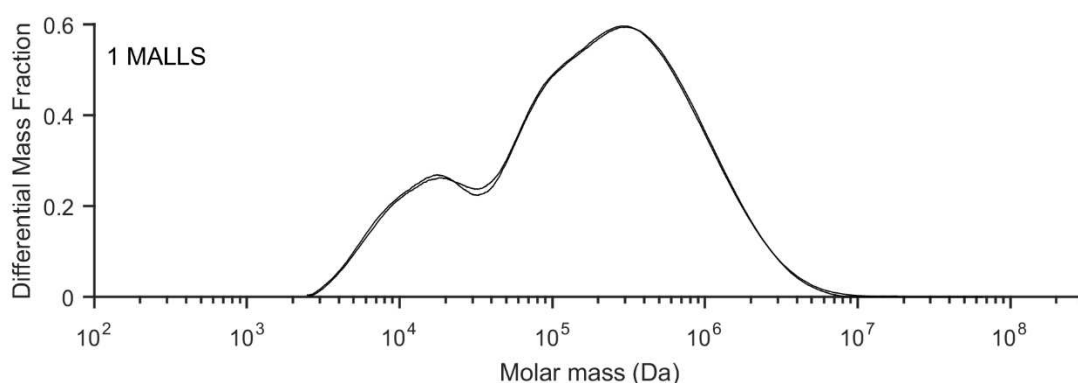


Figure 3-14. Bimodal GPC profile of pretreated pulp showing the hemicellulose (left maximum) and cellulose (right maximum) molecular weight distribution.

The cellulose polymer is known to be homogenous in structure – only consisting of glucose units – but the degree of polymerization varies amongst plant species³⁷ and possibly also amongst genotypes. Interestingly, this was found to be the case for the pulp recovered after pretreatment of 14 willow genotypes investigated in this study (see Figure 3-15).

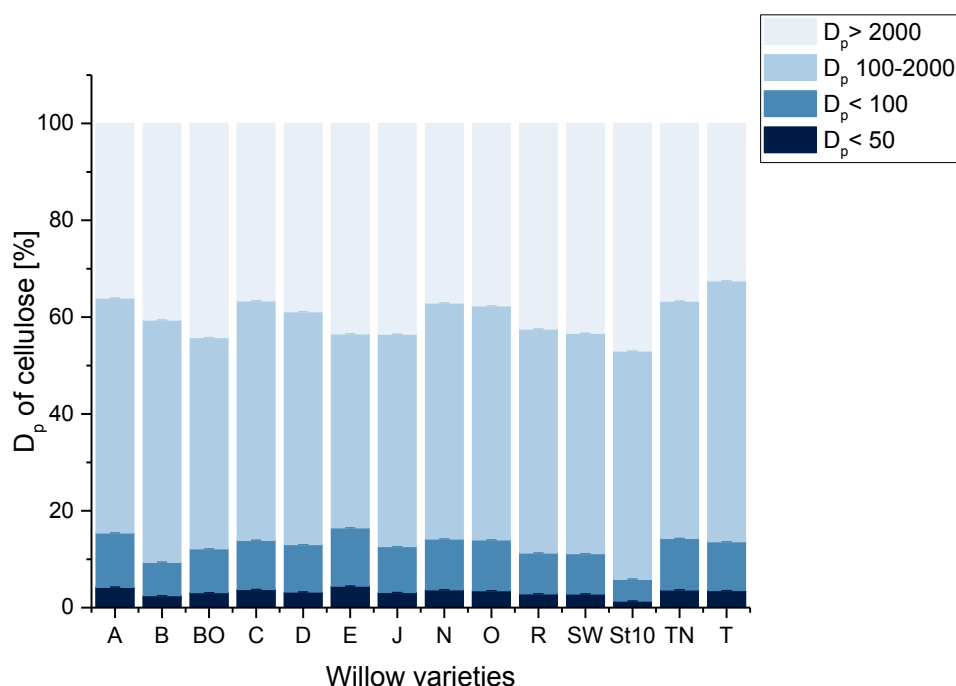


Figure 3-15. Composition of cellulose in terms of chain length present in pulp of 14 genotypes after IL pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

The chain length of the cellulose polymer was categorized into 4 sub-sections according to the molecular weight of the polymer chains, namely (i) $D_p < 50$, (ii) $D_p 50 - 100$, (iii) $D_p 100 - 2000$, and (iv) $D_p > 2000$. Cellulose chains with a D_p below 50 made up the smallest part of the polymer weight distribution with less than 5 % for all the varieties. Slightly larger chains with a D_p of 50 – 100 were found to be more diversely distributed amongst the varieties with the variety Stott 10 containing only 4.5 % of this size fraction, whereas this size fraction made up 12.0 % of the cellulose polymer of Endurance. GPC analysis revealed that most cellulose chains have a D_p of 100 – 2000, with 40.0 % being the least amount (Endurance) and 53.9 % being the highest amount (Tora). The longest chains of $D_p > 2000$ make up the second biggest size fraction, ranging from 32.5% (Tora) to 47.0 % (Stott10). The GPC results suggest that a shorter cellulose chain length could positively influence the glucose yield of enzymatic hydrolysis, since the variety Endurance (second highest glucose yield after

pretreatment) has the highest percentage of $D_p < 50$ and $D_p 50 - 100$ amongst all the tested genotypes. This is in good accordance with studies investigating the depolymerisation of cellulose and its effect on the enzymatic hydrolysis of those substrates.^{38, 39} These studies conclude that a lower degree of polymerization of the cellulose improves enzymatic hydrolysis due to making cellulose more reactive to enzymes *via* increasing the number of cellulose chain reducing ends that are available for the exoglucanases.⁴⁰ Additionally, shorter cellulose chains are easier accessible for cellulases due to the lack of formation of strong hydrogen bonds between chains.⁴⁰

However, the degree of polymerisation of the cellulose in the pulp is not the only property that influences the digestibility of the substrate. The genotype Stott10 was found to have the highest percentage of $D_p 100 - 2000$ and $D_p > 2000$, which would be expected to result in a low saccharification yield. But contrary to that, enzymatic hydrolysis of the pulp yielded 69.1 % of the theoretical maximum of glucose. This shows again, that the yield of saccharification is not controlled by one single property of the substrate. Furthermore, no direct correlation was found between chain length of the cellulose polymer or its different size fractions and glucose release of enzymatic saccharification.

Crystallinity index and crystallite size

The cellulose structure is not only determined by its degree of polymerization but also by the degree of crystallinity and crystallite size of the microfibrils. The cellulose hydroxyl groups form strong inter- and intramolecular hydrogen bonds resulting in four ordered crystalline lattice structures, namely cellulose I, cellulose II, cellulose III and cellulose IV. The most abundant form in nature is cellulose I, which can be transformed into the other cellulose crystal structures.⁴¹ Analysis techniques such as ^{13}C -NMR measurements revealed that the cellulose I crystal structure is formed by two distinct substructures, namely cellulose I_α (triclinic) and cellulose I_β (monoclinic).⁴² Figure 3-16 shows a schematic representation of the two cellulose I substructures.

One has to keep in mind that the cellulose structure is very complex and still not completely understood. Even though cellulose is a very ordered polymer on the molecular level, the cellulose crystallites are imperfect which means that a portion of the cellulose structure is less ordered or amorphous. Thus, a two-phase model has been developed that describes the cellulose structure as a polymer with partly crystalline (ordered) and amorphous (less ordered) regions.⁴⁴ A parameter named crystallinity index (CI) has been suggested to describe the relative amount of crystalline sections in the cellulose polymer.

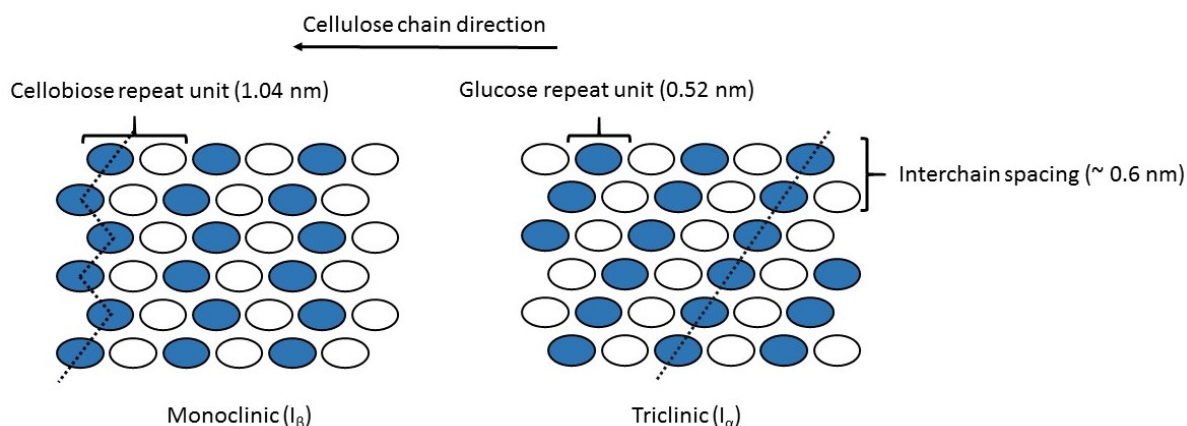


Figure 3-16. Schematic representation of the two cellulose I substructures, namely cellulose I_α (triclinic) and cellulose I_β (monoclinic). The difference in alignment of neighbouring chains leads to either a staggered pattern in the monoclinic unit cell or a diagonal pattern in the triclinic unit cell.⁴³

The initial degree of crystallinity of cellulose has been related to the rate of enzymatic hydrolysis by several studies which found that a completely amorphous cellulose sample is depolymerized at a much higher rate than partially crystalline cellulose.^{40,45,46} This has led to the idea that the amorphous regions are hydrolyzed first, followed by depolymerisation of the crystalline parts which occurs at a slower rate.⁴⁷ The crystallinity of cellulose not only influences the rate of hydrolysis but also the yield of this reaction which is controlled *via* the amount of adsorption of enzymes on the cellulose substrate. It was observed that the maximum enzyme adsorption constant was greatly enhanced at lower crystallinity indices.⁴⁸

Studies on the effect of ionic liquid pretreatment on the crystallinity of lignocellulosic cellulose usually use ionic liquids that dissolve either cellulose or the whole lignocellulose matrix (such as [C₂C₁-im][CH₃COO]). Pretreatment using this ionic liquid results in a decrease of cellulose crystallinity and sometimes even in a transformation to a completely amorphous structure. A correlation between changes in cellulose crystallinity and enhancement of enzymatic glucose release from the pretreated biomass has been established numerous times.^{49–52} It is widely accepted that reduced crystallinity of the cellulose in the pretreated pulp results in a more digestible pulp. Applying cellulose dissolving ionic liquids usually results in limited delignification, but weak correlations between reduced lignin content and cellulose crystallinity were also observed.

In contrast, yields of enzymatic saccharification of pulp recovered after ionoSolv pretreatment typically correlate with reduced lignin content¹⁸, but little is known about the impact of lignocellulose fractionating ILs on the crystallinity of the cellulose of different willow varieties. Therefore, X-ray

diffraction measurements were carried out to better understand whether changes in the ordering of cellulose occur during ionoSolv pretreatment and to determine the apparent cellulose crystallite size.

The crystallinity index is determined *via* X-Ray diffraction patterns using the Segal method.⁵³ An exemplary XRD diffraction pattern of untreated willow (Endurance) and the pretreated pulp is shown in Figure 3-17. The Segal method puts the intensity of the scattering signal of the crystalline region (at *ca.* $22^\circ 2\theta$) into relation to the intensity of the amorphous region (at *ca.* $18^\circ 2\theta$) of the cellulose polymer.

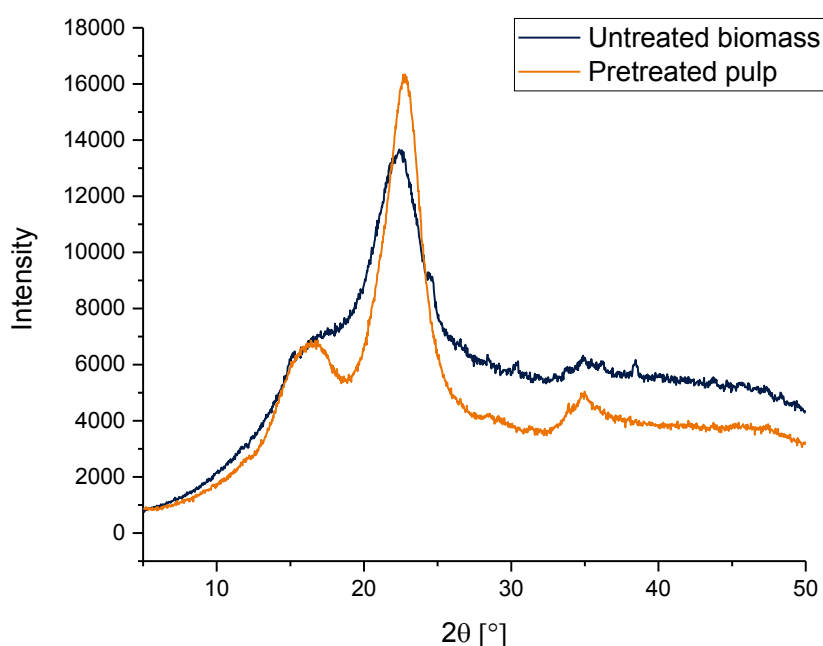


Figure 3-17. Exemplary XRD scatter patterns for willow variety Endurance before and after pretreatment with $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$.

The XRD patterns of the other genotypes are very similar to the one depicted here and they all show typical signals for the crystalline cellulose I polymorph for the untreated biomass sample, with three major peaks visible at $15\text{--}16^\circ 2\theta$, $22^\circ 2\theta$ and $35^\circ 2\theta$, which correspond to the combined $[110] + [1\bar{1}0]$, $[200]$ and $[040]$ crystallographic planes of cellulose I, respectively (see Figure 3-18 schematic depiction). The most intense reflection at $22^\circ 2\theta$ corresponds to the $[200]$ plane of cellulose.

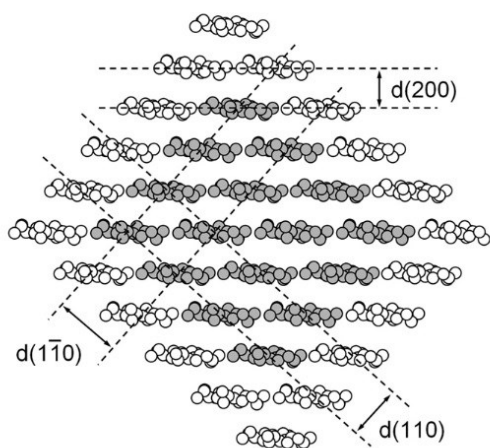


Figure 3-18. Cross-sectional view of a 36-chain cellulose microfibril (only carbon and oxygen atoms are shown). Gray chains are hydrogen bonded to two adjacent chains, whereas white ones are hydrogen bonded to just one adjacent chain. The dashed lines indicate the three crystal planes that contribute the tallest peaks in a XRD diffractogram.⁵⁴

After pretreatment with the ionic liquid solution, changes to the XRD pattern of the pulp were observed even though the three major peaks were still present. Firstly, the scattering signals for untreated biomass were found to be narrower and more defined than for untreated lignocellulose, suggesting that the amount of amorphous material present in the sample was reduced. Additionally, for pretreated pulp, a slight shift of the [200] reflection from around 22.3° 2θ for untreated biomass to around 22.5° 2θ was observed. The shifted position of the major reflection in the pulp indicates that the cellulose structure is significantly more crystalline after ionic liquid pretreatment due to removal of amorphous hemicellulose and lignin and possibly also minor parts of the amorphous cellulose itself. A similar shift of the [200] signal was found for pulp samples of $[N_{2220}][HSO_4]_{80\%}$ pretreated *Miscanthus x giganteus* (unpublished data of the Hallett research group).

The extraction of amorphous material is also reflected by the calculated crystallinity index (CI), which was found to increase after pretreatment with $[N_{2220}][HSO_4]_{80\%}$ for all the willow genotypes (Table 3-3). This is in good agreement with unpublished results of our laboratory, where an increase of CI dependent on pretreatment severity was found for *Miscanthus x giganteus*. The lowest CI of the raw biomass of 41.1 % was observed for Endurance and the highest CI of 50.9 % was found for Terra Nova. The increase of the crystallinity index was found to differ for the 14 varieties with increases ranging from 25.2% for Jorr to 40.9 % for Endurance. After ionic liquid pretreatment, the highest CI was detected for the variety Endurance (69.5 %) and the lowest one for Orm (62.6 %). However, no direct correlation was found for the degree of crystallinity and the saccharification yield of the raw biomass

and the pretreated pulp. This highlights again that the enzymatic hydrolysis is influenced by many properties of the biomass.

In addition to a change in cellulose crystallinity, the width of the diffraction peak was observed to decrease after ionic liquid pretreatment. The full width at half maximum (FWHM) of the diffraction pattern at $22^\circ 2\theta$ can be used to calculate an apparent crystallite size of the cellulose microfibrils.⁵⁵ Interestingly, a decrease of the FWHM was observed for all the pulps compared to the untreated biomass samples which corresponds to an increase in cellulose crystallite size (see Figure 3-19). The crystallite size for untreated samples was calculated to be around 2.5 nm for each variety with the largest crystallite size being 2.86 nm (Terra Nova) and the smallest one being 2.16 nm (Endurance).

Table 3-3. Calculated crystallinity indices of untreated and ionic liquid pretreated *Salix* varieties.

Variety	CI _{untreated}	CI _{pretreated}	Increase in CI [%]
A	44.2	65.8	32.8
B	45.0	67.4	33.3
BO	47.8	64.2	25.5
C	45.9	65.7	30.1
D	45.5	68.4	33.4
E	41.1	69.5	40.9
J	49.7	66.4	25.2
N	46.6	66.1	29.4
O	46.1	62.6	26.4
R	45.5	65.4	30.5
SW	42.1	63.5	33.7
St10	49.3	66.5	25.9
TN	50.9	69.4	26.8
T	40.8	64.6	36.9

As with all the other investigated biomass properties, the increase of crystallite size was dependent on the genotype. For some varieties such as Bowles Hybrid, Orm and Shrubby Willow only a moderate increase of around 20 % was observed, whereas this cellulose property increased significantly for other genotypes after pretreatment. The highest rise of 73 % was found for the variety Endurance, which is also the variety which gave the second highest glucose yield. This indicates that the crystallite size might affect the enzymatic hydrolysis of the pulps. The increase in cellulose crystallites is caused by the removal of hemicellulose and lignin which disrupts the protective layer around the cellulose fibrils, leading to coalescence and annealing of the individual crystallites. A similar increase in cellulose crystallite size accompanied by an increase of saccharification yield was described by Q. Sun *et al.* after

dilute acid pretreatment.⁵⁶ The annealing of the cellulose crystallites might result in an increase of surface area of the polymer, allowing a higher amount of enzymes to access the substrate.

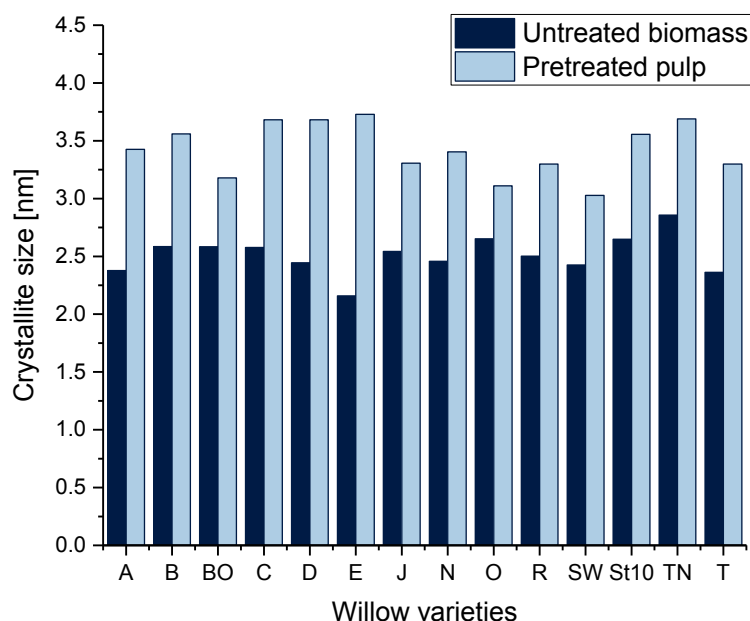


Figure 3-19. Apparent cellulose crystallite size of the 14 willow genotypes before and after ionic liquid pretreatment.

3.2.7 Statistical calculations

To better understand the effects of the individual properties of the biomass and the pulp on the saccharification yield, statistical analysis using a multiple variant approach was performed.

Principal component analysis was performed using the data gathered for pretreatment on all the 14 willow genotypes and no outliers of the dataset could be identified. This means that all the varieties are affected the same by ionic liquid pretreatment.

Additionally, the statistical analysis showed that the saccharification yield of the pretreated pulp is affected by the glucan content, pore volume of the pulp, crystallinity index and crystallite size. Surprisingly, no influence of lignin content of the pulp on the glucose yield was detected.

Interestingly, the yield of enzymatic hydrolysis was also influenced by properties of the untreated lignocellulose. For example, the initial hemicellulose and lignin content were found to correlate with the glucose release of the pretreated samples, whereas a weaker correlation was observed for the initial glucan content and the glucose yield.

3.3 Summary and future work

The study presented in this chapter investigated, for the first time, the effects of protic ionic liquid pretreatment on 14 willow varieties and broadened the understanding of which biomass properties influence the yield of enzymatic hydrolysis. Cellulose hydrolysis is a mixture of several complex reactions that are greatly influenced by the properties of the substrate. One factor that majorly impacts the success of cellulose digestion is the nature of the cellulose environment, with the polymer being embedded in a matrix of hemicellulose and lignin in the native biomass.

Compositional analysis of the untreated samples could show that the 14 willow varieties differ greatly in biomass composition and no correlation of glucan and lignin content was found. Some varieties were found to have very high glucan content (such as Stott10, Terra Nova and Corail). However, a high glucan content does not correspond to a high glucose yield.

Interestingly, the willow varieties not only differed in native biomass composition but also in response to pretreatment with $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$. This study revealed that some varieties are more recalcitrant to pretreatment than others which was demonstrated by the different pulp and lignin recovery. After IL pretreatment, the glucose yields of enzymatic hydrolysis had increased for all the genotypes and the highest yield was found to be 79.1 % for Terra Nova. However, the highest increase of glucose yield of 43.2 % after pretreatment was observed for the variety Resolution and the lowest one of 1.7 % for Shrubby Willow. Statistical analysis of the data could show that the initial lignin content of the genotypes did not play a role in the glucose release for both untreated and pretreated varieties. This is a surprising finding with regards to research in biotechnology and plant breeding that aims to reduce the lignin content of bioenergy crops to improve glucose yields of enzymatic hydrolysis.

It was found that IL pretreatment alters the composition of the willow genotypes and leads to an increase of the relative glucan content of the pulp. The extraction of hemicellulose and lignin differed for the varieties, suggesting genetic control of the structure of these polymers. The highest lignin removal of 59 % was observed for Endurance, whereas Orm only showed delignification of 47 %. The removal of structural polymers from the biopolymer matrix was not the only biomass change induced by ionic liquid pretreatment.

The pore volume and specific surface area were both measured to increase after pretreatment. The largest pore volume of 23.8 nm was detected for the variety Resolution which also had the second highest glucose yield. Another property crucial for successful saccharification is known to be the degree of polymerization of the cellulose. Interestingly, the willow varieties differed in the D_p of the cellulose. The genotype Endurance was found to have the highest percentage of short cellulose chains

and Shrubby Willow the lowest one. Those two varieties were also found to be on the opposing end of saccharification yields which supports the hypothesis that the cellulose chain length plays a significant role in enzymatic hydrolysis. Additionally, the crystallinity index and crystallite size of the variety Endurance was found to be the highest after ionic liquid pretreatment.

It could be shown that the saccharification yield of the pretreated pulp was dominantly influenced by several properties of the raw biomass (namely initial hemicellulose and lignin content) and of the pulp itself. Interestingly, the lignin content of the pulp did not play a role in this respect but the accessibility of the cellulose reflected by properties such as glucan content, pore volume of the pulp, crystallinity index and crystallite size as prominently shown by the variety Endurance.

This and the previous chapter of this thesis could show that the hardwood willow is a promising candidate for bioethanol production due to good response to ionic liquid pretreatment which results in high pulp digestibility and glucose yields. However, a biorefinery would not be commercially competitive if the only product sold is bioethanol meaning that investigations into novel applications of lignin are needed. Willow lignin generally shows a high S/G ratio corresponding to a relatively linear polymer structure which makes the lignin of this hardwood an interesting subject for several applications such as carbon fibres⁵⁷. The following chapters of this thesis will focus on studying lignin modifications for potential material applications.

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Chapter 4- Lignin modification part I: Production of lignin-PFA copolymers

4.1 Introduction

Resins are widely used in industrial applications such as waste water treatment¹, coatings² and adhesives³. One widely used resin in the wood adhesive industry is phenol-formaldehyde (PF) which is used in applications such as particle boards, finger-jointing fields, plywood, cement mould boards and container boards.^{4,5} These resins are synthesised in an acid or base catalysed process from phenol and formaldehyde (Figure 4-1).⁶

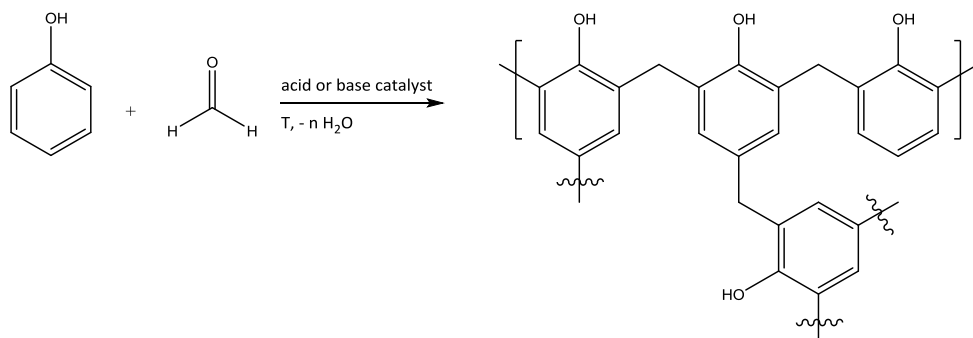


Figure 4-1. Schematic representation of the synthesis of phenol-formaldehyde resin.⁶

Phenol-formaldehyde resins are well known for their beneficial properties such as mechanical strength, resistance to moisture, chemicals and weather, as well as good thermal stability.⁶ However, they also face several disadvantages. Firstly, the starting materials (phenol and formaldehyde) are derived from fossil resources. It is well known that the era of cheap supply of fossil resources will come to an end⁷, hence relying on fossil resources for chemicals and materials production is problematic. Secondly, phenol is typically synthesised at industrial scale using the Hock process which involves oxidation of cumene to cumene hydroperoxide followed by the cleavage of cumene hydroperoxide to phenol and acetone applying a strong mineral acid as catalyst.⁸ The cumene utilized in the production of phenol is synthesised from benzene and propylene *via* a Friedel-Crafts-Alkylation.⁹ Benzene is known as being very toxic and a human carcinogen¹⁰ and thus use of this compound should be strictly limited. Additionally, studies have found that formaldehyde-based resins can thermally degrade releasing hazardous and carcinogenic formaldehyde in the process causing concerns amongst users of

those resins.^{11, 12} For this reason, the removal of formaldehyde from consumer goods has been advocated in recent years.

This has led to the development of alternative resins where formaldehyde was substituted by furfuryl alcohol to create a phenol-furfuryl alcohol resin with similar properties to phenol-formaldehyde resins.^{13, 14}

In recent years the focus of research has shifted to design more sustainable and environmentally friendly resins with lignin being a prime candidate to substitute phenol in resins due to the structural similarity of polymerized phenol in resins and the biopolymer. This approach is not only beneficial in terms of reducing the reliability of resin production on the availability and cost of fossil resources but also because lignin is currently considered a waste product of the biorefinery and mostly burned for energy production.¹⁵ Several studies have reported the successful substitution of phenol with lignin in the production of phenol resins.^{16,17,18–20} However, the majority of these resins are typically synthesised using formaldehyde and the lignin is used to only partially (up to 50 wt%) replace phenol.²¹

The concerns about the utilization of compounds derived from fossil resources in the resin production have led to a renewed interest in producing a resin synthesised completely of bio-derived compounds.^{22, 23, 24} One possible resin solely synthesised from renewable resources is polyfurfuryl alcohol (PFA) which contains only repeating units of the monomer furfuryl alcohol (FA).

Furfuryl alcohol is a so-called platform chemical which is derived from lignocellulosic biomass, more specifically from the hemicellulose biopolymer. Generally, xylans are acid-hydrolysed to the monomer xylose which is then cyclodehydrated into furfural (Figure 4-2).²⁵

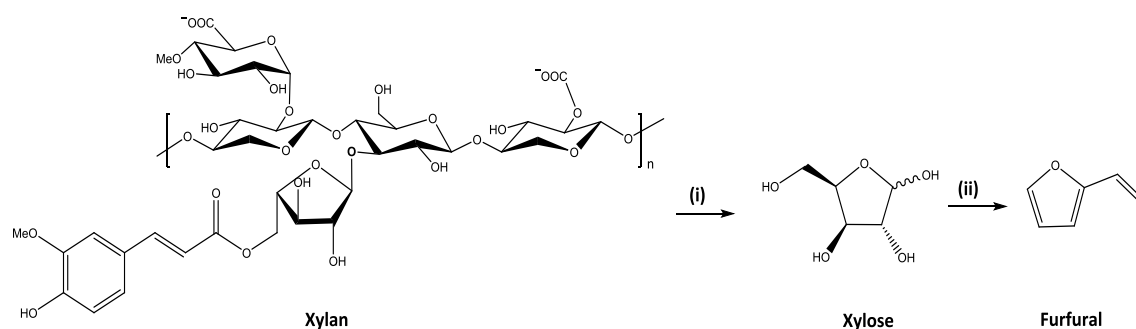


Figure 4-2. Simplified reaction scheme of the production of furfural from xylan *via* (i) acid hydrolysis and (ii) dehydration.²⁵

Furfural is a versatile platform chemical which is used for the production of a variety of chemicals (e.g. furan, 2-methyl tetrahydrofuran, levulinic acid, maleic anhydride) and fuels (e.g. furfuryl acetate biofuel, 1,5-pentanediole, ethyl furfuryl ether biofuel) with furfuryl alcohol being the most important one.¹² Furfuryl alcohol is produced *via* hydrogenation of furfural using hydrogen and a catalyst either in the gas or liquid phase (Figure 4-3).²⁶

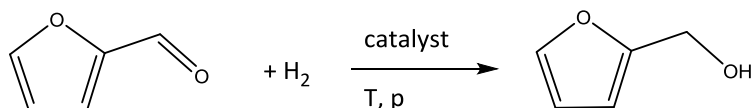


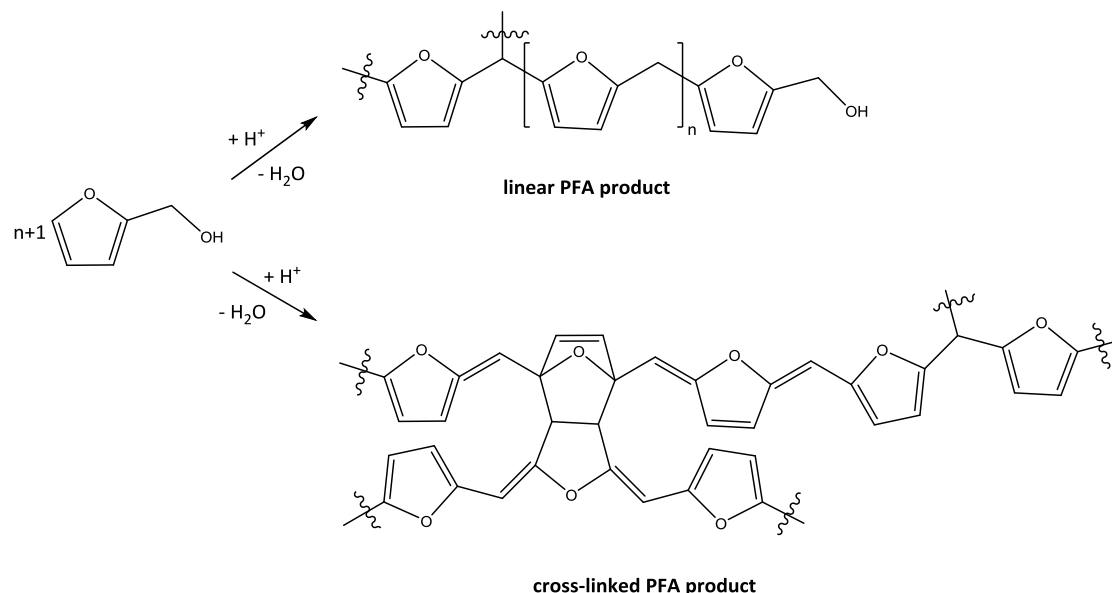
Figure 4-3. Conversion of furfural to furfuryl alcohol.²⁶

Furfuryl alcohol is mainly used for the production of thermoset resins such as polyfurfuryl alcohol which are used as high-quality cores and moulds for metal casting in the foundry industry. The compound is also used as a reactive solvent for phenolic resins in the refractory industry, to reduce the viscosity of epoxy resins as well as in the production of polyurethane foams and polyesters. Additionally, it is used as a monomer in the synthesis of tetrahydrofurfuryl alcohol (THFA).²⁷

PFA is synthesised from furfuryl alcohol in an acid-catalysed step growth polymerization.²⁸ Theoretically, the polycondensation of FA to PFA should yield linear colourless thermoplastic polymers. However, the isolated polymeric products are usually dark brown or black in colour and contain cross-linked structures and chromophores.²⁸ Reports on the isolation of linear PFA have been published, however, the reaction was stopped very early in the polymerisation process (D_p of PFA = 2 – 5).^{29–32} Interestingly, these studies also mentioned a mixture of products already containing more complex and cross-linked structures.³²

The polymerization of furfuryl alcohol has been the subject of research using various experimental conditions in water or organic solvents.^{33–37} The actual mechanism of the formation of PFA is still not understood in detail due to its complexity. The polymerization involves many steps that most likely have different activation energies.³⁸ However, consensus has been reached that the polymerization can be divided into two phases. The first stage consists of the condensation of the methylol group of one furan unit with the C5 position of another furan ring *via* the abstraction of one water molecule.²⁸ This step results in the formation of linear furan oligomers which are then cross-linked to the final polymeric product in the second stage of the polymerization. The formation of the complex network is realised *via* condensation reactions of the methylol group of one furan ring with the methylene

bridge of the polymer^{33,34} and *via* Diels-Alder cycloadditions between furan rings (acting as the diene) and chromophore structures present in the PFA (acting as the dienophile)^{28,39}. Figure 4-4 depicts the formation of a linear and cross-linked polyfurfuryl alcohol product.



Figure

4-4. Schematic representation of the polymerization of furfuryl alcohol to polyfurfuryl alcohol under acidic conditions.

The polymeric product derived from furfuryl alcohol is known for good chemical, mechanical and thermal properties which lead to the application of polyfurfuryl alcohol in many fields such as coatings with high resistance to corrosion, concretes, fibre-reinforced plastics, adhesives and binders, low flammability materials or wood protection. Additionally, carbonization of PFA gives rise to products such as carbonaceous electrodes, capacitors or materials for the preparation of desalination membranes.^{12, 28, 40}

In the last decade the synthesis of lignin-polyfurfuryl alcohol copolymers and blends has gained interest in the scientific community. The combination of those two compounds will create a material that is solely bio-derived and can aid to making biorefineries cost competitive by utilizing the waste product lignin and converting it into a value-added product in addition to decreasing the cost of production of PFA resins. Several studies have investigated the formation of blends and copolymers from lignin and PFA. The first study reported on the synthesis of these new resin materials was authored by N. Guigo *et al.*⁴¹ in 2010. The authors report the production of two lignin-PFA copolymers using plasticized lignin and FA (FA/lignin ratio of 80/20 and 70/30). The prepared materials were

characterised and it was indicated that covalent bonds formed between the two polymers. New signals in the ^{13}C -NMR spectra assigned to ether or ester links between lignin and PFA corresponding to links between terminal hydroxymethyl groups of furan and carboxylic acids of lignin were described. Additionally, spectroscopic evidence for the formation of a CH_2 bridge *via* condensation of the hydromethyl group of FA on a free position of a lignin aromatic subunit was described. The materials were also characterized for their thermal stability, thermo-mechanical behaviour and morphology. Addition of PFA to lignin increased the thermal stability of the biopolymer as well as the glass transition temperature (T_g) and the damping capacity (stiffer material).

The preparation of a resin composed of Organosolv lignin and FA supported by cellulose fibres was reported by J. Pin *et al.*⁴² The polymerization was induced by addition of maleic anhydride (MA) as catalyst and a weight ratio of FA:lignin:MA of 40:55:5 was used. The tensile modulus and strength of the lignin PFA matrix were compared to the synthesised PFA resin and found to be similar. This means that addition of lignin to PFA did not negatively impact the mechanical properties such as the brittleness of the composite. The authors argued that this can be attributed to the rigid aromatic structure of lignin which does not allow flexibility in the polymeric network.

Another publication by H. Deka *et al.*⁴³ also discussed the formation of a blend of lignin and PFA. For the synthesis, lignin was directly dispersed in FA and the polymerization was catalysed by *p*-toluenesulfonic acid monohydrate (PTSA). The prepared blends contained 0, 5, 10 and 20 wt% lignin and the resins were tested for their mechanical properties. It was found that the flexural strength and modulus as well as the storage modulus increased with an increase in lignin content in the matrix. The optimum amount of lignin in the blends was determined to be 20 wt% with regards of the mechanical properties of the material.

Spent liquor, a waste product of the magnefite pulping process containing 60 % ligno-sulfonates was mixed with furfuryl alcohol and sulphuric acid in water (with different ratios) to synthesize a PFA copolymer for use as wood adhesive for the production of wood panels (Figure 4-5).⁴⁴ The authors reported the observation of covalent bonds between lignin and the furan polymer based on FTIR analysis and solubility tests. The adhesive properties of the lignin-PFA resins strongly depended on the curing conditions and the ratio of lignin:FA:catalyst used. When high temperatures were used for the curing, satisfactory solid wood bonding was described. However, the authors also mention difficulties in the handling and continuity of the performance of the lignin-PFA adhesives.



Figure 4-5. Particleboards manufactured from wood chips and a bio-based adhesive consisting of spent-liquor and furfuryl alcohol with 10% and 15% of glue.⁴⁴

Another study investigated the formation of lignin-PFA copolymers in order to substitute phenol-formaldehyde resins as wood adhesives.⁴⁵ Acid hydrolysis lignin was used and a mixture of hydrochloric acid and formic acid acted as polymerization catalyst for furfuryl alcohol. The molecular weight of the lignin-PFA polymer was found to have increased compared to the native lignin and the authors argued that this indicates the formation of covalent bonds between lignin and polyfurfuryl alcohol. The effect of different ratios of the starting materials on the properties of the final product was examined as well as the adhesive strength after formation of adhesive reinforced fibre glass. Comparison of the tensile strength of the synthesised resin with phenol-formaldehyde showed that the new material could reach 50 – 90 % of the industrially used PF resin depending on the reaction and curing conditions.

Lignin has become a widely studied polymer for the production of materials in recent years. However, the research field is relatively novel and commercialisation of polymeric products is still sparse. One promising application of lignin is the use as bio-based resins for adhesive purposes. This chapter discusses the synthesis of polyfurfuryl alcohol (PFA) and lignin-PFA copolymers in protic ionic liquids and the characterization of these resins. The novelty of this approach lies in the use of the protic ionic liquid as a combined catalyst and solvent system.

First, the synthesis of PFA from furfuryl alcohol in protic ionic liquids was studied to confirm the suitability of the IL reaction system for the synthesis of lignin-PFA copolymers. The ionic liquid chosen was selected in regard of several functions it needs to fulfil. First, a relatively high acid strength is required to induce protonation of the hydroxyl group of the FA which is the first step in the polycondensation reaction of FA to PFA. Secondly, with respect of lignin functionalization, the selected ionic liquid needs to be able to dissolve lignin in order to minimize diffusion and mass transfer issues that would occur if lignin was not dissolved in the reaction medium.

4.2 Results and discussion

4.2.1 Synthesis of PFA in protic ionic liquids

PFA is generally synthesised in dichloromethane (DCM) with the aid of acidic catalysts (such as *p*-toluenesulphonic acid or trifluoroacetic acid)^{33, 34} via a polycondensation reaction (Figure 4-6). It is generally accepted that the first phase of the polymerization of FA to PFA consists of condensation reactions of the hydroxyl group with either the hydrogen atom at the 5-position in the furan ring (yielding a head-to-tail structure) or with another hydroxyl group (resulting in a head-to-head structure). Under strong acidic conditions the polymer containing dimethyleneoxide bridges is not stable and abstracts formaldehyde and transforms into a polymer mainly containing methylene bridges.²⁸

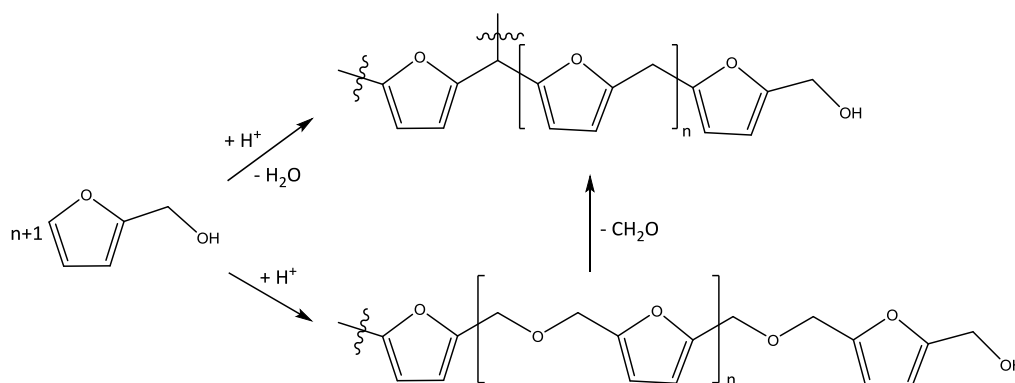


Figure 4-6. Simplified synthesis pathway of polyfurfuryl alcohol from furfuryl alcohol with acid catalyst to yield head-to-tail (top structure) or head-to-head (bottom structure) polymers.²⁸

The established PFA synthesis routes face disadvantages such as the use of toxic and harmful solvents (DCM) and the need for neutralisation of the reaction mixture at the end of the polymerization process which produces salt as a waste product of the reaction. The use of protic ionic liquids that function as both solvents and catalyst for the reaction has the potential to improve these issues.

In this study, PFA was first synthesised in the protic ionic liquid $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$ at 120 °C for 1 hour and 2 hours, respectively. A brown solid product was precipitated using water as anti-solvent for each reaction condition and the yield of the polymerization is reported in Table IV-1.

Table 4-1. Reaction conditions of polymerization of FA into PFA in $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$ and product yield.

Experiment	Temperature [°C]	Reaction time [h]	Starting material [g]	Product recovered [g]	Yield [%]
1	120	1	0.565	0.324	57.27
2	120	2	0.565	0.319	56.46

Interestingly, the yield of the polymerization was found to be independent of the reaction time when the temperature was kept constant and a yield of *ca.* 59 % was observed indicating that long reaction times might lead to degradation of the product. Further analysis needs to be conducted to confirm the structure of the recovered product. The fastest technique for structure analysis is ^1H -NMR and an exemplary spectrum is shown in figure 4-7 and a detailed peak list is given in Table 4-2.

Analysis of the recorded spectrum showed that the product recovered is indeed polyfurfuryl alcohol. The signals present in the spectrum are in good accordance with literature reports of successful polymerisation of FA to PFA.^{33, 39} This confirms that the synthesis of the polymer is possible in a protic ionic liquid system. The protic ionic liquid fulfils the role of both solvent and catalyst in this reaction. Two possible scenarios could be the reason for this behaviour: (i) a slight excess of sulfuric acid is present in the ionic liquid solution due to inaccuracies during the IL synthesis or (ii) partial dissociation of the protonated cation $[\text{N}_{2220}]^+$ occurs at elevated temperatures which leads to the protonation of the $[\text{HSO}_4]^-$ anion and the formation of minor amounts of sulphuric acid. The formed sulphuric acid then induces the protonation of the FA monomer which is the first step of the polymerisation reaction.

The spectrum of the PFA product differs significantly from the spectrum of the FA monomer (Figure A-8 in the appendix). The proton signals of the polymer are generally broader and less resolved due to signal overlap of the repeating units in the polymer and slow relaxation because of the larger mass of the polymer.⁴⁶ Additionally, the polycondensation results in a polymer with a methylene bridge

between the furan rings and the loss of the OH group attached to the methylene bridge in the monomer. This change in environment of the methylene bridge is represented by an upfield shift of the corresponding NMR signal compared to the signal in the monomer.

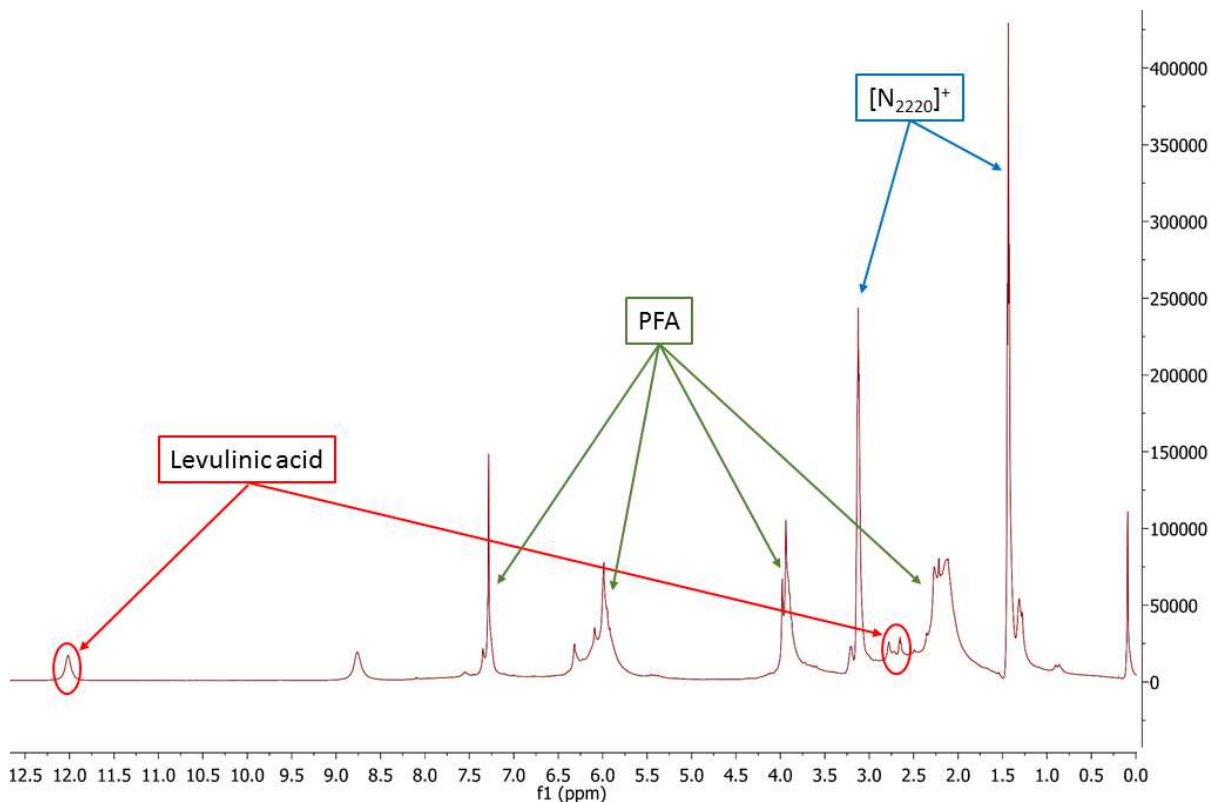


Figure 4-7. Proton spectrum of product recovered from polymerization of FA in $[N_{2220}][HSO_4]_{80\%}$ at 120 °C for 1 hour. The NMR solvent used is $CDCl_3$.

Table 4-2. Peaks shifts and corresponding assignments for product recovered from polymerization of FA in $[N_{2220}][HSO_4]$.

Peak shift δ_H [ppm]	Peak assignment
11.86 (s)	OH levulinic acid
7.34 (m)	$H_{5, \text{arom.}}$ PFA
6.41 (m)	$H_{4, \text{arom.}}$ PFA
6.15 (m)	$H_{3, \text{arom.}}$ PFA
6.03 (m)	$H_{4', \text{arom.}}$ and $H_{3', \text{arom.}}$ PFA
3.92 (m)	CH_2 PFA
3.13 (p)	CH_2 $[N_{2220}]^+$
2.74 (t)	CH_2 levulinic acid
2.62 (q)	CH_2 levulinic acid
2.21 (m)	CH_3 levulinic acid and PFA
1.44 (t)	CH_3 $[N_{2220}]^+$

However, in addition to the signals of the desired polymeric product several other signals were also observed. The methylene and methyl groups of the $[N_{2220}]^+$ cation were clearly detected, showing that the product contains impurities of the solvent used in the polymerisation. Additionally, signals of carboxylic acid ($\delta = 11.86$ ppm), methylene group ($\delta = 2.60$ ppm) and methyl group ($\delta = 2.21$ ppm) were also detected. These signals can be assigned to the compound levulinic acid (LA), which is the product of the hydrolysis reaction of furfuryl alcohol occurring *via* ring-opening under aqueous acidic conditions.⁴⁷ The reaction mechanism of the transformation of FA to LA is shown in figure 4-8.

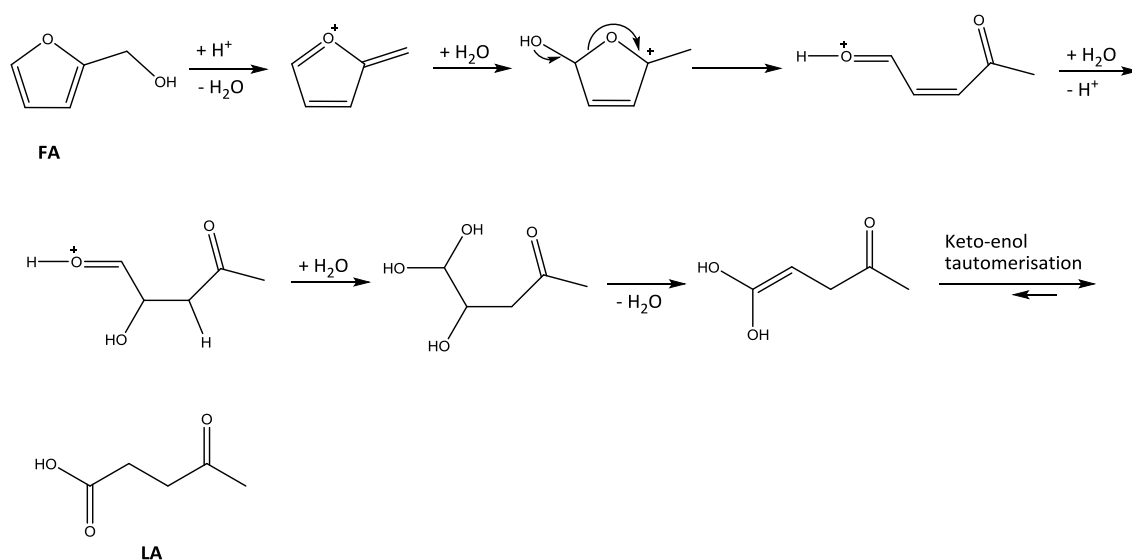


Figure 4-8. Pathway of conversion of furfuryl alcohol (FA) to levulinic acid (LA) under aqueous acidic conditions.

The ring opening hydrolysis reaction of furfural is an undesired side-reaction in the synthesis of polyfurfuryl alcohol since it lowers the yield of the polymeric product. Changing the reaction conditions (i.e. temperature and polymerization time) did not result in preventing the hydrolysis reaction. In order to produce resins from the PFA polymer, the product is required to have a certain degree of purity. Thus, several improvements to the synthesis of PFA in protic ionic liquids need to be undertaken. Firstly, the reaction medium of the polymerization needs to be considered. The ionic liquid used needs to display a certain acidic strength to act as a catalyst for the polymerization reaction since protonation of the hydroxyl group of furfuryl alcohol is the first step of the reaction. On the other hand, the ionic liquid acid strength should not be too high, otherwise FA hydrolysis to LA will take place.

Following the proposed reaction mechanism of the synthesis of PFA a linear polymeric product should be obtained and the ratio of the proton signal of the methylene bridge between furan rings and the two aromatic protons on the furan ring (H_3' and H_4') should be 1 (Figure 4-9).

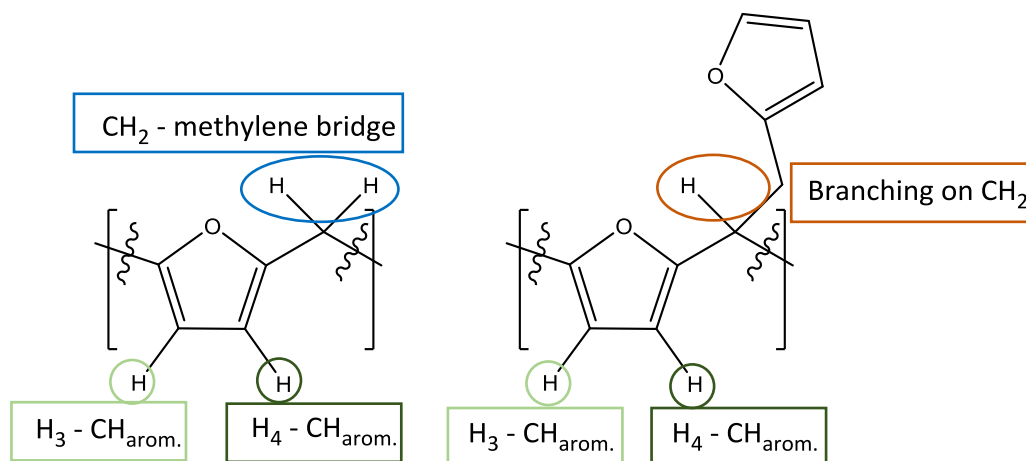


Figure 4-9. Expected proton signals in NMR spectra in linear PFA (left) and branched PFA (right).

However, integral analysis of the PFA products showed that the ratio of the above mentioned proton signals is lower, namely 0.65 and 0.69 for the product recovered after synthesis of 1 hour and 2 hours, respectively (Table 4-3). This indicates that the polymer does not possess a solely linear backbone but is branched to a certain degree with the branching occurring on the methylene bridge between two furan rings. The presence of branches in the polymer structure was also previously described by M. Principe *et al.*³⁴ However, complete understanding of the mechanism that favours the formation of branches on the methylene bridge is still lacking. Interestingly, the reaction time seems to have an impact on the branching of the polymer and a slightly lesser degree of branching was observed for longer polymerisation times. This indicates that the linear chain growth does occur at a higher reaction rate than the branching.

Table 4-3. Degree of branching of PFA synthesized in $[N_{2220}][HSO_4]_{80\%}$.

Entry	Reaction time [h]	$I(CH_2)/I(H_3+H_4)$
1	1	0.65
2	2	0.69

Using the calculated ratio of the NMR signals of the methylene bridge and protons of the furan ring allows for a rough estimate of the degree of branching of the polymer. It was found that more than every second methylene bridge would be functionalized. PFA synthesised by M. Principe *et al.*³⁴ showed branching at every third or fourth methylene bridge of the polymer backbone and comparing these findings to the branching analysis of PFA undertaken here suggests that the calculated degree of branching seems very high. Another possible explanation for the low ratio of $\text{CH}_2/\text{CH}_{\text{arom.}}$ proton signals of the synthesised PFA was given by M. Choura *et al.*³⁹ The authors investigated the colour formation during the acid-catalysed synthesis of PFA and concluded that the formation of conjugated structures in the polymer is responsible for the blackening of the reaction mixture. The following mechanism was suggested in literature to explain the formation of these conjugated systems. The self-condensation of FA monomers produces oligomers which can undergo hydride-ion exchanges with the protonated chain ends of growing polymer chains (Figure 4-10).

This hydride-ion transfer results in methyl-terminated oligomers and carbenium ions (the positive charge is shared by the carbon atom on the methylene bridge and the two adjacent furan rings). The loss of a proton then yields the corresponding conjugated structure. In turn, the furanic-dihydrofuranic moiety can again abstract a hydride ion and eventually give rise to further conjugation. The authors describe that this sequence of events can occur multiple times which enhances the degree of conjugation after each occurring cycle. It is important to note that the monomer does not seem to play a major role in this event because the hydride-ion and proton exchange can take place between FA oligomers. Additionally, the transfer reaction and creation of each new conjugated moiety will result in the formation of a methyl group terminated FA chain. Interestingly, the proton spectra of the PFA products synthesised in $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$ show a strong signal at $\delta = 1.70 - 2.21$ ppm attributed to the methyl group attached to a furan ring and thus delivering further evidence of the presence of conjugated structures in the synthesised polymer. Additionally, a colour change of the reaction mixture from light yellow (FA monomer in IL) to dark brown was observed during the polymerization which also indicates the formation of conjugated structures in the polymer.

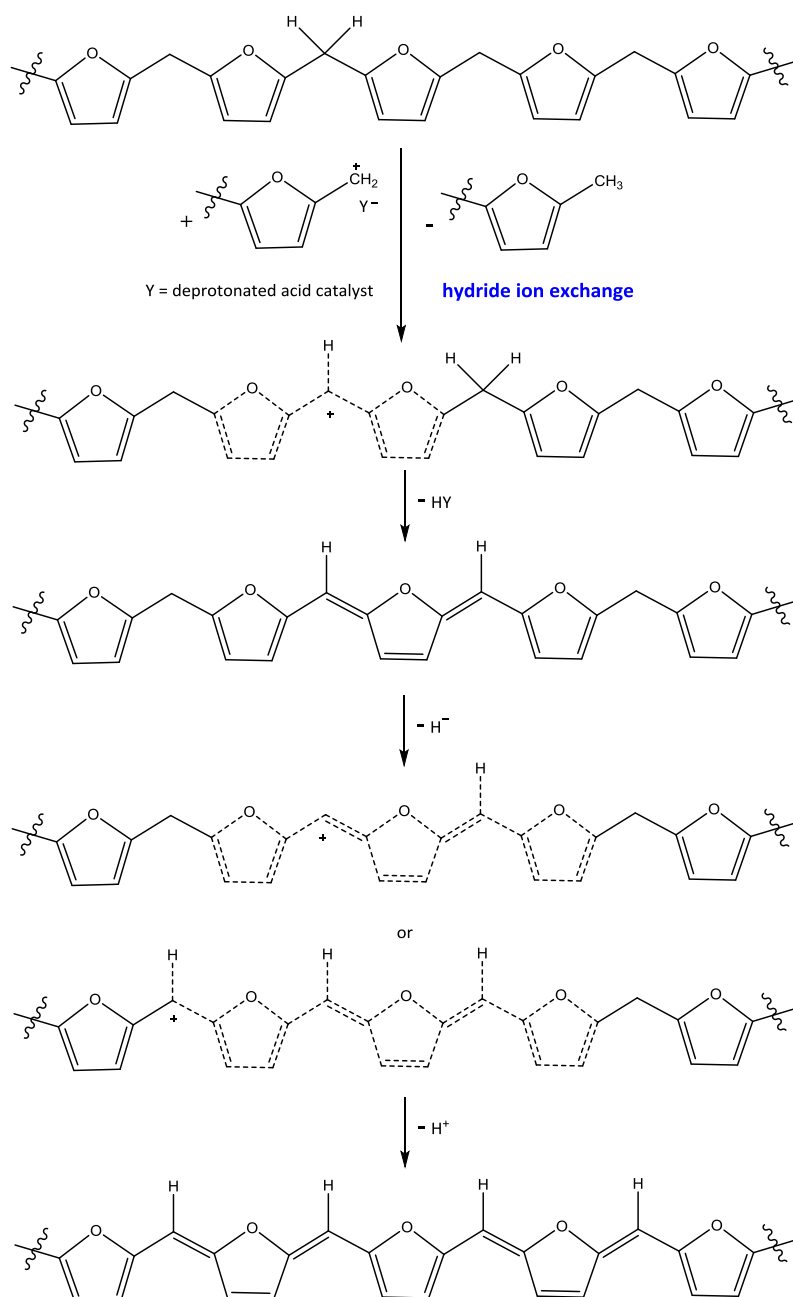


Figure 4-10. Proposed mechanism of formation of conjugated species during polymerization of FA via hydride ion exchange.³⁹

The successful synthesis of PFA in $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$ did prove that acidic ionic liquid catalyst/solvent system is suitable for the polymerisation of FA, however, the incorporation of the IL in the product mixture and the formation of LA during the reaction are clearly undesired outcomes. To prevent this, a different protic ionic liquid solution was applied for the polymerisation.

The acid strength of a protic ionic liquid is not only controlled by the anion (typically $[\text{HSO}_4]^-$ or $[\text{CH}_3\text{COO}]^-$) but also by the cation. Protic ionic liquids are synthesised *via* a simple acid base reaction. Acid base reactions are known to exist in equilibria meaning that the reaction can reverse to the native acid and base. In the case of protic ionic liquids, this equilibrium is influenced by the acid strength (pK_a) of the cation amongst other factors.

Another typical ionic liquid used in biomass fractionation and thus able to dissolve lignin is $[\text{HC}_4\text{im}][\text{HSO}_4]$. The imidazolium based cation $[\text{HC}_4\text{im}]^+$ has a larger pK_a value compared to the $[\text{N}_{2220}]^+$ cation (Table 4-4) thus being a weaker acid. This means that the $[\text{N}_{2220}]^+$ cation is more able to protonate the $[\text{HSO}_4]^-$ anion which results in H_2SO_4 which then can initiate the hydrolysis of FA to LA. For this reason, a new set of experiments of polymerization of FA to PFA was carried out in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$.

Table 4-4. pK_a values of triethylammonium (N_{222}) and 3-butylimidazole (HC_4im) and pK_b values of the corresponding protonated compounds.

	N_{222}	C_4im
pK_a	11.01	7.09
pK_b	2.99	6.91

The polymerization of FA in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ was carried out at two different temperatures, namely 120 °C and 80 °C. The polymerization of FA under acidic conditions occurs via protonation of the hydroxyl group of the molecule followed by abstraction of a water molecule which creates a carbocation as reactive species (Figure 4-11).

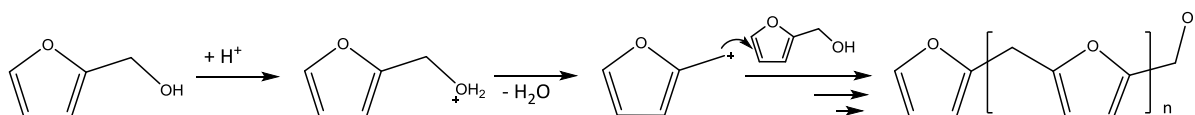


Figure 4-11. Formation of the carbocation during FA polymerisation under acidic conditions.⁴⁸

It is hypothesised that a lower reaction temperature leads to a lesser amount of carbocations being formed thus resulting in polymer chains with a lower molecular weight.

The product yield of the polymerization of FA in [HC₄im][HSO₄] is shown in Table IV-5. Interestingly, the yield was not affected by the catalyst/solvent system chosen for the polymerization and the same yield was observed for synthesis of PFA in [N₂₂₂₀][HSO₄]_{80%} and [HC₄im][HSO₄]_{80%}. Additionally, the yield of the isolated product does not seem to be significantly influenced by the reaction temperature or time.

Table 4-5. Reaction conditions of polymerization of FA into PFA in [HC₄im][HSO₄]_{80%} and product yield.

Experiment	Temperature [°C]	Reaction time [h]	Starting material [g]	Product recovered [g]	Yield [%]
1	120	1	0.565	0.311	55.10
2	120	2	0.565	0.312	55.19
3	80	1	0.565	0.324	57.40
4	80	2	0.565	0.333	58.97

Product characterisation *via* ¹H-NMR revealed that the recovered product was indeed polymeric PFA (Figure 4-12). Interestingly, no signals of levulinic acid are present in the NMR spectrum of the product synthesised in [HC₄im][HSO₄]_{80%}. This suggests that the ring-opening hydrolysis of FA to LA did not occur during FA polymerisation in this ionic liquid solution. This might be due to the differences in acidic strength of the catalyst/solvent system applied as well as to stabilisation of the furan ring *via* π - π -stacking occurring between the furan ring and the imidazolium based cation.⁴⁹

As with the product recovered after polymerization of FA in [N₂₂₂₀][HSO₄]_{80%} branching analysis of the polymer *via* comparison of the NMR integrals of the protons of the methylene bridge and the aromatic ring was undertaken. For an overview of these calculations see Table 4-6. Choosing a different ionic liquid for the polymerization did not significantly impact the degree of branching of PFA. The PFA isolated after reaction at 120 °C displayed a slightly lesser amount of branching compared to the one polymerized under the same reaction conditions but in [N₂₂₂₀][HSO₄]_{80%}. Interestingly, reducing the reaction temperature to 80 °C did seem to yield polymers with an even more linear structure, especially at short polymerization times (see entry 1) which suggests that the condensation reaction is favoured in harsher polymerization conditions. This is in good agreement with research undertaken previously which described the polymerization of FA to be a two-step process with the second step consisting of the condensation of linear oligomers to a branched polymer.²⁸

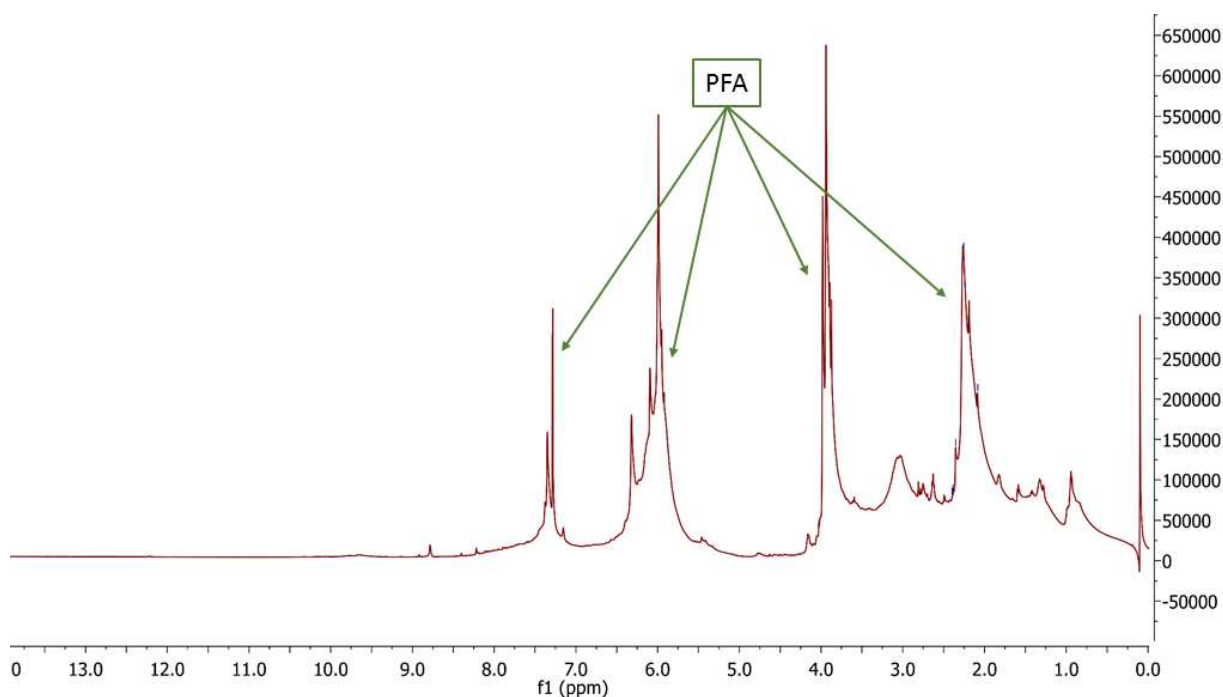


Figure 4-12. Proton spectrum of product recovered from polymerization of FA in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ at 120 °C for 1 hour. The NMR solvent used is CDCl_3 .

Table 4-6. Degree of branching of PFA synthesized in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$.

Entry	Temperature [°C]	Reaction time [h]	$I(\text{CH}_2)/I(\text{H}_3+\text{H}_4)$
1	80	1	0.75
2	80	2	0.70
3	120	1	0.69
4	120	2	0.72

The polymerisation of FA in protic ionic liquids clearly resulted in the formation of PFA. However, a polymer is not only characterised by its molecular structure, but also by properties such as degree of branching and molecular weight.

It was found that the molecular weight of the PFA synthesized in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ depended on the reaction conditions applied (Figure 4-13). An increase in the polymerization time at 80 °C resulted in an increase of the weight average molecular weight of the polymer from *ca.* 2500 g mol^{-1} to *ca.* 4500 g mol^{-1} , however, the number average molecular weight remained constant. This shows that several new short chains were formed during the second hour of the polymerization.

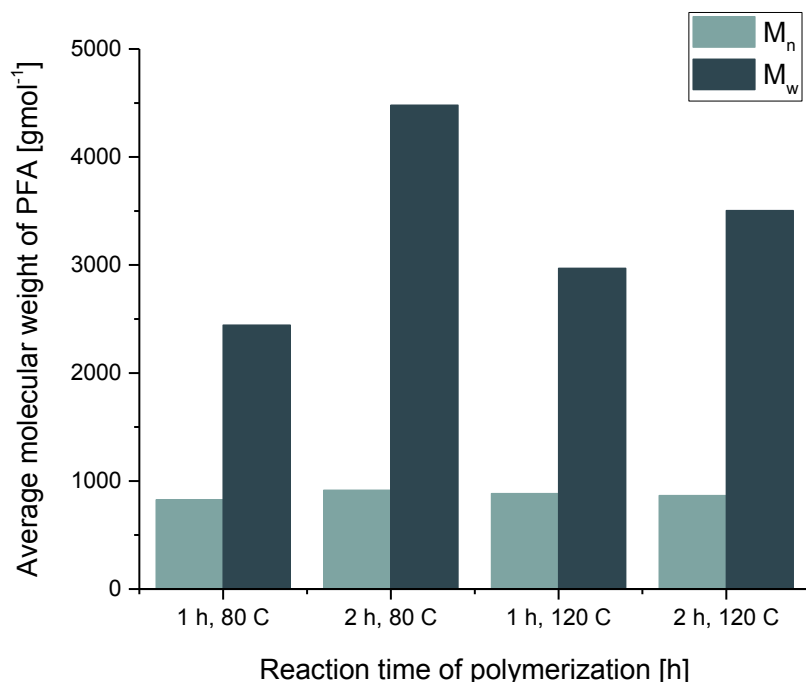


Figure 4-13. Number average molecular weight ($\bar{M}_n$) and weight average molecular weight ($\bar{M}_w$) of PFA synthesized in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ at 80 °C and 120 °C for 1 hour and 2 hours.

Surprisingly, an increase in the reaction temperature to 120 °C only led to an increase in the molecular weight of the PFA for the shorter reaction time where the $\bar{M}_w$ was found to be *ca.* 3000 gmol⁻¹. Elongating the reaction time to 2 hours gave a polymer with a weight average molecular weight of *ca.* 3500 gmol⁻¹ which is lower than the one recovered for the same reaction time at 80 °C. This might indicate that using these severe conditions did result in partial degradation of the polymer. Comparing the molecular weight data obtained here with data already published in the literature shows that the chain lengths of the polymers synthesized in ionic liquid solutions are in the range of the ones synthesized using conventional solvents and catalysts. PFA polymerized in DCM or THF adding *p*-toluenesulfonic acid or trifluoroacetic acid as catalyst was reported to have molecular weight between 2200 and 3500 gmol⁻¹.³³

4.2.2 Characterisation of lignin extracted with ionic liquid pretreatment

The synthesis of lignin-PFA copolymers in protic ionic liquids has never been attempted before. This means that reactive sites and resulting structure of the product are still unknown. Typically, lignin

modification is undertaken *via* the reactive hydroxyl groups of the lignin polymer⁵⁰, however, the hydroxyl groups are suspected to undergo elimination reactions in protic acidic ionic liquids at elevated temperatures meaning that they will most likely not be available as reactive centres under these conditions. The abstraction of the hydroxyl group of lignin in protic ionic liquids leads to the formation of a carbocation on the α carbon of the β -O-4 aryl ether bond which could attack the furan ring to form a copolymer.⁵¹

The polymerisation of FA to PFA occurs *via* a cationic polycondensation mechanism (Figure 4-11) where the formed carbocation attacks the electron rich furan ring. This suggests that modification of lignin with FA/PFA might occur *via* a similar reaction mechanism, where the produced FA carbocation attacks the electron rich aromatic rings of the lignin polymer. It is hypothesised that successful synthesis of lignin-PFA copolymers and the responsible reactive sites for the reaction will largely depend on the structure of the extracted lignin (i.e. amount of aryl ether linkages present, S/G ratio and degree of cross-condensation of the aromatic subunits). The structure of the lignin recovered *via* the IonoSolv pretreatment is determined by the applied pretreatment severity (see chapter 1 of this thesis). For this reason, lignin was isolated from willow Endurance using [N₂₂₂₀][HSO₄]_{80%} using 3 different pretreatment conditions, namely 1 hour at 120 °C, 150 °C and 170 °C.

The different severity of the IL pretreatment was also reflected in the physical appearance of the isolated lignin (Figure 4-14). The recovered product appeared noticeably darker with increasing pretreatment severity, suggesting a greater degree of cross-condensation of the aromatic subunits. NMR analysis was performed to verify this hypothesis.



Figure 4-14. Lignins isolated with different pretreatment severity: 120 °C (left), 150 °C (centre) and 170 °C (right).

In addition to the change in colour of the extracted lignin, the yield was also affected by the pretreatment conditions (Table 4-7). A higher pretreatment severity (represented by the higher pretreatment temperature) resulted in a higher lignin recovery yield. This should be kept in mind for future applications where a high lignin yield of a batch pretreatment is beneficial.

Table 4-7. Yield of recovered lignin after IL pretreatment with different severity.

Pretreatment condition	Lignin yield [% of BM]
1 h at 120 °C	6.09
1 h at 150 °C	15.91
1 h at 170 °C	23.00

HSQC NMR spectroscopy was used to obtain information about the structural changes of the extracted lignin with focus on the relative amounts of the aromatic subunits, the degree of condensation of the aromatic subunits as well as the amount of inter-unit linkages. NMR analysis revealed that the amount of aryl ether linkages present in the isolated lignin were significantly reduced when a pretreatment with higher severity was applied (Figure 4-15). The amount decreased from 0.43 bond/C₉ unit (for 120 °C) to 0.06 bonds/C₉ unit (for 170 °C) with hydrolysis reactions of the aryl ether linkages taking place under acidic conditions (as described elsewhere).⁵² This means that the structure of the recovered lignin differs significantly, with mild pretreatment resulting in lignin that is rich in aryl ether bonds, whereas harsh pretreatment conditions yield lignin that mainly consists of aromatic subunits that are possibly also condensed. The amount of β - β and phenylcoumaran linkages was found to be relatively stable and independent of the applied pretreatment conditions.

It is important to note that this difference in lignin structure will most likely also affect the reactivity towards grafting furfuryl alcohol from the lignin polymer in respect of available reactive sites on the lignin.

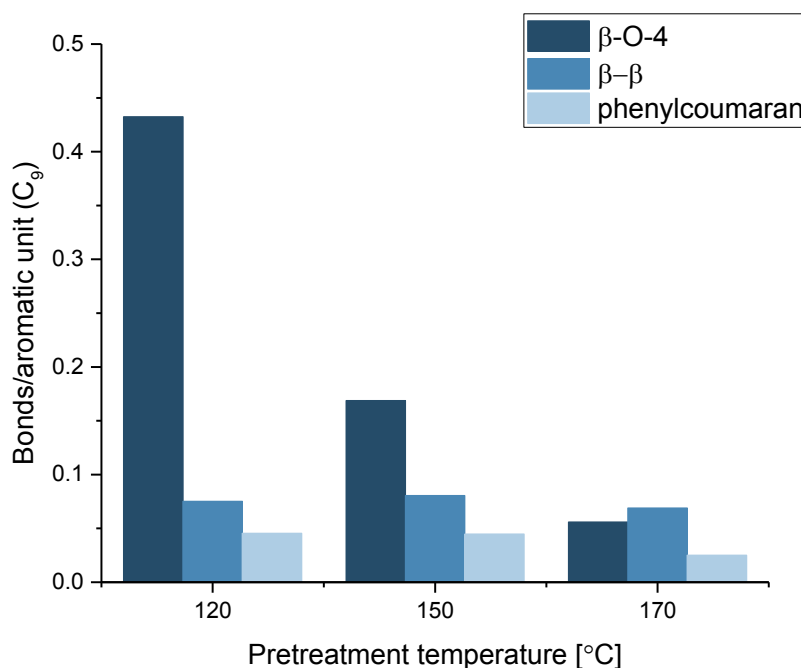


Figure 4-15. Relative amount of inter-unit ether linkages present in lignin after isolation with $[N_{2220}][HSO_4]_{80\%}$.

The inter-unit linkages were not the only structural feature of lignin that was affected by the pretreatment severity. Another interesting lignin property to consider is the S/G ratio, which represents the ratio of S subunits to G subunits. The S/G ratio was found to increase from 2.7 to 4.6 with increasing pretreatment temperature, showing that the lignin isolated at higher temperatures is richer in S subunits compared to lignin isolated at very mild pretreatment conditions (Table 4-8). This is indeed interesting, as S rich lignin is known to show a greater linearity compared to G rich lignin⁵³ which might also affect the reactivity of the polymer towards modification and the final structure of the copolymer. It is hypothesised that a more linear polymer displays less steric hindrance and is thus more reactive towards chemical modification.

Table 4-8. S/G ratio and degree of condensation of aromatic subunits of isolated lignin.

Temperature [°C]	S/G ratio	S [uncond./cond.]	G [uncond./cond.]
120	2.7	1.4	no condensation
150	4.6	0.8	5.4
170	4.6	0.5	3.1

Extraction of lignin under acidic conditions often leads to formation of a carbocation on the aryl ether linkage, followed by condensation reactions of the aromatic subunits with this carbocation.⁵¹ This leads to a significant change in the structure and properties of the isolated lignins. NMR signal integration of the isolated lignins showed that the degree of condensation of both the S and G subunits increased continuously with pretreatment severity. Interestingly, it was observed that the S subunits were more prone to condensation reactions than the G subunits. This can be explained with the S subunits being more electron rich compared to the G subunits due to the additional methoxy group that has an electron donating effect.⁵¹ For lignin extracted at 120 °C, no signals indicating condensation of the G units were observed, and a significant amount of S subunits were not condensed. Harsher pretreatment conditions at higher temperature then led to condensation reactions, resulting in lignin where the amount of condensed S subunits were twice as high as the uncondensed ones (Table 4-8). A more condensed lignin structure is thought to be more suitable for replacing phenol in phenol formaldehyde resins due to a greater structural similarity of the polymers which will result in comparable properties.

Another important lignin property is the molecular weight of the extracted polymer. The weight average molecular weight ($\bar{M}_w$) of the lignin was observed to first decrease with increasing pretreatment temperature from *ca.* 4200 g mol⁻¹ (120 °C) to *ca.* 3700 g mol⁻¹ (150 °C) and then increase again to *ca.* 5900 g mol⁻¹ (170 °C) as shown in figure 4-16. This suggests that first hydrolysis of the aryl ether linkages results in a depolymerisation and shortening of the lignin polymer chain length as described earlier elsewhere⁵² and as was also observed by the NMR analysis of the lignin. Condensation reactions do occur under severe pretreatment conditions, resulting in an increase of the molecular weight of the lignin when isolated at 170 °C. Interestingly, the number average molecular weight of lignin was found to stay almost constant with increasing pretreatment temperature, resulting in an increase of polydispersity of the lignin polymer, which suggests that the lignins isolated at higher temperature are more heterogeneous in chain length. The molecular weight of a polymer is known to affect its properties, such as melting point, solution and melt viscosity, solubility, mechanical strength and moduli.⁴ A higher molecular weight will result in higher mechanical strength which is a desired property for resins.

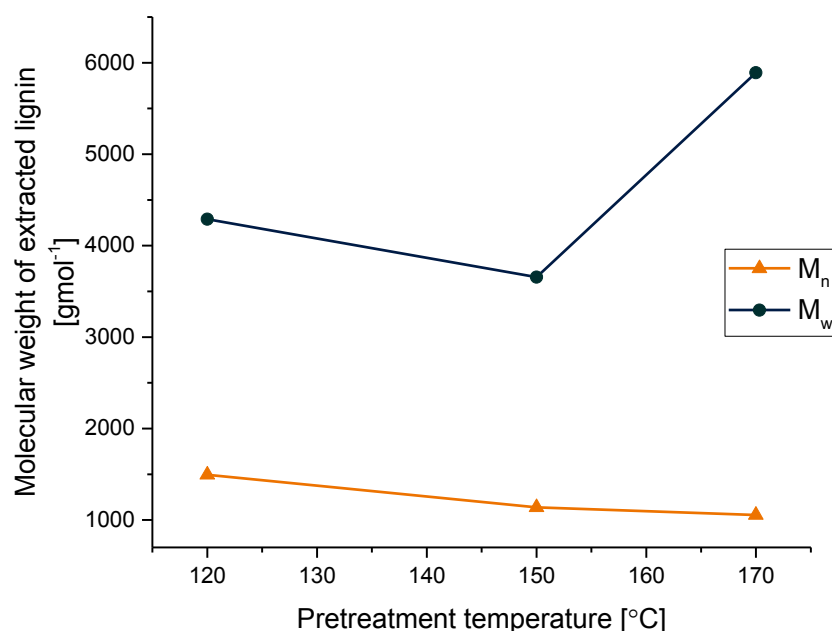


Figure 4-16. Measured number average molecular weight (M_n) and weight average molecular weight (M_w) of lignin extracted with $[N_{2220}][HSO_4]_{80\%}$.

The high molecular weight and highly condensed structure of the lignin isolated at 170 °C suggests that this will be the most promising candidate for the task to replace phenol in resins.

4.2.3 Copolymerisation of lignin and FA in protic ionic liquids

The lignin/PFA polymers were synthesized using the ionic liquid solution $[HC_4im][HSO_4]_{80\%}$ based on the findings of using the ionic liquid solutions $[N_{2220}][HSO_4]_{80\%}$ and $[HC_4im][HSO_4]_{80\%}$ for the synthesis of PFA. It is hypothesized that copolymers of lignin and PFA are formed under these conditions using the so-called grafting from approach where FA/PFA is grafted from the lignin polymer (Figure 4-17 for illustration). This idea of the grafting from is based on reactions occurring in acidic media involving both the FA polymerization and transformation of lignin. Firstly, the polymerization of FA is well known to proceed *via* protonation of the hydroxyl group on the furan ring, followed by abstraction of a water molecule and thus the creation of a primary carbocation. The said carbocation then attacks the electron rich furan ring of a second molecule to form a dimer. This reaction is repeated multiple times to form the polyfurfuryl alcohol polymeric product.^{39, 54} Secondly, it is also well established that lignin undergoes the formation of a carbocation *via* protonation of the hydroxyl group at the α carbon of the β -O-4 aryl ether followed by elimination of a water molecule in acidic media. This carbocation then

attacks an aromatic subunit to form a new carbon-carbon bond resulting in cross-linking of lignin.⁵¹ The formation and reactivity of the carbocation of the β -O-4 aryl ether lignin bond is responsible for the undesired side-reaction of condensation in lignin but can also be viewed as a potential reactive site of the lignin polymer.

Based on the above mentioned reactions occurring under acidic conditions, the grafting of FA/PFA from lignin should theoretically be possible to create a lignin-PFA copolymer. Additionally, functionalization of lignin isolated at mild pretreatment conditions such as 120 °C with many intact β -O-4 should result in a copolymer with a high number of attached PFA chains compared to a copolymer with lignin extracted at 170 °C with a small amount of aryl ether linkages present was used.

The properties of the final polymeric product will not only depend on the ratio of lignin to PFA but also on the properties of the lignin used for functionalization which are controlled by the lignin structure. To get a better understanding of how the lignin structure influences the reactivity towards grafting FA from lignin as well as the properties of the copolymer lignin isolated at three different degrees of pretreatment severity was used.

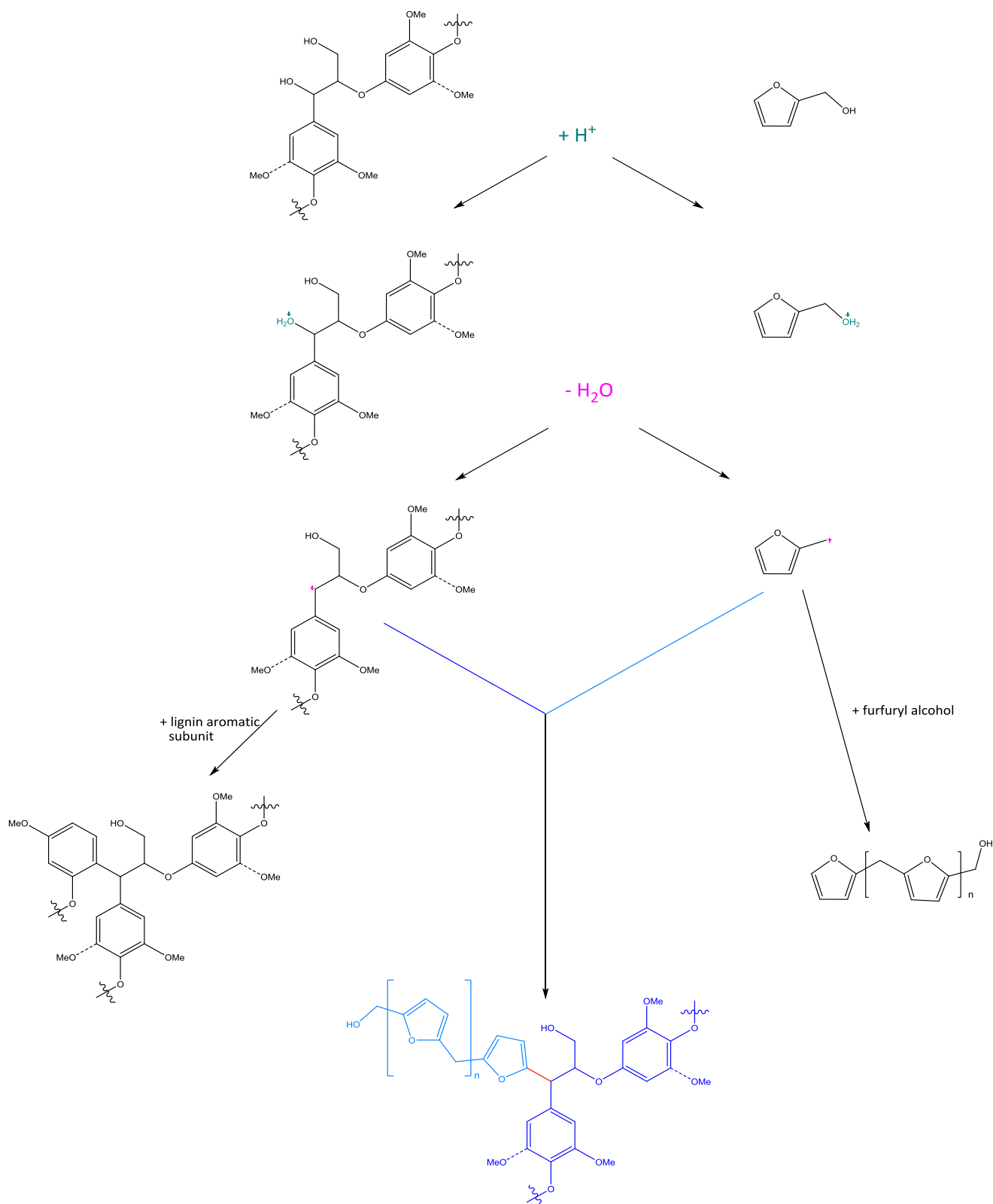


Figure 4-17. Illustration of hypothesized grafting of FA/PFA from lignin.

4.2.3.1 Yield of polymeric product

The reaction was carried out in the acidic ionic liquid solution $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ which acts as the solvent for both lignin and FA and simultaneously as the proton donor to catalyse the polymerization of FA and ideally the coupling between FA/PFA and lignin.

The reaction was carried out with the following molar ratios of lignin to furfuryl alcohol: (i) 1:1, (ii) 1:2 and (4) 1:4 at 120 °C for 1 hour or 2 hours. The amount of recovered product after the reaction was quenched with DI water is shown in figure 4-18.

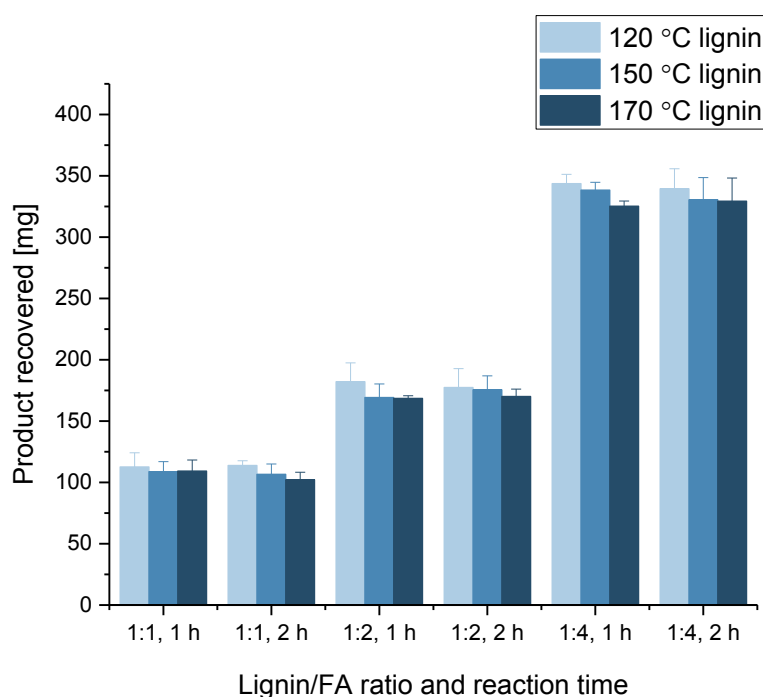


Figure 4-18. Weight of product recovered of functionalization of lignin with FA using lignin isolated at 120 °C (light blue), 150 °C (medium blue) and 170 °C (dark blue) with different lignin/FA ratios and reaction times.

The amount of product recovered was found to be dependent on the lignin to FA ratio as well as the lignin used for functionalization. Interestingly, the time of reaction did not play a role in the amount of product formed, indicating that the reaction rate of the FA polymerisation is fast. The amount of product isolated increased linearly with the increase in FA ratio in the reaction mixture but slightly decreased with an increase in pretreatment severity used to isolate the lignin. This might suggest that more reactive sites are available in the lignin extracted under mild pretreatment conditions since it is unlikely that the chain length of the PFA is influenced by the lignin used for modification.

For each lignin functionalization experiment 100 mg of lignin were used and the amount of product recovered using a 1:1 lignin:FA ratio was around 100 mg for the different lignins and both reaction times. This might first seem surprising, but one has to keep in mind that lignin depolymerisation does occur in acidic reaction conditions at elevated temperatures.^{51 52} Additionally, full conversion of the FA monomer to PFA is unlikely since a yield of 55 – 60 % was observed for FA polymerization in [HC₄im][HSO₄]_{80%}. This suggests that a large percentage of the FA reacted to low molecular weight oligomers which did not get precipitated upon the addition of the anti-solvent.

4.2.3.2 Molecular weight analysis

The products recovered from modification of lignin with FA were subjected to molecular weight analysis to get an insight to whether the reaction resulted in a covalent coupling of lignin and FA/PFA or in a blend of the two polymers. Theoretically, a successful production of a lignin/PFA copolymer should result in an increase of molecular weight of the lignin/PFA copolymer compared to the lignin polymer and PFA. It is known that lignin undergoes condensation reactions in acidic ionic liquids at elevated temperatures^{51, 52} which lead to an increase in molecular weight of the recovered lignin. To be able to exclude this effect and distinguish an increase in molecular weight caused by the formation of lignin/PFA copolymers, the three types of lignin used for functionalization were also subjected to the same reaction conditions used for lignin modification with FA.

The weight average molecular weight ($\bar{M}_w$) and number average molecular weight ($\bar{M}_n$) of the extracted lignin used as starting material for the synthesis of lignin/PFA polymers depended on the pretreatment severity applied for lignin isolation. The $\bar{M}_w$ was found to be *ca.* 4200 gmol⁻¹, *ca.* 3700 gmol⁻¹ and *ca.* 5900 gmol⁻¹ for pretreatment at 120 °C, 150 °C and 170 °C, and the $\bar{M}_n$ was measured to be 1500 gmol⁻¹, 1100 gmol⁻¹ and 1000 gmol⁻¹ respectively (Figure 4-19 and 4-20). It was found that both the $\bar{M}_w$ and $\bar{M}_n$ of those three different lignins changed after being heated in [HC₄im][HSO₄]_{80%} at 120 °C for 1 hour. Interestingly, the $\bar{M}_w$ slightly decreased from *ca.* 4200 gmol⁻¹ to *ca.* 3800 gmol⁻¹ and the $\bar{M}_n$ decreased from *ca.* 1500 gmol⁻¹ to 1400 gmol⁻¹ for lignin isolated after pretreatment at 120 °C, indicating that depolymerisation of the lignin *via* cleavage of the aryl ether bonds^{15, 17} was the dominant alteration of lignin structure occurring here. In contrast, the molecular weight of lignin extracted at 150 °C and 170 °C was observed to slightly increase from *ca.* 3700 gmol⁻¹ to *ca.* 4100 gmol⁻¹ ($\bar{M}_w$) and *ca.* 1100 gmol⁻¹ to *ca.* 1500 gmol⁻¹ ($\bar{M}_n$) as well as *ca.* 5900 gmol⁻¹ to *ca.* 6300 gmol⁻¹ ($\bar{M}_w$) and *ca.* 1000 gmol⁻¹ to *ca.* 1600 gmol⁻¹ ($\bar{M}_n$), respectively. The lignin isolated using a more severe

pretreatment already underwent depolymerisation during the isolation process with the ionic liquid solution. However, a small amount of β -O-4 aryl ether linkages is still present in the polymer (see section 3.3.2) and can participate in further condensation reaction to form lignin condensates with higher molecular weights.

GPC analysis of the products recovered from lignin functionalization showed a higher molecular weight of those polymers compared to the unmodified lignin and the lignin control, indicating the successful production of a true lignin/PFA copolymer. The weight average molecular weight values and number average molecular weight values for the products obtained with a lignin:FA ratio of 1:1 and 1:2 are shown in figure 4-19 and IV-20. Dissolution of the products synthesised with a lignin:FA ratio of 1:4 in the GPC solvent (DMSO) proved to be an issue and full dissolution was not possible, thus prohibiting GPC analysis of those samples.

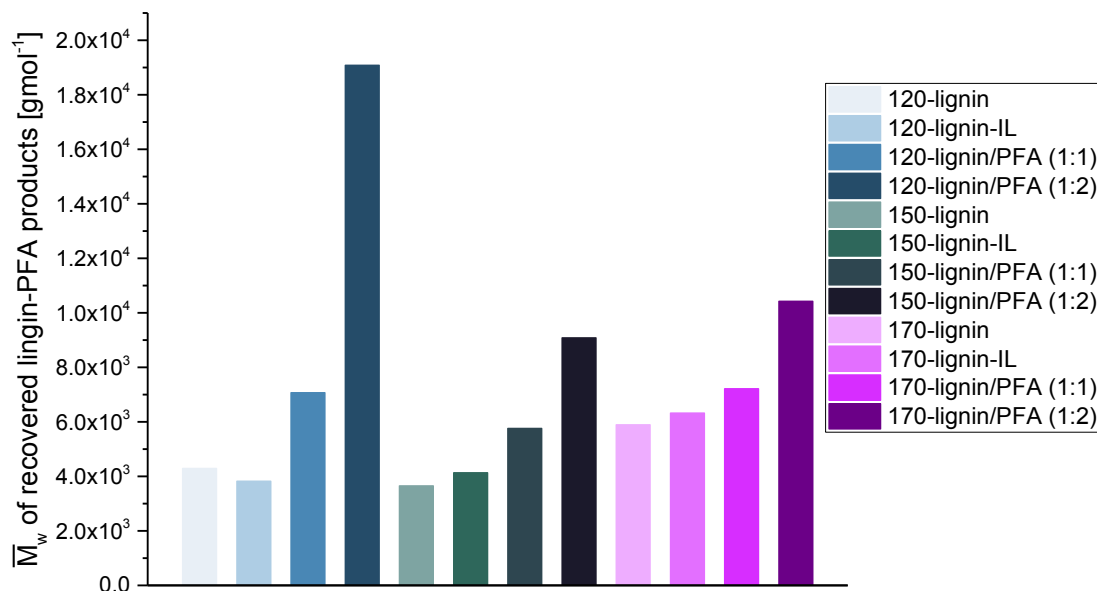


Figure 4-19. Weight average molecular weight ($\bar{M}_w$) of lignin isolated at 120 °C (120-lignin), 150 °C (150-lignin) and 170 °C (170-lignin), these lignins heated in [HC₄im][HSO₄]_{80%} at 120 °C for 1 h (1x0-lignin-IL) and the polymeric products recovered after functionalization of different lignin with PFA in [HC₄im][HSO₄]_{80%} at 120 °C for 1 h with a ratio of lignin:PFA of 1:1 (1x0-lignin/PFA 1:1) and 1:2 (1x0-lignin/PFA 1:2).

The molecular weight of the final product was found to depend on the type of lignin used and the amount of FA used in the reaction. In general, a lower lignin:FA ratio resulted in a higher molecular weight of the polymeric product showing that more FA in the reaction mixture leads to longer PFA

chains grafted from the lignin. Interestingly, the molecular weight of the polymers synthesised using lignin isolated at 120 °C displayed higher molecular weight compared to the ones where lignin extracted at 150 °C and 170 °C was used. These copolymers were found to have a weight average molecular weight of *ca.* 7000 g mol⁻¹ (lignin:PFA = 1:1) and *ca.* 19000 g mol⁻¹ (lignin:PFA = 1:2) compared to 5700 g mol⁻¹ (lignin:PFA = 1:1) and 9000 g mol⁻¹ (lignin:PFA = 1:2) as well as 7200 g mol⁻¹ (lignin:PFA = 1:1) and 10400 g mol⁻¹ (lignin:PFA = 1:2) for copolymers synthesised with lignin extracted at 150 °C and 170 °C, respectively. This might suggest that more reactive sites are present on the lignin polymer resulting in a greater number of PFA chains growing from the polymer resulting in an overall higher weight average molecular weight. Interestingly, it was observed that the $\bar{M}_n$ of the lignin-PFA copolymers did not depend on the ratio of the starting materials and was measured to be *ca.* 1600 g mol⁻¹ (120 °C-lignin) and *ca.* 1400 g mol⁻¹ for 150 °C-lignin and 170 °C-lignin.

Additionally, the increase in molecular weight after lignin functionalization was found to be the highest for the grafting of FA/PFA from 120 °C-lignin when compared to the lignin subjected to heating at 120 °C for 1 or 2 hours. An increase of 84 % (lignin:PFA = 1:1) and 361 % (lignin:PFA = 1:2) compared to 39 % (150 °C, lignin:PFA = 1:1) and 119 % (150 °C, lignin:PFA = 1:2) as well as 14 % (170 °C, lignin:PFA = 1:1) and 64 % (170 °C, lignin:PFA = 1:2) was found indicating once more that the lignin isolated using the mildest pretreatment conditions seems to contain the highest amount of reactive sites for the coupling between lignin and FA to occur. This might indicate that the coupling of FA to lignin does take place at the carbocation formed at the C_α of the β-O-4 aryl ether bond since the amount of those linkages present in the lignin polymer used for modification significantly decrease in the following order: 120 °C-lignin > 150 °C-lignin > 170 °C-lignin. However, this hypothesis needs still to be confirmed. For this reason, extensive NMR characterization of the product was carried out and will be discussed later on in this chapter.

Using lignin extracted at 170 °C for the grafting of furfuryl alcohol always yielded products with a higher molecular weight compared to the ones where lignin isolated after pretreatment at 150 °C was used independent of the lignin:FA ratio in the reaction mixture. This might be due to the more condensed structure of the 170 °C-lignin and the resulting higher molecular weight of the lignin in the copolymer. After successful grafting of FA/PFA from 170 °C-lignin a higher molecular weight of the products should be expected.

The significant difference in calculated increase in weight average molecular weight of the lignin/PFA copolymers for different lignin:FA ratios illustrates that a higher amount of FA in the reaction mixture does indeed yield a product with longer chains (presumably stemming from PFA). This hypothesis is

supported by the fact that none of the copolymers recovered after synthesis with a lignin:FA ratio of 1:4 were soluble in the GPC solvent, most likely due to their very high molecular weights.

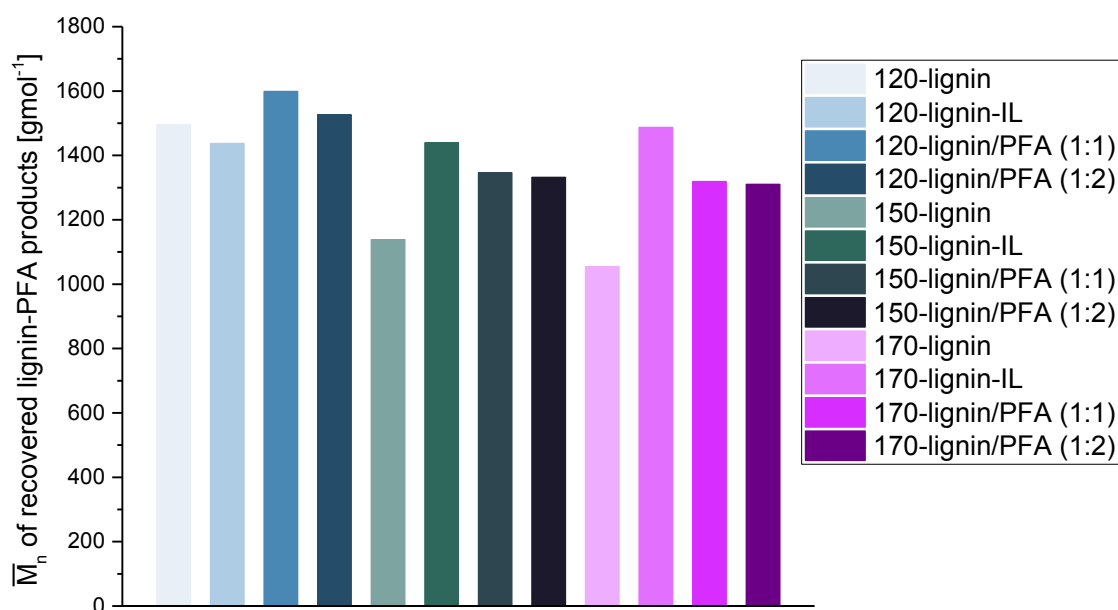


Figure 4-20. Number average molecular weight ($\bar{M}_n$, right) of lignin isolated at 120 °C (120-lignin), 150 °C (150-lignin) and 170 °C (170-lignin), these lignins heated in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ at 120 °C for 1 h (1x0-lignin-IL) and the polymeric products recovered after functionalization of different lignin with PFA in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ at 120 °C for 1 h with a ratio of lignin:PFA of 1:1 (1x0-lignin/PFA 1:1) and 1:2 (1x0-lignin/PFA 1:2).

A study conducted by P. Dongre *et al.*⁴⁵ discussed the synthesis of lignin-PFA adhesives and also analysed the molecular weight of the resulting material. Unfortunately, only the raw data of the size exclusion chromatography measurements was given, showing a broad distribution of molecular weight of the copolymers ranging from around 50000 g mol^{-1} to *ca.* 600 g mol^{-1} with two maxima around 9000 g mol^{-1} and 2000 g mol^{-1} . For the production of these adhesives, spent liquor rich in lignin was reacted with FA at elevated temperatures using hydrochloric acid or sulphuric acid as catalyst. Even though this method is able to produce a small amount of high weight polymeric products the polydispersity of the product is much higher than the one of the copolymers synthesised here which might be not beneficial for applications.

The ratio of lignin to FA is not the only factor that influences the molecular weight of the final polymeric product. It was found that the chain length is also controlled by the reaction time as shown in figure 4-21. An increase of molecular weight (both $\bar{M}_n$ and $\bar{M}_w$) of the products recovered using a

1:1 ratio of starting materials was observed when the lignin functionalization was carried out for 2 hours instead of 1 hour. This suggests that the longer reaction time did indeed lead to longer polymer chains.

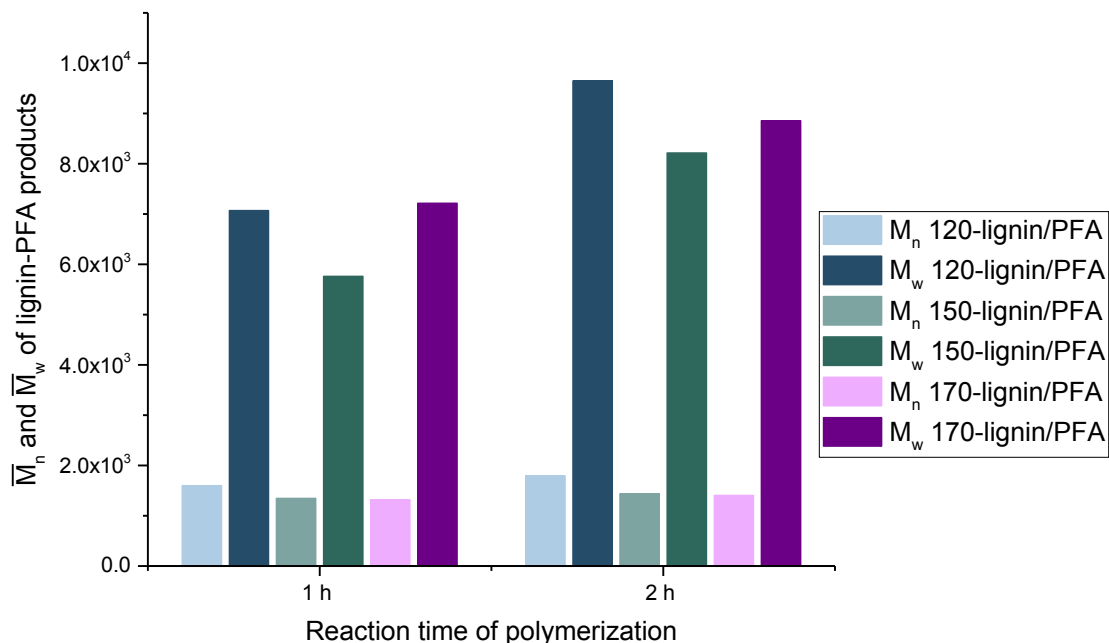


Figure 4-21. Number average molecular weight ($\bar{M}_n$) and weight average molecular weight ($\bar{M}_w$) of products recovered after lignin functionalization with FA/PFA in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ for 1 hour and 2 hours with a lignin:FA ratio of 1:1.

The weight average molecular weight was found to increase from *ca.* 7000 gmol^{-1} to *ca.* 9600 gmol^{-1} (120 °C-lignin), *ca.* 5700 gmol^{-1} to *ca.* 8200 gmol^{-1} (150 °C-lignin) and *ca.* 7200 gmol^{-1} to *ca.* 8800 gmol^{-1} (170 °C-lignin). Interestingly, the increase in molecular weight for polymers derived from functionalization of 120 °C-lignin and 150 °C-lignin was *ca.* 2500 gmol^{-1} or 26 furfuryl alcohol repeat units. The increase in weight average molecular weight could be explained by several reasons. Firstly, not all the possibly available reactive sites present in lignin were functionalized with furfuryl alcohol during a reaction time of 1 hour and an increase in reaction time led to a higher degree of functionalization. Secondly, further chain growth of the PFA chains already grafted from the lignin would also explain an increase in molecular weight as also observed for the synthesis of PFA in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$.

Surprisingly, the increase of weight average molecular weight for the product formed after modification of 170 °C-lignin was only by *ca.* 1600 gmol^{-1} or 16 furfuryl alcohol repeat units. This could

be due to the very low amount of aryl ether linkages present in the lignin extracted at 170 °C which significantly limits the reactive sites for coupling of lignin with furfuryl alcohol.

The influence of reaction time on the molecular weight of the polymeric product was found to be more pronounced when a lignin:FA ratio of 1:2 was used (Figure A-9 in the appendix). After a polymerization of 1 hour, the isolated product showed a weight average molecular weight of *ca.* 19000 gmol^{-1} (120 °C-lignin), 9000 gmol^{-1} (150 °C-lignin) and 10400 gmol^{-1} (170 °C-lignin). However, after reaction of 2 hours the $\bar{M}_w$ of the lignin-PFA product was found to have increase significantly to *ca.* 28000 gmol^{-1} (120 °C-lignin), 25000 gmol^{-1} (150 °C-lignin) and 23400 gmol^{-1} (170 °C-lignin). This shows that the conversion of FA to PFA is not limited by the concentration of the starting materials and the chain length of the lignin-PFA polymer can be controlled by the ratio of the reactants and the reaction time.

4.2.3.3 Structural analysis *via* NMR techniques

The GPC analysis of the synthesised lignin-PFA polymers already suggested successful coupling between lignin and FA/PFA. However, a thorough confirmation of covalent binding between those two polymers is still needed. For this reason, various NMR techniques such as HSQC and DOSY were applied.

The synthesis of the lignin-PFA copolymer was carried out in the acidic ionic liquid solution $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ which is known to induce structural changes in the lignin polymer.^{51, 52} The lignin utilized for functionalization with FA was subjected to the reaction conditions used for the synthesis of the lignin-PFA polymers (heating in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ at 120 °C for 1 hour) in order to be able to distinguish the changes to the lignin structure derived from the acidic reaction medium (such as cleavage of β -O-4 bonds and condensation at the aromatic ring) and the changes occurring during grafting of FA from lignin. HSQC NMR analysis was then used to compare the two sets of lignin (extracted *via* the IonoSolv process and subjected to heat treatment) with regard to analysing the amount of interunit linkages and the S/G ratio. Figure 4-22 shows the amount of three main structural linkages present in lignin in relation to an aromatic subunit (C_9), namely β -O-4, β - β and phenylcoumaran. Interestingly, the heat treatment did not significantly alter the structure of the lignin.

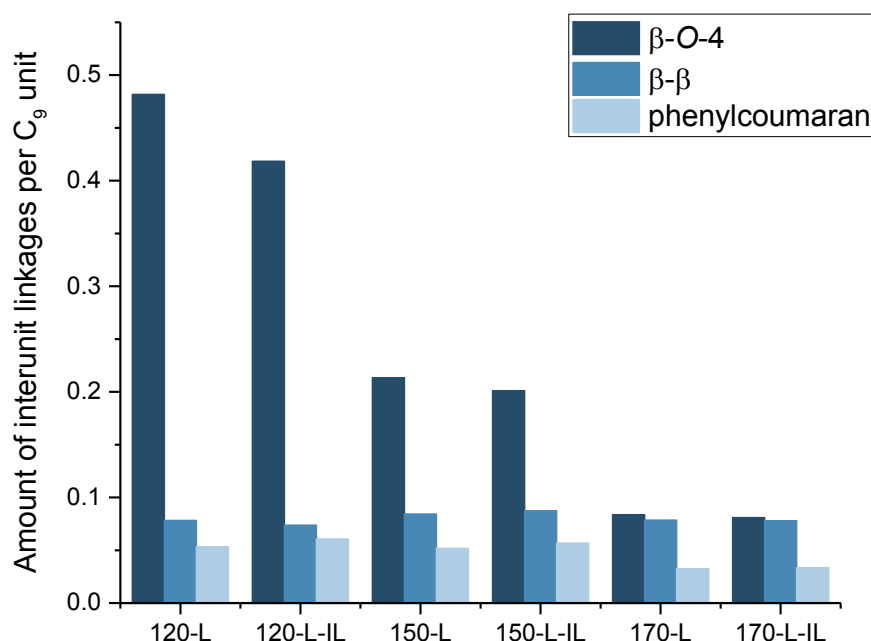


Figure 4-22. Amount of interunit linkages of lignin isolated at 120 °C, 150 °C and 170 °C (1x0-L) and lignin isolated at these pretreatment temperatures heated in [HC₄im][HSO₄]_{80%} at 120 °C for 1 hour (1x0L-IL).

The lignin isolated at 120 °C showed the highest number of β-O-4 aryl ether linkages (0.48 per C₉ unit) and it was found that cleavage of that bond occurred during heating of the lignin in [HC₄im][HSO₄]_{80%} and the amount of that linkage decreased to 0.42 per C₉ unit, showing that the lignin was depolymerized, as also observed during GPC analysis (see section 3.3.3.2). However, the β-β and phenylcoumaran linkages were not affected and the amount was observed to stay constant. Lignin extracted at more severe pretreatment conditions (e.g. 150 °C and 170 °C) already showed a much reduced amount of easily hydrolysed β-O-4 bonds, namely 0.21 and 0.08 per aromatic ring. A repeated exposure to heat and the acidic ionic liquid solution did not result in further cleavage of any of the analysed interunit linkages, showing that the structure was mainly unaffected (as was the molecular weight as describe in the section above). This suggests that the lignin isolated at harsh pretreatment conditions should theoretically only undergo modification with FA/PFA and not any further structural changes.

Another important characterization of the structure of lignin is the S/G ratio of the polymer. However, the S/G ratio did not seem to be affected by the subsection of the lignin to the reaction conditions of the synthesis of lignin-PFA polymers (Figure 4-23).

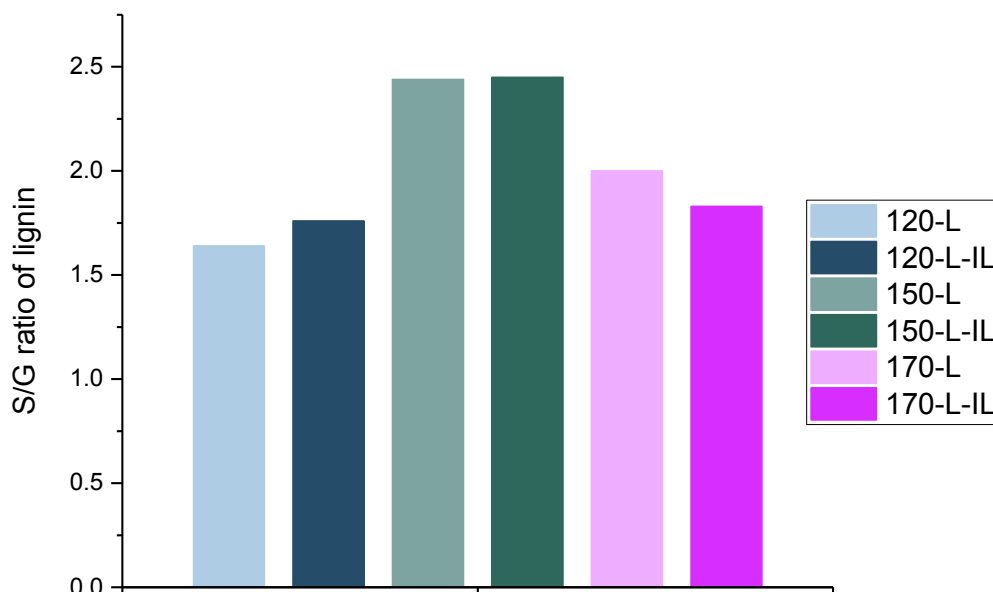


Figure 4-23. S/G ratio of lignin isolated at 120 °C, 150 °C and 170 °C (1x0-L) and lignin isolated at these pretreatment temperatures heated in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ at 120 °C for 1 hour (1x0-IL).

NMR analysis of the polymeric products isolated after lignin functionalization with FA was performed and the spectra were compared with the ones measured of unmodified lignin subjected to the polymerization conditions (Figure 4-24 for two exemplary spectra).

The NMR spectra of lignin functionalized with PFA did differ quite significantly from the spectra of unmodified lignin. In addition to the lignin signals new C-H cross signals for the PFA aromatic protons of the furan ring and protons for the methylene bridge were observed. Interestingly, no signal for the C5-H5 bond of the terminal furan ring of the polymer was visible, suggesting that this part of the furan ring is involved in the coupling to the lignin polymer and a covalent bond is indeed formed between the furan ring and lignin.

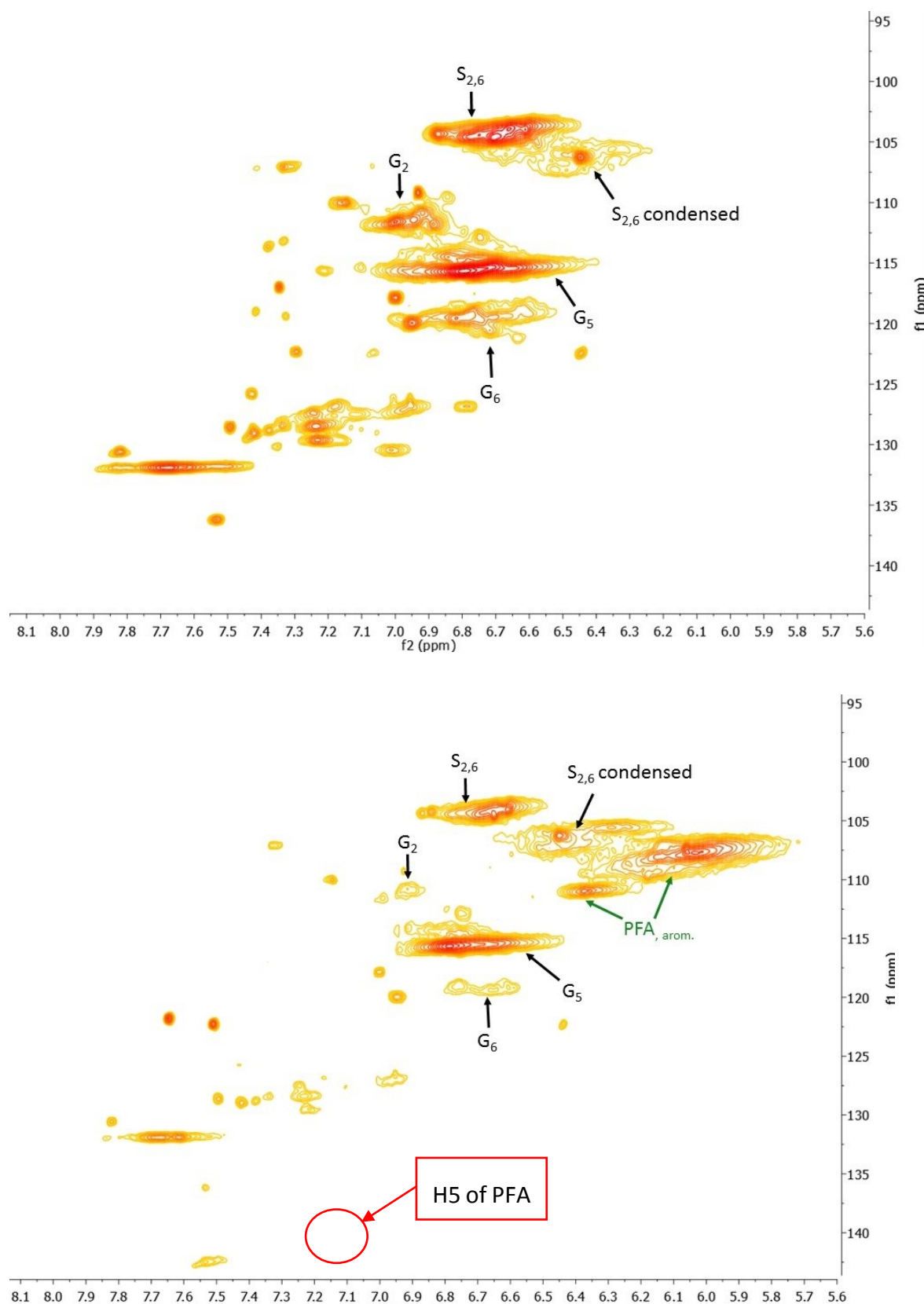


Figure 4-24. HSQC-NMR spectra (solvent used: DMSO-d_6) of aromatic region of 120 °C-lignin heated in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ (top) and product of polymerization of 120 °C-lignin and FA in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ at 120 °C for 1 hour with a starting material ratio of 1:1 (bottom).

It was assumed earlier in this chapter that the coupling between lignin and PFA would occur on the α carbon of the β -O-4 aryl ether bond, however, detailed analysis of the NMR spectrum of the lignin-PFA products did not confirm this hypothesis. A successful coupling between the furan ring and the aryl ether linkage of lignin would have resulted in a downfield shift of the signal corresponding to the C_{α} -H $_{\alpha}$ of said linkage (Figure 4-25). However, no such shift was observed indicating that this part of the lignin polymer was not involved in a coupling reaction of lignin and FA.

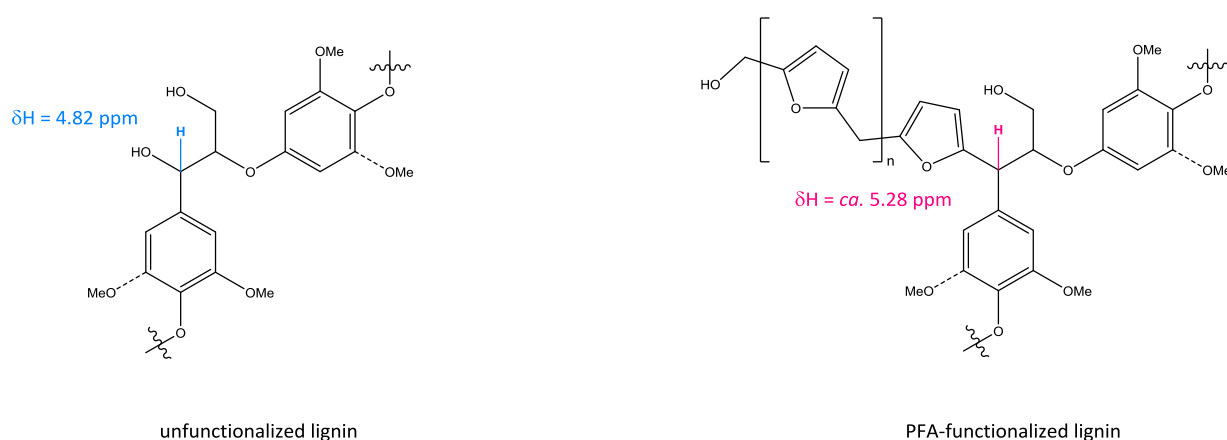


Figure 4-25. Observed shift of proton on the α carbon of the β -O-4 linkage of lignin (left) and expected shift of the same proton if coupling of PFA to lignin occurred on the aryl ether linkage.

Further analysis of the aromatic region of the spectra revealed a decrease in signal intensity of the uncondensed G_2 and G_6 C-H signals of the lignin aromatic subunits for the products isolated after functionalization with FA. Interestingly, the signal intensity of the uncondensed lignin aromatic subunits decreased further with an increase of the ratio of lignin:FA used in the modification. Obviously, increasing the concentration of FA in the reaction mixture will lead to the synthesis of proportionally more PFA (compared to lignin) and thus weaker lignin signals compared to the ones for the PFA. However, this does not explain the selective decrease in signal intensity for the uncondensed aromatic subunits, whereas no such decrease was observed for the condensed ones. It is therefore proposed that the lignin aromatic subunits participate as reactive sites in the grafting of FA/PFA onto lignin. The 2 and 6 position on the guaiacyl aromatic subunit are known to have increased electronic density due to the resonance effect of the electron pairs on the methoxy group at the 5 position (Figure 4-26) and the induction effect of the alkyl group on the 1 position.⁵⁷

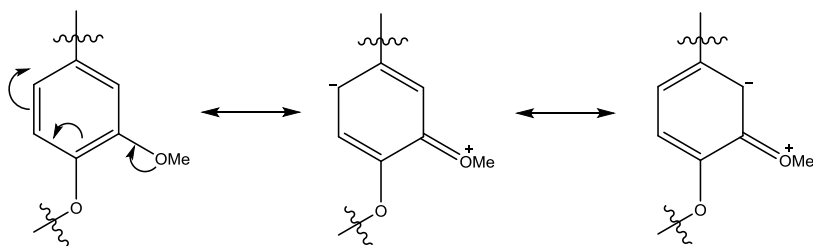


Figure 4-26. Resonance structures of the guaiacyl lignin aromatic subunit induced by the electron pairs on the methoxy group at the 5 position.

These findings suggest that the furanic carbocation reacts with the aromatic rings of lignin to create the copolymer. However, it is very important to note that the shift of the C₅-H₅ signal for the condensed guaiacyl unit overlaps with the signal of the C₄-H₄ of the furan ring in PFA. Unfortunately, this means that accurate quantification of the ratio of condensed aromatic subunits to uncondensed ones is not possible. A possible reaction mechanism for the grafting of FA onto lignin is shown in figure 4-27.

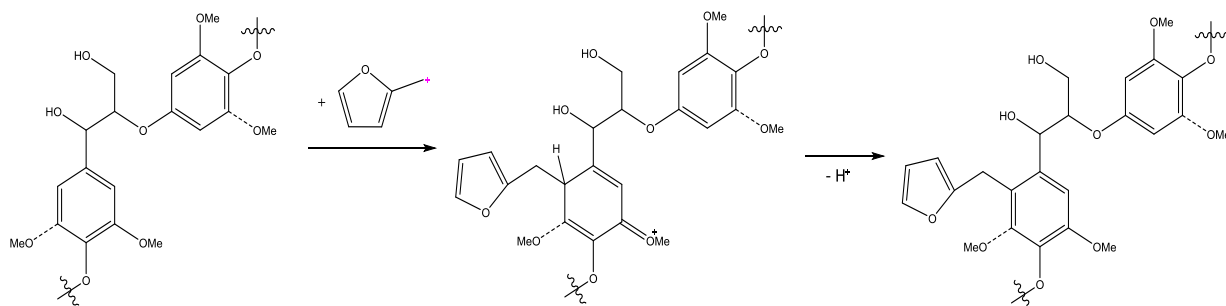


Figure 4-27. Schematic representation of grafting of FA onto lignin *via* electrophilic attack of the furanic carbocation on the aromatic subunit of lignin.

The lignin isolated at 120 °C was found to yield lignin/PFA copolymers with the highest molecular weight which suggests that this type of lignin has the most reactive sites available for functionalization with PFA. The reason for this can be found in the structure of the lignin isolated under mild pretreatment conditions. As discussed above, this lignin is characterized by the lowest S/G ratio as well as the lowest degree of condensation of the aromatic subunits.

A similar mechanism of the reaction of furfuryl alcohol with lignin model compounds was proposed by L. Nordstierna *et al.*⁵⁸ where the authors investigated the reactivity of creosol, 4-methyl syringol

and a non-phenolic arylglycerol- β -ether towards furfuryl alcohol. The authors reported clear spectroscopic evidence for the formation of a covalent bond between the lignin aromatic ring and the methylene bridge of furfuryl alcohol.

However, the lignin polymer used here for functionalization displays a much more complex structure compared to the model compounds used in the above mentioned study which most likely also influences the reactivity of the polymeric system. This calls for the need of more spectroscopic evidence to prove definitely the formation of a covalent bond between FA/PFA and lignin during the modification of lignin in ionic liquids.

Hence, Diffusion Ordered Spectroscopy (DOSY) was used to confirm the formation of lignin/PFA conjugates. The concept behind DOSY measurements is the following: molecules of different molecular weight will diffuse at different speed and thus have different diffusion coefficients in solution when a gradient of the pulse field strength during an NMR measurement is applied. The product recovered from polymerisation of FA was found to have a lower molecular weight than the product recovered after reaction of FA and lignin in acidic ionic liquids. This means that lignin/PFA conjugates which have a higher molecular weight than the homopolymers (either PFA or lignin) will diffuse slower.

Figure 4-28 displays the DOSY spectrum of the product recovered after the reaction of lignin isolated at 120 °C and FA with a ratio of the starting materials of 1:2 ($M_w = ca. 19000 \text{ g mol}^{-1}$) which clearly shows signals for both PFA and lignin. In fact, two species that both show signals for PFA and lignin with similar diffusion coefficients ($D_1 = 6.70 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ and $D_2 = 7.24 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$) were present in the isolated product. This finding can be interpreted in two ways. Either lignin and FA did not form a copolymer, but FA still polymerized to PFA with a very similar molecular weight of the two polymers (and thus the same diffusion coefficient) or the copolymer was successfully formed and the two different diffusion coefficients correspond to polymeric species with different molecular weights. Taking the molecular weight data discussed above into account, the second option seems to be the more likely one. In addition to the signals observed for the lignin-PFA copolymer, a third species with signals of only PFA and a diffusion coefficient of $D_3 = 9.16 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ was observed which showed that not all the FA present in the reaction mixture reacted with the lignin but rather also formed a homopolymer.

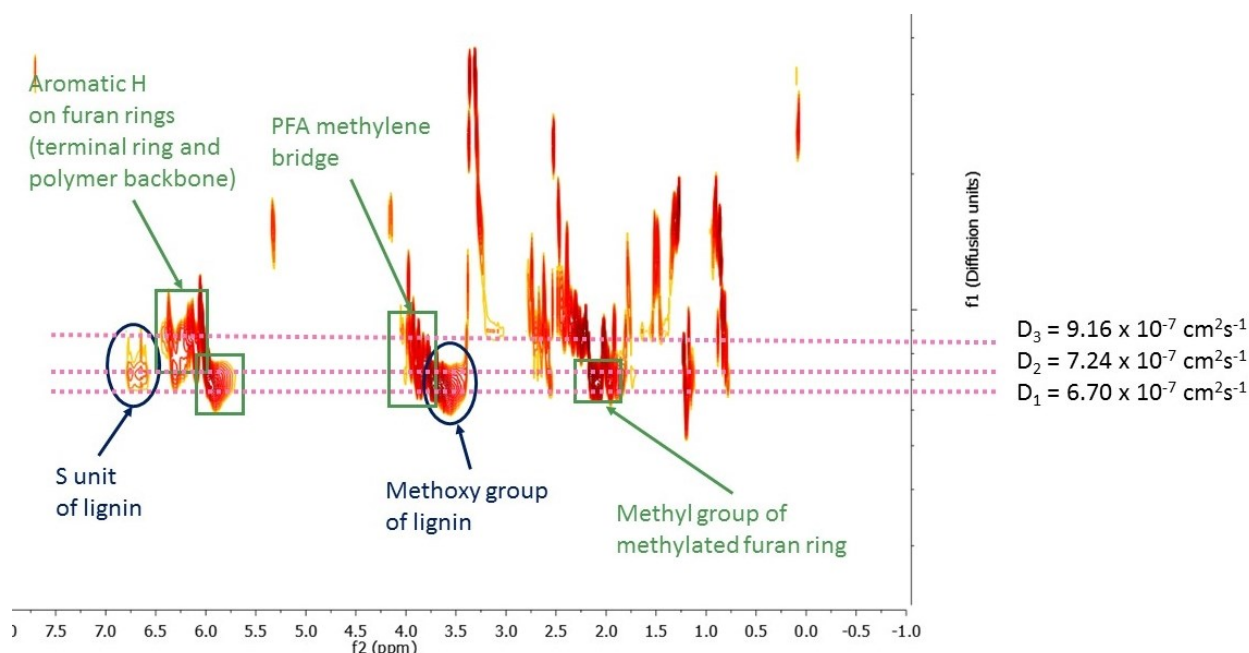


Figure 4-28. DOSY-NMR spectrum of product recovered from lignin/PFA coupling reaction with a ratio of the starting materials of 1:2 carried out in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ at 120 °C for 1 h.

To confirm fully the formation of a copolymer between lignin and PFA another DOSY experiment was conducted. For this experiment a mixture of lignin subjected to the polymerization conditions and previously synthesised PFA in a 1:1 wt/wt ratio was dissolved in the NMR solvent and the spectrum was recorded under the same conditions as the one above. Interestingly, the second recorded DOSY spectrum did not show two individual diffusion coefficients for lignin and PFA (Figure 4-29, bottom) but one diffusion coefficient which was calculated to be $D_4 = 8.31 \times 10^{-7} \text{ cm}^2\text{s}^{-1}$.

This suggests that the molecular weight of the polymers is very similar, rendering them indistinguishable with regard to the measured diffusion coefficient. However, the diffusion coefficient of the lignin-PFA mixture was found to be higher than the ones calculated for the product recovered from the lignin functionalization reaction. Both spectra were measured under the same experimental conditions and should have the same viscosity (the diffusion coefficient for the solvent DMSO was found to be the same for both spectra) which implies that the lower diffusion coefficient observed for the lignin-PFA copolymer in the first DOSY spectrum does indeed have a higher molecular weight as the individual polymers analysed in the second DOSY experiment.

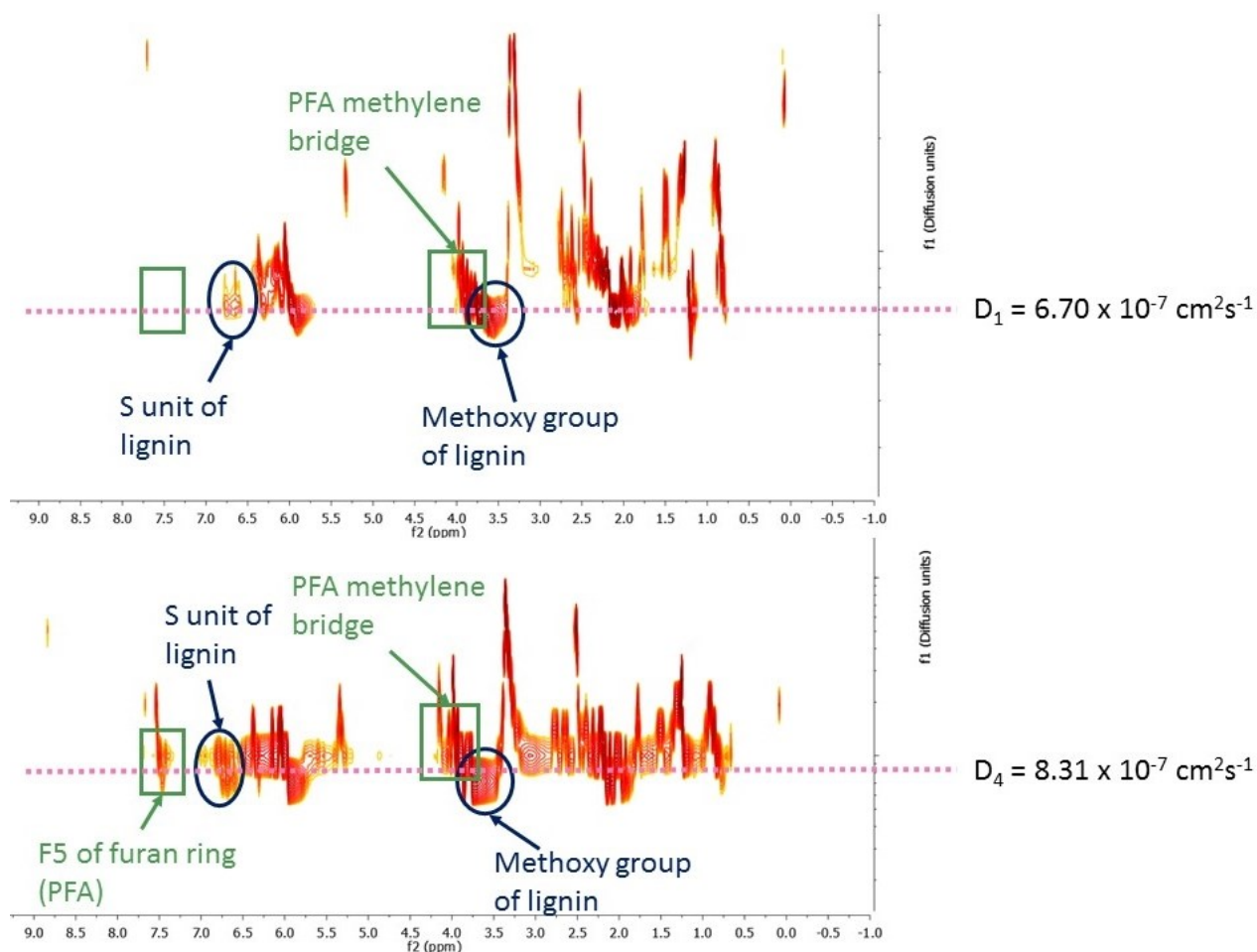


Figure 4-29. Comparison of DOSY-NMR spectra of product from lignin/PFA copolymer (lignin/FA ratio = 1:2) (top) and mixture of PFA and unmodified lignin subjected to the conditions of the FA polymerization reaction (bottom).

Additional DOSY spectra for products of the lignin modification with FA using lignin isolated at different pretreatment conditions and different ratios of the starting materials were also recorded (see appendix for spectra). Even though the diffusion coefficient for the copolymers were found to be different for each isolated polymeric product they were always observed to be higher than $D_4 = 8.31 \times 10^{-7} \text{ cm}^2\text{s}^{-1}$ for the polymer mixture proving that the functionalization of lignin with FA did indeed result in the formation of a covalently bound copolymer.

4.2.4 *In-Situ* modification of lignin with FA during IL pretreatment

The successful synthesis of lignin-PFA copolymers in an acidic ionic liquid solution has led to the idea of eliminating the lignin extraction step via ionic liquid pretreatment and directly modify the lignin during isolation with ionic liquids. To realize this, FA was dissolved in the IL solution $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ and this mixture was used for pretreatment of willow Endurance for 1 hour at 120 °C and 150 °C. It was found that the addition of FA to the ionic liquid during pretreatment led to an increase of the weight of the recovered lignin fraction from 5.46 wt% to 13.85 wt% for pretreatment at 120 °C and 14.12 wt% to 17.90 wt% for pretreatment at 150 °C, respectively (Figure 4-30). However, the isolated product could just be a mixture of PFA and lignin since it is now known that FA polymerizes in acidic ionic liquid solutions showing the need for further characterisation of the recovered polymer.

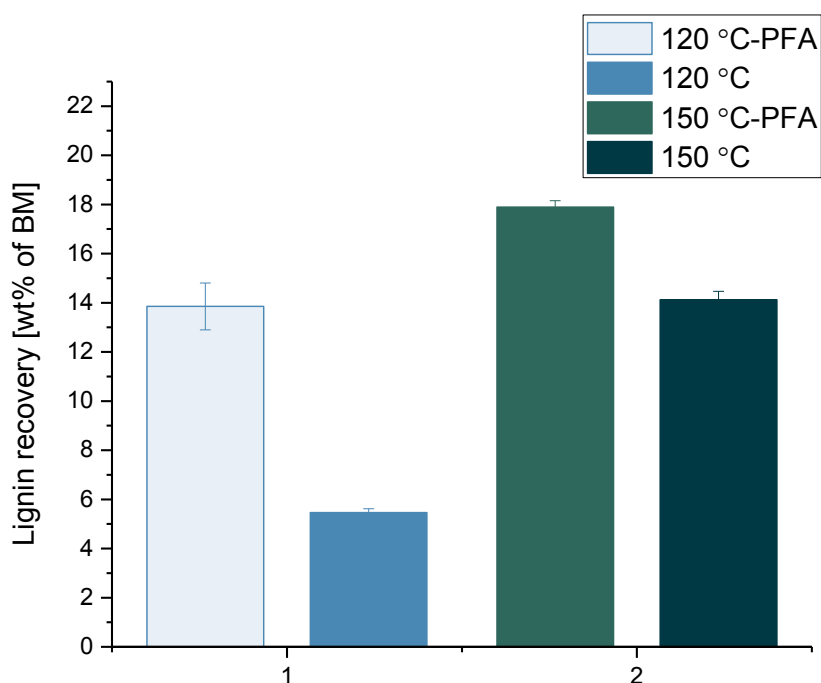


Figure 4-30. Lignin recovery of *in situ* functionalization of lignin with FA/PFA during pretreatment of willow Endurance with $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ for 1 hour at 120 °C and 150 °C.

GPC analysis was used to measure the molecular weight of the products recovered from *in-situ* modification of lignin with FA and the data was compared to the molecular weight data from lignin isolated at the same pretreatment conditions (Figure 4-31). Interestingly, it was found that the applied pretreatment conditions did affect the chain length of the product. Using mild pretreatment conditions (e.g. 120 °C and 1 hour) resulted in an increase of the weight average molecular weight

from *ca.* 4000 gmol^{-1} to *ca.* 5000 gmol^{-1} and a slight increase of the number average molecular weight indicating that the formation of a lignin-PFA copolymer was successful. However, the molecular weight of the product recovered here is a lot lower compared to the lignin-PFA copolymer synthesized using already extracted lignin (see 3.3.3.2). This clearly shows that even though the *in-situ* modification seems possible this route might not be the synthesis technique of choice due to the low molecular weight of the resulting polymers. However, the desired properties of the polymeric product such as molecular weight do depend on the final application of the material and a shorter chain length might not be an issue if further crosslinking to produce a resin can be achieved.

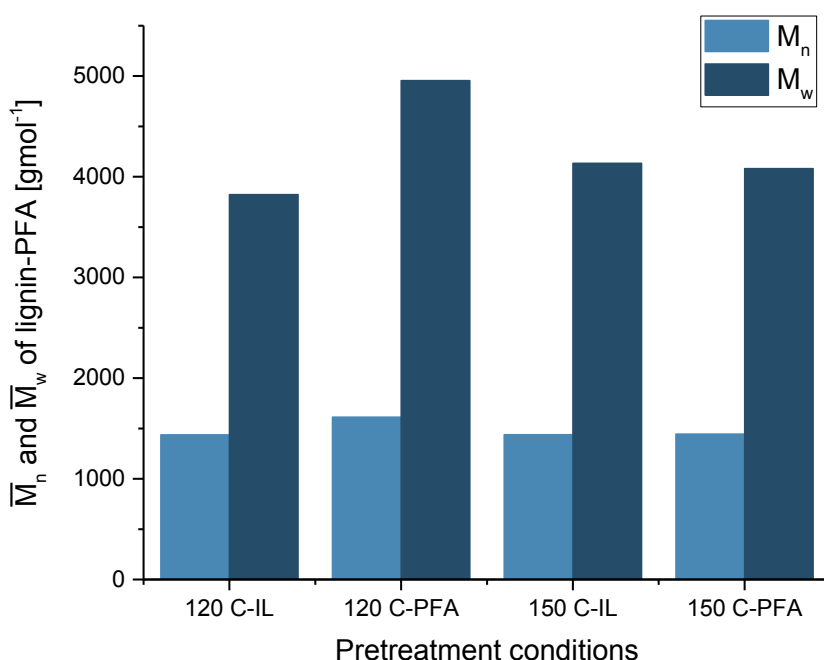


Figure 4-31. Number average molecular weight ($\bar{M}_n$) and weight average molecular weight ($\bar{M}_w$) of product recovered after *in-situ* modification of lignin with FA.

Surprisingly, no difference in molecular weight was observed when 150 °C were applied for pretreatment of lignin and the *in-situ* modification. This might be caused by the harsh reaction conditions that led to ring opening of the furfuryl alcohol and formation of levulinic acid which does not polymerize under these conditions or by degradation of the formed lignin/PFA product.

The extraction of lignin from biomass is a very complex process with many reactions occurring in parallel such as cleavage of the lignin-carbohydrate complexes and lignin aryl ether linkages as well as lignin condensation. Since the FA is dissolved in the ionic liquid and most likely won't penetrate the

wood matrix the reaction of FA with lignin can only take place after the lignin has been extracted from the secondary cell wall and the reaction is most likely hindered by mass transfer and diffusion in the very viscous solid-liquid reaction mixture resulting in the formation of low molecular weight products.

The HSQC NMR spectra recorded of both the products recovered after *in-situ* functionalization of lignin showed the typical peaks of the lignin polymer and additional signals that correspond to PFA showing that the polymerization of FA did occur during the ionic liquid pretreatment (Figure A-10 and A-11 in the appendix). However, the signal intensities for PFA were found to be a lot stronger for the product isolate after pretreatment at 120 °C compared to pretreatment at 150 °C. Taking the molecular weight data into account it can be assumed that coupling between FA and lignin did occur for the milder pretreatment at 120 °C and for a lesser extent for the pretreatment at 150 °C.

The *in-situ* functionalization of lignin with FA not only modifies the lignin fraction recovered from pretreatment but might also impact the pulp composition and yield of enzymatic hydrolysis. Given that the pulp and bioethanol production is and probably will still be the most important revenue of a biorefinery it is important to investigate how the addition of FA to the ionic liquid pretreatment affects those pulp properties. It can be clearly seen that the presence of FA in the ionic liquid solution during pretreatment affects the composition of the recovered pulp (Figure 4-32). Surprisingly, it was found that the glucan content was lower for pretreatment with addition of FA compared to regular IL pretreatment. Pretreatment at 120 °C resulted in a pulp with a glucan content of 35.14 % (compared to 39.68 %) and an increase in temperature to 150 °C also led to a further increase in glucan degradation with a resulting glucan content of 29.78 % (compared to 36.82 %). The ideal pretreatment should preserve as much glucan in the pulp as possible in order to maximise the yield of enzymatic hydrolysis and the subsequent yield of bioethanol. The addition of FA in the ionic liquid solution however has the opposite effect which introduces doubt about this alteration of the ionic liquid pretreatment. However, a lower glucan content in the pulp does not always correlate with an overall lower glucose yield because the accessibility of the glucan by enzymes is not only determined by the amount of glucan present in the pulp.

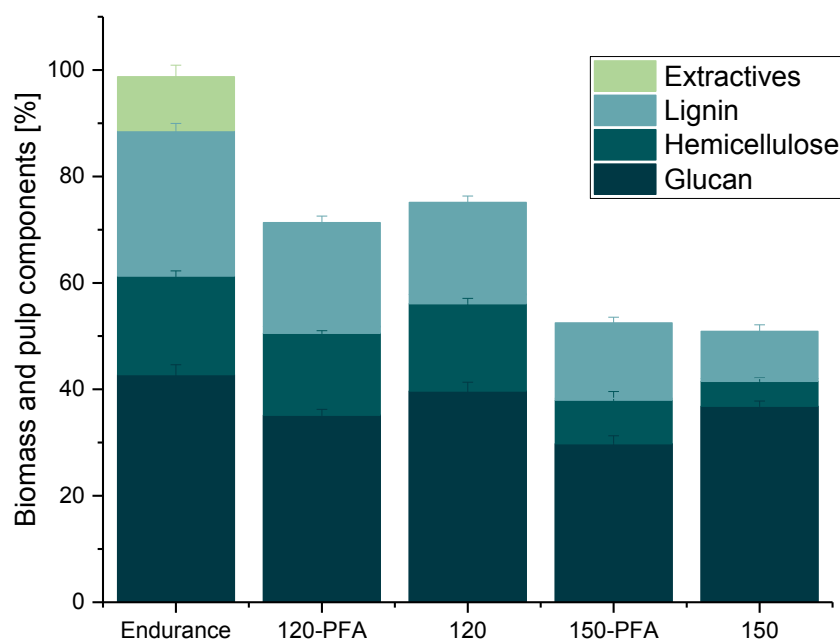


Figure 4-32. Biomass and pulp components as measured *via* compositional analysis of untreated willow Endurance, pulp pretreated using the ionicSolv pretreatment at 120 °C and 150 °C for 1 hour and pulp pretreated under the same conditions with addition of FA to the ionic liquid solution.

The amount of lignin in the pulp was found to be one of the factors influencing the pulp digestibility.⁵⁹ The *in-situ* modification of lignin with FA unfortunately resulted in an increase of lignin-like material in the pulp compared to pulps recovered after regular IL pretreatment from 19.04 % to 20.76 % and 9.38 % to 14.52 % for pretreatment at 120 °C and 150 °C, respectively. This increase can be explained by precipitation of PFA on the pulp during the pulp washing step with ethanol. Compositional analysis is a useful technique to investigate the changes in composition of biomass occurring during pretreatment, however, the lignin content is measured only gravimetrically and thus it is not possible in this case to distinguish between lignin present in the pulp and lignin/pseudolignin or PFA which re-deposited back on the pulp.

The increased amount of lignin-like material on the pulp did indeed influence the pulp digestibility as can be seen in figure 4-33. Interestingly, the addition of FA to the ionic liquid solution did not affect the glucose yield of enzymatic hydrolysis when mild pretreatment conditions (120 °C, 1 h) were used showing that the pulp digestibility was not affected by the *in-situ* modification of lignin.

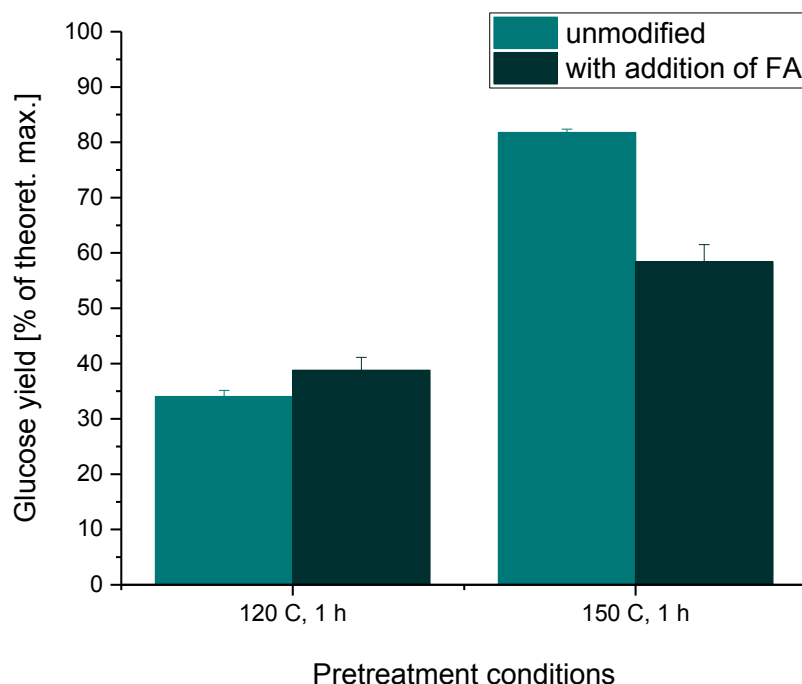


Figure 4-33. Glucose yields of pulp isolated after regular ionic liquid pretreatment and pretreatment with addition of FA.

Applying harsher pretreatment conditions led to a significant decrease in glucose release from the pulp from 81.7 % for regularly pretreated biomass to 58.4 % for the pulp recovered after *in-situ* modification with FA. This does highlight that the amount of lignin-like material on the pulp after pretreatment does indeed impact the pulp digestibility. Additionally, the data presented here suggests that polyfurfuryl alcohol redeposited on the pulp does hinder enzymatic saccharification to a greater extent than redeposited lignin does.

4.2.5 Thermal stability of the lignin-PFA copolymers

The application of resins in areas where high temperatures are applied (e.g. as moulds for metal casting in the foundry industry) renders testing the thermal stability of the material necessary. The TGA profiles of neat PFA, lignin isolated at 120 °C, 150 °C and 170 °C using the ionoSolv pretreatment and lignin-PFA copolymers synthesised with the different lignins and different lignin:FA ratios are shown in figure 4-34 and 4-35. DSC profiles were measured simultaneously to verify that the sample

mass loss corresponds to an exothermic event (e.g. degradation of the material). Figure A-12 to A-14 in the appendix for DSC profiles.

It can be seen clearly that the PFA polymer has the highest thermal stability amongst all the tested polymers and two decomposition steps are observed. The first step at 180 °C can be assigned to the evaporation of volatiles present in the PFA, which is followed by degradation of the methylene bridges around 315 °C and finally decomposition of the furan ring at 550 °C.⁶⁰ The extracted lignin also displays a two-step degradation pattern, however, thermal stability was found to increase with increasing pretreatment temperature applied for lignin isolation. This is due to the change of structure of the polymer such as cleavage of aryl ether bonds and condensation during acidic ionic liquid extraction. The first decomposition step around 250 °C is correlated to the degradation of the interunit linkages in lignin and the second one at 400 °C to the degradation of the aromatic subunits.

Interestingly, the thermal stability of all the copolymers was found to be enhanced compared to the corresponding extracted lignin as also observed in studies published in the literature.^{41, 43} However, the thermal behaviour of these new polymers was influenced by its ratio of lignin and PFA.

Using a ratio of the starting materials of 1:2 resulted in a copolymer with slightly increased thermal stability where the degradation of the interunit linkages starts at 270 °C (120 °C-lignin), 300 °C (150 °C-lignin) and 305 °C (170 °C-lignin) suggesting that the PFA forms a protective layer around the lignin in parts of the copolymer. However, the decomposition of the aromatic rings is largely unaffected and occurs around 400 °C. This shows that the thermal behaviour is largely influenced by the lignin part of the copolymer, indicating that the majority of the polymer matrix consists of lignin.

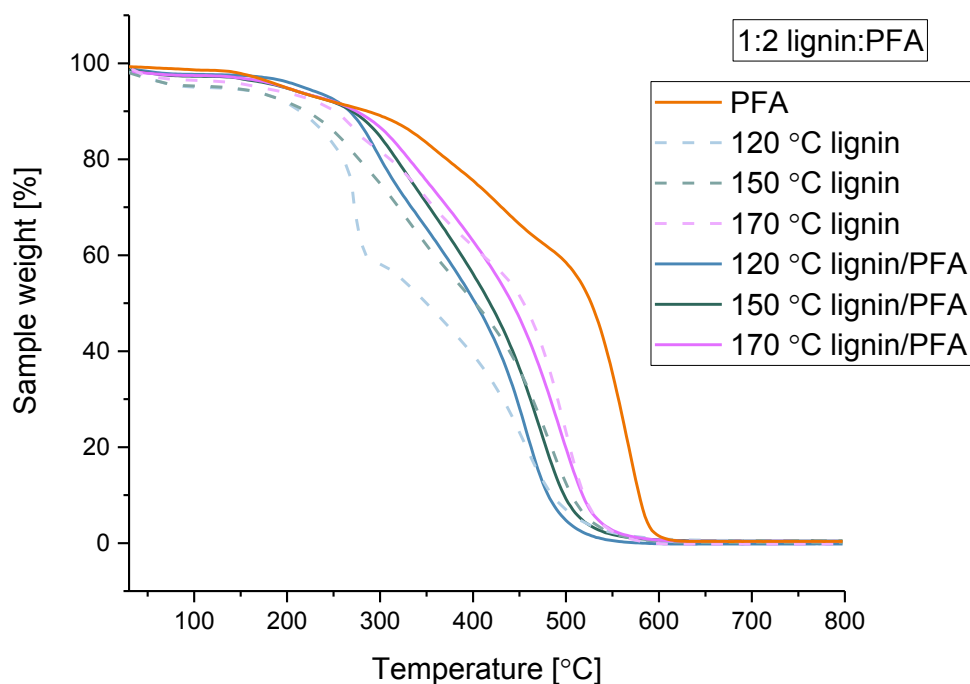


Figure 4-34. TGA profile of PFA, lignin extracted at different temperatures using the ionoSolv pretreatment and lignin-PFA copolymers synthesized with $[\text{HC}_{4}\text{im}][\text{HSO}_4]_{80\%}$ using a ratio of 1:2 of the starting materials.

When a ratio of lignin and FA of 1:4 was used, the thermal stability of the copolymers was observed to increase significantly and the degradation pattern completely resembled that of the neat PFA. This indicates that these copolymers predominantly consist of PFA with lignin being a minor part of the polymer matrix.

The synthesized lignin-PFA copolymers do compare well in terms of thermal stability with similar copolymers published in literature.^{41,43} However, given that the temperatures of molten aluminium and molten iron (the main metals used in the foundry industry) are 660 °C and above 1200 °C, respectively, it can be said that the materials presented here will not find application in that specific field.

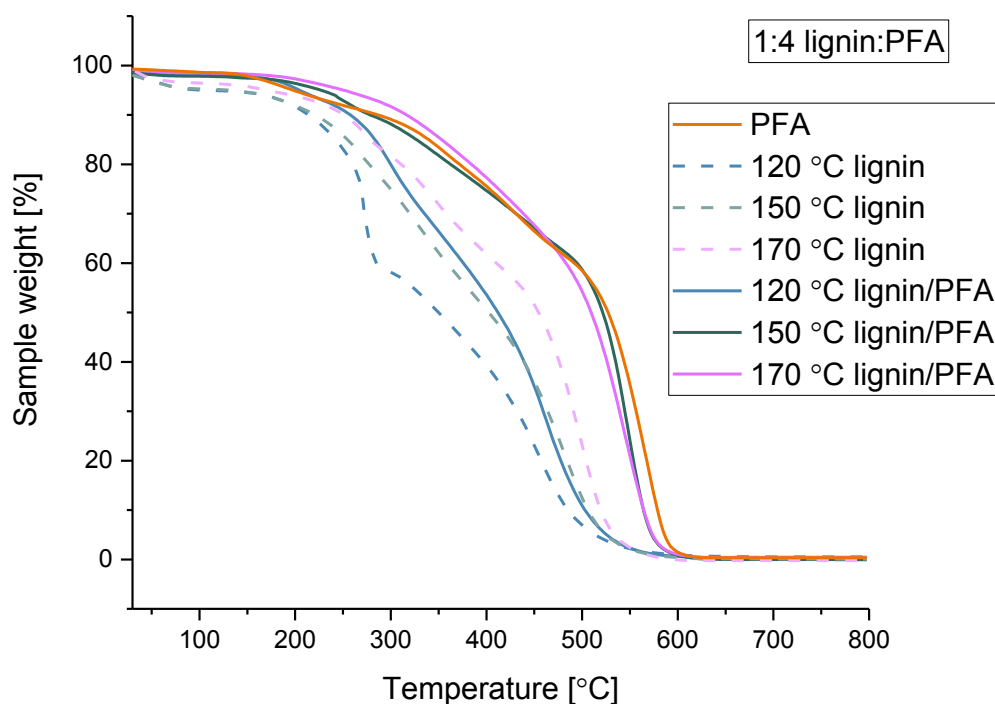


Figure 4-35. TGA profile of PFA, lignin extracted at different temperatures using the ionoSolv pretreatment and lignin-PFA copolymers synthesized with $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ using a ratio of 1:4 of the starting materials.

4.2.6 Elemental analysis

Another promising application for the lignin-PFA copolymers lies in the area of carbon materials such as glassy carbon and carbon fibres. The manufacture of those materials from pure lignin is very challenging due to the short chain length and brittleness of lignin.^{61, 62} The lignin-PFA copolymers show high molecular weight as well as high carbon content (Table 4-9). It was found that PFA has a high carbon content of around 69 %. Grafting of PFA onto lignin resulted in an increase of carbon content of the copolymers depending on the amount of the PFA present in the copolymer. Interestingly, the structure of the lignin also plays a role in the amount of carbon present in the lignin-PFA and harsher pretreatment conditions (e.g. less interunit linkages) resulted in a polymer matrix with comparable or even higher carbon content than PFA.

Table 4-9. Carbon, hydrogen and oxygen content of PFA and different lignin-PFA copolymers as determined *via* elemental analysis.

Entry	Temperature [°C]	Lignin:PFA	Lignin	C [%]	H [%]	O [%]
1	80	1	-	69.25	5.32	24.76
2	120	1	-	68.36	5.37	23.35
3	120	1:1	120 °C	65.48	6.19	25.46
4	120	1:2	120 °C	65.04	5.98	24.81
5	120	1:4	120 °C	68.19	5.68	23.60
6	120	1:4	150 °C	68.46	5.54	23.80
7	120	1:4	170 °C	70.41	5.39	23.33

Carbon fibers in industry are made from polymers such as polyacrylonitrile which has a carbon content of 68 %⁶³ showing that the here synthesized copolymers are very promising candidates for these applications. To the best of my knowledge, no one has studied these novel lignin-PFA copolymers for the production of carbon fibres. However, projects to utilize this material for the purpose of carbon fibre productions are undergoing in the Hallett and Talbot-Brandt labs.

4.3 Summary and future work

This chapter discussed the use of lignin as a resource for the production of a value-added material *via* the formation of a lignin-polyfurfuryl alcohol copolymer in an acidic ionic liquid solvent/catalyst system.

For this purpose, the synthesis of PFA in this solvent/catalyst system was studied first and it was found to be successful in both tested IL solutions [N₂₂₂₀][HSO₄]_{80%} and [HC₄im][HSO₄]_{80%}. However, several issues were faced in the form of IL impurities and the formation of levulinic acid observed when [N₂₂₂₀][HSO₄]_{80%} was used. These were then avoided by changing the IL to [HC₄im][HSO₄]_{80%} which has a lower acid strength. Structural investigations showed that the synthesised PFA polymer is branched on the methylene bridge between two furan rings and that formation of chromophores occurred during the polymerization. The molecular weight of the here synthesized polymer was found to be comparable to PFA reported in literature showing that this novel synthesis route is promising in terms of both product properties and environmental aspects.

Polymerization of FA in the presence of lignin was undertaken in the acidic ionic liquid solution [HC₄im][HSO₄]_{80%} and products with high molecular weight (up to 28000 gmol⁻¹) were recovered

suggesting successful synthesis of a lignin/PFA copolymer. The molecular weight of the products was found to be determined by the molecular structure of the lignin used for modification as well as the reaction time and ratio of lignin:FA in the reaction mixture. Polymers with the highest molecular weights were isolated when lignin extracted at 120 °C was used (compared to lignin extracted at 150 °C and 170 °C) suggesting that this type of lignin bears more reactive sites for functionalization with FA. The formation of a covalent bond between PFA and lignin was confirmed by various NMR experiments and the lignin aromatic subunits were identified to be the reactive sites in the modification reaction of lignin.

In-situ modification of lignin during IL pretreatment at 120 °C and 150 °C was investigated and was found to result in the formation of lignin-PFA copolymers. However, these copolymers did have a much lower molecular weight compared to the ones synthesised using previously extracted lignin. The addition of FA to the ionic liquid solution did result in a higher amount of lignin-like material on the recovered pulp most likely due to deposition of PFA on the pulp. This did negatively affect the glucose release for the pulp isolated after pretreatment at 150 °C showing that this approach should not be preferred to the synthesis of lignin-PFA using extracted lignin since the glucose yield and subsequent production of bioethanol is the biggest revenue of a biorefinery.

The thermal behaviour of lignin, PFA and lignin-PFA copolymers was investigated and it was found that the PFA has the highest thermal stability followed by the copolymers and lastly by lignin. The ratio of lignin:FA in the reaction mixture does influence the thermal properties of the resulting copolymers with a higher amount of FA leading to a copolymer that shows the same thermal stability as pure PFA. The high carbon content of the PFA and lignin-PFA copolymers makes these materials promising candidates to be used in applications such as carbon fibres and glassy carbon.

As for future work, the synthesized lignin-PFA copolymers should be tested for their tensile strength, flexural properties and Young's modulus in order to understand the suitability of these polymers as adhesive resins. Additionally, the formation of carbon materials such as glassy carbon and carbon fibres should be investigated.

This data discussed in this chapter has shown that the functionalization of lignin in acidic ionic liquids is successful and leads to the formation of promising materials. The uniqueness of the reaction system which induces structural changes to the lignin polymer gives rise to other possible lignin modification reactions which will be discussed in the following chapter.

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Chapter 5: Functionalization of lignin for the synthesis of electrically conducting polymers

5.1 Introduction

The electrical properties (i.e. electrical conductivity) of a material are determined by its electronic structure. The band theory has been developed to describe the different electrical behaviours of insulators, semiconductors and conductors. A schematic representation of the band theory is given in Figure 5-1.¹

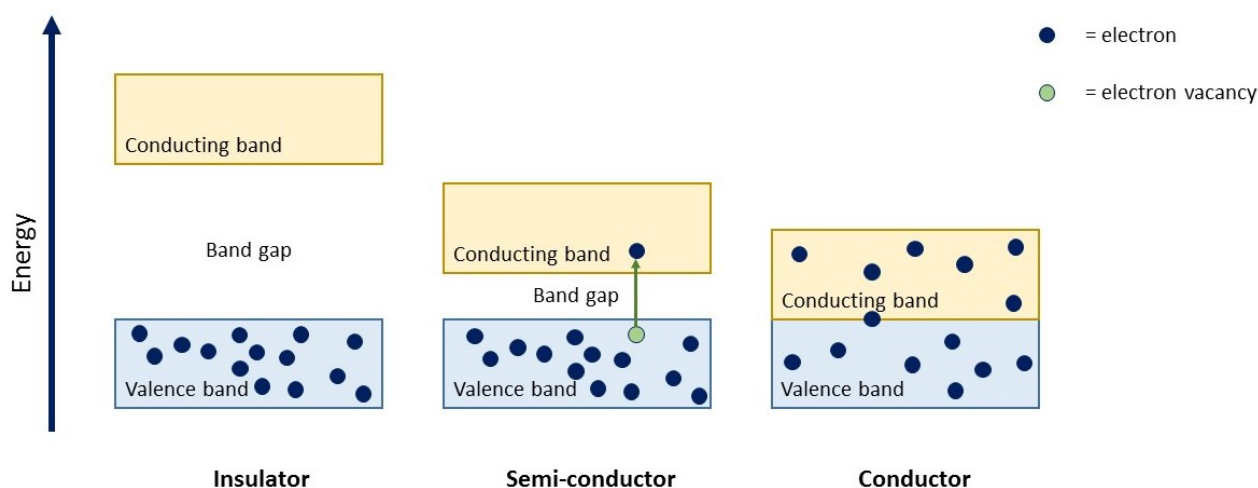


Figure 5-1. Model of band structure for an insulator, semi-conductor and conductor.¹

The energy spacing between the highest occupied energy level (e.g. valence band) and the lowest unoccupied energy level (e.g. conduction band) is defined as the band gap. The band gap in metals is non-existing which corresponds to those materials having high electron mobility, i.e. electrical conductivity. Semi-conducting materials have a narrow band gap of around 2.5 – 1.5 eV, which allows conductivity to occur only *via* excitation of electrons from the valence band to the conduction band. This can be achieved for example by heating the material. Electron excitation becomes difficult if the band gap is larger than 3 eV, meaning that the electrons are unable to cross the band gap and the material is known as an insulator.¹ Electrical conductivity ranges from 10^{-18} to 10^{-8} Scm^{-1} for insulators to 10^{-8} to 10^3 Scm^{-1} for semiconductors and 10^3 to 10^8 Scm^{-1} for conducting materials.²

Typical examples for conducting materials are metals such as copper or silver³ but in the last decades the development of electrically conducting organic polymers has been successful. These conductive polymers are all categorised by the presence of an extended system of conjugated $-C=C-$ carbon-carbon bonds in the backbone of the polymer structure. Electrically conducting organic materials can be divided into three main groups which are polyenes, poly- heterocycles, and polyaminoaromatic compounds. Well known examples of conductive polymers are polyphenylene, polyacetylene, polythiophene or polypyrrole (Figure 5-2).¹

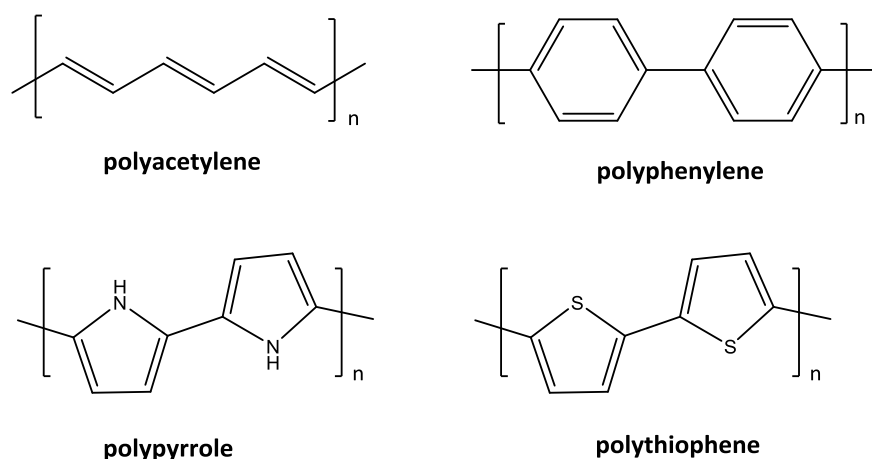


Figure 5-2. Chemical structures of electrically conducting polymers.

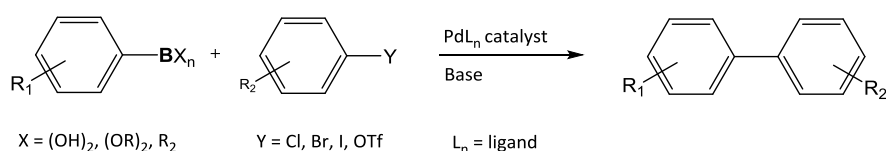
The above discussed concept of band gaps does not sufficiently explain the behaviour of electrically conducting organic polymers. A new theory involving the presence of polarons (radical cation that is partially delocalized over several monomer units) and dipolarons (diradical dication) in the organic materials has been developed.⁴ Both species are introduced to the polymer matrix *via* doping and are able to move along the polymer chain. Doping is carried out to transform insulating polymers into conducting ones by the formation of charge-transfer complexes either by electron donors such as sodium or potassium (so-called n-doping or reduction) or by electron acceptors such as I_2 , AsF_5 , or $FeCl_3$ (so-called p-doping or oxidation).¹ This results in the polymer backbone becoming positively or negatively charged and the dopant forms the opposite charge.⁴

After doping, the electrical conductivity of polymers lies in the range of the one of metals. Besides being conductive some of those polymers also display other interesting properties such as magnetic, wetting, optical, mechanical and microwave-absorbing properties.⁵ This allows for a wide range of applications for example as chemical and bio-sensors^{6, 7}, field-effect transistors⁸, field emission and

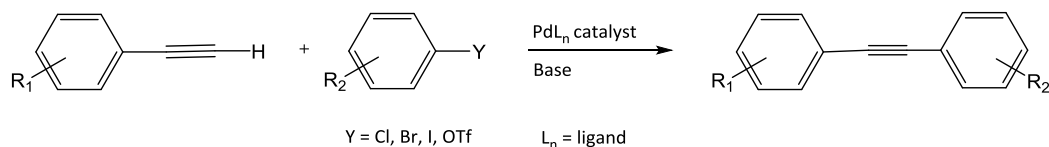
electrochromic display devices⁸, supercapacitors⁹, actuators and separation membranes⁹, light emitting diodes⁷, etc.

Conducting polymers can be synthesized either *via* chemical polymerization or *via* electrochemical polymerization.¹⁰ In this chapter, conducting polymers were synthesized using the first approach, thus only this one will be discussed here. Conducting polymers can be chemically synthesized using many different reaction systems. Modern synthesis routes typically involve palladium-catalysed cross-coupling reactions such as Suzuki–Miyaura, Sonogashira, Heck, and Stille reactions (Figure 5-3).¹¹

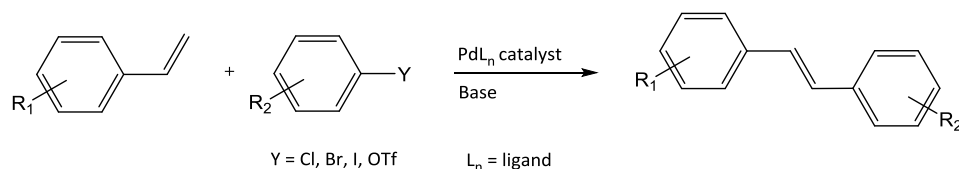
1) Suzuki-Miyaura coupling



2) Sonogashira coupling



3) Heck coupling



4) Stille coupling

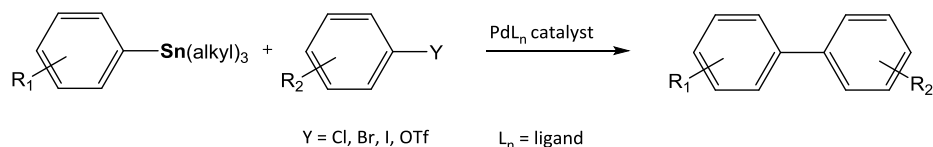
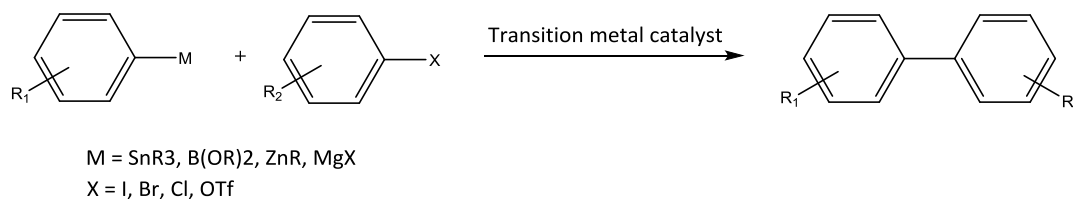


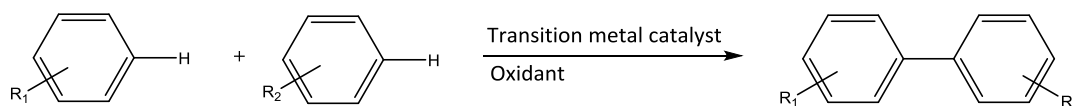
Figure 5-3. Example reaction schemes of the most popular palladium catalysed carbon-carbon coupling reactions.

Research in the area of aryl C-C bond formation has led to the development of alternative methods for the carbon coupling reaction (Figure 5-4). The coupling of two unsubstituted arenes *via* oxidative activation using a Pd-catalyst has been investigated. The drawback of this approach is that the C-H bonds are relatively inert and a directing group is required to activate them. The low reactivity of the reactants leads to a low selectivity of the coupling reaction.¹²

1) Typical cross coupling



2) Oxidative coupling



3) Direct (Hetero)Arylation

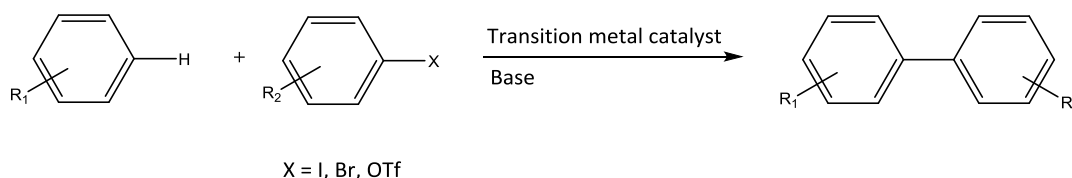


Figure 5-

4. Three different approaches of the formation of a new carbon-carbon bonds using transition metal catalysts.¹³

A combination of the traditional organometallic synthesis route and the oxidation protocol is the so-called direct (hetero)arylation. It is an atom-economical and environmentally benign synthesis route to create a new carbon-carbon bond. This approach involves the coupling of pre-functionalized halogenated (hetero)arenes with simple (hetero)arenes with an activated C-H bond.¹⁴ The lack of functionalization of the monomers with organometallic moieties decreases the overall production cost of conjugated polymers.¹⁵ In this reaction system a base is added to assist in the C-H bond activation and to neutralize the stoichiometric amount of acid formed during the coupling. This

method can be applied for various monomers¹⁴ and is particularly popular for the synthesis of polythiophenes due to the high yield achieved.¹³ However, optimization of the reaction conditions (e.g. solvent system, phosphine ligand, carboxylate additives, temperature and reaction time) is needed for efficient C-H bond reactivity of each monomer. Once optimized, the coupling reaction yields polymers with elevated molecular weights and properties that are comparable to polymers synthesized using the standard organometallic approaches. Another advantage of this method is the possibility to synthesize polymers that were previously inaccessible due to the instability of certain organometallic monomers.¹³

Even though the mechanism of the direct arylation polymerization (DAP) has been studied intensely both experimentally and computationally, the mechanistic pathway is still under discussion. Possible pathways include (i) electrophilic aromatic substitution, (ii) Heck-type coupling and (iii) concerted metalation-deprotonation (CMD).^{16, 17}

However, thiophenes are believed to follow the third mechanism. CMD can follow two different mechanistic cycles depending whether carboxylates are used as additives in the reaction system or not.¹³ In this chapter, carboxylates were utilized and thus only this mechanism will be discussed in detail. The catalytic cycle involves several steps starting with the oxidative addition of the carbon-halogen bond to the Pd(0) (Figure 5-5). This is followed by exchange of the halogen ligand for the carboxylate anion and complex **1** is formed. The carboxylate ligand then assists in the deprotonation of the thiophene substrate while a metal-carbon bond is simultaneously formed and the catalytic complex goes through transition state **TS-1**.¹⁷ The catalytic mechanism can then follow two different pathways. The first one involves the re-coordination of either the phosphine ligand or the solvent to the metal centre in the next step. The second pathway is characterized by the fact that the carboxylate group remains coordinated throughout the entire pathway.¹⁸ The final step of the catalytic cycle is then the reductive elimination of the aryl-coupled product and the regeneration of the Pd(0) species.¹³

One disadvantage of this reaction method has to be addressed, which is the low selectivity (especially for thiophene substrates) which can result in undesired cross-linking of the polymer. This is known to have a negative impact on the properties of the material since high conductivity is only ensured by linear polymers with high molecular weight. This issue is thought to be reduced by further optimization of the reaction conditions such as temperature and reaction time.¹³ Research focussed on this problem in recent years has led to significant improvement of the defect free synthesis of conjugated polymers *via* DAP.¹⁵

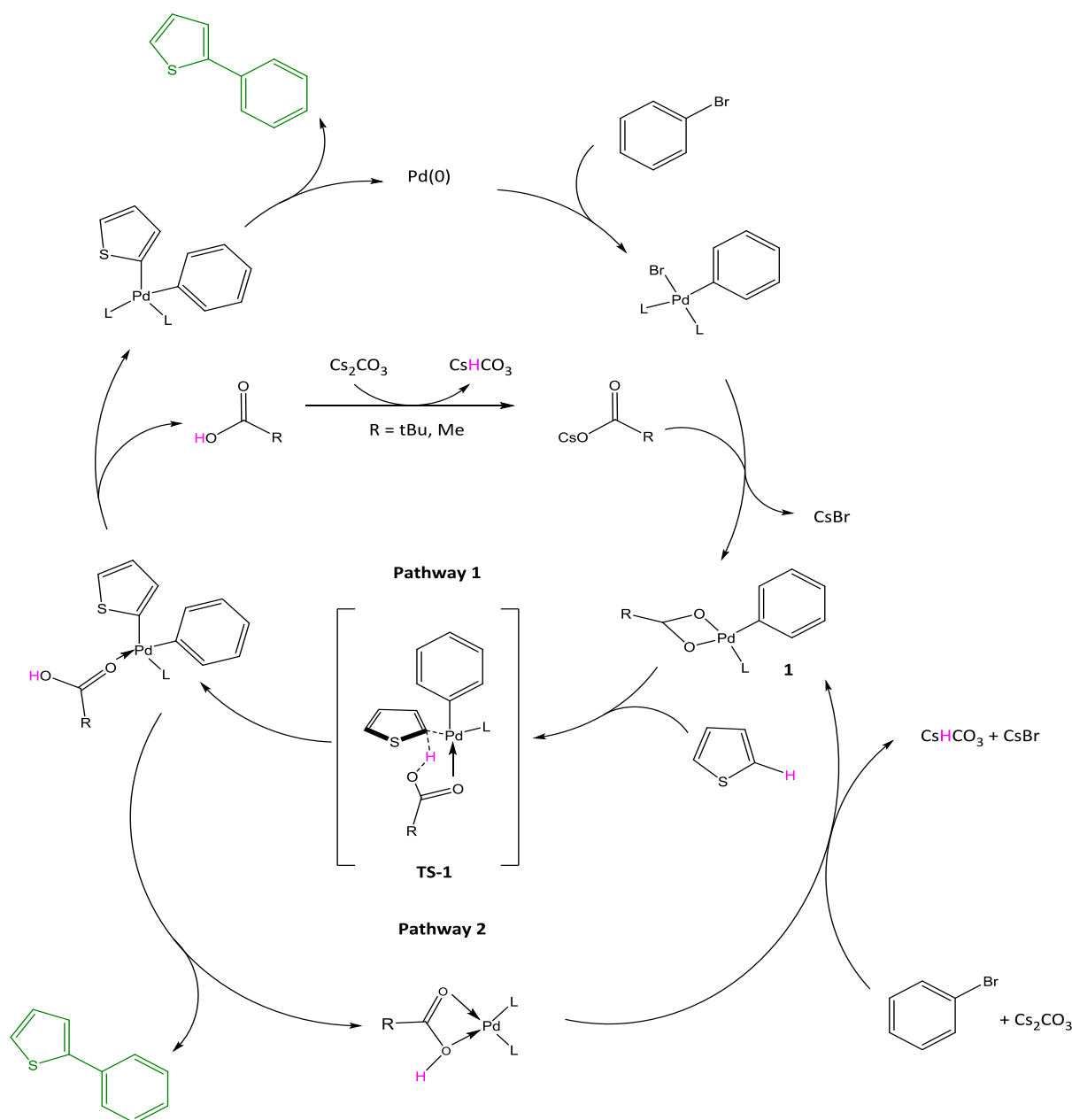


Figure 5-5. Catalytic cycle of the carboxylate assisted direct (hetero)arylation polymerization resulting in the formation of a new carbon-carbon bond.¹³

The conductive or semi-conductive properties of conjugated polymers arise from the π -conjugation of the polymer which allows for effective delocalization of the π -electrons along the polymer backbone (and one delocalized π -electron per carbon atom) resulting in charge transport along the polymer chain.¹⁹ The electronic and optical properties of the polymer can be easily tuned *via* doping, providing a transition of the material from insulator to semi-conductor to metal.^{20–22} Polythiophenes and derivatives are known for their good properties such as high stability of the undoped and doped

states, ease of structural modification and solution processability and are thus of great interest in device applications.^{23,24} The areas of application of polythiophenes are numerous and span from classical uses such as light emitting diodes²⁵, field-effect transistor^{26,27} and photovoltaics^{28,29} to more unconventional ones such as resistive memory devices^{30,31}, hydrogen storage³² and biosensors (e.g. DNA, RNA and negatively charged particles) for water purification³³.

Conductive polymers are usually synthesized from fossil resources but the general trend in chemistry research to search for bio-based alternatives has also arrived in the field of conductive polymers. The most promising approach to (partially or fully) substitute oil-based conductive polymers is to use lignin as a macromonomer due to the possibility to design its structure *via* the extraction process³⁴ and the low cost of the biopolymer³⁵.

The first study investigating the use of lignosulfonate as a polymeric template for the synthesis of a conductive molecular complex containing polyaniline (PANI) and lignosulfonate was published by S. Roy³⁶ *et al.* in 2002. The authors aimed to improve the water solubility of PANI in addition to introducing new properties to the PANI polymer such as processability, corrosion protection, and biodegradability. The PANI was successfully synthesized using a PEG-hematin catalyst in the presence of lignosulfonate to form a matrix containing both polymers. Interestingly, the product of this reaction showed a conductivity of 10^{-3} Scm^{-1} and thus falls in the category of semiconducting materials. This study proved that lignosulfonate can be effectively used as a matrix for conducting polymers. Lignosulfonate was also used as a template as well as a dopant to synthesize a molecular complex of the biopolymer with polypyrrole and a good conductivity of 2.7 Scm^{-1} was measured for the optimized system.³⁷ The development of a novel synthesis route to lignosulfonate PANI molecular complexes led to materials with a conductivity of up to 5.0 Scm^{-1} which is significantly higher than previously reported.³⁸

The success of using lignosulfonate as dopant in the synthesis of complexes with conducting polymers such as PANI and polypyrrole inspired the utilization of the biopolymer in the production of redox-active films of lignosulfonate and poly(3,4-ethylenedioxythiophene) (PEDOT).³⁹ PEDOT is a very interesting polymeric material known for its high conductivity, great stability, and high transparency in thin film.⁴⁰ However, pure PEDOT is insoluble in any solvent and is infusible. This means that the polymer cannot be effectively processed, which significantly limits its application.⁴¹ Thus, PEDOT is often mixed with other polymers, typically poly(styrene sulfonic acid) (PSS). The aim to find more sustainable alternatives for chemical compounds and polymers has inspired the use of lignosulfonate as a substitute for PSS in material applications. A composite material of PEDOT:lignosulfonate was tested as a sensor in the detection of uric acid, which is of importance for clinical diagnosis. It was

found that the synthesized lignosulfonate-PEDOT material showed a positive response at lower uric acid concentrations compared to the standard PEDOT material.³⁹

PEDOT is not only used as a material for sensor applications but also often as a p-type semiconducting material, especially in the form of composite materials (such as PEDOT:PSS). An article published in 2015 by Y. Li⁴² *et al.* discussed the substitution of PSS with lignosulfonate as a more sustainable alternative material for organic solar cells. The composite material was synthesized *via* a simple reaction where EDOT is first dispersed in a water/lignosulfonate mixture and then polymerized using an ammonium persulfate solution as oxidant. The proposed structure of the final product is shown in Figure 5-6.

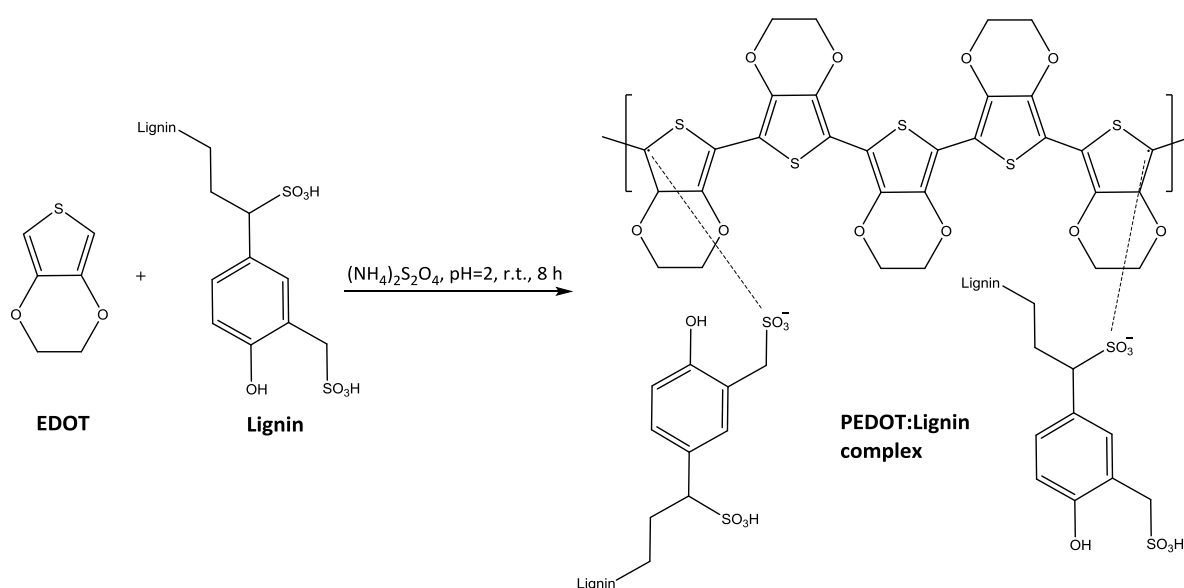


Figure 5-6. Proposed structure of the PEDOT:Lignin complex formed after polymerization of EDOT in the presence of lignosulfonate.⁴²

The synthesized PEDOT:lignosulfonate material unexpectedly showed comparable hole transport properties to the standard material PEDOT:PSS. The authors claim that the good hole transport property of the lignosulfonate composite originated in the electron transfer process taking place during the oxidation of electron-rich phenol derivatives in the lignosulfonate as well as the good aggregation properties of the biopolymer.

Several synthesis methods have been tested to produce PEDOT:lignosulfonate composite materials. F. N. Ajjan⁴³ *et al.* reported the formation of a PEDOT:lignosulfonate composite *via* oxidative chemical and electrochemical polymerization. The synthesized bio-composite was tested as an electrode material and it was found that it showed double the capacitance compared to reference PEDOT

electrode materials. The authors concluded that the enhanced energy storage performance is a consequence of the additional pseudo capacitance generated by the quinone moieties in lignin. Those moieties give rise to faradaic reactions showing that lignin acts as a dopant in the composite material. Additionally, it could be shown that PEDOT:lignosulfonate is a highly stable composite material, which retained about 83% of its activity after 1000 charge/discharge cycles.

The existing literature contains examples that demonstrate the promising potential of lignin as a dopant in conductive polymeric materials. However, the lignin applied in these studies is typically isolated using the sulphite pulping process and already contains sulphur groups in the structure which increases the conductive properties of the biopolymer. The aim of this chapter is to develop a method to use lignin extracted *via* the ionoSolv process (which does not contain sulphur) as a macromonomer in the synthesis of electrically conducting polymers.

5.2 Results and Discussion

5.2.1 Functionalization of lignin with aromatic compounds in protic ionic liquids

In order to be able to use lignin as a macro-monomer in the production of electrically conductive polymers the structure of the biopolymer needs to be altered. Native lignin typically contains a large number of aliphatic linkages that connect the aromatic subunits. From a perspective of electrical conductivity, this structure will hinder the free electron flow across the polymer backbone and thus result in low conductivity. However, the molecular structure of lignin can be altered to a certain extent by the applied extraction process. The ionoSolv pretreatment was found to lead to cleavage of the β -O-4 aryl ether bond and condensation reactions between the α carbon of the aryl ether linkage and an aromatic subunit.⁴⁴ The condensation reaction is believed to occur *via* the protonation of the hydroxyl group located on the α carbon followed by the abstraction of one water molecule. This creates a carbocation at this position which is then attacked by an electron rich aromatic subunit of lignin. The condensation reaction yields a lignin polymer that is richer in aromatic units and has fewer ether linkages, which is beneficial for electron mobility.

The formation of the carbocation also introduces the possibility to use it as a reactive site for lignin functionalization. Previously, the addition of aromatic compounds as lignin scavengers to autohydrolysis pretreatment has been studied to decrease lignin repolymerization and redeposition on the pulp with the aim to increase the glucose yield of enzymatic hydrolysis.⁴⁵ However, this study did not investigate the effects on the lignin structure and properties of this modification. The chapter in this thesis discusses the use of protic ionic liquids as a reaction medium to functionalize lignin with

halogenated aromatic compounds in order to increase the aromatic subunit content in lignin and to introduce a reactive functionality for further lignin modification.

5.2.1.1. Functionalization with 6-Bromo-2-naphthol

One of the compounds used to decrease lignin repolymerization in the above mentioned study⁴⁵ is 2-naphthol (a reactive aromatic molecule) which was found to be the most effective compound to suppress lignin repolymerization. The popularity of 2-naphthol as a lignin scavenger does not only lie in its high reactivity but also in the fact that it undergoes a single electrophilic substitution with lignin. This is based on the ability of the hydroxyl group to stabilise the positive charge created when 2-naphthol is substituted in the 1 position.⁴⁶

One derivative of 2-naphthol which bears a bromine functionality is 6-bromo-2-naphthol which was the first compound to be tested for lignin functionalization in this study (Figure 5-7). The compound selected to increase the aromatic subunit content in lignin needs to be a strong enough nucleophile to react with the carbocation at a faster rate than a lignin aromatic subunit would. The additional bromine functionality is known to be a strong electron withdrawing group but can also donate a free electron pair to the aromatic ring system and activates the ortho and para positions. It is therefore hypothesized that 6-bromo-2-naphthol will react not only ortho to the hydroxyl moiety but also ortho to the bromine substituent.

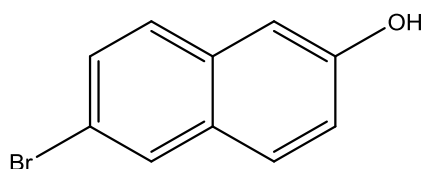


Figure 5-7. Structure of 6-bromo-2-naphthol **B-1**.

The lignin modification reaction was conducted in the protic ionic liquid solution $[N_{2220}][HSO_4]_{80\%}$ using 100 mg of lignin (extracted at 120 °C and 4 hours using the ionoSolv pretreatment) and the following different equivalents of 6-bromo-2-naphthol (**B-1**) per C9 unit: (i) 0.2, (ii) 0.4, (iii) 0.6, (iv) 0.8 and (v) 1.0. The purpose of the lignin functionalization was to eliminate the majority of aryl ether linkages, to increase the aromatic content of the biopolymer (Figure 5-8) and to add a bromine functionality to create a precursor for the synthesis of electrically conducting polymers.

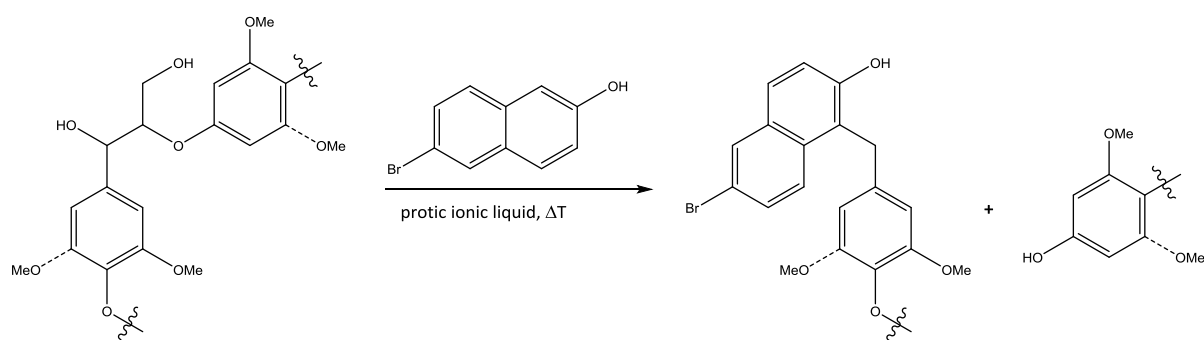


Figure 5-8. Schematic representation of lignin functionalization with 6-bromo-2-naphthol.

5.2.1.2 Influence of temperature and loading of 6-bromo-2-naphthol on lignin recovery and lignin structure

The modification of lignin with 6-bromo-2-naphthol was conducted at 170 °C for 30 minutes and 1 hour, respectively. The harsh reaction conditions were chosen to achieve the above mentioned goals regarding the change in lignin structure. Control experiments with no equivalents of **B-1** were also performed to be able to compare the changes in lignin structure induced by the functionalization with **B-1**. The amount of lignin recovered after the reaction is summarized in Figure 5-9. Surprisingly, the lignin recovery was generally relatively low with 60 % for the control experiment and similar yields for the functionalized lignin. This can be explained by the harsh reaction conditions applied that most likely led to the formation of low molecular weight lignin compounds that are soluble in the IL solution and don't precipitate after the addition of water to the reaction mixture. Addition of **B-1** to the IL solution did result in an increase of recovered product with an increasing amount of **B-1** present. The product was washed very carefully to remove any unreacted **B-1**, which indicates that the increasing product yield is due to incorporation of the aromatic compound into the lignin structure.

The reaction time was found to impact the amount of the product, with an increase of reaction time from 30 minutes to 1 hour resulting in a lower yield of the recovered product. The longer reaction times lead to a higher amount of conversion of the lignin polymer into low-molecular products thus resulting in a lower yield of the polymeric product.

2-D HSQC NMR spectra of the recovered products were recorded to investigate the structural changes of the lignin occurring during modification with **B-1** (Figure 5-11) and compared to the spectrum of lignin subjected to the same reaction conditions but without the addition of **B-1** (Figure 5-10). Several interesting observations were made during analysis of the NMR spectra. First, no signals of the typical linkages present in lignin (such as β -O-4, β - β or phenylcoumaran) were present suggesting that heating the previously extracted lignin for 30 minutes at 170 °C in the acidic IL solution [N₂₂₂₀][HSO₄]_{80%} did

nearly quantitatively hydrolyse the lignin linkages resulting in a polymer that mainly contains aromatic subunits.

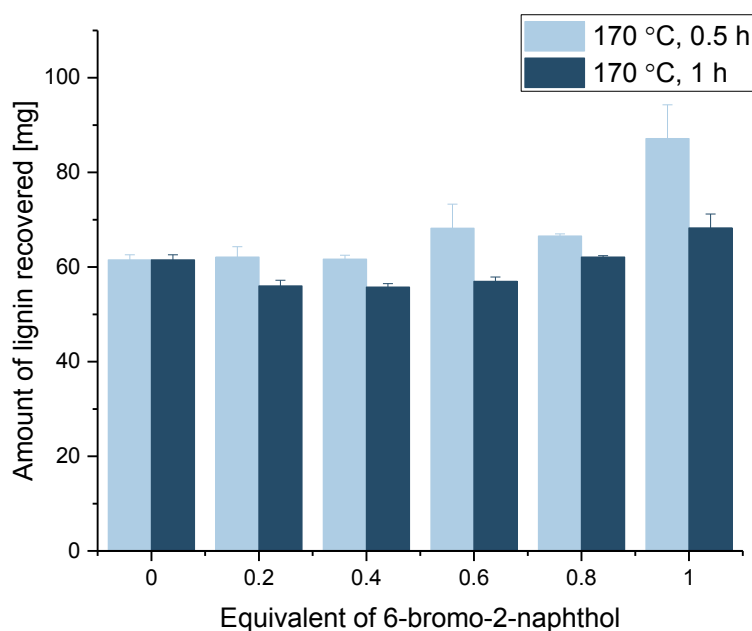


Figure 5-9. Yield of recovered lignin after modification with 6-bromo-2-naphthol in $[N_{2220}][HSO_4]_{80\%}$.

Additionally, the signal for the G_2 subunit was not observed, instead the signal for the condensed G_2 subunit was present in the spectrum and the signal of the uncondensed S subunit integrates to a much lower value than the condensed S subunit. This suggests that the lignin structure is highly condensed, which will be beneficial for the use as a precursor of electrically conducting polymers since the condensed structure might allow for better electron mobility. These changes in the structure were also found for the lignin that was subjected to the reaction conditions of the functionalization, but without the addition of **B-1**, showing that those were induced by heating in the acidic ionic liquid mixture.

The spectrum of the **B-1** functionalized lignin did show additional signals of the added aromatic compound. The measured GPC chromatograms of the recovered lignin after modification did not show any peak with the molecular weight of the monomer, suggesting that the functionalization was indeed successful. This shows that lignin modification with aromatic compounds in acidic ionic liquids is a new route to lignin functionalization which differs significantly from the already established methods. Typically, lignin is modified using the aliphatic and aromatic OH groups as reactive sites and in some cases, the functionalization occurs on the aromatic subunit.^{47,48}

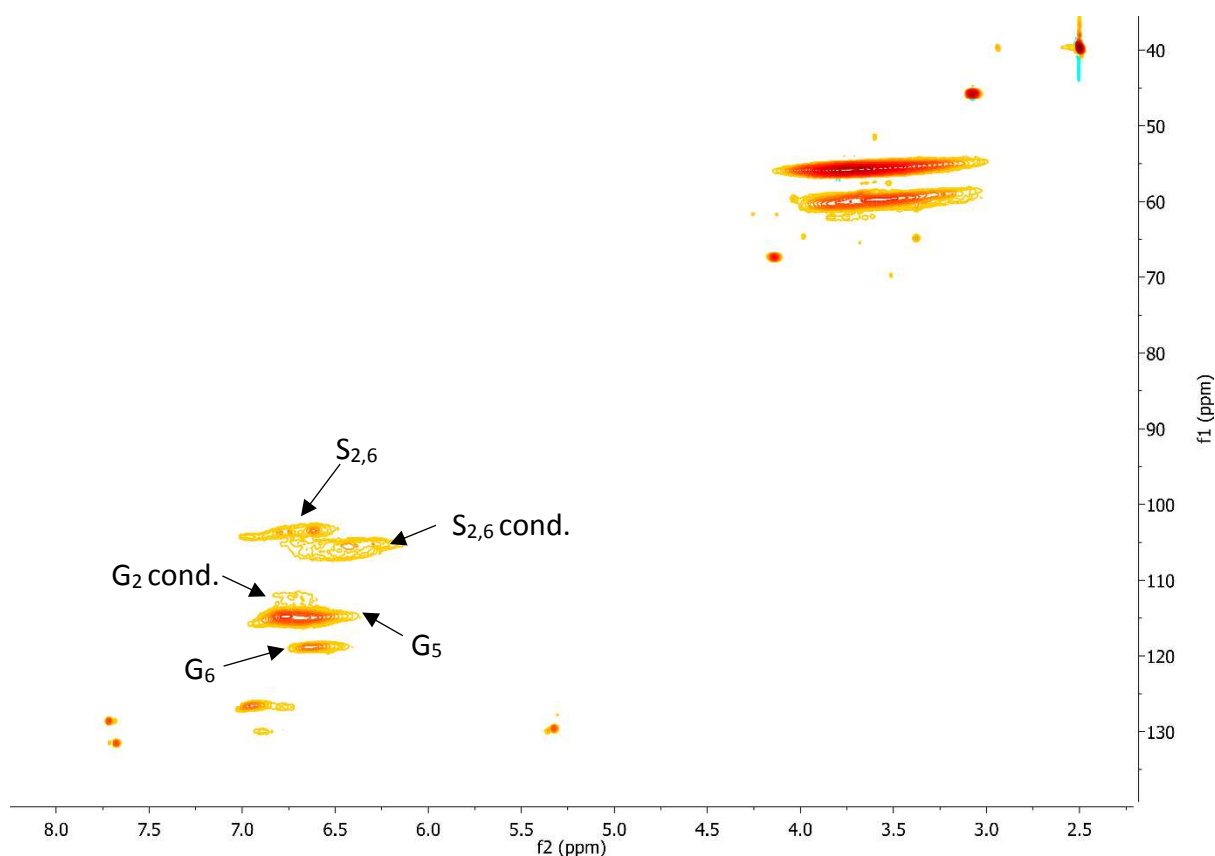


Figure 5-10. HSQC NMR spectrum of extracted lignin in DMSO- d_6 subjected to the following reaction conditions: 0.5 hours at 170 °C.

However, surprisingly signals for all six protons of **B-1** were found in the HSQC spectrum (δ_H/δ_C = H6: 7.14/108.5, H1: 7.14/119.5, H2: 7.49/128.6, H4: 7.67/128.0, H5: 7.76/128.4, H3: 8.03/129.3). This suggests that either the coupling reaction was not successful due to the *in-situ* formed carbocation preferably reacting with a lignin aromatic subunit or the reaction of the carbocation with **B-1** is not selective with regard to the position on the aromatic compound. It is thought that 2-naphthol is primarily electrophilically attacked at the 1 position taking the stabilisation of the positive charge and the intactness of the aromaticity of the molecule into account.⁴⁵ The presence of the very electronegative bromine functionality in 6-bromo-2-naphthol seems to influence the reactivity of the here-used compound significantly.

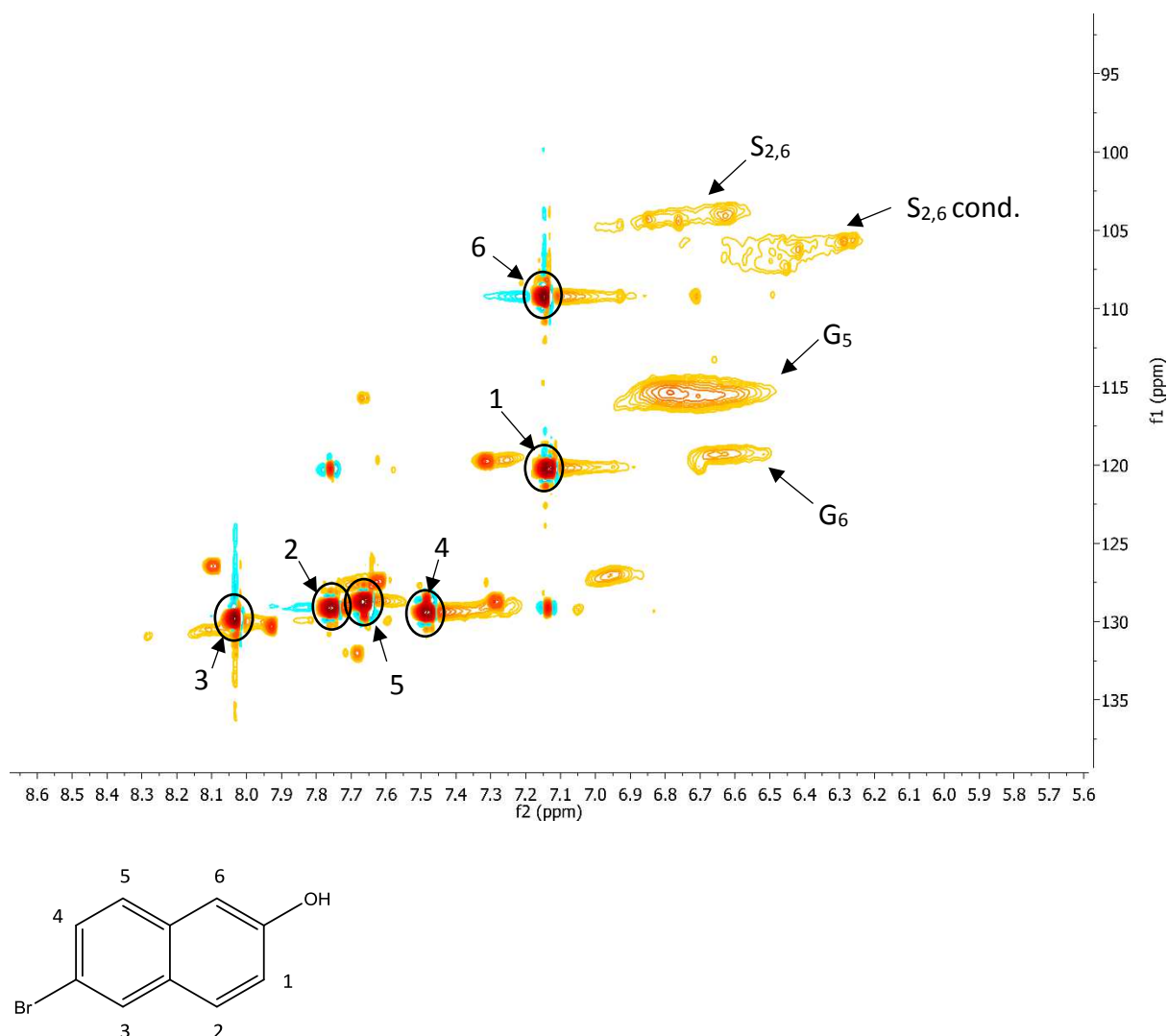


Figure 5-11. HSQC NMR spectrum of lignin modified with 1.0 equivalents of 6-bromo-2-naphthol (reaction conditions: 0.5 hours at 170 °C) in DMSO-d_6 .

In order to be able to confirm the successful functionalization of lignin with **B-1**, heteronuclear multiple bond correlation (HMBC) spectra were recorded (Figure 5-12). HMBC experiments show correlations between carbon atoms and protons which are not directly linked but separated by two or three bonds while direct one bond correlations are suppressed. In conjugated systems, the correlation between carbon atoms and protons separated by four bonds can be visible. This method allows for information of connectivity that goes beyond that offered by HSQC experiments.⁴⁹

The HMBC spectrum clearly shows the correlation of an aliphatic proton at 4.66 ppm with carbon atoms at 119.6 ppm, 132.2 ppm and 152.1 ppm. These carbon atoms can be assigned to carbons in the 2, 3 and 10 position of 6-bromo-2-naphthol and the 6 position of a lignin aromatic subunit. 6-Bromo-2-naphthol does not contain any aliphatic proton which could explain the correlation between

the carbon atoms of **B-1** and the proton at 4.66 ppm. The proton must be part of an aliphatic linkage of lignin, most likely at the α position of a now hydrolysed β -O-4 bond. The reaction between the *in-situ* generated carbocation on this position and the **B-1** resulted in a change of the electron density of the proton on the α position which resulted in slight deshielding and a slight upfield shift from 4.86 ppm⁵⁰ to the here observed 4.66 ppm. The analysis of the HMBC spectrum thus confirms the successful covalent incorporation of 6-bromo-2-naphthol into the lignin polymer. This adds a bromine functionality which can now be investigated for the use as a reactive site for further lignin functionalization.

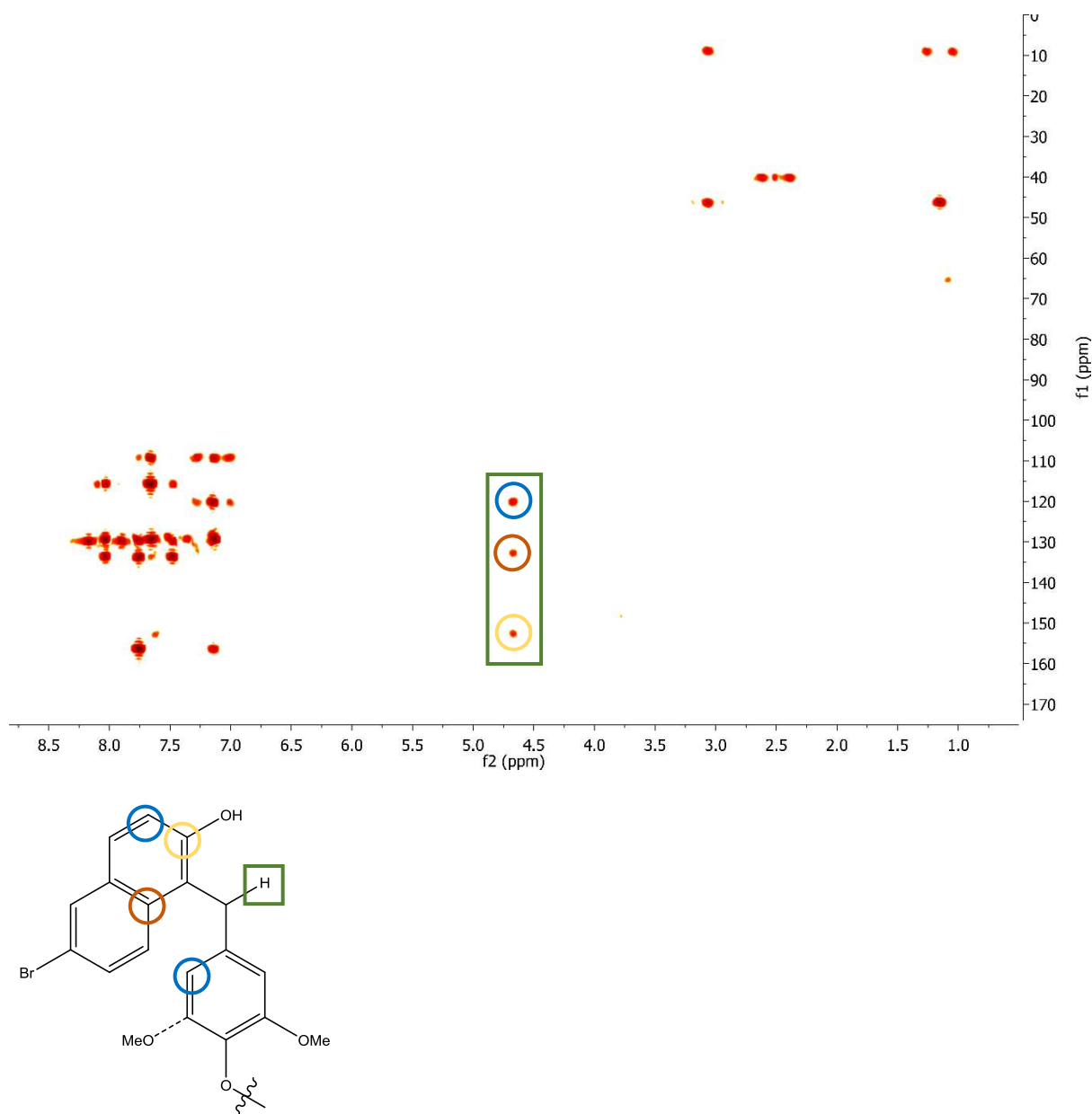


Figure 5-12. HMBC spectrum of lignin modified with 1.0 equivalents of 6-bromo-2-naphthol (reaction conditions: 0.5 hours at 170 °C) in DMSO- d_6 .

GPC analysis was performed to understand the effect of the modification with **B-1** on the molecular weight of lignin (Figure 5-13 and 14). Subjecting extracted lignin to heating at 170 °C in $[N_{2220}][HSO_4]_{80\%}$ for 30 minutes or 1 hour leads to condensation on the aromatic subunits, which was reflected by an increase in molecular weight as also reported in Chapter 4 of this thesis. It was found that the reaction time did influence the degree of condensation and polymers with a higher weight average molecular weight were recovered for longer reaction times (*ca.* 8000 $gmol^{-1}$ after 1 hour and *ca.* 6500 $gmol^{-1}$ after 30 minutes).

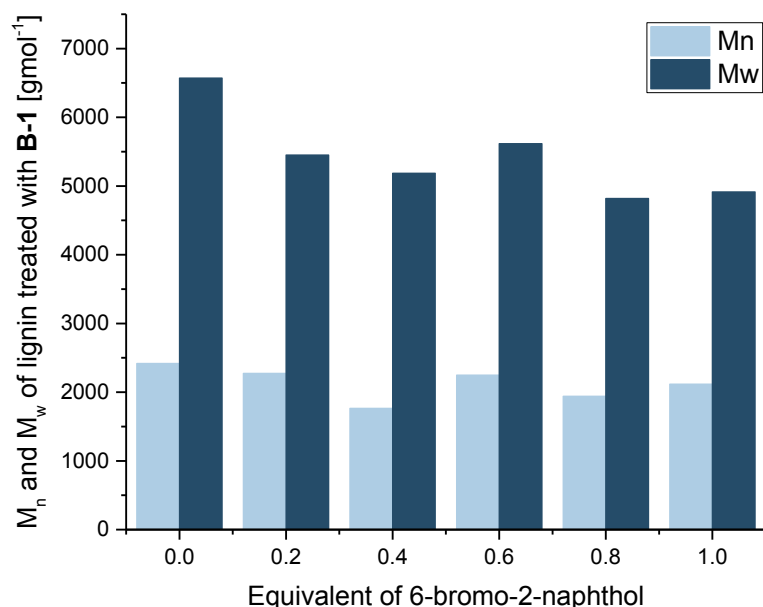


Figure 5-13. Number average molecular weight ($\bar{M}_n$) and weight average molecular weight ($\bar{M}_w$) of lignin subjected to the modification reaction conditions and lignin functionalized with 6-bromo-2-naphthol using different equivalents of the aromatic compound. Reaction conditions used: 0.5 hours at 170 °C.

It was observed that the addition of **B-1** to the reaction mixture did result in a decrease of the weight average molecular weight of the polymer to around 5500 g mol⁻¹ compared to the control experiment. This can be assigned to **B-1** acting as a scavenger for the *in-situ* formed lignin carbocation and thus preventing the intermolecular condensation of lignin as also suggested elsewhere for 2-naphthol.⁴⁵ Interestingly, the weight average molecular weight and number average molecular weight of the functionalized lignin were found to not depend on the reaction time in contrast to the molecular weight of the unmodified lignin subjected to the same reaction conditions. This suggests that the addition of **B-1** to the reaction mixture effectively suppresses condensation reactions of the lignin polymer even at very harsh reaction conditions. The addition of 0.8 and 1.0 equivalents of **B-1** to the reaction mixture did yield functionalized lignin polymers with a slightly lower molecular weight compared to the addition of lower amounts of the aromatic compound. These results indicate that the degree of incorporation of **B-1** into the lignin polymer does rely on the availability of it in the reaction mixture.

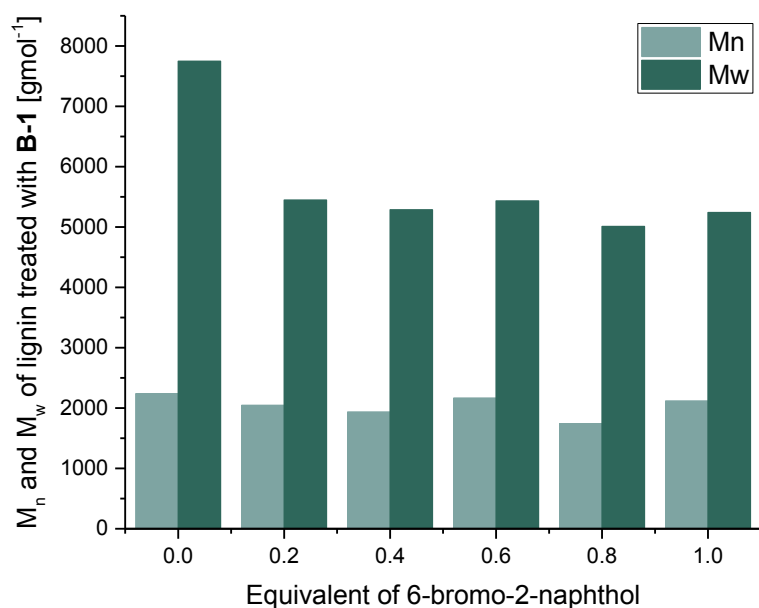


Figure 5-14. Number average molecular weight ($\bar{M}_n$) and weight average molecular weight ($\bar{M}_w$) of lignin subjected to the modification reaction conditions and lignin functionalized with 6-bromo-2-naphthol using different equivalents of the aromatic compound. Reaction conditions used: 1 hour at 170 °C.

HSQC and GPC analysis confirmed the incorporation of **B-1** into the lignin polymer, however, those analysis techniques do not provide insight in the amount of bromine present in the material. Thus, elemental analysis was performed to better understand this. It was found that the amount of bromine in the product did increase with increasing equivalent of **B-1** added to the IL solution from 1.51 % for 0.2 equivalents to 2.78 % for 1.0 equivalent, respectively (Figure 5-15). The recovered lignin was washed multiple times with two different solvents to ensure the complete removal of unreacted monomer. The increase in bromine content in the lignin after functionalization clearly shows that the incorporation of **B-1** is controlled by the amount of the aromatic compound available for functionalization. However, the incorporation of **B-1** does not follow a linear trend and when using 0.6 equivalents of **B-1** the bromine content already reached 92 % compared to using 1.0 equivalent of **B-1**. This suggests that not all of the **B-1** added to the reaction mixture reacted with the lignin. This can be explained by looking at the structure of lignin. Not every C9 unit is connected to an aryl ether linkage (which is believed to be the precursor of the reactive site for the functionalization with **B-1**) but is connected *via* β - β or phenylcoumaran linkages or *via* cross-condensation. The data presented

here clearly shows that **B-1** only reacts with the β -O-4 bond of the lignin and not with any other part of the polymer.

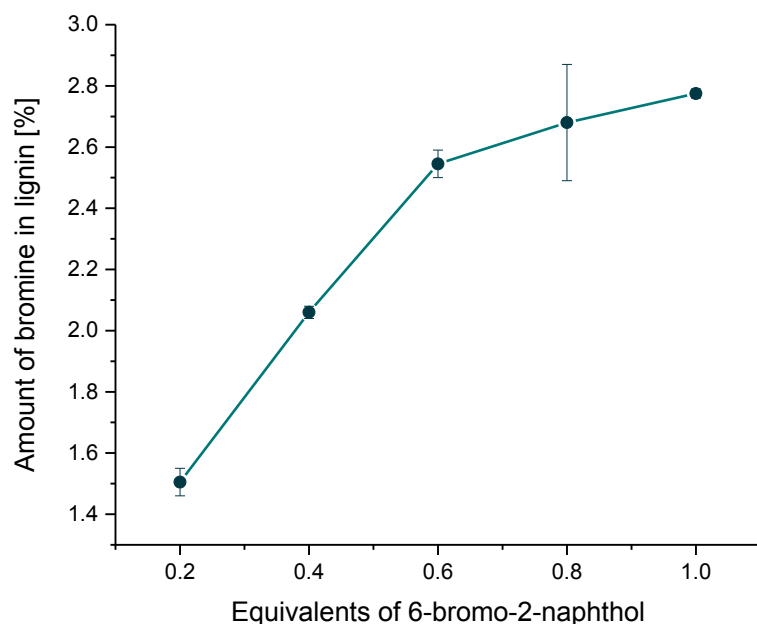


Figure 5-15. Bromine content as determined by elemental analysis in lignin modified with different equivalents of 6-bromo-2-naphthol in $[N_{2220}][HSO_4]_{80\%}$.

5.2.1.3 Functionalization with other aromatic compounds

The successful functionalization of lignin with the aromatic compound 6-bromo-2-naphthol has sparked the idea to try to functionalize lignin with other aromatic compounds containing more aromatic rings to increase the amount of aromatic subunits in lignin even further (Figure 5-16). If successful, this modification would benefit the properties of lignin as a macromonomer for the synthesis of electrically conducting polymers.

The aromatic compound with a similar structure and reactivity to 6-bromo-2-naphthol is 6-6'-dibromo-1,1'-bi-2-naphthol (**B-2**) which was selected to test the functionalization of lignin with a structurally more complex compound. The reaction was performed at 170 °C for 1 hour in $[N_{2220}][HSO_4]_{80\%}$ based on the positive results of the lignin modification with **B-1** under these conditions. NMR analysis of the recovered product was performed to understand the structural changes occurring to lignin during the reaction as well as to investigate where the coupling between the lignin and **B-2** occurred. The HSQC spectrum did show signals corresponding to the aromatic subunits of lignin (S and G) as well as 4 new signals in the aromatic region that can be assigned to proton-carbon correlations of **B-2** (Figure 5-17).

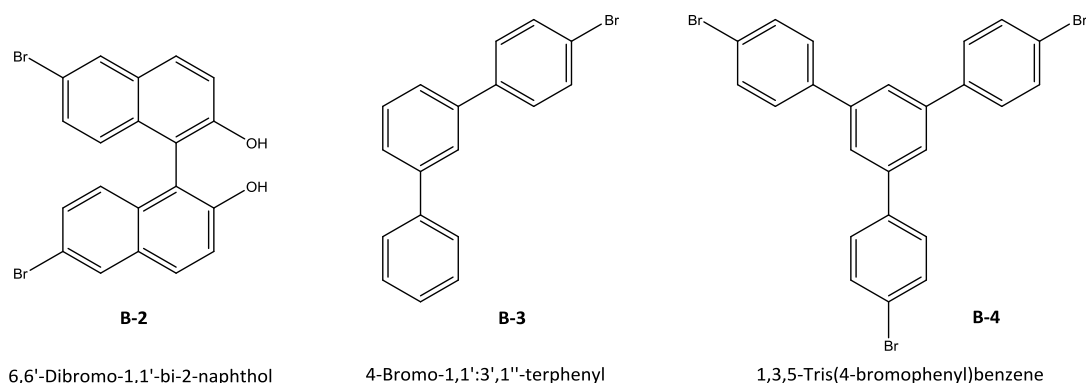


Figure 5-16. Aromatic compounds used to functionalize lignin in the protic ionic liquid solution $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$.

The signals were assigned to the proton-carbon correlations as follows: $\delta_{\text{H}}/\delta_{\text{C}} = 4+4'$: 7.31/128.5, $5+5'$: 7.35/119.4, $2+2'$: 7.87/127.9, $3+3'$: 8.12/129.3. Interestingly, no signal for the $1+1'$ correlation was observed, suggesting that lignin reacted with **B-2** on those two positions. Interestingly, only four signals of **B-2** were present in the spectrum showing that the symmetry of the aromatic compound was kept intact after reaction with lignin, indicating that lignin indeed reacted with both the 1 and $1'$ position of **B-2**.

The molecular weight of the lignin recovered after the reaction with **B-2** was measured and compared to lignin functionalized with **B-1** and unmodified lignin subjected to the same reaction conditions (Figure 5-18). Interestingly, the molecular weight was found to be influenced by the aromatic compound chosen for the modification. The highest molecular weight of *ca.* 8000 gmol^{-1} was observed for the unmodified lignin after heating in the ionic liquid solution due to condensation reactions occurring under these conditions.⁴⁴ Modification of lignin with **B-2** resulted in a decrease of weight average molecular weight to *ca.* 6900 gmol^{-1} compared to the unmodified biopolymer. However, the weight average molecular weight and number average molecular weight of lignin + **B-2** was found to be higher than the ones of the polymer recovered after functionalization with **B-1** (6900 gmol^{-1} and 2400 gmol^{-1} compared to 5300 gmol^{-1} and 1900 gmol^{-1}), respectively. This indicates that **B-2** did indeed react with probably 2 lignin fragments on both the 1 and $1'$ positions of the aromatic molecule, resulting in the formation of polymers with a higher molecular weight than after the reaction of lignin with **B-1**. This result is also supported by the NMR data discussed above and highlights the difference in reactivity of the two aromatic compounds used for lignin functionalization.

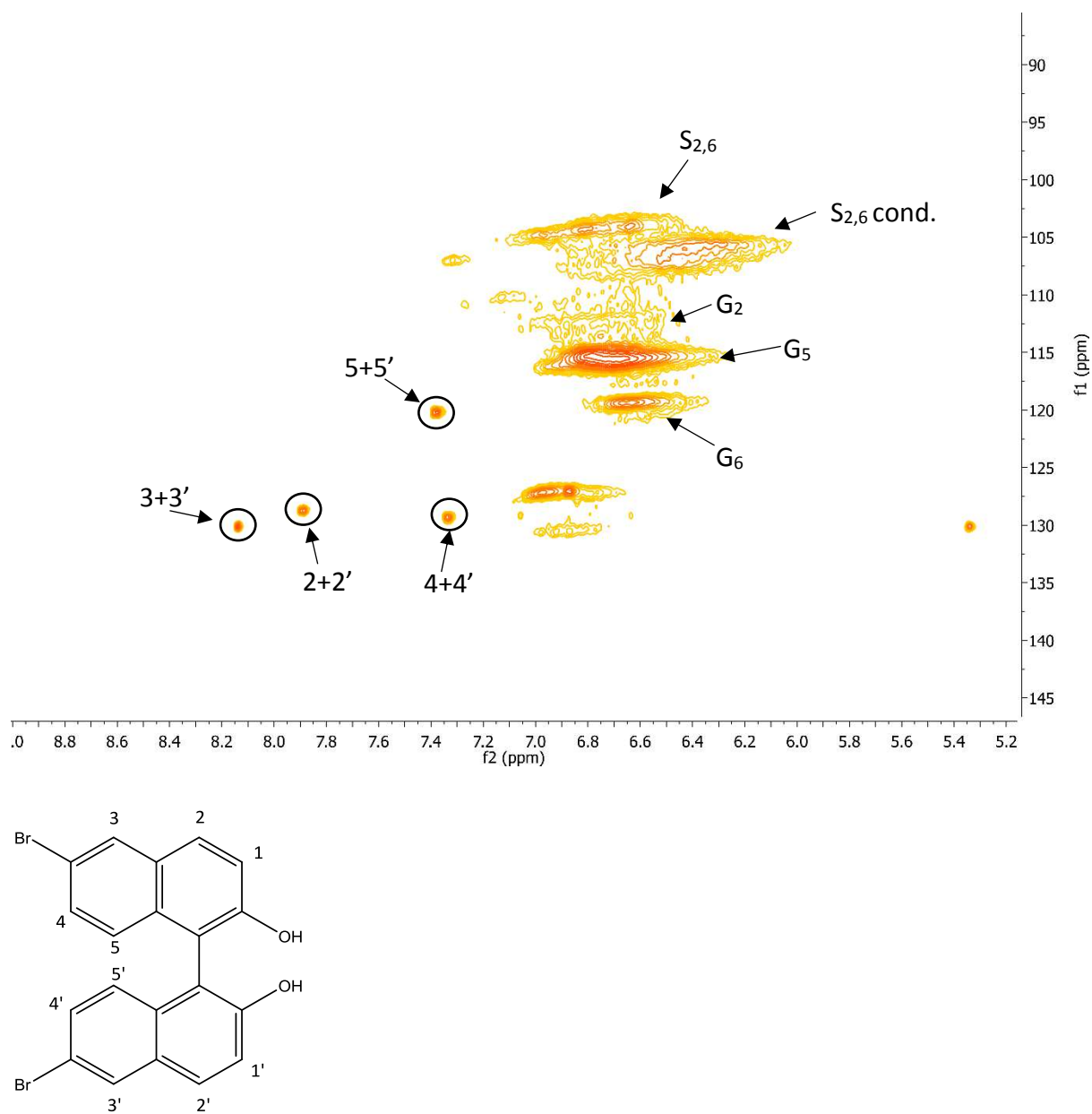


Figure 5-17. HSQC NMR spectrum of lignin modified with 0.2 equivalents of **B-2** (reaction conditions: 1 hour at 170 °C) in DMSO- d_6 .

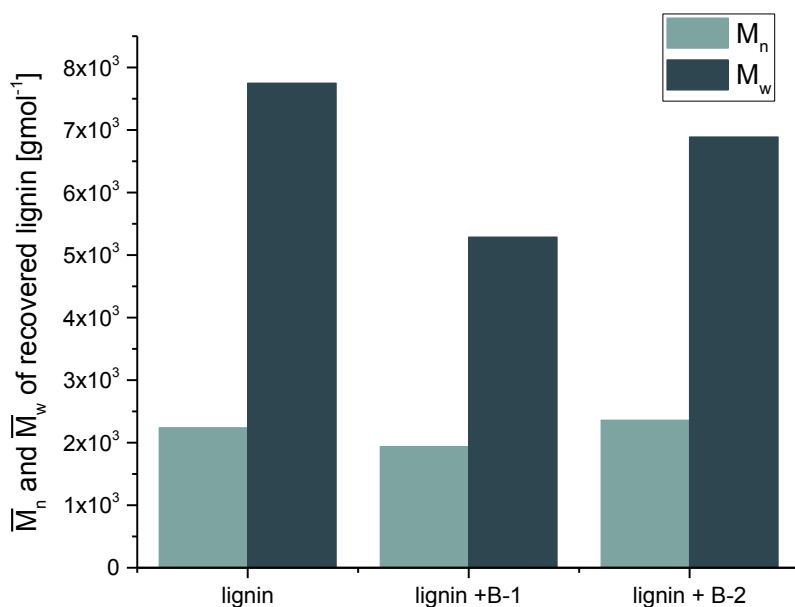


Figure 5-18. Number average molecular weight ($\bar{M}_n$) and weight average molecular weight ($\bar{M}_w$) of lignin functionalized with **B-1** and **B-2** and lignin subjected to the same reaction conditions without the addition of an additive. Reaction conditions: 1 hour at 170 °C in $[N_{2220}][HSO_4]_{80\%}$, 0.4 equivalents of **B-1** or **B-2**.

The functionalization of lignin with **B-3** and **B-4** was carried out at 170 °C for 1 hour using $[N_{2220}][HSO_4]_{80\%}$ and 0.2 equivalents of the aromatic compounds. The product recovered after modification with **B-3** did show the corresponding signals in the NMR spectrum, however, the GPC spectrum did show a large peak with the molecular weight in the range of the **B-3** monomer. The product was then washed again several times with various solvents to remove the **B-3** monomer impurity but the monomer peak was still visible in the subsequent GPC analysis. It can thus be concluded, that the reaction between lignin and **B-3** was not successful. As for lignin modification with **B-4** it was found that the solubility of the aromatic compound was an issue. To solve this problem, **B-4** was dissolved in 0.5 mL of toluene and then mixed with the ionic liquid solution prior to the reaction. After the desired reaction time, the lignin was precipitated from solution by adding water as an anti-solvent which also resulted in the precipitation of white crystals which could be identified to be **B-4**. NMR analysis of the isolated lignin then showed no peaks that can be assigned to **B-4** thus proving that the reaction was not successful. These results indicate that the reason for the low reactivity of lignin towards **B-3** and **B-4** is most likely because of the absence of OH-groups on the aromatic compounds which changes the reactivity of the molecule. The positive charge which forms after the electrophilic attack of the lignin carbocation on the aromatic ring of **B-3** and **B-4** could not be stabilized. This clearly shows that the structure of the aromatic compound does significantly influence its reactivity toward ionic liquid lignin.

5.2.2 Synthesis of lignin-thiophene copolymers

The synthesis of lignin-thiophene copolymers was attempted using the previously bromine functionalized lignin, which enabled the use of traditional synthesis techniques developed for conjugated polymers. The classical synthesis methods such as Suzuki-Miyaura, Heck or Stille coupling all utilize a halogenated compound as one reagent in the coupling reaction.¹¹ The introduction of a bromine moiety attached to an aromatic ring system into the lignin structure results in the lignin acting as a macromonomer and it is hypothesized that grafting of thiophene polymer chains from the lignin polymer is theoretically possible. Lignin modified with 6-bromo-2-naphthol was used for all of the following grafting experiments.

The direct arylation polymerization (DAP) was developed as an alternative synthesis route to the standard synthesis methods of conjugated polymers and applies palladium based catalysts for the coupling reaction using carboxylic acids and potassium carbonate as additives. The standard organometallic coupling methods often require harsh conditions or are utilizing harmful or difficult to handle reactants.¹³ DAP was chosen for the synthesis of lignin-thiophene copolymers for the following reasons: (i) the solvent used in the reaction is typically dimethylacetamide (DMA) or dimethylformamide (DMF) – both of which are known to dissolve lignin and thus decrease issues regarding phase separation during the reaction, (ii) DAP was shown to yield polythiophene in good yield with high purity and a low amount of β -defects, and (iii) this synthesis method applies mild reaction conditions and does not use compounds containing organometallic moieties.⁵¹

The synthesis protocol applied in the coupling of **B-1** functionalized lignin with 3-hexylthiophene was based on publications by A. Rudenko^{51,52} *et al.* For this study, the polymerization of 3-hexylthiophene was conducted under the optimized conditions (60 °C for 48 h and 0.25 mol% catalyst loading) reported in the literature⁵¹ and poly(3-hexylthiophene) (P3HT) was successfully synthesized (see appendix for spectra). The synthesized P3HT was analysed and used as a baseline reference for comparison purposes for future products recovered from lignin-thiophene copolymerization.

The reaction between bromine functionalized lignin and 3-hexylthiophene is depicted in Figure 5-19.

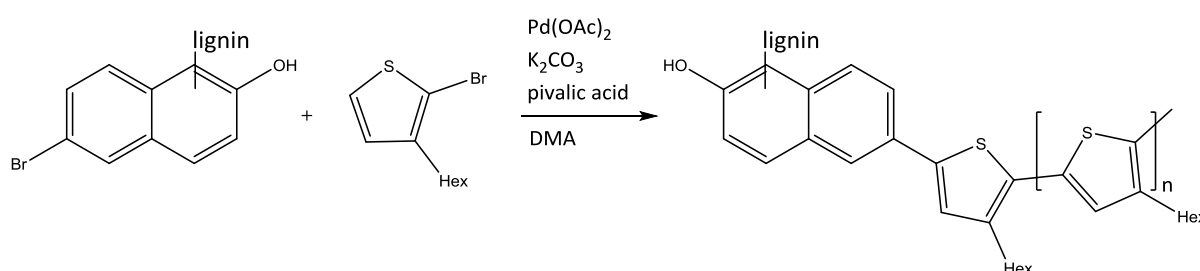


Figure 5-19. Schematic representation of the synthesis of copolymers consisting of 6-bromo-2-naphthol functionalized lignin and 3-hexylthiophene.

The first attempt to synthesize lignin-polythiophene copolymers was performed using the optimized reaction conditions as already tested for the synthesis of poly(3-hexylthiophene) (entry 1 in Table 5-1). The addition of methanol as anti-solvent after the reaction resulted in the precipitation of a solid material. Visual examination of the recovered solid showed the presence of two separate phases resembling P3HT and lignin. However, separation of the two distinct phases was very difficult and NMR analysis of the material gave no distinct result. Interestingly, the presence of lignin in the reaction mixture did not seem to hinder the polymerization of 3-hexylthiophene to P3HT. This indicates that an optimization of the reaction conditions might result in a successful formation of a lignin-polythiophene copolymer.

Table 5-1. Reaction conditions of DArP coupling between **B-1** functionalized lignin and 3-hexylthiophene.

Entry	temperature [°C]	reaction time [h]	catalyst loading [mol%]
1	60	48	0.25
2	100	24	1
3	60 than 100	24	1
4	60 than 100	24	10

With this in mind, the reaction conditions were adjusted and a higher catalyst loading and higher reaction temperature were applied (Entry 2, Table 5-1). The bromine moiety attached to the lignin polymer is most likely more sterically hindered compared to typical small organic compounds used in the synthesis of conjugated polymers. A higher catalyst loading was chosen in order to counteract this phenomenon. The more complex structure of the brominated lignin compared to the typical small halogenated reactant might mean that a higher activation energy is needed for addition of the brominated lignin to the metal centre of the catalyst and thus a higher reaction temperature is required. Unfortunately, the changes in reaction conditions did not lead to a successful coupling of **B-1** functionalized lignin and 3-hexylthiophene and a mixture of P3HT and lignin were recovered after the reaction.

The first step in the coupling reaction is known to be the oxidative addition of the halogenated compound to the metal centre of the catalyst.¹³ The much larger size of the functionalized lignin and the resulting steric hindrance of the bromine functionality might decrease the effectiveness of the coupling of the catalyst with the halogenated reactant. Keeping this in mind, the synthesis protocol was altered again into a two-step synthesis procedure. First, the bromine modified lignin, potassium carbonate, pivalic acid and Pd(OAc)₂ were dissolved in DMA and heated at 60 °C for several hours to

facilitate the oxidative addition of brominated lignin to the metal centre of $\text{Pd}(\text{OAc})_2$ (Entry 3 and 4 in Table 5-1). This was followed by the addition of 3-hexylthiophene dissolved in DMA. However, this approach also proved to be not successful in the synthesis of lignin-polythiophene copolymers and no thiophene signals were observed in the analysed lignin recovered after the reaction.

5.3 Summary and future work

Lignin was functionalized with 6-bromo-2-naphthol **B-1** in different equivalents (0.2, 0.4, 0.6, 0.8 and 1.0) with the aim to increase the aromatic content in lignin to create a lignin macromonomer for the synthesis of electrically conducting polymers. The modification was carried out in the protic ionic liquid solution $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$ under harsh reaction conditions (30 min and 1 hour at 170 °C) to cleave most aliphatic lignin linkages and alter the lignin structure to form a very condensed aromatic polymer. The yield of the recovered products after functionalization with **B-1** was found to be around 60 % which is most likely due to depolymerisation reactions of the lignin polymer occurring in protic ionic liquid solution under these reaction conditions. However, it was observed that the yield slightly increased with an increasing amount of **B-1** present in the reaction mixture. NMR analysis of the isolated products showed that the lignin structure was significantly altered after heating in $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$. Additionally, almost quantitative cleavage of aliphatic linkages was found along with a high degree of aromatic subunit condensation of the lignin (for lignin both modified with **B-1** and unmodified). The NMR spectra of **B-1** functionalized lignin also showed the typical signals of the aromatic compound. HMBC analysis was performed and confirmed that a covalent bond between lignin and **B-1** was formed. This shows that lignin was successfully functionalized with the aromatic compound 6-bromo-2-naphthol in a protic ionic liquid solution. It was found that the molecular weight of the lignin was influenced by the incorporation of **B-1** and a decrease in M_w was observed compared to the control experiment. This shows that **B-1** does act as a lignin scavenger and prevents lignin repolymerization. Bromine elemental analysis revealed that an increase of **B-1** in the reaction mixture does result in a higher degree of incorporation of the aromatic compound into the lignin structure. However, the observed trend was not linear, thus suggesting that **B-1** only reacts with the carbocation created on the β -O-4 linkage of lignin.

Based on the successful modification of lignin with **B-1**, lignin modification with other aromatic compounds was investigated. The second tested compound was 6,6'-dibromo-1,1'-bi-2-naphthol (**B-2**) and the reaction conditions were chosen based on the successful functionalization of lignin with **B-1**. NMR and GPC analysis of the recovered product confirmed the successful modification of lignin with **B-2** and additionally showed that **B-2** reacted with two lignin fragments instead of one. Lignin

functionalization with structurally even more complex aromatic compounds was also performed but was found to be not successful.

The synthesis of lignin-polythiophene copolymers was attempted using the direct (hetero)arylation approach. Several catalyst loadings and reaction temperatures were studied, however, the synthesis of a copolymer was not successful.

The research discussed in this chapter showed that lignin functionalization in protic ionic liquids is a promising approach to synthesize new lignin materials. Interestingly, the approach used here for the first time of the *in situ* created lignin carbocation as reactive site opens up a whole new reaction pathway for lignin modification that does not rely on the reactivity of lignin hydroxyl groups or other reactive sites introduced into the lignin polymer via reactions on the lignin hydroxyl groups.

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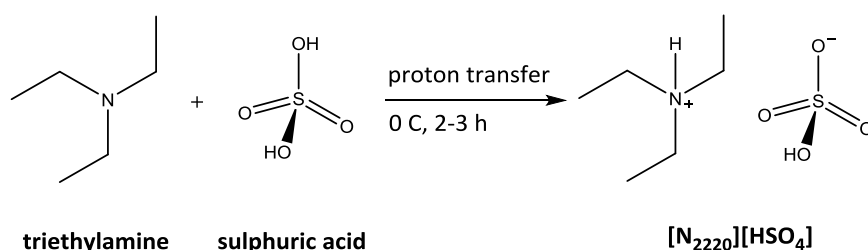
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Chapter 6: Materials and Methods

6.1 Materials

Willow chips were grown and supplied by Rothamsted Research, ground and sieved to a size range of 180 – 850 μm (US mesh scale -20/+80) at Imperial College London. All other materials or reagents were purchased from Sigma Aldrich, VWR International or Fluorochem, unless stated otherwise. Triethylamine was purchased with a purity of above 99 % and sulphuric acid was obtained as a 5 M solution. All commercial reagents and solvents were used as received without further purification unless stated otherwise.

6.2 Synthesis of triethylammonium hydrogen sulfate $[\text{N}_{2220}][\text{HSO}_4]$ with different acid/base ratio



The ionic liquid solution $[\text{N}_{2220}][\text{HSO}_4]$ was synthesised with an acid/base ratio of 1.02 and 0.98 for ionic liquid pretreatment of biomass.

Triethylamine (126.49 g, 1.25 mol) was weighed into a round bottom flask and cooled in an ice bath and sulphuric acid solution (256 mL, 5 mol L^{-1} , 1.28 mol for $a/b = 1.02$ or 246 mL, 5 mol L^{-1} , 1.23 mol for $a/b = 0.98$) was added drop-wise under stirring over 1 hour. The reaction mixture was then stirred for 3 hours at 0 °C. Residual water was removed under vacuum from the colourless liquid until a water content of below 20 wt% was achieved. The water content was measured using a Karl-Fischer titrator and then adjusted via addition of water to 20 wt%.

6.3 Acid/base ratio of the IL solution

To determine the acid/base ratio, the synthesized ionic liquid solution $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$ was characterized using two different methods, namely pH measurements and density measurements, using a Metler Toledo DM40 density meter at 25 °C and a Jenway 3510 pH meter, respectively.

For measurements of the pH, a 1% solution of IL in water was prepared. For this, 100 mg of dry IL $[\text{N}_{2220}][\text{HSO}_4]$ (125 mg of $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$) was weighed into a 10 mL volumetric flask and the flask was filled to the 10 mL mark with purified water. The mixture was mixed well to guarantee equal concentration of the ionic liquid/water mixture and the pH was measured using the pH probe until a stable reading was achieved. All measurements were performed in triplicates.

The acid/base ratio, defined as the ratio of the protonated triethylammonium $[\text{NEt}_3\text{H}]^+$ and the hydrogen sulfate $[\text{HSO}_4]^-$ anion, was calculated from the density measurement. The acid/base ratio of the synthesized ionic liquid was determined using a method developed by M. S. Y. Jennings¹ using the following equation:

$$y = 0.772x + 1.1135 \quad (1)$$

where y is the density of the IL solution and x is the acid/base ratio of IL solution.

Table 1 shows exemplary pH and density for $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$ with different acid/base ratios. The pH and density of each batch of synthesised $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$ were measured before use for pretreatment and adjusted by addition of triethylamine or sulphuric acid if needed.

Table 6-1. Examples of pH measurement and density measurement results for synthesized $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$.

measurement	pH	density $[\text{g mL}^{-1}]$	acid/base ratio
1	1.52	1.1925	1.02
2	1.53	1.1923	1.02
3	1.52	1.1924	1.02
4	1.65	1.1888	0.98
5	1.66	1.1887	0.98
6	1.65	1.1889	0.98

6.4 Moisture content of untreated *Salix* and pulp

All experiments are based on the oven-dried weight of the biomass, which makes knowledge of the moisture content crucial. To determine the moisture content of untreated *Salix* and pulp, 50 – 100 mg of ground air-dried material was weighed onto a pre-weighed piece of aluminium foil, folded into a small package to prevent loss of biomass and dried in an oven (VWR Venti-Line 115) at 105 °C overnight. The samples were transferred into a desiccator equipped with activated silica and weighed after 20 min. The moisture content was calculated according to the following equation:

$$\text{moisture content} = \frac{m(\text{air dried}) - m(\text{oven dried})}{m(\text{oven dried})} \cdot 100 \% \quad (2)$$

6.5 Ionic liquid pretreatment for biomass fractionation

The fractionation experiments to isolate ionoSolv lignin and cellulose-rich pulp were carried out using a standard operating protocol developed in our laboratory. All experiments were performed in triplicates with a 20wt% loading of dry biomass in the IL and the standard deviation was calculated for each data point.

For pretreatment, 2.00 g of ground willow was weighed into a 15 mL Ace pressure tube with screw-cap and Teflon lining, followed by addition of 10.00 g of $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$. The pressure tubes were sealed well and the mixture was vortexed for 1 min to guarantee homogeneous mixing of biomass and IL. The samples were incubated without stirring in an oven at different temperatures (120 °C, 150 °C and 170 °C) for various length of time. After pretreatment, the samples were cooled to room

temperature, transferred to a 50 mL Falcon tube, mixed with 40 mL of abs. EtOH and left to equilibrate for 1 h. The mixture was then centrifuged at 3000 rpm for 50 min. The supernatant containing the ionic liquid and dissolved lignin was decanted in a 250 mL round bottom flask leaving the pulp in the Falcon tube. The washing procedure was repeated 3 more times. The pulp was subjected to soxhlet extraction for 20 h set to 135 °C and using *ca.* 180 mL abs. EtOH per sample and subsequently air-dried for 2 days. The supernatant (still containing EtOH) was dried under reduced pressure and the residue was combined with the EtOH from the soxhlet extraction and dried again under reduced pressure until total removal of EtOH and water (from the ionic liquid) was achieved. To recover the lignin, 40 mL of anti-solvent (in this case DI water) was added to the solid IL/lignin mixture and the mixture was transferred into a 50 mL Falcon tube and left to incubate for 1 h. The mixture was then centrifuged at 3000 rpm for 25 min, after which the supernatant was decanted into a 250 mL round bottom flask. The lignin was washed 2 more times with 40 mL DI water and finally freeze-dried. To determine the yields of pulp and lignin, both biopolymer fractions were weighed on aluminium foil. The air-dried pulp yield was determined by weighing the recovered biomass after air-drying. The oven-dried yield was determined by calculating the amount of oven-dried pulp obtained as a percentage of the amount of oven-dried biomass used for the pretreatment. The air-dried pulp was then subjected to saccharification and compositional analysis to evaluate the effectiveness of the pretreatment. The recovered lignin was analysed via NMR Spectroscopy and Gel Permeation Chromatography.

6.6 Enzymatic hydrolysis of untreated *Salix* and pulp

The enzymatic saccharification of raw *Salix* and pulp was performed according to the protocol “Enzymatic Saccharification of Lignocellulosic Biomass” published by the NREL (National Renewable Energy Laboratory).² In brief, 100±3 mg untreated or pretreated biomass (oven-dried weight) was weighed into a 50 mL Falcon tube, followed by addition of the enzyme master mix. The enzyme master mix consisted of 5 mL 1M sodium citrate buffer (at pH 4.8), 4.8 mL water, 40 µL tetracycline antibiotic solution (10 mg/mL in 70% ethanol), 30 µL cycloheximide solution (10 mg/mL in purified water) and 50 µL of a commercial enzyme mixture (*Trichoderma reesei* ATCC 26921 and Cellobiase from *Aspergillus niger*) per biomass sample. The mixture was incubated in a Stuart Orbital Incubator (S1500) for 7 days at 50 °C under shaking at 250 rpm. If seen important, various time point samples were taken (usually at 2 h, 4 h, 6 h, 8 h, 24 h, 3 days and 5 days after start of incubation) to determine the initial hydrolysis rate of the hydrolysis reaction. For time point sampling, the plastic vials containing the biomass and enzyme mixture were taken out of the incubator and immediately put on ice to interrupt the enzymatic hydrolysis. Then, 500 µL of the enzyme master mix and a small amount of untreated

biomass/pulp were pipetted from the reaction mixture using an Eppendorf pipette with a cut plastic tip. This mixture was transferred into 1.5 mL microcentrifuge tubes and centrifuged at 13.3 rpm for 10 min. Then, 200 µL of the supernatant were pipetted into a 1.5 mL HPLC vial with a 250 µL insert. Sample preparation for analysis after 7 days of saccharification was performed as follows: 1 mL of the enzyme master mix was transferred into a 1 mL plastic syringe and then filtered into a 1.5 mL HPLC vial using a 0.22 µm syringe filter. The samples were analysed for monomeric sugars (glucose, xylose, arabinose, mannose and galactose) using a Shimadzu HPLC system with refractive index detector equipped with an Aminex HPX-87P column (Biorad). The mobile phase was DI water (18.8 mΩ), the flow rate 0.6 mLmin⁻¹, the column oven set to 85 °C and the acquisition time 20 min. For retention times of the sugar monomers, see table 2. Calibration standards with concentrations of 0.1, 1, 2 and 4 mg/mL of each of glucose, xylose, mannose, arabinose and galactose, and 8 mg/mL of glucose were used. Monomeric sugar yields were calculated based on the glucose and hemicellulose contents of the untreated biomass.

Table 6-2. Retention times of monomeric sugars analysed after enzymatic saccharification and compositional analysis.

Monomeric sugar	Retention time [min]
Glucose	12.79
Xylose	13.82
Galactose	14.55
Arabinose	15.62
Mannose	16.39

The glucose and xylose yields were calculated using the following equations:

$$\%Glucose\ yield(t) = \frac{c(HPLC,t) \cdot V \cdot 0.9 \cdot Y(pulp)}{G(0) \cdot ODW(sample)} \cdot 100 \quad (3)$$

$$\%Xylose\ yield(t) = \frac{c(HPLC,t) \cdot V \cdot 0.88 \cdot Y(pulp)}{G(0) \cdot ODW(sample)} \cdot 100 \quad (4)$$

where $c(HPLC, t)$ is the sugar concentration detected by HPLC after saccharification time t had elapsed, V is the initial volume of the solution in mL (10.00 mL), $Y(pulp)$ is the pulp yield recovered as a proportion of initial biomass dry weight before pretreatment (100% for untreated biomass and around 40–70% for pretreated pulps), $G(0)$ is the glucan content in untreated biomass determined by compositional analysis, and $ODW(sample)$ is the oven-dried weight of the biomass sample before saccharification in mg.

6.7 Compositional analysis of untreated *Salix* and pulp

For untreated biomass, determination of extractives³ was included in the protocol, whereas this step was not performed for pretreated pulp due to the assumption that all extractives will be dissolved in the ionic liquid during pretreatment.

For determination of the composition of biomass/pulp, the protocols “Extractives in biomass” and “Determination of Structural Carbohydrates and Lignin in Biomass” published by the NREL (National Renewable Energy Laboratory) were followed and all experiments were performed in triplicates.⁴

For compositional analysis of untreated biomass, *ca.* 4 g of untreated biomass (oven-dried weight) was subjected to soxhlet extraction for 24 h using 190 mL of abs. EtOH per sample, subsequently air-dried and then the weight determined. The EtOH was removed under reduced pressure and the round bottom flask containing the extractives dried in a vacuum oven over night at 45 °C, then placed in a desiccator to cool to room temperature and weighed to determine the mass of extractives. The mass of extractives was calculated using equation 5:

$$\% \text{ Extractives} = \frac{\text{Weight}(\text{flask plus extractives}) - \text{Weight}(\text{flask})}{ODW(\text{sample})} \cdot 100 \quad (5)$$

where $\text{Weight}(\text{flask plus extractives})$ is the weight of the round bottom flask containing the extractives after soxhlet extraction and drying in the oven, $\text{Weight}(\text{flask})$ is the weight of the empty round bottom flask before soxhlet extraction and $ODW(\text{sample})$ is the oven-dried weight of the biomass sample before soxhlet extraction.

In the following step, 300 ± 3 mg (oven dried weight) of extracted biomass or pulp after pretreatment were weighed into a 100 mL Ace pressure tube with a screw-cap and Teflon lining and 3 mL of 72 %

sulphuric acid were added per sample and each sample was carefully stirred using a Teflon stir rod to guarantee homogenous mixing of the biomass sample and sulphuric acid. The pressure tubes were placed in a water bath preheated to 30 °C for 1 hour and stirred every 10 min, followed by the addition of 84 mL of DI water and incubated in an autoclave (Sanyo Labo Autoclave ML5 3020 U) for 1 hour at 121 °C. The samples were then filtered through ceramic crucibles of known weight, the filtrate collected and subjected to UV-Vis spectroscopy ($\lambda = 240$ nm) using a Perkin Elmer Lambda 650 UV/Vis spectrometer (for determination of acid soluble lignin).

The acid soluble lignin content (ASL) was calculated using the following equation:

$$\%ASL = \frac{A}{l \cdot \epsilon \cdot c} \cdot 100 = \frac{A \cdot V(\text{filtrate}) \cdot \text{dilution}(\text{sample})}{l \cdot \epsilon \cdot ODW(\text{sample})} \cdot 100 \quad (6)$$

where A is the UV absorbance at 240 nm, $V(\text{filtrate})$ is the volume of the filtrate in mL and is equal to 86.73 mL, $\text{dilution}(\text{sample})$ is the dilution of the supernatant to guarantee an absorbance below 1, l is the path length of the cuvette in cm (1 cm in this case), ϵ is the extinction coefficient (12 L/g cm), c is the concentration in mg/mL and $ODW(\text{sample})$ is the oven-dried weight of the sample.

The filtrate was also subjected to HPLC analysis for monomeric sugars (glucose, xylose, arabinose, mannose and galactose) after neutralisation to pH around 5 using calcium carbonate. The samples were analysed using a Shimadzu HPLC system with refractive index detector equipped with an Aminex HPX-87P column (Biorad). The mobile phase was DI water (18.8 mΩ), the flow rate 0.6 mLmin⁻¹, the column oven set to 85 °C and the acquisition time 20 min. For retention times of the sugar monomers, see table 6-2. Calibration standards with concentrations of 0.1, 1, 2 and 4 mg/mL of each of glucose, xylose, mannose, arabinose and galactose, and 8 mg/mL of glucose were used.

Sugar recovery standards (SRS) were made as 10 mL aqueous solutions close to the expected concentration of each monomeric sugar in the samples to determine the degradation of the sugars during autoclave incubation and transferred to pressure tubes. Sulphuric acid (278 µL, 72%) was added, the pressure tube closed and autoclaved for 1 hour at 121 °C and the sugar content determined as described above. The sugar recovery standard coefficient (SRC) was determined according to the following equation:

$$SRC = \frac{c(\text{HPLC}, t) \cdot V}{\text{initial weight}} \quad (7)$$

where $c(HPLC, t)$ is the sugar concentration detected by HPLC at retention time t , V is the initial volume of the solution in mL (10.00 mL for the sugar recovery standards) and *initial weight* is the mass of the sugars weighed in (grams).

The sugar content of each analysed sample was calculated using the following equation:

$$\%Sugar = \frac{c(HPLC, t) \cdot V \cdot corr(anhydro)}{SCR \cdot ODW(sample)} \cdot 100 \quad (8)$$

where $c(HPLC, t)$ is the sugar concentration detected by HPLC at retention time t , V is the initial volume of the solution in mL (10.00 mL for the sugar recovery standards and 86.73 mL for the samples), $corr(anhydro)$ is the correction for the mass change during hydrolysis of polymeric sugars (0.90 for C₆ sugars glucose, galactose and mannose and 0.88 for C₅ sugars xylose and arabinose) and $ODW(sample)$ is the oven-dried weight of the sample.

The acid insoluble residue (AIR) in the crucible was dried overnight in a convection oven (VWR Venti-Line 115) set to 105 °C, placed in a desiccator to cool to room temperature, the weight determined and fired in a muffle oven (Nabertherm + controller P 330) to determine the ash content of the sample. The lignin content in the sample was calculated according to the following equation:

$$\%AIR = \frac{weight(crucible + AIR) - weight(crucible)}{ODW(sample)} \quad (9)$$

where AIR is the acid insoluble residue, $weight(crucible + AIR)$ is the weight of the crucible containing the AIR after drying in an oven over night, $weight(crucible)$ is the weight of the empty crucible and $ODW(sample)$ is the oven-dried weight of the sample.

The total lignin content in the untreated *Salix* and pulp was calculated by addition of the acid soluble lignin content and acid insoluble residue content of each sample:

$$\% \text{ lignin} = \% \text{ AIR} + \% \text{ ASL} \quad (10)$$

The delignification of the samples was calculated using the following equation:

$$\text{Delignification} = \frac{\text{Lignin}(\text{untreated}) - (\text{Lignin}(\text{pulp}) \cdot Y(\text{pulp}))}{\text{Lignin}(\text{untreated})} \quad (11)$$

where *Lignin(untreated)* is the lignin content in untreated *Salix*, *Lignin(pulp)* is the lignin content in the pulp and *Y(pulp)* is the oven-dried yield of pulp.

The glucan recovery after pretreatment was calculated using the following equation:

$$\text{Glucan recovery} = \frac{\text{Glucan}(\text{untreated}) - (\text{Glucan}(\text{pulp}) \cdot Y(\text{pulp}))}{\text{Glucan}(\text{untreated})} \quad (12)$$

where *Glucan(untreated)* is the glucan content of untreated *Salix*, *Glucan(pulp)* is the glucan content in the pulp and *Y(pulp)* is the oven-dried yield of pulp.

The removal of hemicelluloses from the biomass during pretreatment was calculated using the following equation:

$$\text{Hemicellulose removal} = \frac{\text{Hem}(\text{untreated}) - (\text{Hem}(\text{pulp}) \cdot Y(\text{pulp}))}{\text{Hem}(\text{untreated})} \quad (13)$$

where *Hem(untreated)* is the hemicellulose sugar content in untreated *Salix*, *Hem(pulp)* is the hemicellulose content in the pulp and *Y(pulp)* is the oven-dried yield of pulp.

6.8 2D HSQC and HMBC NMR spectroscopy of extracted lignin

For NMR measurements, 20 – 23 mg of freeze dried lignin were weighed into a 1.5 mL glass vial and dissolved overnight in 250 μ L DMSO- d_6 , then transferred to a Shigemi NMR microtube using a 1 mL plastic syringe and needle. Heteronuclear single quantum correlation (HSQC) measurements were carried out on a Bruker 600 MHz NMR machine using the following parameters: pulse sequence *hsqcetgpsi2*, spectral width of 12 ppm in F2 (^{1} H) with 2048 data points and 160 ppm in F1 (^{13} C) with 256 data points, 16 scans and 1.2 s interscan delay.

Heteronuclear Multiple Bond Correlation (HMBC) experiments were carried out on a Bruker 600 MHz NMR machine using the following parameters: pulse sequence *hmbcgp1pndqf*, spectral width of 16 ppm in F2 (^{1} H) with 2048 data points and 220 ppm in F1 (^{13} C) with 128 data points, 64 scans and 1.2 s interscan delay.

The acquired spectra were analysed using MestReNova and referenced to the solvent peak.

6.9 DOSY measurements

For the measurements, 20 – 23 mg of sample was weighed into a 1.5 mL HPLC glass vial and dissolved overnight in 250 μ L DMSO- d_6 . The solution was transferred to a NMR tube using a 1 mL plastic syringe and needle. For DOSY experiments, the spectra were recorded with the Bruker standard pulse programs “*step1s1d*” (1D) and “*step1s*” (2D). After 90° ^{1} H pulse calibration, two one dimensional ^{1} H-DOSY spectra of different magnetic field gradient strength (GPZ6=2% and 95%) were recorded. The signal intensity in the high magnetic field gradient spectrum was set to ca. 5% - 10% of the intensity in the low magnetic field gradient spectrum by adjusting the diffusion delay (d20) and the duration of the magnetic field gradient pulse (p30) in both 1D experiments simultaneously. The d20 values ranged from 200 – 600 ms, and the p30 values ranged from 1 – 2 ms. Once the desired intensity ratio of both spectra was obtained, a 2D ^{1} H DOSY spectrum was recorded by using the obtained values for d20 and p30.

6.10 Scanning electron microscopy

A JEOL JSM 5610 LV scanning electron microscope (SEM) was used to image untreated and pretreated samples. Samples were coated with a thin layer of gold and images were captured with 10 kV accelerated voltage.

6.11 Gel permeation chromatography of extracted lignin

For determination of weight average molecular weight (M_w), number average molecular weight (M_n) and polydispersity (PDI) of the isolated lignin, 20 mg of lignin were weighed into a 1.5 mL glass vial and dissolved in DMSO/LiBr (LiBr concentration = 1 mg mL^{-1}) and filtered using a $0.22 \mu\text{m}$ syringe filter and plastic syringe. The measurements were performed on an Agilent 1260 Infinity instrument equipped with a Viscotek column set (AGuard, A6000M and A3000M) with an Agilent 1260 Infinity RID detector being used for detection. HPLC grade DMSO containing LiBr (1 mg mL^{-1}) was used as eluent at a flow rate of 0.4 mL/min at 60°C . Ten pullulan standards (Agilent calibration kit with molecular weights in the range of 180 to 780,000) were used to calibrate the instrument. The data was processed using the software supplied by Agilent.

6.12 Low-Temperature Nitrogen Adsorption Measurements

The untreated biomass and pretreated pulp were analysed for surface area and pore size using the TriStar surface area and porosity analyser (micromeritics). For the measurements, about 200 mg of air-dried untreated Salix or freeze-dried pulp were weighed into the sample vial and degassed using nitrogen over night at 45°C . The adsorption-desorption isotherm was measured at 77 K and high-purity N_2 was used as adsorbate. The surface area, pore size and pore volume was calculated by the manufacturer's software based on the adsorption-desorption data using the Braunauer-Emmett-Teller model of multilayer absorption for all the samples.

6.13 X-Ray Powder Diffraction

The samples (raw biomass and pulp) were ground into a powder and sieved to a particle size of below 45 μm . The samples were analysed using a X'PERT PRO PANalytical powder X-Ray diffractometer with $\text{Cu K}\alpha$ radiation ($\lambda = 1.542 \text{ \AA}$) operating at 40 kV and 40 mA. Diffractograms were collected in the range of $2\theta = 5 - 50^\circ$ with a step size of 0.033423° and a step time of 70 s.

The crystallinity index (CI) of the untreated biomass and pulp samples was calculated according to the method by Segal⁵ *et al.* using the following equation:

$$CI = \frac{(I_{002} - I_{am})}{I_{am}} \cdot 100 \quad (14)$$

where I_{002} corresponds to the intensity of the diffraction signal assigned to the crystalline portion of cellulose in raw biomass or pulp (at position $2\theta = 22.5^\circ$) and I_{am} corresponds to the intensity of the diffraction signal of the amorphous portion (i.e., hemicellulose, lignin, and amorphous cellulose) at position of $2\theta = 18^\circ$.

The crystallite size was calculated from the diffractogram according to the Scherrer⁶ equation using the full width at half maximum value (FWHM) of the peak at position $2\theta = 22.5^\circ$. The correction factor for instrument broadening was included in the calculation.

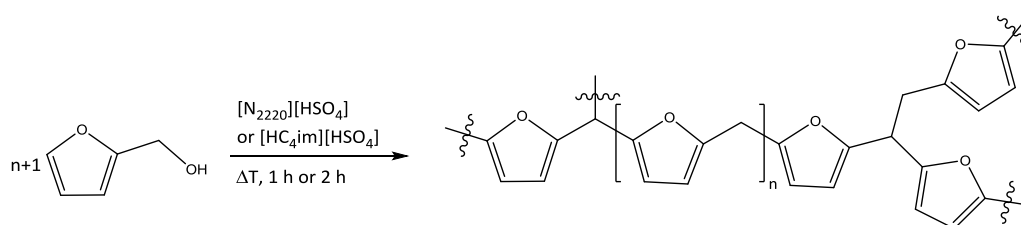
$$\tau = \frac{k \cdot \lambda}{\beta \cdot \cos\theta} \quad (15)$$

where k is a constant depending on the shape of the crystallites (here 0.94 for spherical crystals with cubic symmetry), τ is the average size of the cellulose crystallite, λ is the X-Ray wavelength of 0.1542 nm, β is the line broadening at full width at half maximum (FWHM) after subtracting the instrumental line broadening (in radians) and θ is the Bragg angle (in degrees).

6.14 Gel permeation chromatography of cellulose

Prior to the analysis, the samples were grinded into particles less than 60 mesh and de-lignified by sodium chlorite aqueous solution according to the method of Wise et al.¹ The delignified samples were pre-activated by a water/acetone/ N,N-dimethylacetamide (DMAc) solvent exchange sequence. The samples were then dissolved in a 90 g/L lithium chloride (LiCl)/DMAc mixture at room temperature. The solution was diluted to 9 g/l LiCl/DMAc and filtered through a 0.2 mm syringe filter and analyzed by using a Dionex Ultimate 3000 system with refractive index (RI) detection (Shodex RI-101).

6.15 Synthesis of polyfurfuryl alcohol in acidic ionic liquid



The synthesis of polyfurfuryl alcohol from furfuryl alcohol was carried out in 15 mL Ace pressure tube with screw-cap and Teflon lining equipped with a stirrer bar. Two ionic liquids were tested for the polymerisation reaction, namely $[N_{2220}][HSO_4]_{80\%}$ and $[HC_4im][HSO_4]_{80\%}$. The IL (5.0 mL) was transferred into the pressure tube using a 5 mL Eppendorf pipette and furfuryl alcohol (500 μ L, 5.67 mmol, 10v%) was added using a 1 mL Eppendorf pipette. The mixture was vortexed for 30 seconds to guarantee homogeneity and then subjected to a preheated oil bath and heated under stirring for 1 or 2 hours at a constant temperature. The reaction mixture was then transferred to a 50 mL Falcon tube and the polymeric product was precipitated via addition of anti-solvent (DI water). The mixture was incubated for 1 hour and then centrifuged for 30 min at 3000 rpm. The water/IL phase was carefully decanted and the washing step repeated another 3 times. The product was freeze-dried for 2 days and a light to medium dark brown powder was recovered. To determine the yields of the polyfurfuryl alcohol polymers the products were weighed on aluminium foil.

¹ L. E. Wise, M. Murphy, A. A. D'Addieco, Pap. Trade J. 1946, 122, 35– 42

6.16 Synthesis of lignin-PFA copolymers from extracted lignin in acidic ionic liquid

The synthesis of lignin-PFA copolymers was carried out similarly to the homopolymerisation of furfuryl alcohol. Lignin (100 mg) isolated from *Salix* under three different pretreatment severities (1 hour at 120 °C, 150 °C and 170 °C) was weighed into a 15 mL Ace pressure tube with screw-cap and Teflon lining equipped with a stirrer bar. Two ionic liquids were tested for the polymerisation reaction, namely [N₂₂₂₀][HSO₄]_{80%} and [HC₄im][HSO₄]_{80%}. The IL (5.0 mL) was transferred into the pressure tube using a 5 mL Eppendorf pipette. The mixture was vortexed for 30 seconds to guarantee homogeneity and then put into a water bath preheated to 30 °C for 30 min to guarantee complete dissolution of the lignin in the IL. Three different amounts of furfuryl alcohol (Table 6-3) were added to the lignin/IL mixture and the reaction mixture was vortexed again for 30 seconds and subjected to a preheated oil bath and heated under stirring for 1 or 2 hours at a constant temperature.

Table 6-3. Lignin and furfuryl alcohol amounts for the synthesis of lignin-PFA copolymers.

Lignin [mg]	Furfuryl alcohol [μL]	n(FA) [mmol]	Lignin/FA ratio [wt%]
100	88	1.01	1:1
100	176	2.02	1:2
100	352	4.05	1:4

The reaction mixture was then transferred to a 50 mL Falcon tube and the polymeric product was precipitated via addition of anti-solvent (DI water). The mixture was incubated for 1 hour and then centrifuged for 30 min at 3000 rpm. The water/IL phase was carefully decanted and the washing step repeated another 3 times. The product was freeze-dried for 2 days and a light to medium dark redbrown powder was recovered. To determine the yields of the lignin-PFA copolymers the products were weighed on aluminium foil.

6.17 Thermal stability of extracted lignin, copolymers and resins

The thermal stability of lignin, lignin-PFA copolymers and resins was tested using a [STA 449 F5 Jupiter](#) instrument under 50 mLmin⁻¹ N₂ flow and 50 mLmin⁻¹ O₂ flow. Prior to measurements, the samples were freeze-dried for 72 hours to avoid any water artefacts. For each measurement, 20 – 30 mg of sample were weighed into a pre-weighed fused silica sample pan and the weight was recorded. Additionally, an empty reference pan was weighed and the weight recorded. Both pans were placed

on the sample table, the instrument was closed and the measurements started. DSC and TGA data was collected simultaneously and the following heating profile was applied: 30 °C to 800 °C with a heating rate of 5 °C/min. The data was analysed using the software supplied by the manufacturer of the instrument.

6.18 Elemental analysis of lignin-PFA copolymers

Elemental analysis was carried out on the lignin-PFA copolymers to determine their composition. Measurements were carried out on a Vario MICRO CUBE instrument equipped with a combustion column using tungsten (IV) oxide (operating at 1150 °C), a reduction column using reduced copper wires (operating at 850 °C) and an adsorption column (operating from 40 – 210 °C) as well as a TCD detector. The instrument uses Helium as carrier and flushing gas and the combustion is carried out by pulse injection of oxygen. For each measurement, 2.00 mg of sample or sulfanilamide standard were weighed into pre-weighed tin boats for elemental microanalysis and the boats were subsequently folded into small rectangular packages paying attention to remove all of the air from the packages. The tin boats containing the samples were then placed in the instrument sample rack and analysed separately. Experiments were carried out in triplicates and sulfanilamide standards were used. The data was analysed using the software supplied by the manufacturer of the instrument.

6.19 Elemental analysis of modified lignin

CHNSBr analysis of the modified lignins was performed in duplicate by MEDAC Ltd. (Chobham, UK) by dynamic flash combustion analysis and thermal conductivity detection. Oxygen content was obtained by difference. Accuracy is $\pm 0.30\%$ absolute.

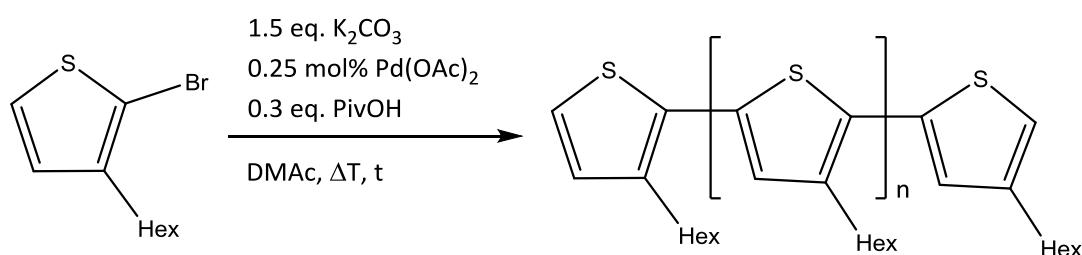
6.20 General working procedure of the modification of lignin with bromine containing organic compounds in ionic liquid solution

Several novel lignin compounds containing bromine functionalities were synthesized in the ionic liquid solution $[N_{2220}][HSO_4]_{80\%}$. The lignin used in these experiments was isolated via the ionSolv pretreatment from the *Salix* varieties Shrubby Willow and Bowles Hybrid under mild pretreatment

conditions (120 °C for 4 hours) using the IL solution $[N_{2220}][HSO_4]$. For details of the lignin extraction see section 6.5. The synthesis of the modified lignin was carried out in 15 mL Ace pressure tube with screw-cap and Teflon lining equipped with a stirrer bar and the IL solution $[N_{2220}][HSO_4]_{80\%}$ was used as solvent. All experiments were performed in triplicates and lignin (100 mg) in 2.5 mL IL was used as a control experiment. Lignin (100 mg) was weighed into the pressure tubes, the IL (1.5 mL) was added using a 5 mL Eppendorf pipette and the mixture was vortexed for 30 seconds. The lignin/IL mixture was subjected to a pre-heated water bath (30 °C) and sonicated for 30 min to guarantee complete dissolution of the lignin. The organic compounds were weighed into another pressure tube in different equivalents (0.2, 0.4, 0.6, 0.8 and 1.0) to the lignin (assuming that the molecular weight of a lignin C_9 unit equals 185 g mol^{-1}) and dissolved in IL (1.0 mL) in the water bath for 20 min or until completely dissolved. The two IL solutions were combined in one pressure tube and subjected to an oven at a specific temperature for a specific reaction time. The reaction mixture was then transferred to a 50 mL Falcon tube and the polymeric product was precipitated via addition of 40 mL anti-solvent (DI water). The mixture was incubated for 1 hour and then centrifuged for 30 min at 3000 rpm. The water/IL phase was carefully decanted and the washing step repeated another 3 times. The lignin was then washed with 20 mL diethylether 5 times to remove any unreacted small organic compound from the product. The product was freeze-dried for 2 days and a light to medium dark brown powder was recovered. To determine the yields of the polymers the products were weighed on aluminium foil.

6.21 General working procedure for the polymerisation of 2-Bromo-3-hexylthiophene via

DArP



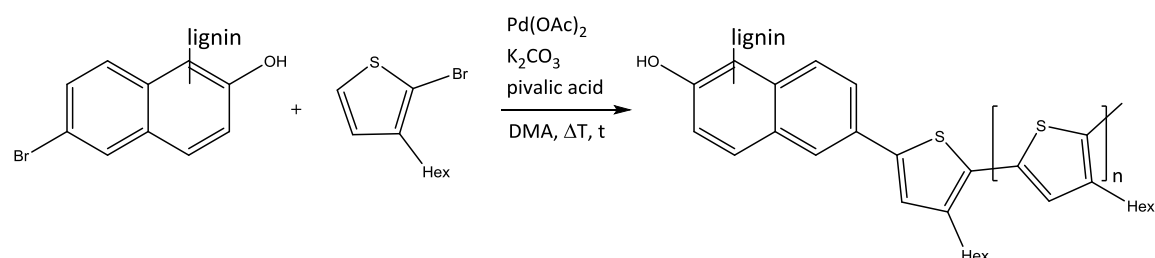
All steps of the synthesis of 3-hexylpolythiophene (P3HT) were carried out under inert gas atmosphere and the glassware was flame-dried and flushed with N_2 three times before use. All reagents and the solvent were stored in the glovebox. In a glovebox, a stock solution of the $Pd(OAc)_2$ catalyst (0.25 mol% in DMAc) was prepared. 3-Hexylthiophene (247 mg, 1.47 mmol, 1.0 eq.), pivalic acid (31 mg, 0.30 mmol, 0.3 eq.) and K_2CO_3 (207 mg, 1.50 mmol, 1.5 eq.) were weighed into a separate Schlenk

round bottom flask and dissolved in 20 mL DMAc. Then, the catalyst stock solution was added to the reaction mixture, the Schlenk flask was sealed well and the mixture was removed from the glovebox and subjected to a pre-heated aluminium heating block (60 °C) and heated under stirring 48 hours. After two days, the mixture was cooled to room temperature and poured into 200 mL dry MeOH to precipitate the polymer. The product was filtered off and soxhleted with MeOH (190 mL), followed by hexanes (*n*-hexane:cyclohexane 1:1 v/v, 190 mL) and finally CHCl₃ (190 mL) for 22 hours each time. The MeOH and hexane phases were discharged of and the CHCl₃ was removed under vacuum. The purple product was recovered in a yield of 35 %.

To characterise the product, ¹H and ¹³C NMR spectra were recorded on a Bruker 600 MHz spectrometer. Chemical shifts (δ) of the recovered product are reported in ppm below.

¹H NMR: δH (600 MHz, CDCl₃)/ppm: 6.98 (s, 1H, **CH_{arom.}**), 2.81 (t, J = 8.1 Hz, 2H, **CH₂**), 1.71 (q, J = 7.7 Hz, 2H, **CH₂**), 1.31 – 1.48 (m, 6H, **CH₂**), 0.92 (t, J = 6.5 Hz, 3H, **CH₃**). ¹³C NMR: δC (150.9 MHz, CDCl₃)/ppm: 139.9 (**C_q**, **C_{arom.}**), 133.7 (**C_q**, **C_{arom.}**), 130.5 (**C_q**, **C_{arom.}**), 128.6 (**CH**, **C_{arom.}**), 31.7 (**CH₂**, hexyl chain), 30.5 (**CH₂**, hexyl chain), 29.5 (**CH₂**, hexyl chain), 29.3 (**CH₂**, hexyl chain), 22.7 (**CH₂**, hexyl chain), 14.1 (**CH₃**, hexyl chain).

6.22 General working procedure of the synthesis of lignin-P3HT copolymer via DArP



All steps of the synthesis of the copolymer of 6-B-2-N modified lignin and 3HT were carried out under inert gas atmosphere and the used glassware was flame-dried and flushed with N₂ three times before use. The modified lignin was dried on the Schlenk line overnight and stored in the glove box. All reagents and the solvent were stored in the glovebox. In a glovebox, a stock solution of the Pd(OAc)₂ catalyst (0.25 mol%, 1 mol% and 10 mol% in DMAc) was prepared. Lignin (250 mg, 1.0 wt eq.), 3-Hexylthiophene (247 mg, 1.47 mmol, 1.0 eq.), pivalic acid (31 mg, 0.30 mmol, 0.3 eq.) and a base (either K₂CO₃ (207 mg, 1.50 mmol, 1.5 eq.) or NaOH (101 mg, 2.5 mmol, 2.5 eq.)) were weighed into a separate Schlenk round bottom flask and dissolved in 10 mL DMAc. Then, the catalyst stock solution was added to the reaction mixture, the Schlenk flask was sealed well and the mixture was removed

from the glovebox and subjected to a pre-heated aluminium heating block (60 °C or 100 °C) and heated under stirring for a certain amount of time. After this, the mixture was cooled to room temperature and poured into 200 mL dry MeOH or diethylether to precipitate the polymer. The product was filtered off and soxhleted with hexanes (*n*-hexane:cyclohexane 1:1 v/v, 190 mL) and CHCl₃ (190 mL) for 22 hours each time and finally washed again with diethylether. The brown solid material was recovered by removing the solvent under vacuum.

7. Final Conclusions

The work of this thesis investigated to use of the hardwood willow (*Salix*) for the production of the platform chemical glucose and value-added lignin composite materials using ionic liquids as both the solvent for willow pretreatment *via* biomass fractionation and as solvent for lignin functionalization.

In my opinion, a cost competitive biorefinery needs to fulfil several requirements:

- **Selection of appropriate feedstock** – The hardwood willow is very good raw material to be used in commercial biorefineries because it shows high resistance to pests, provides high biomass yield, has a low requirement for fertilizer and water and can be grown on sparse land which is not in competition with food production. Additionally, it can be grown in short rotation coppice to maximise biomass output.
- **Pretreatment conditions** – Optimisation of the pretreatment conditions is needed to find the sweet spot between maximum glucose yield and energy/material input. Enzymatic hydrolysis of the pretreated lignocellulose yields the platform chemical glucose (and xylose) which can be used to produce either bioethanol or other chemicals, e.g. lactic acid, glycols, or furfurals. In order to guarantee a cost competitive biorefinery, a thorough market study and the selection of appropriate products from sugars is needed.
- **Use of protic ionic liquids** – The protic ionic liquid/water mixture $[N_{2220}][HSO_4]_{80\%}$ is a promising solvent for biomass deconstruction due to its simple synthesis from low-cost, readily available starting materials. However, further studies such as up-scaling and corrosion tests are required to fully understand the use of this novel green solvent in industrial processes.
- **Utilization of lignin** – All streams of the biorefinery need to be valorised to be cost competitive in an industrial scale. Here, the focus should also lie on creating value added products from sulphur-free lignin which can be recovered from IL pretreatment with $[N_{2220}][HSO_4]_{80\%}$. Two possible routes to utilize lignin can be considered: 1) production of aromatic chemicals and 2) lignin functionalization for the production of materials. The first route requires good catalytic processes and downstream purification, which is very cost intensive. The second route was studied in this thesis and showed promising results. The creation of functional materials from lignin is possible and should be in the focus of the process design of any lignocellulosic biomass based biorefinery.

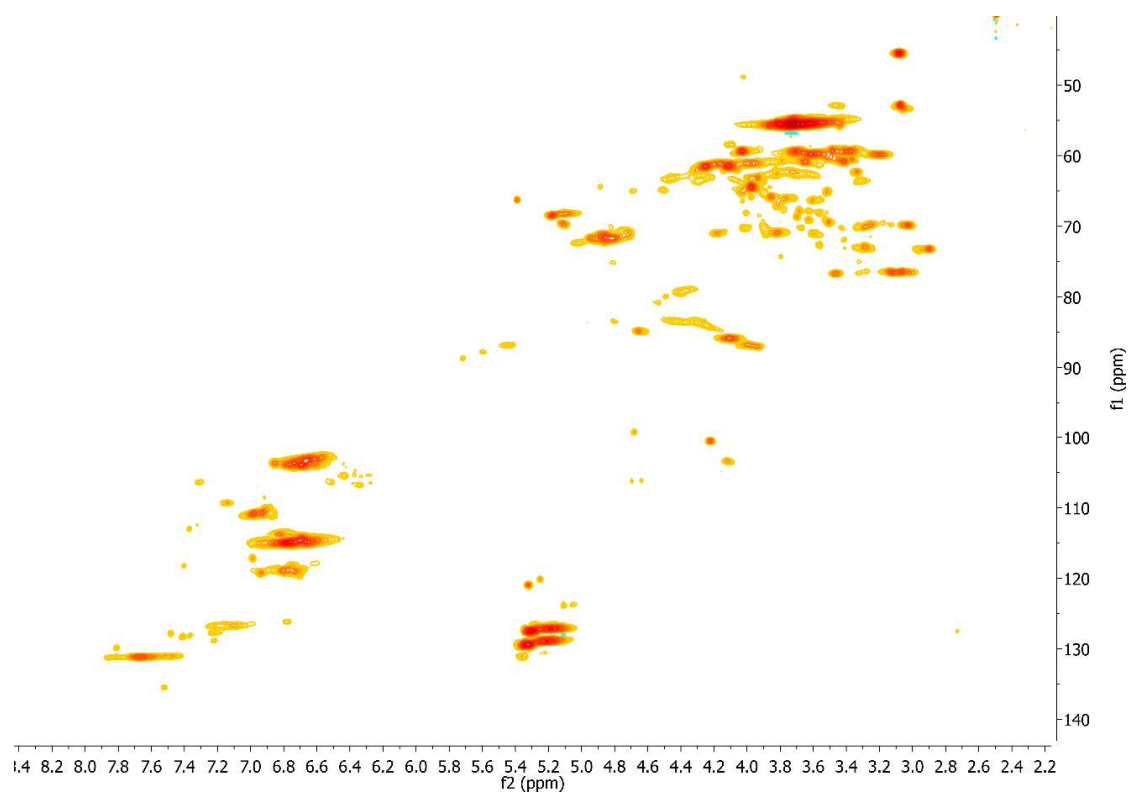


Figure A-1. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 120 °C for 0.5 hours with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 1.02$.

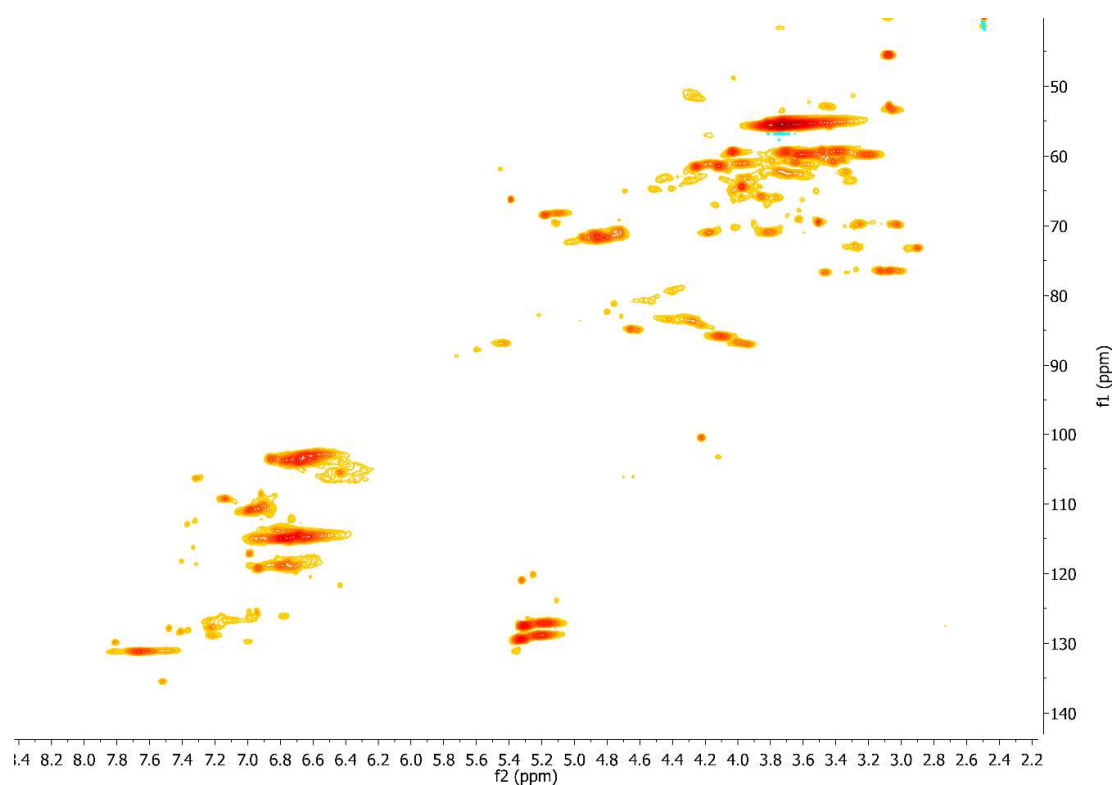


Figure A-2. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 120 °C for 1 hour with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 1.02$.

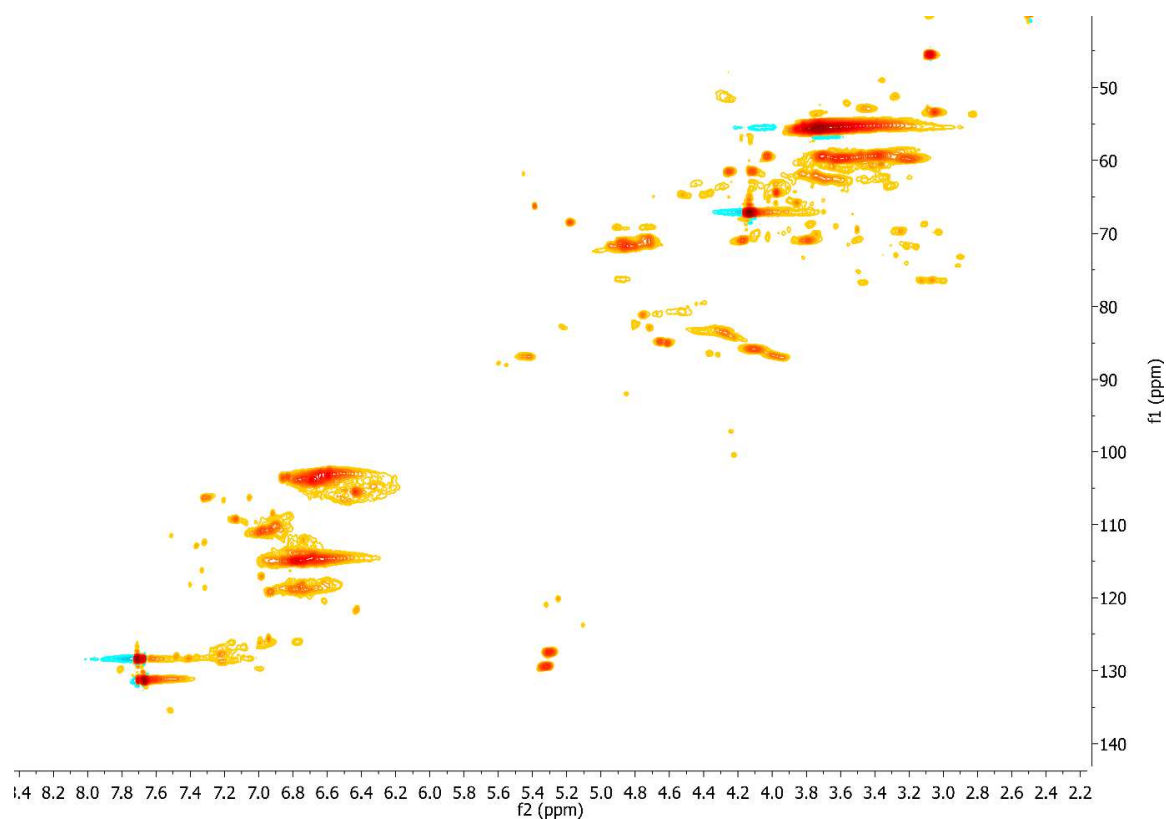


Figure A-3. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 120 °C for 2 hours with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 1.02$.

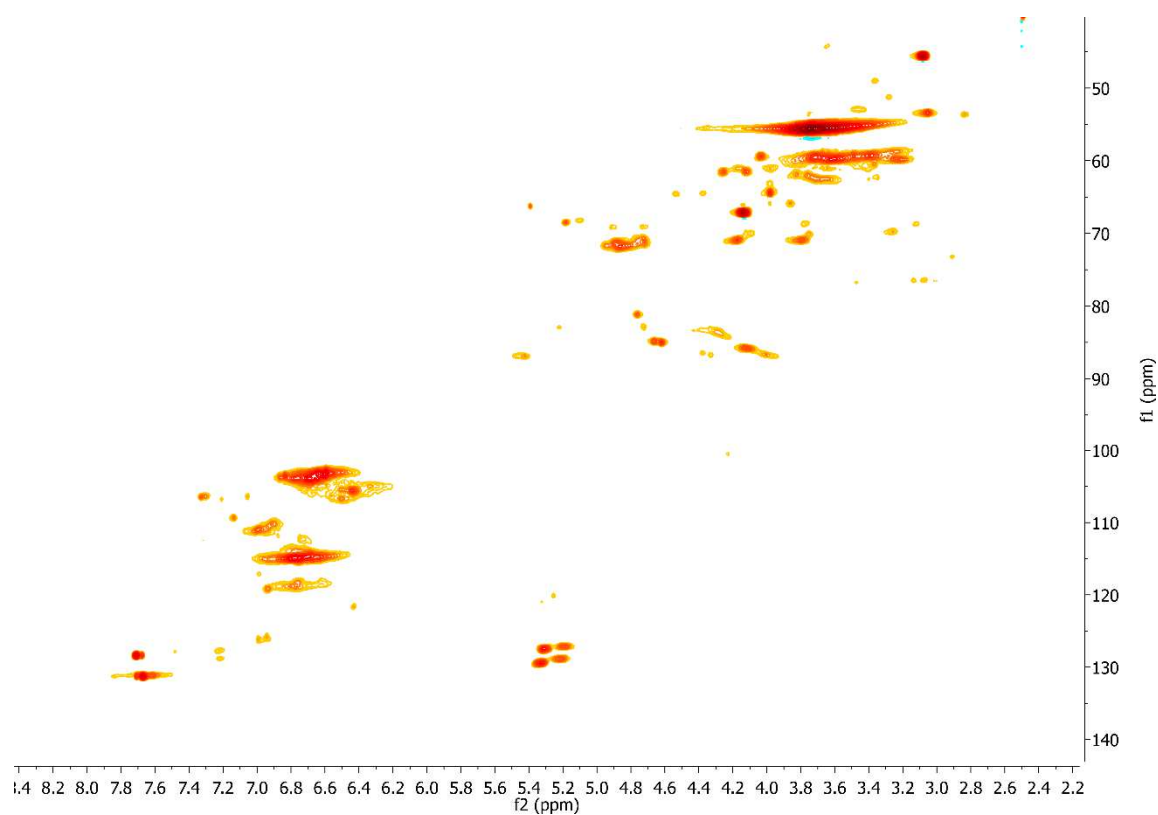


Figure A-4. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 120 °C for 4 hours with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 1.02$.

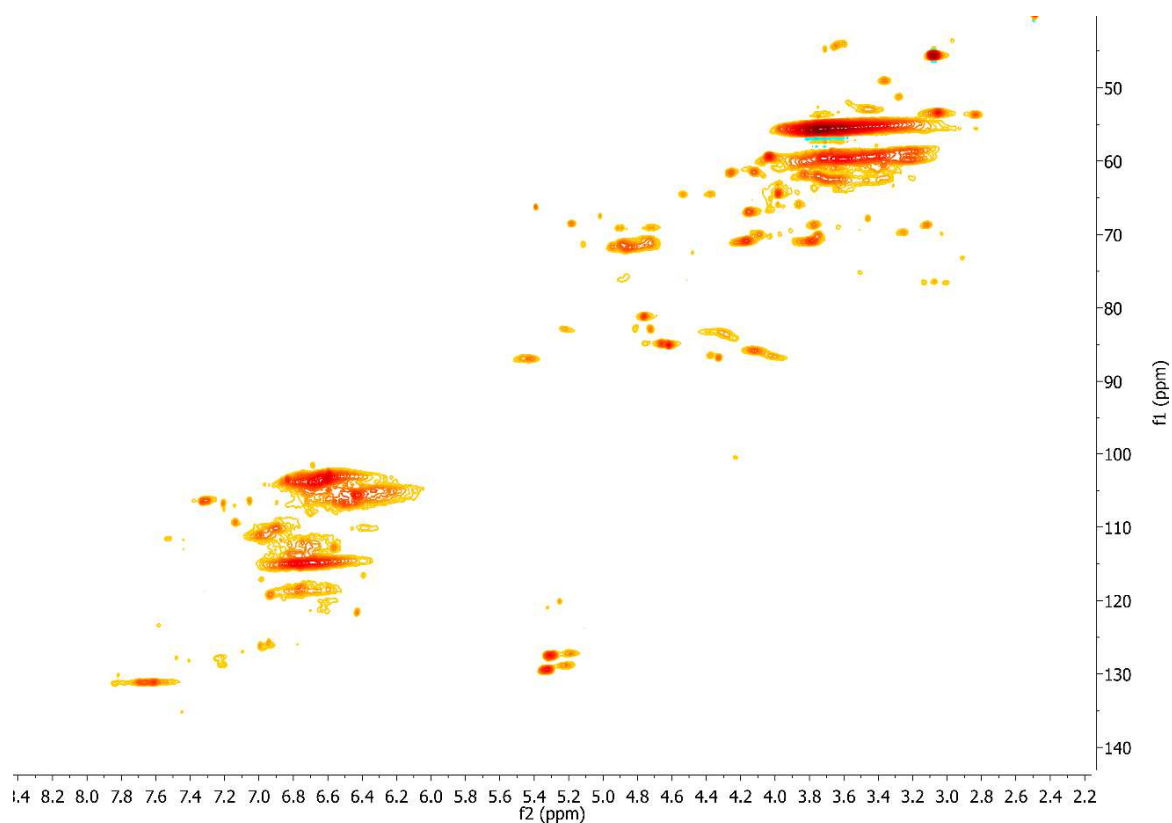


Figure A-5. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 120 °C for 8 hours with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 1.02$.

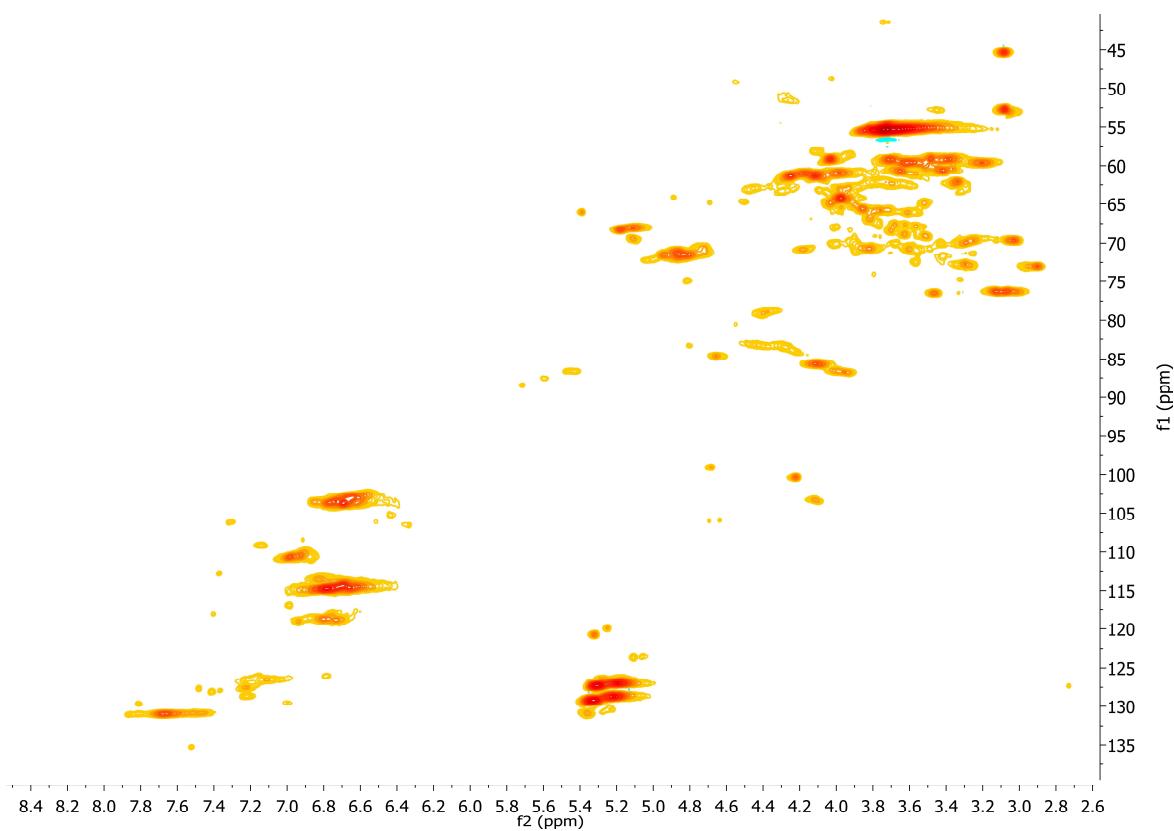


Figure A-6. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 120 °C for 0.5 hours with $[N_{2220}][HSO_4]_{80\%}$ $a/b = 0.98$.

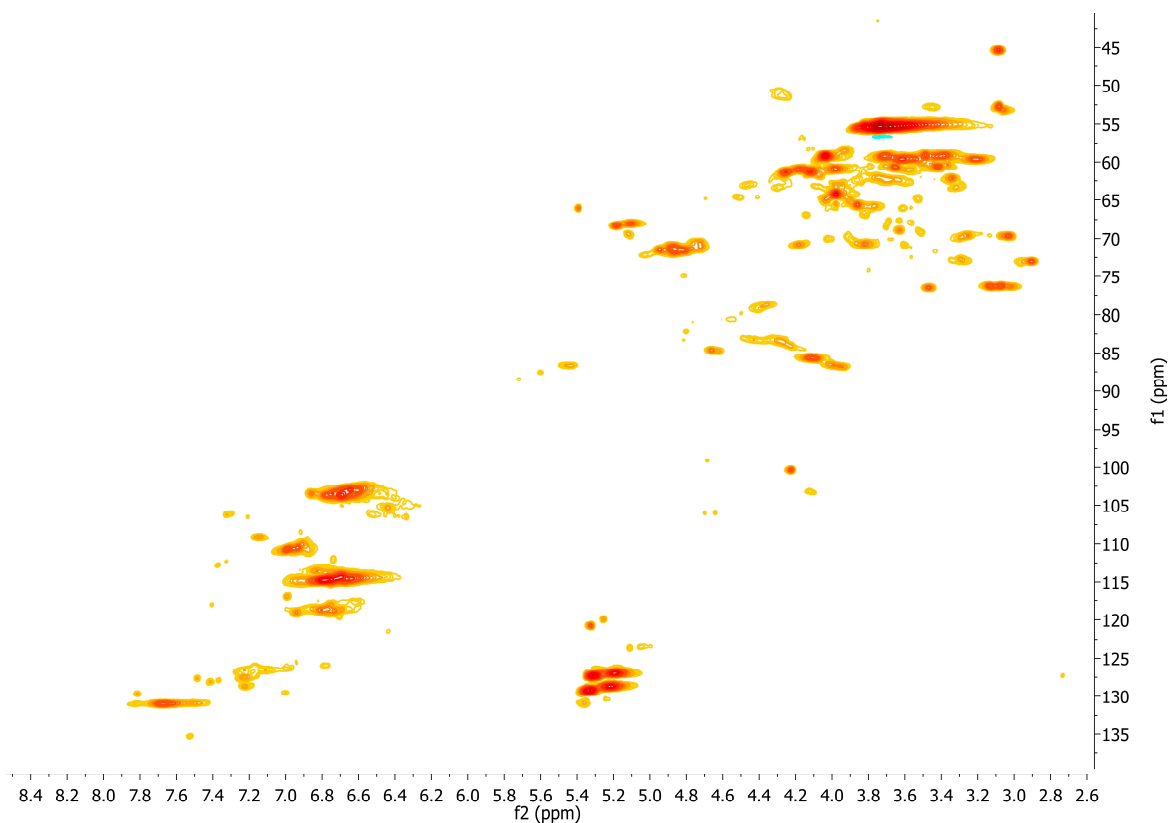


Figure A-7. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 120 °C for 1 hour with $[N_{2220}][HSO_4]_{80\%}$ $a/b = 0.98$.

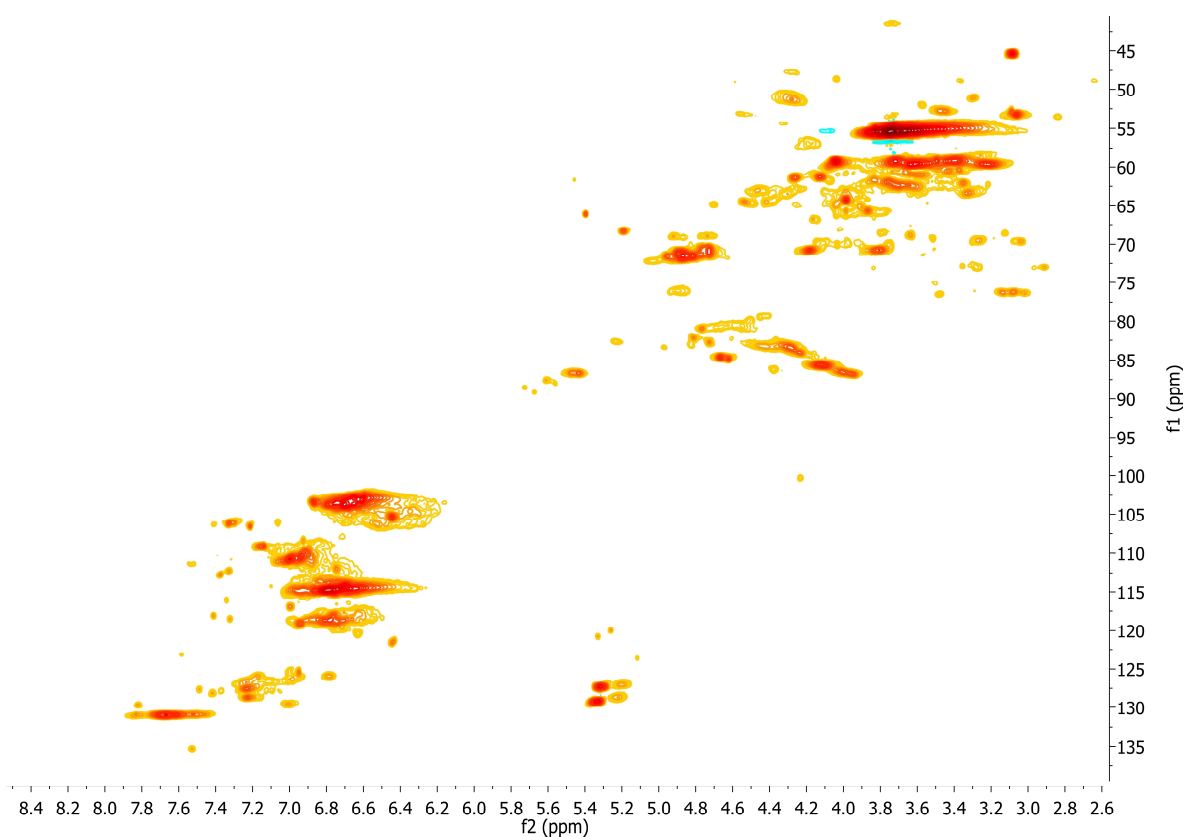


Figure A-8. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 120 °C for 2 hours with $[N_{2220}][HSO_4]_{80\%}$ $a/b = 0.98$.

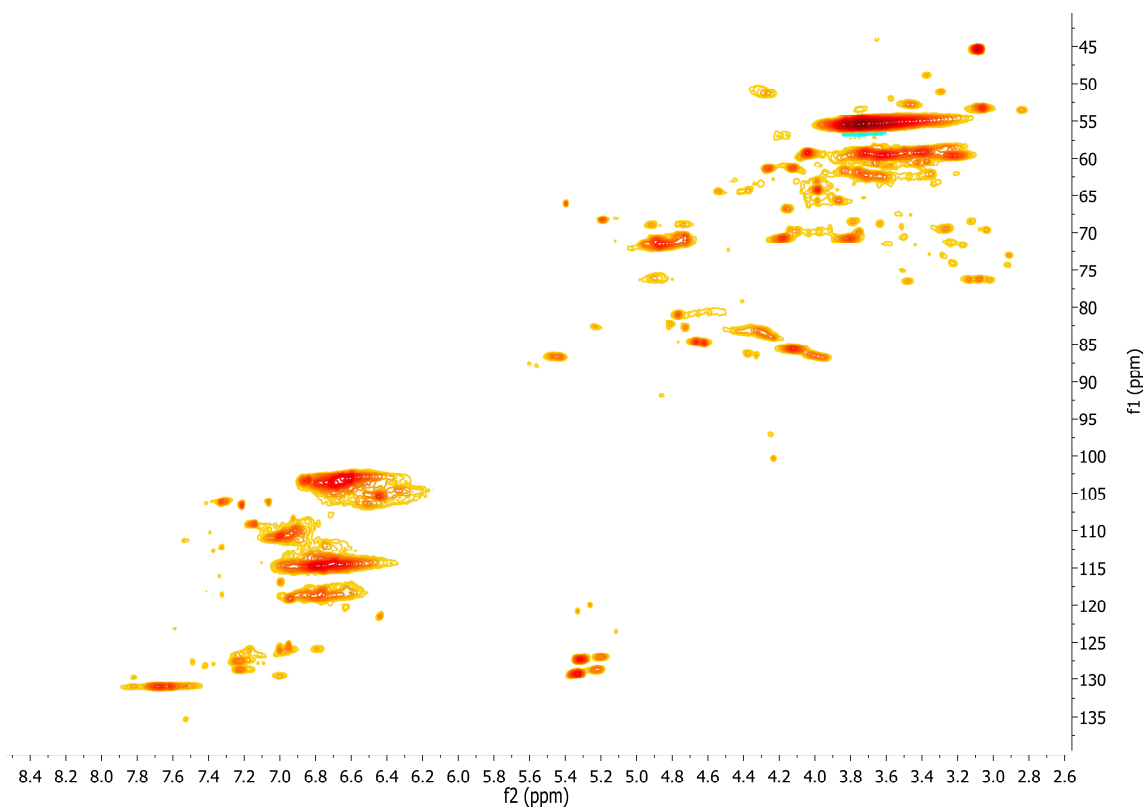


Figure A-9. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 120 °C for 4 hours with $[N_{2220}][HSO_4]_{80\%}$ a/b = 0.98.

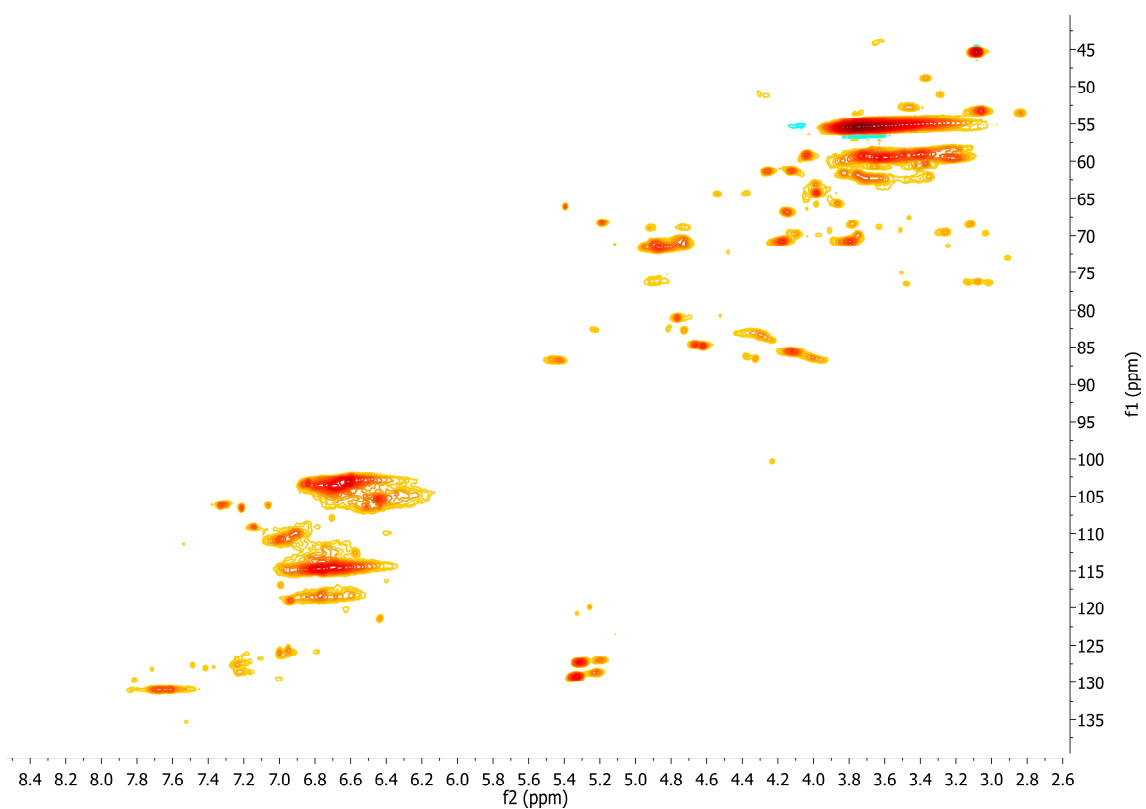


Figure A-10. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 120 °C for 8 hours with $[N_{2220}][HSO_4]_{80\%}$ a/b = 0.98.

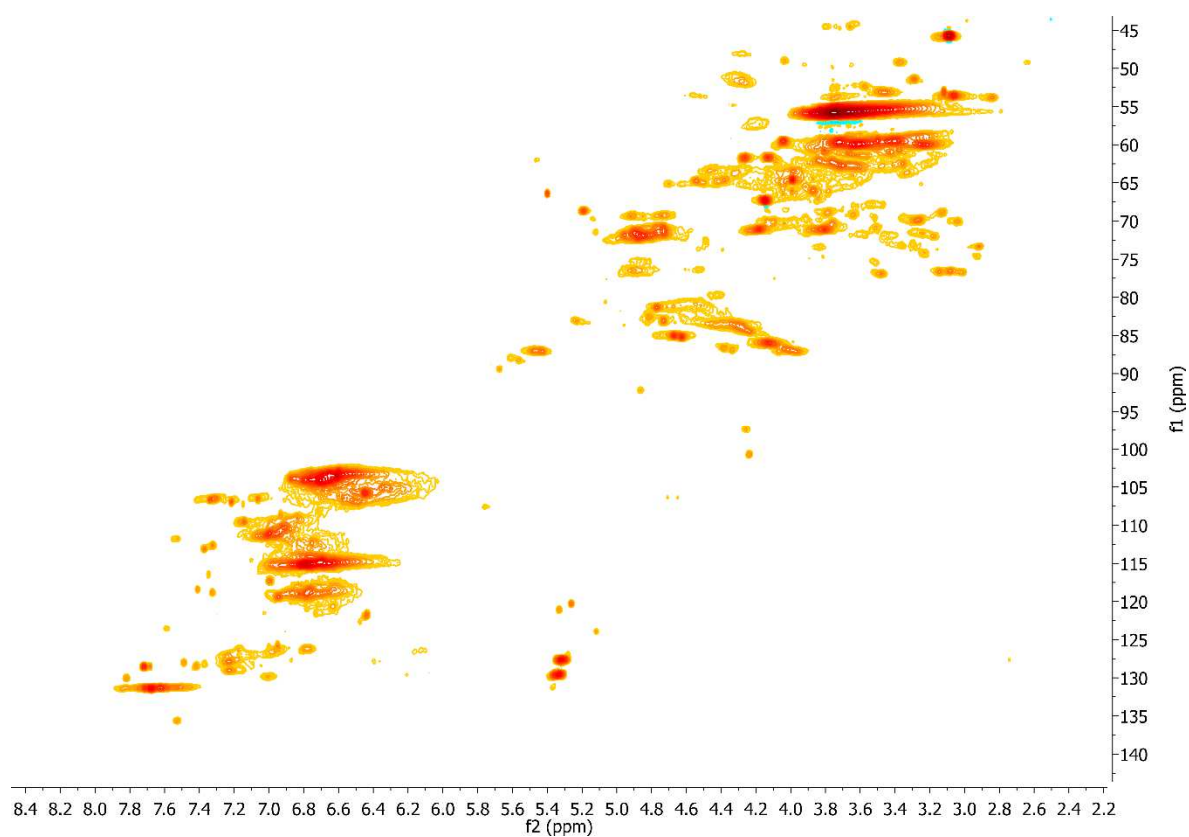


Figure A-11. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 150 °C for 0.5 hours with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 1.02$.

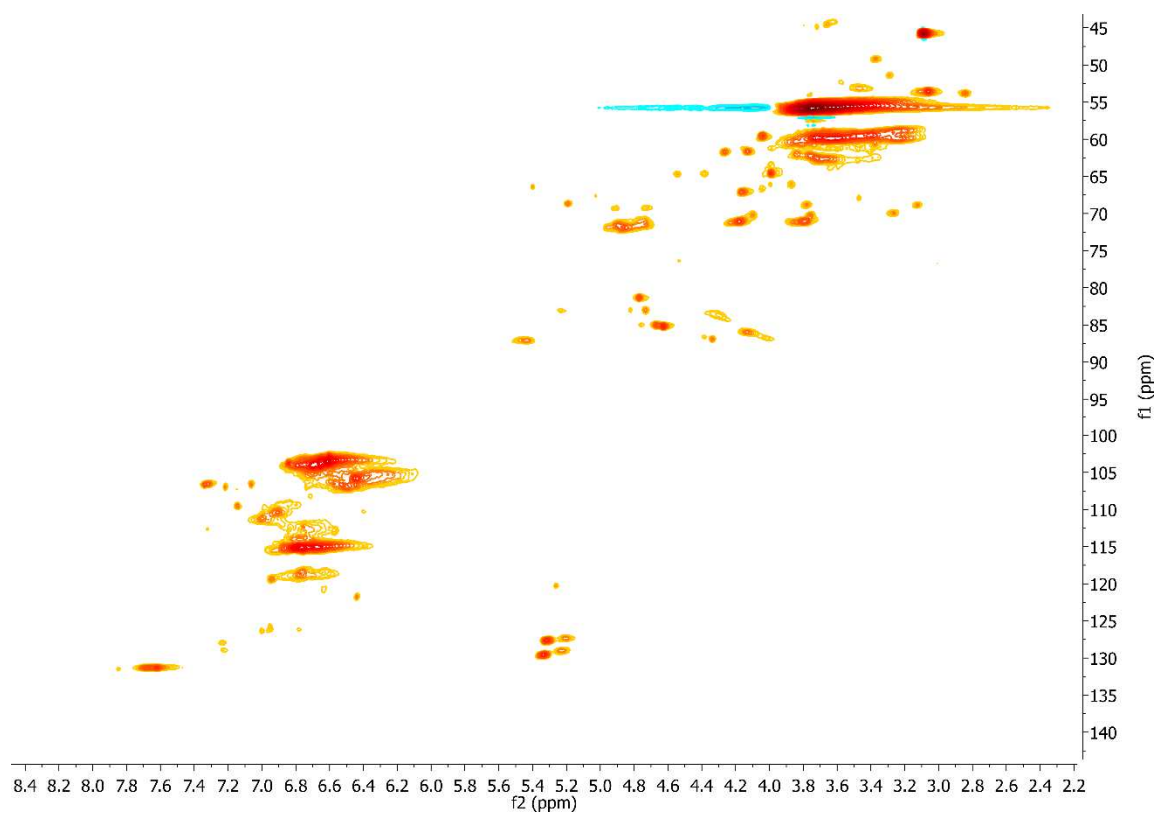


Figure A-12. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 150 °C for 1 hour with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 1.02$.

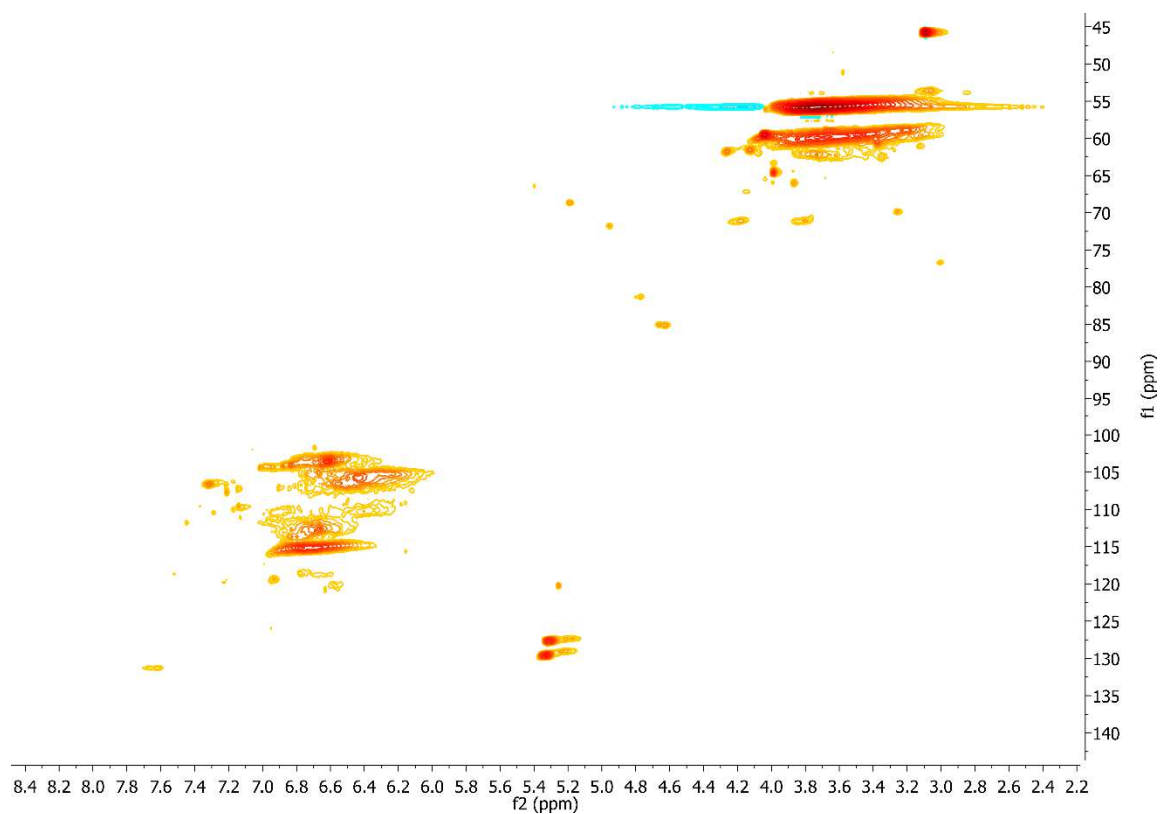


Figure A-13. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 150 °C for 2 hours with [N₂₂₂₀][HSO₄]_{80%} with a/b = 1.02.

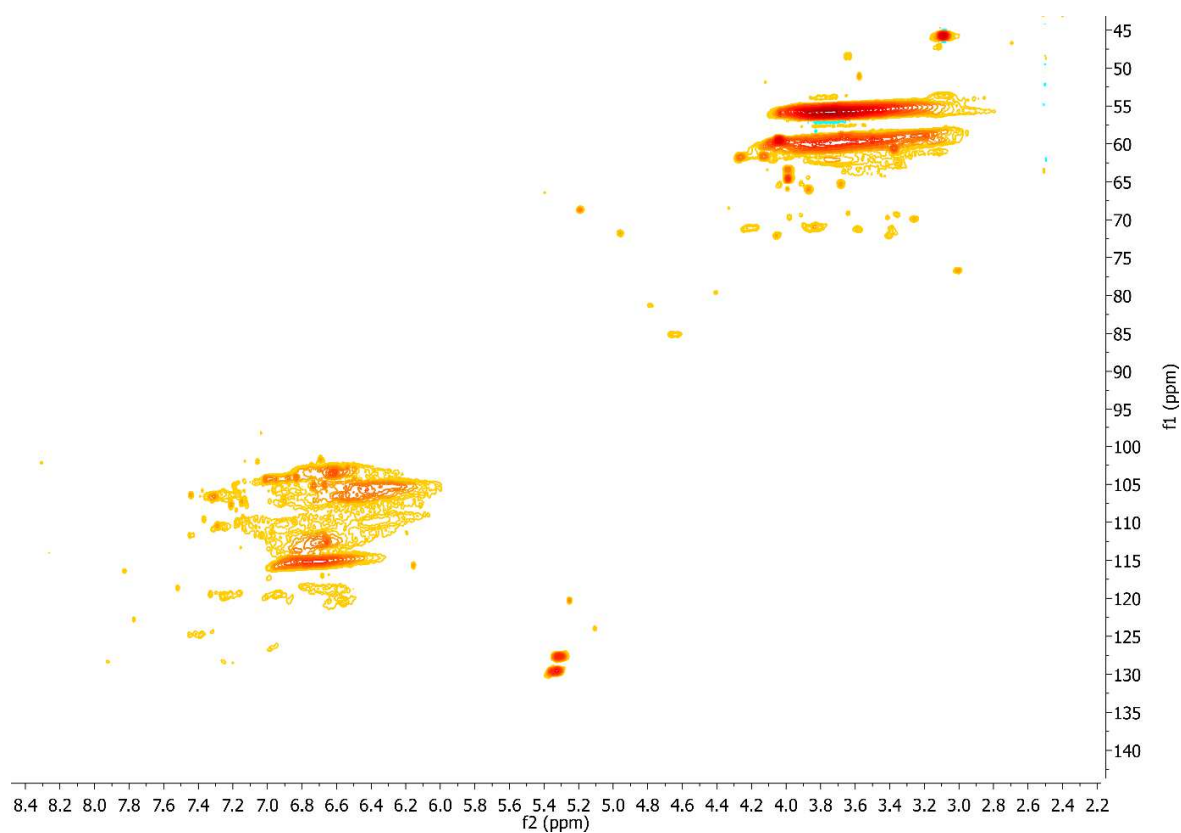


Figure A-14. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 150 °C for 4 hours with [N₂₂₂₀][HSO₄]_{80%} with a/b = 1.02.

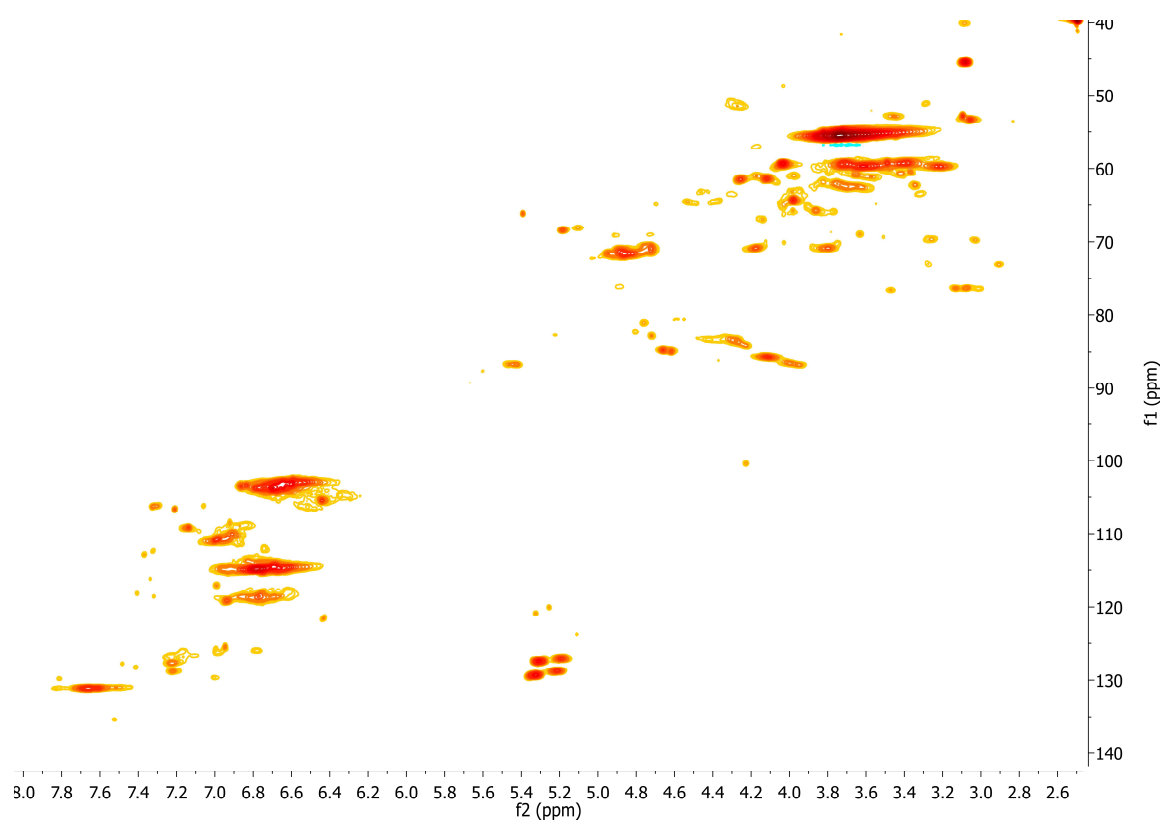


Figure A-15. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 150 °C for 0.5 hours with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 0.98$.

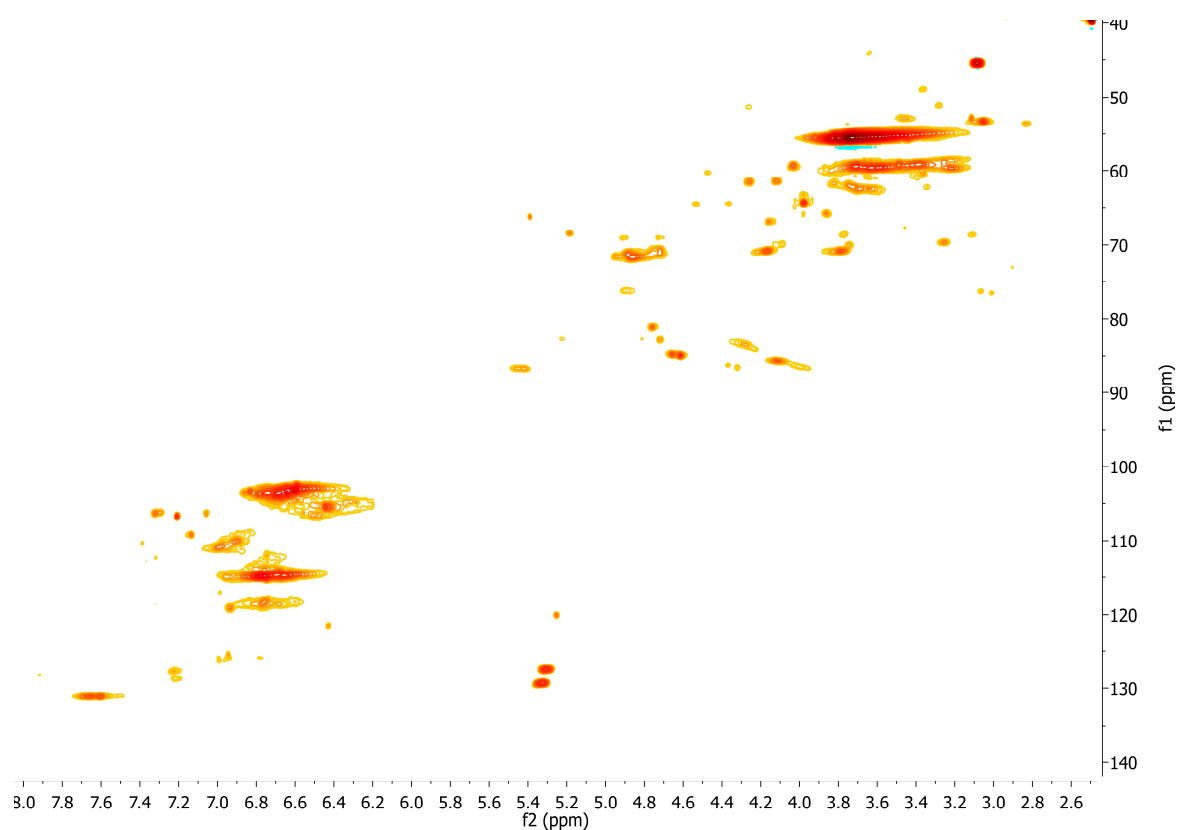


Figure A-16. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 150 °C for 1 hour with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 0.98$.

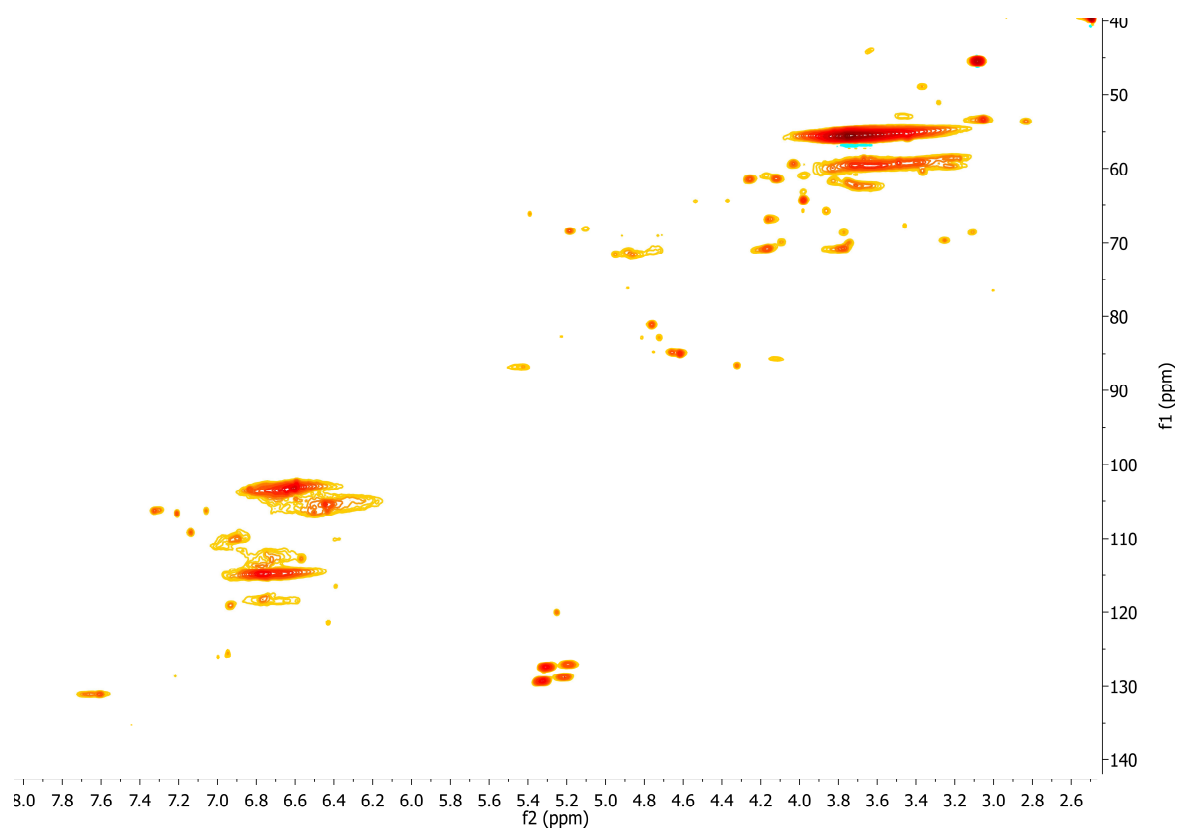


Figure A-17. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 150 °C for 2 hours with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 0.98$.

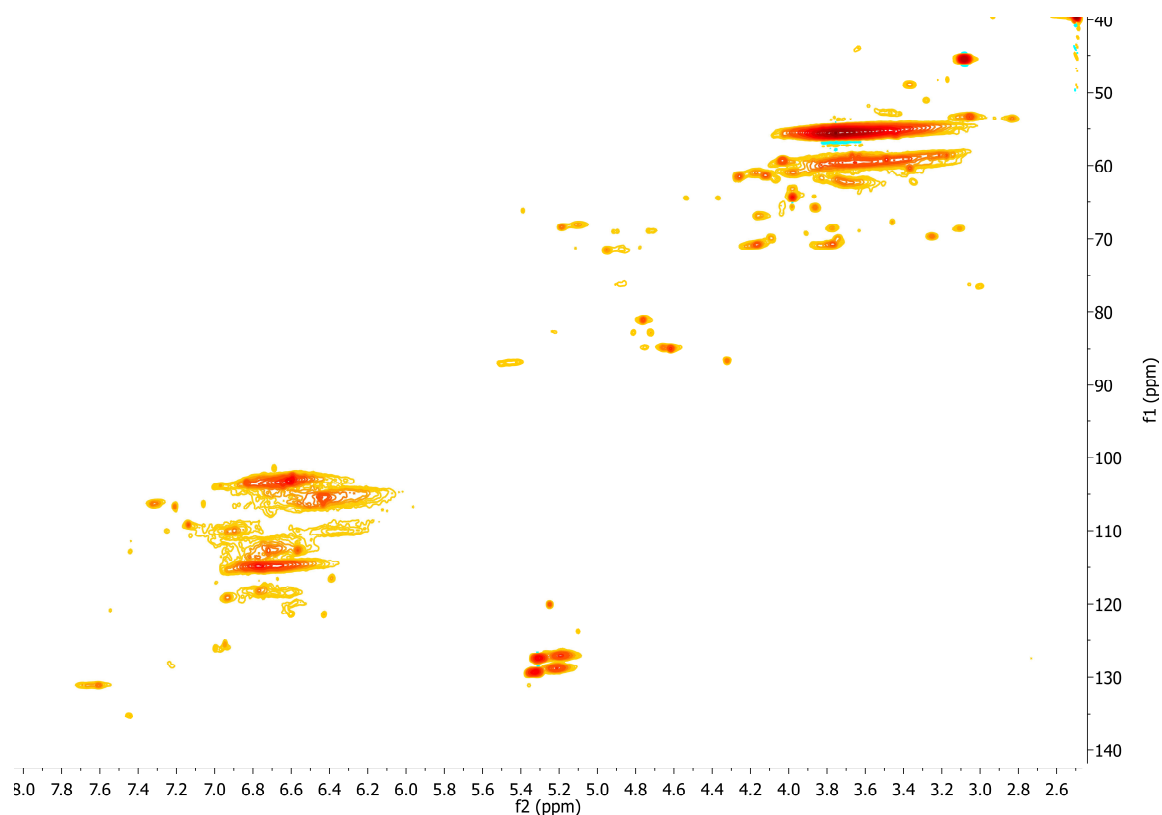


Figure A-18. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 150 °C for 4 hours with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 0.98$.

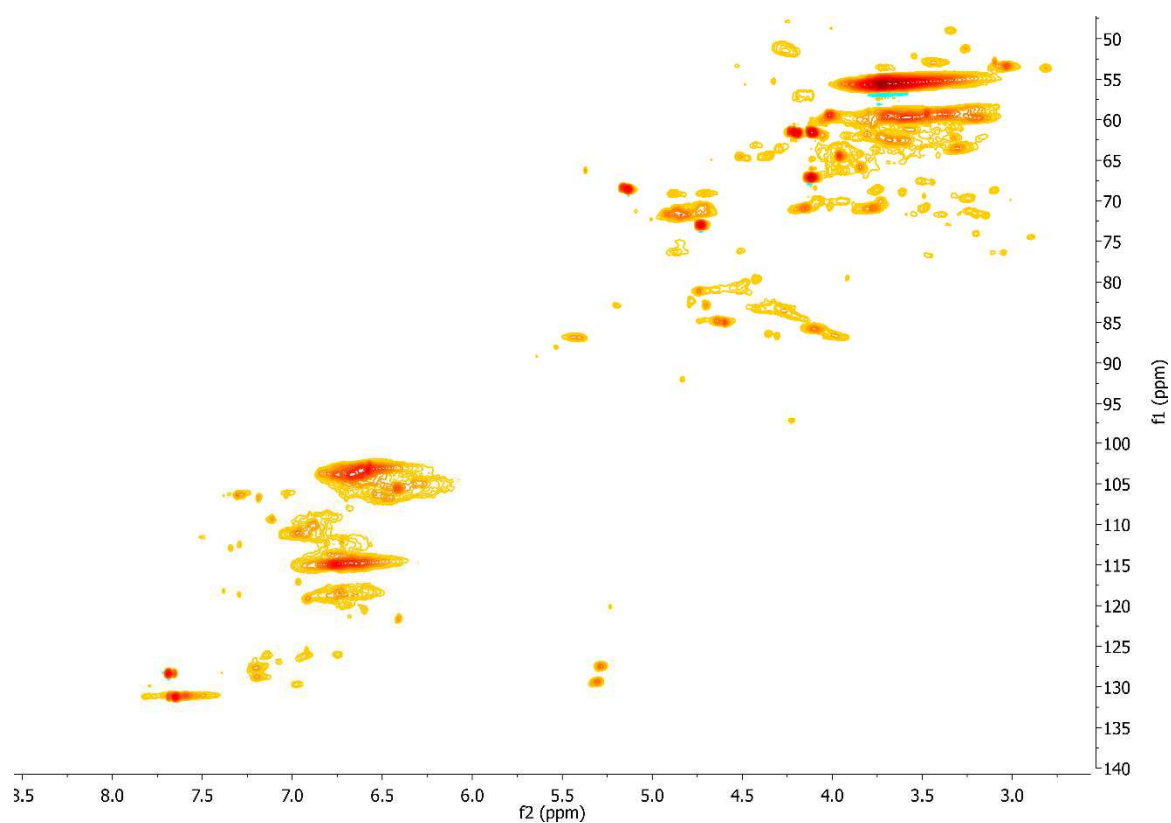


Figure A-19. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 170 °C for 0.33 hours with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 1.02$.

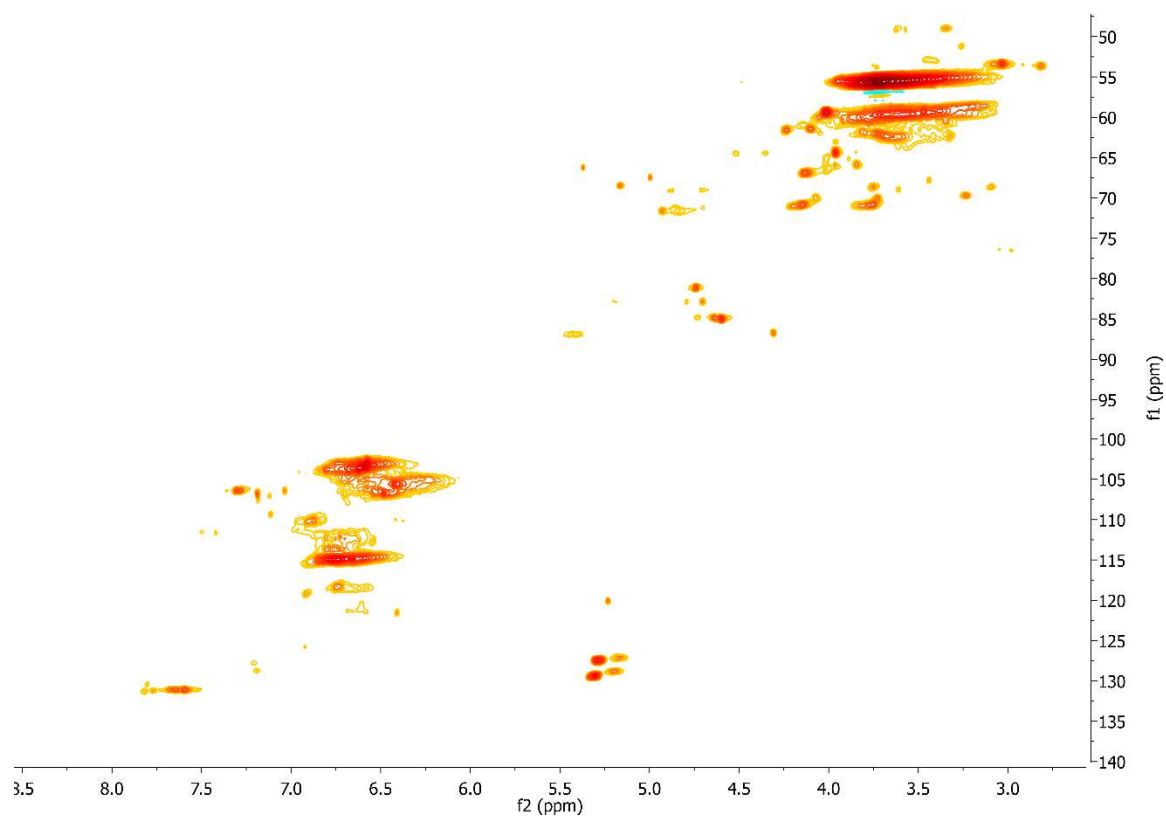


Figure A-20. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 170 °C for 0.5 hours with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 1.02$.

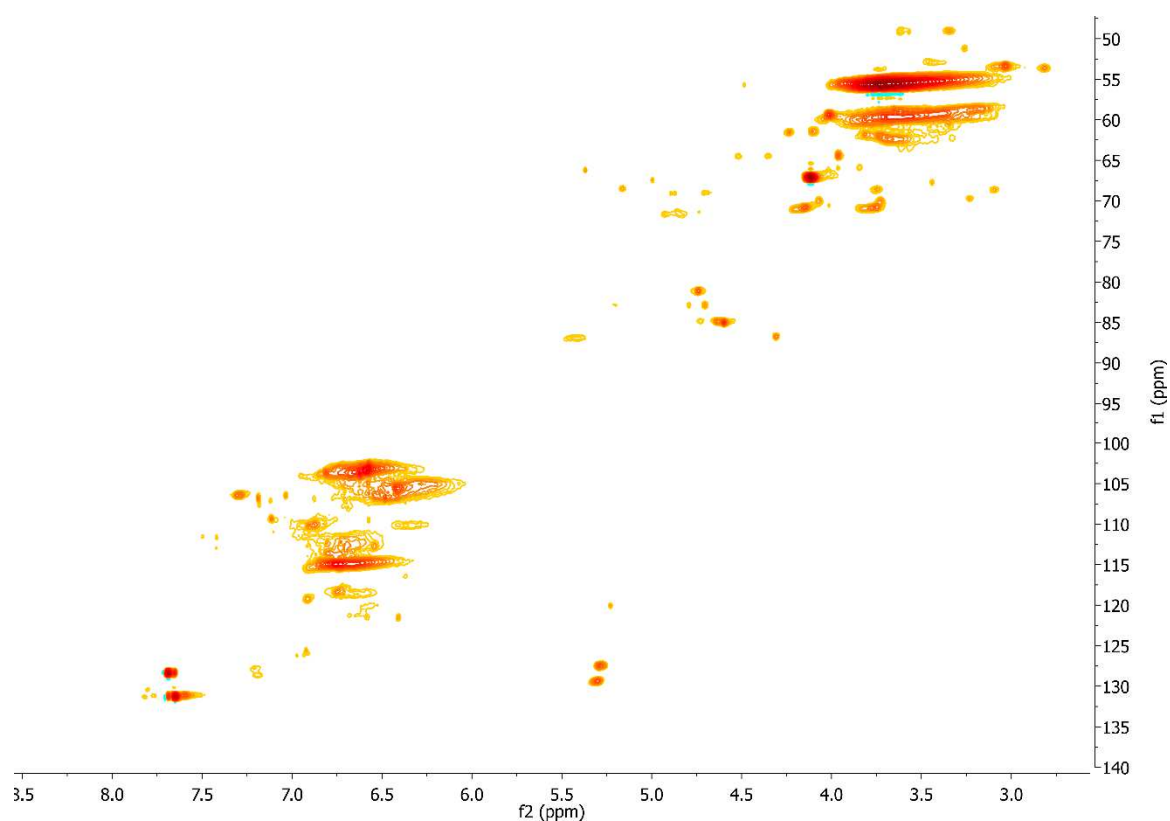


Figure A-21. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 170 °C for 0.66 hours with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 1.02$.

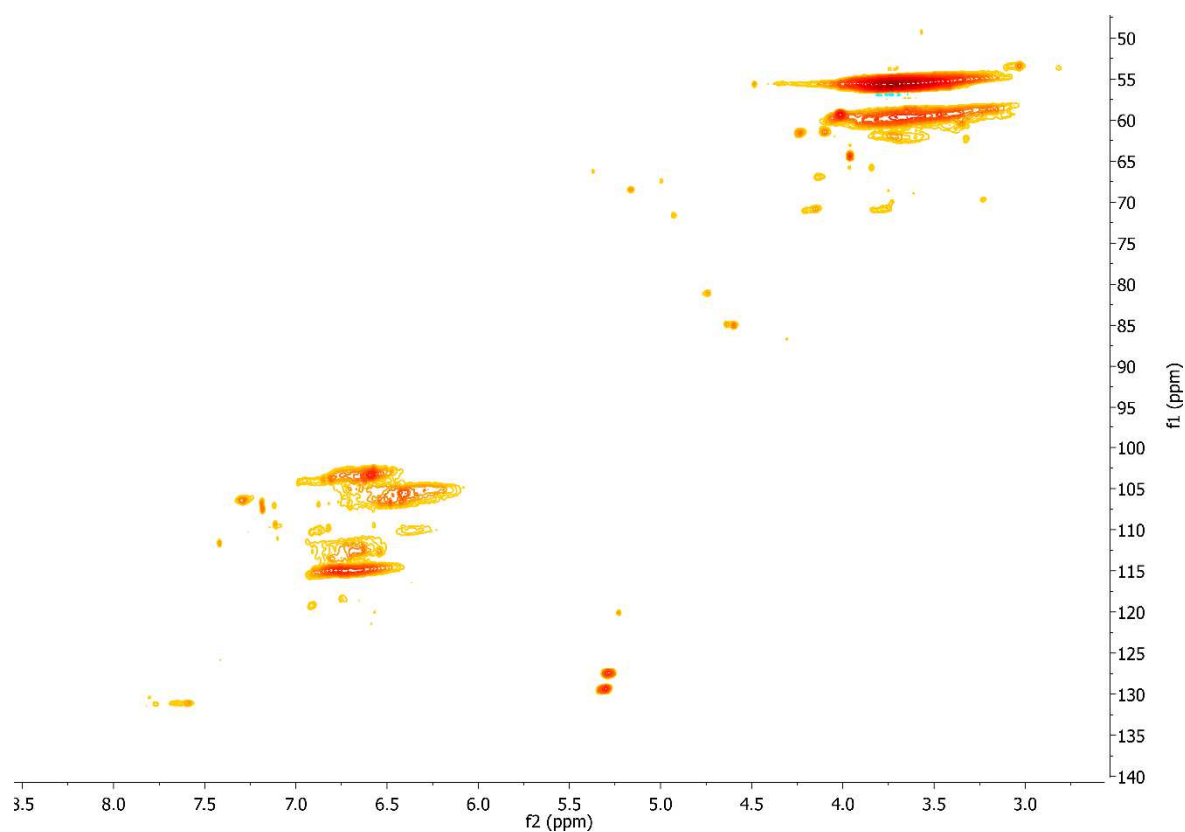


Figure A-22. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 170 °C for 1 hour with $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 1.02$.

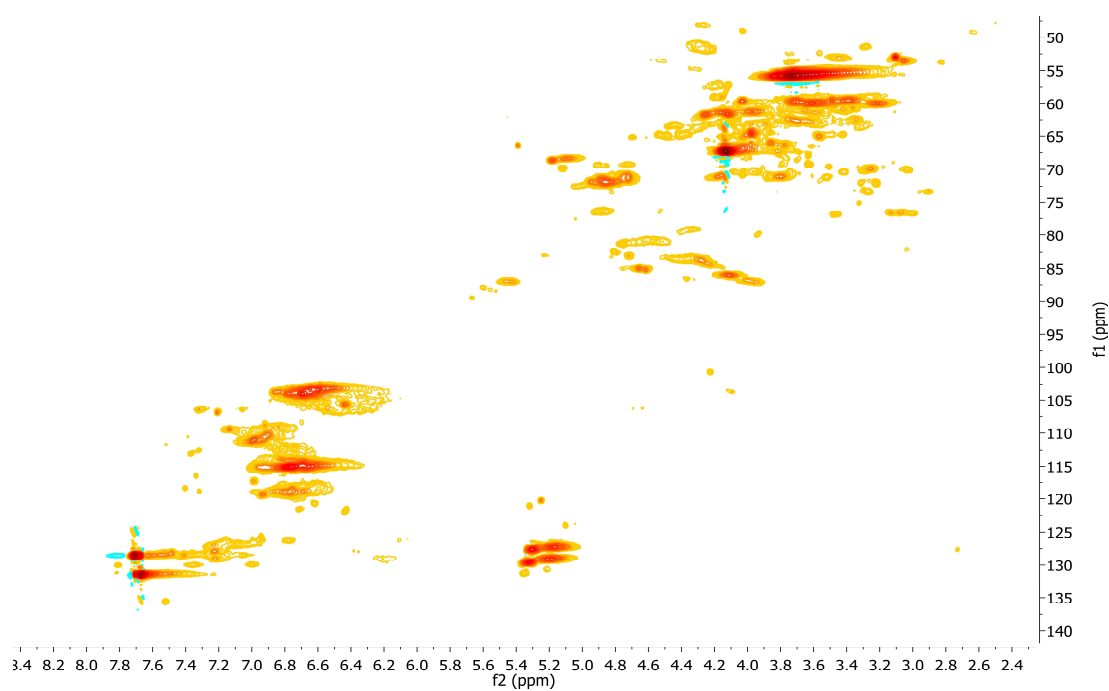


Figure A-23. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 170 °C for 0.33 hours with $[N_{2220}][HSO_4]_{80\%}$ a/b = 0.98.

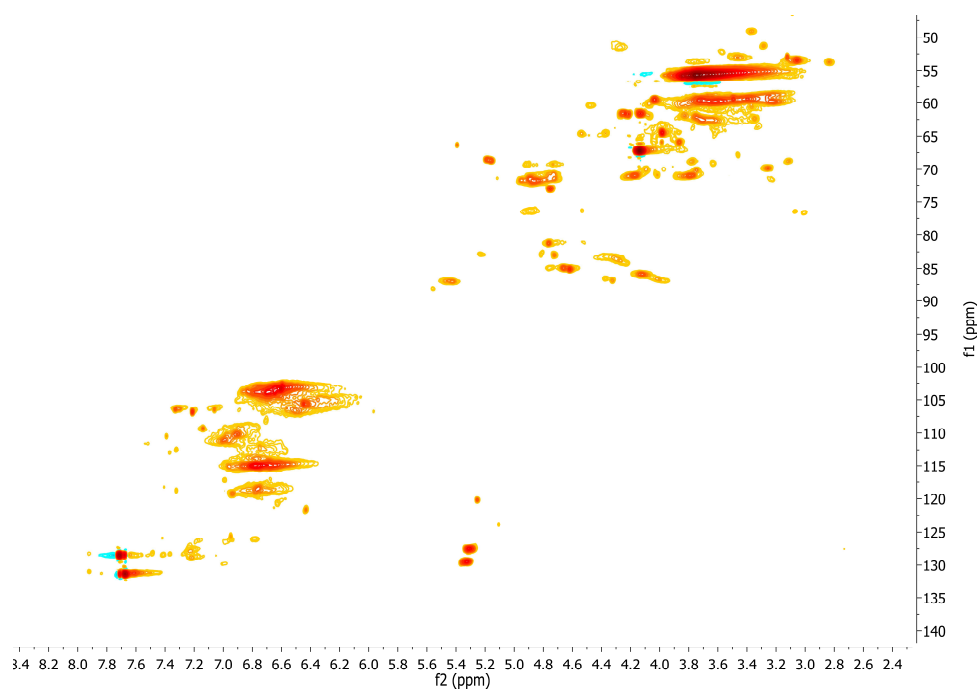


Figure A-24. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 170 °C for 0.5 hours with $[N_{2220}][HSO_4]_{80\%}$ a/b = 0.98.

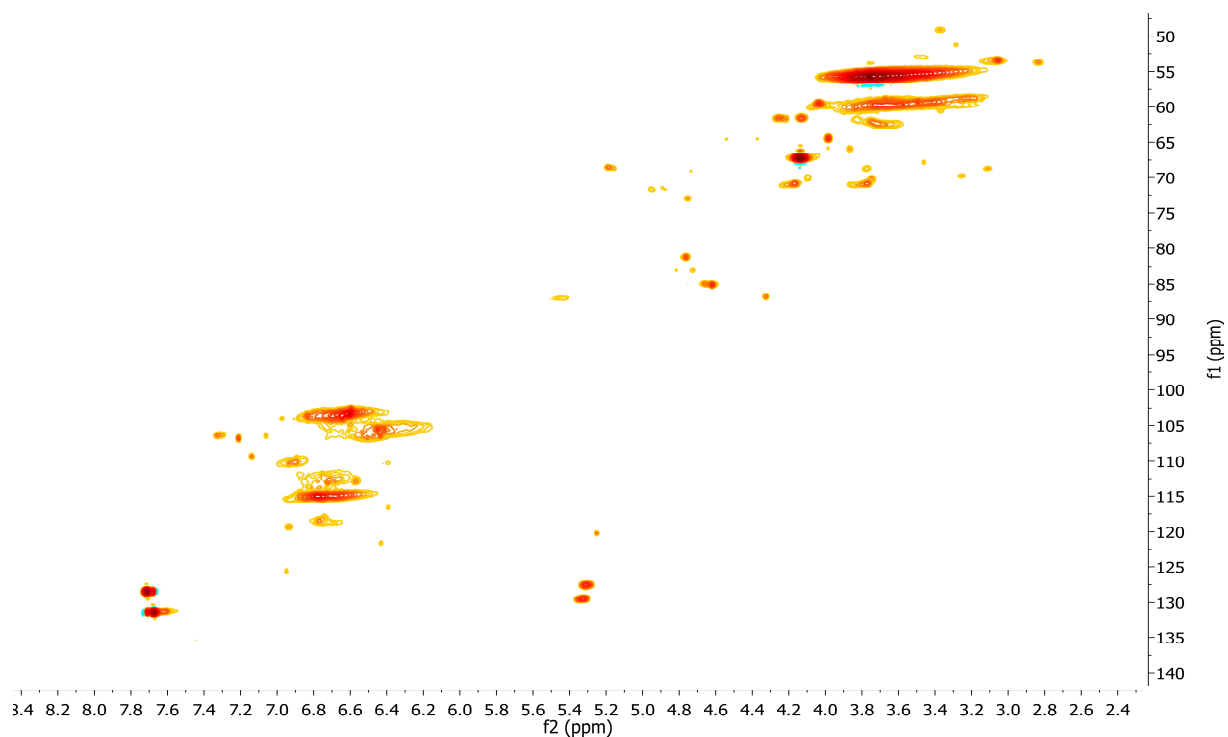


Figure A-25. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 170 °C for 0.66 hours with $[N_{2220}][HSO_4]_{80\%}$ a/b = 0.98.

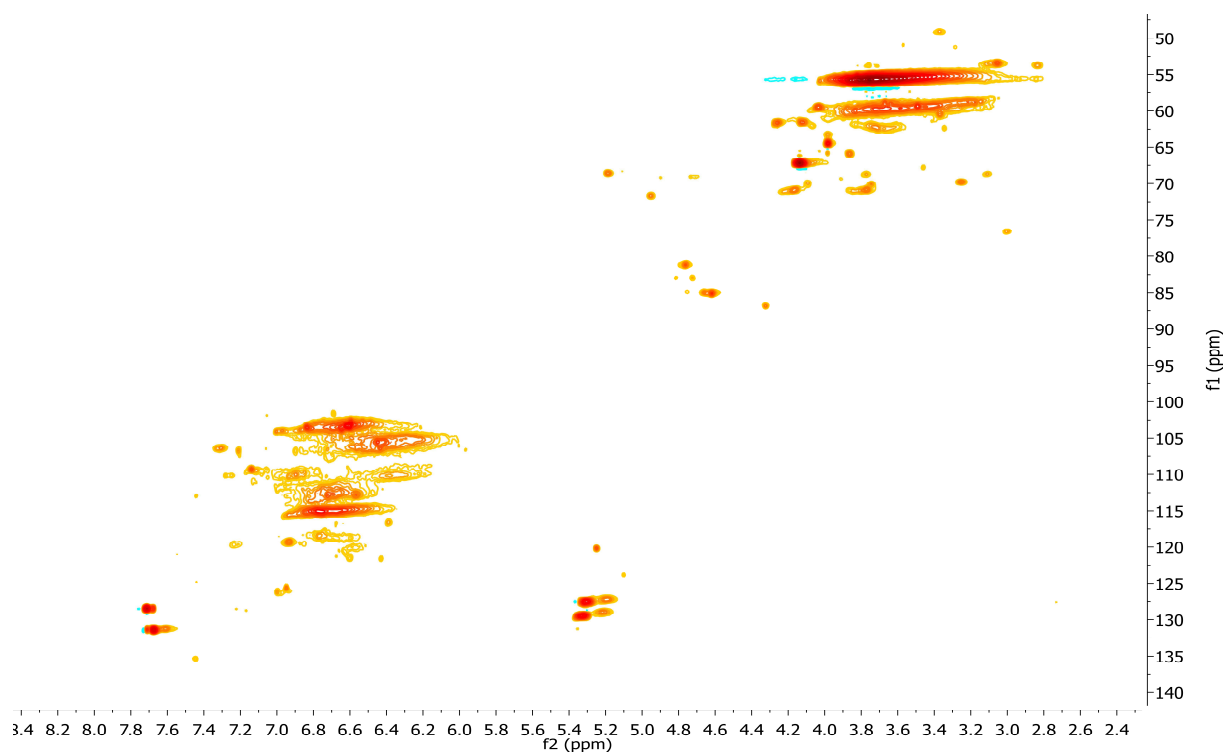


Figure A-26. HSQC spectrum of lignin recovered after pretreatment of *Salix* Endurance at 170 °C for 1 hour with $[N_{2220}][HSO_4]_{80\%}$ a/b = 0.98.

Table A-1. Summary of weight average molecular weight (M_w), number average molecular weight (M_n) and polydispersity (PDI) of lignin isolated under various pretreatment conditions with the IL solution $[N_{2220}][HSO_4]_{80\%}$ with $a/b = 1.02$ and $a/b = 0.98$.

Entry	Temperature [°C]	time [h]	a/b = 1.0			a/b = 0.98		
			M_w	M_n	PDI	M_w	M_n	PDI
1	120	0.5	8953	1133	7.9	8831	1164	7.6
2		1	8270	1110	7.5	6678	1216	5.5
3		2	6529	1117	5.8	6822	1360	5.0
4		4	6243	1068	5.8	7414	1376	5.4
5		8	6826	1151	5.9	7188	1255	5.7
6	150	0.5	5798	1024	5.7	5919	1207	4.9
7		1	5235	930	5.6	4969	1090	4.6
8		2	7174	1037	6.9	5236	1009	5.2
9		4	5221	907	5.8	5676	966	5.9
10	170	0.3	5854	1187	4.9	6375	1174	4.6
11		0.5	5988	1055	5.7	4941	995	5.0
12		0.7	6563	944	7	5267	994	5.3
13		1	5877	1022	5.8	5559	995	5.6

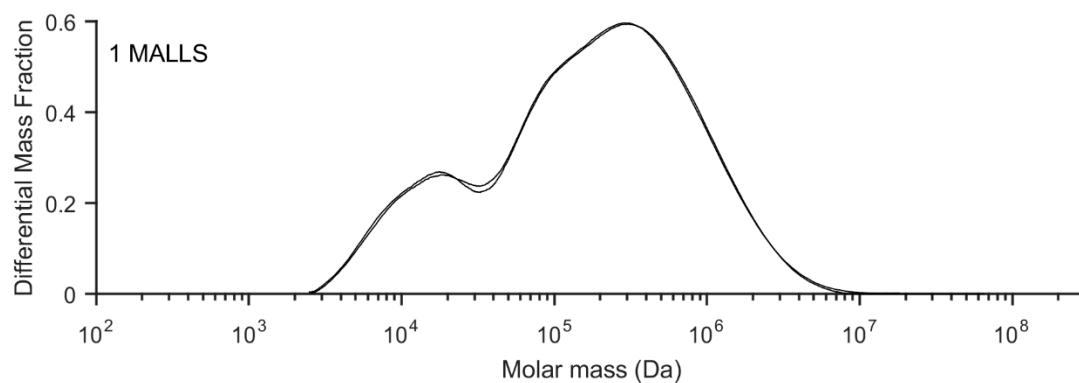


Figure A-27. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix Asgerd* after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

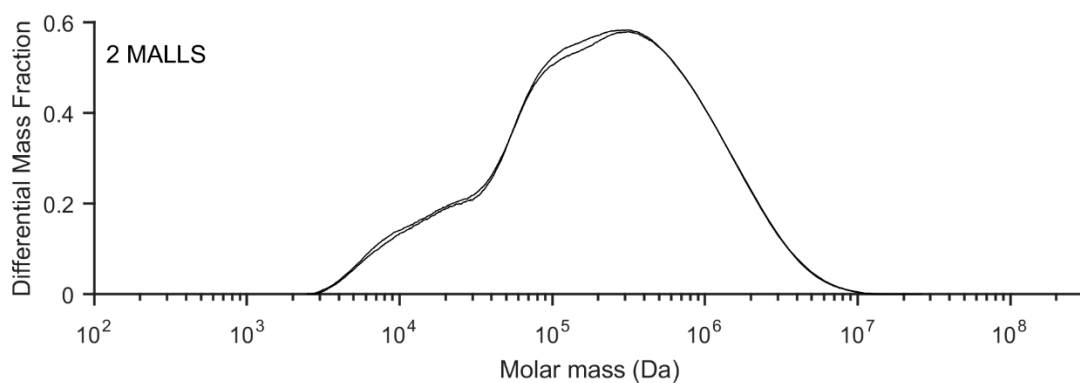


Figure A-28. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix Baldwin* after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

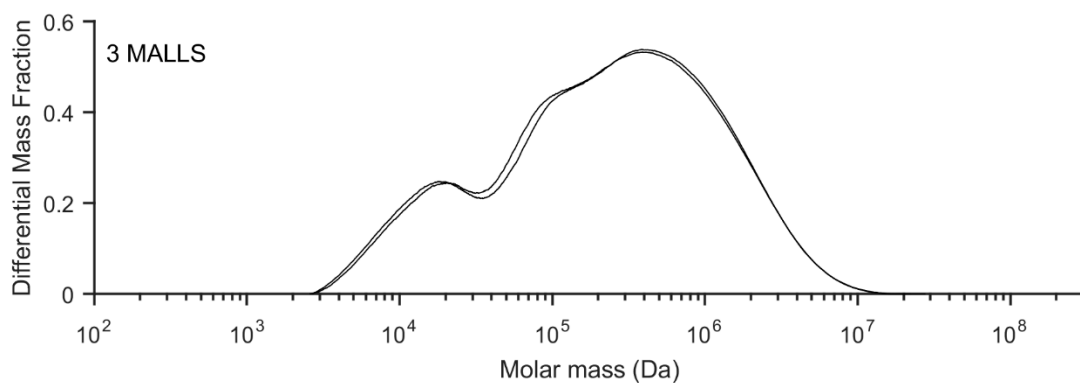


Figure A-29. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix Bowles Hybrid* after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

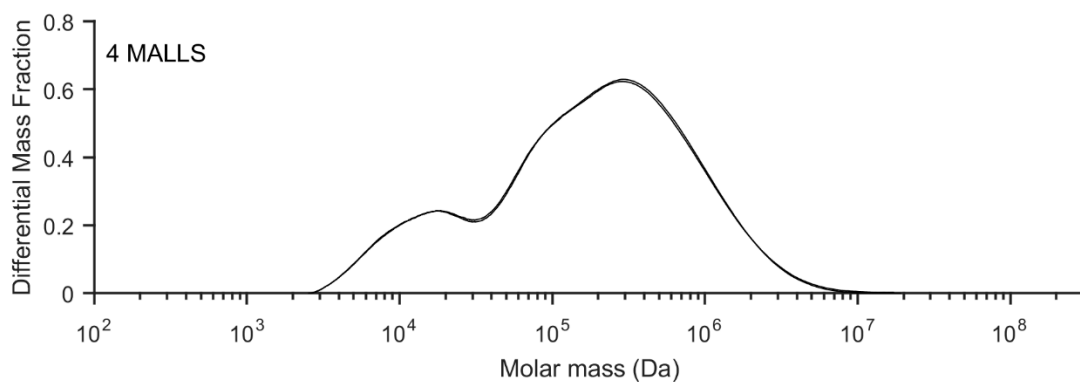


Figure A-30. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix Corail* after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

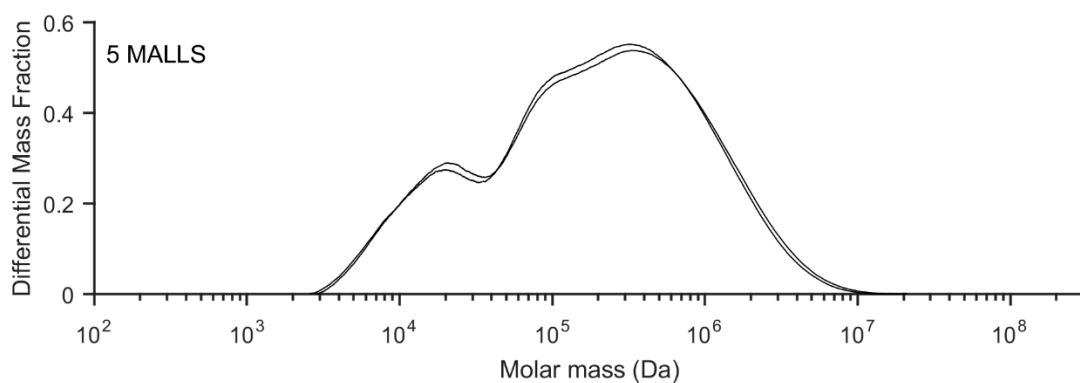


Figure A-31. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix* Discovery after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

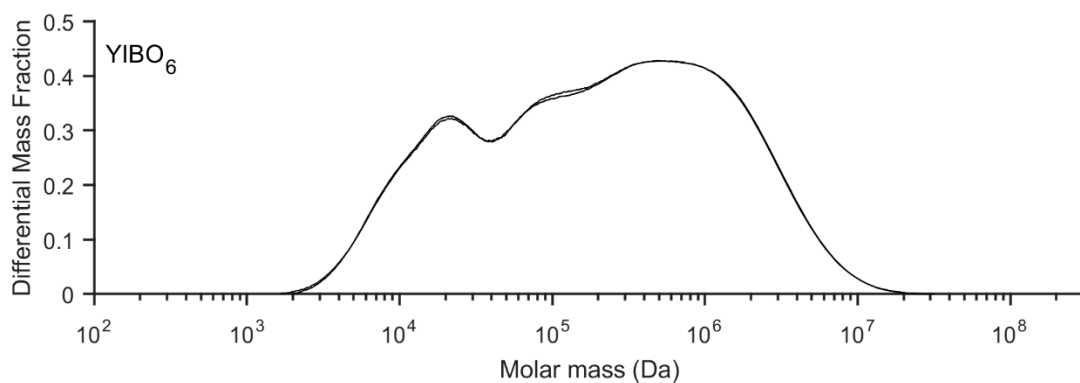


Figure A-32. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix* Endurance after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

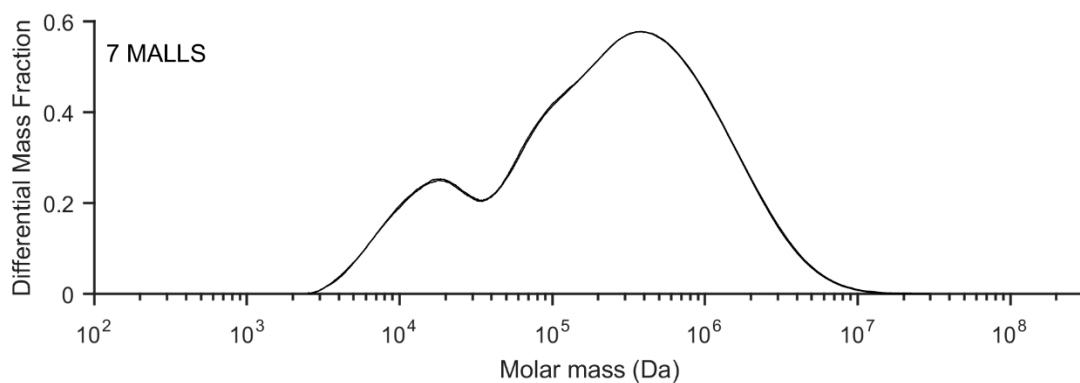


Figure A-33. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix* Jorr after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

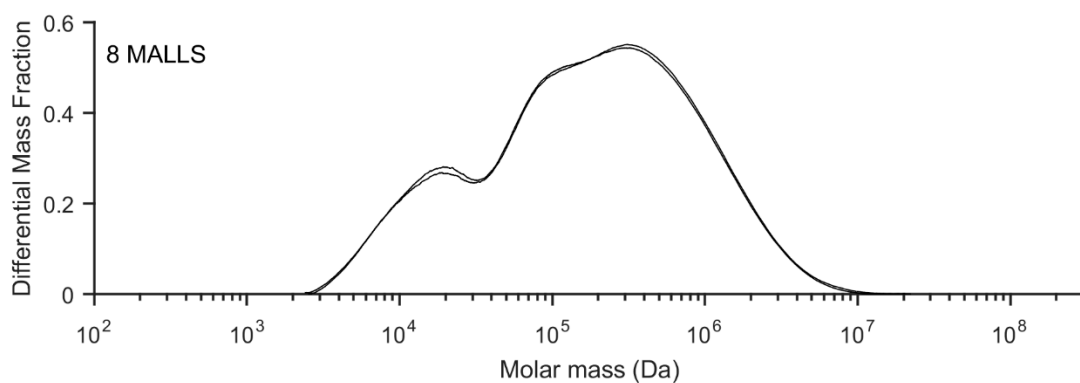


Figure A-34. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix Nimrod* after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

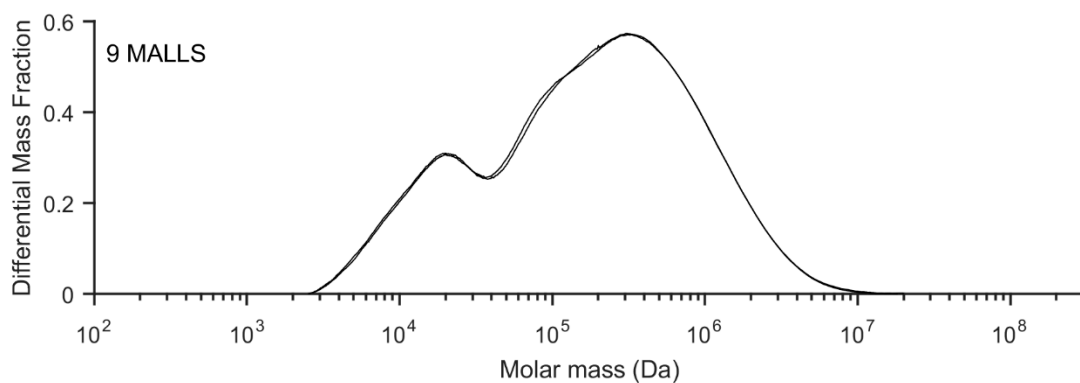


Figure A-35. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix Orm* after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

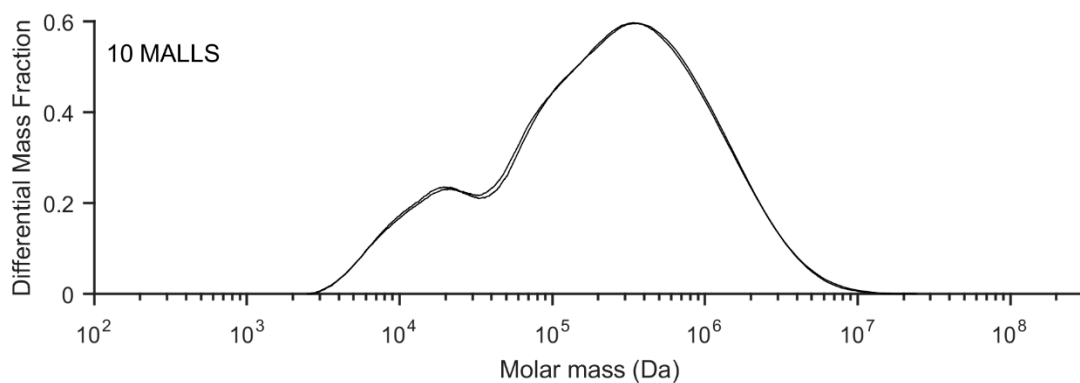


Figure A-36. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix Resolution* after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

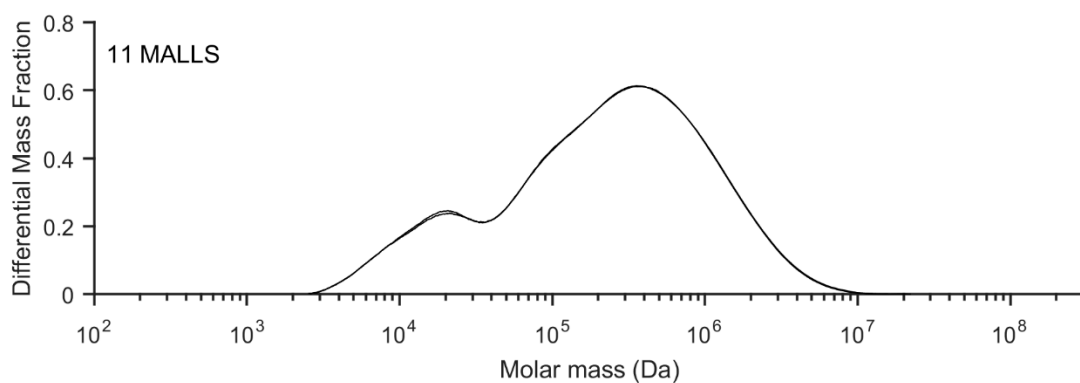


Figure A-37. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix Shrubby Willow* after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

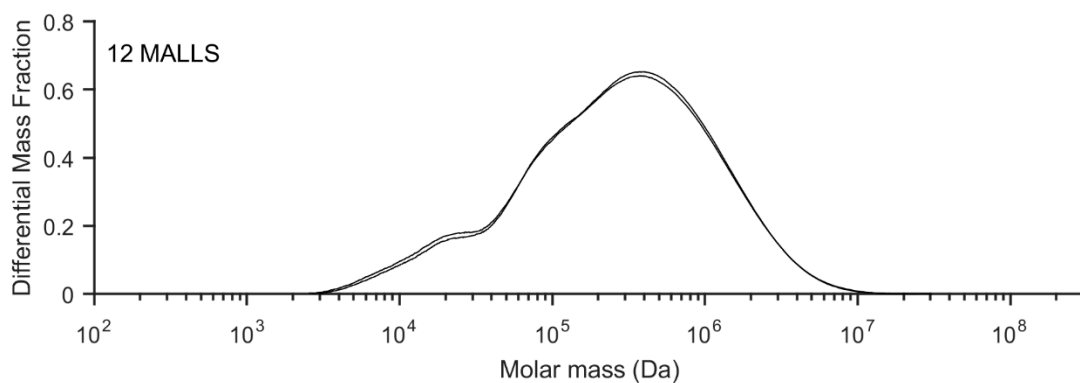


Figure A-38. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix Stott10* after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

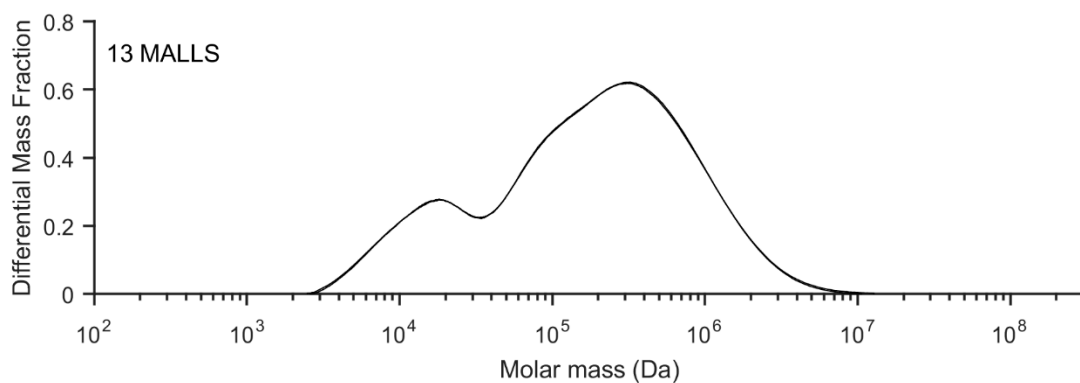


Figure A-39. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix Terra Nova* after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

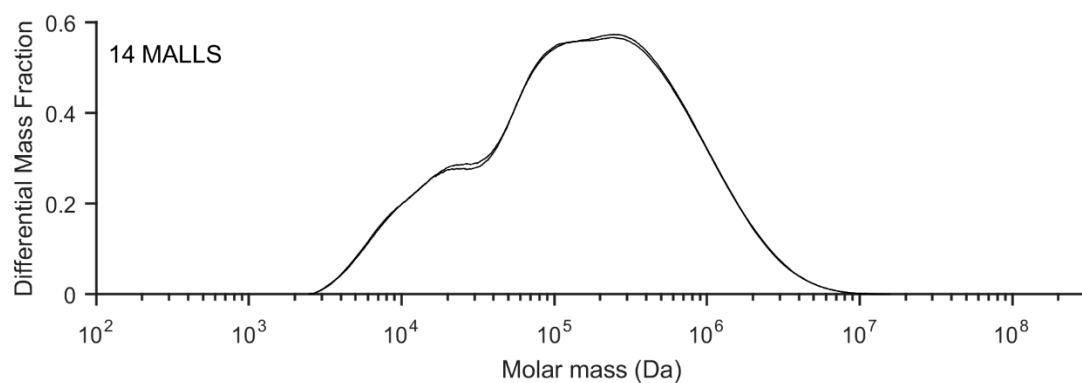


Figure A-40. GPC profile of cellulose and hemicellulose polymers isolated from pulp of *Salix Tora* after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

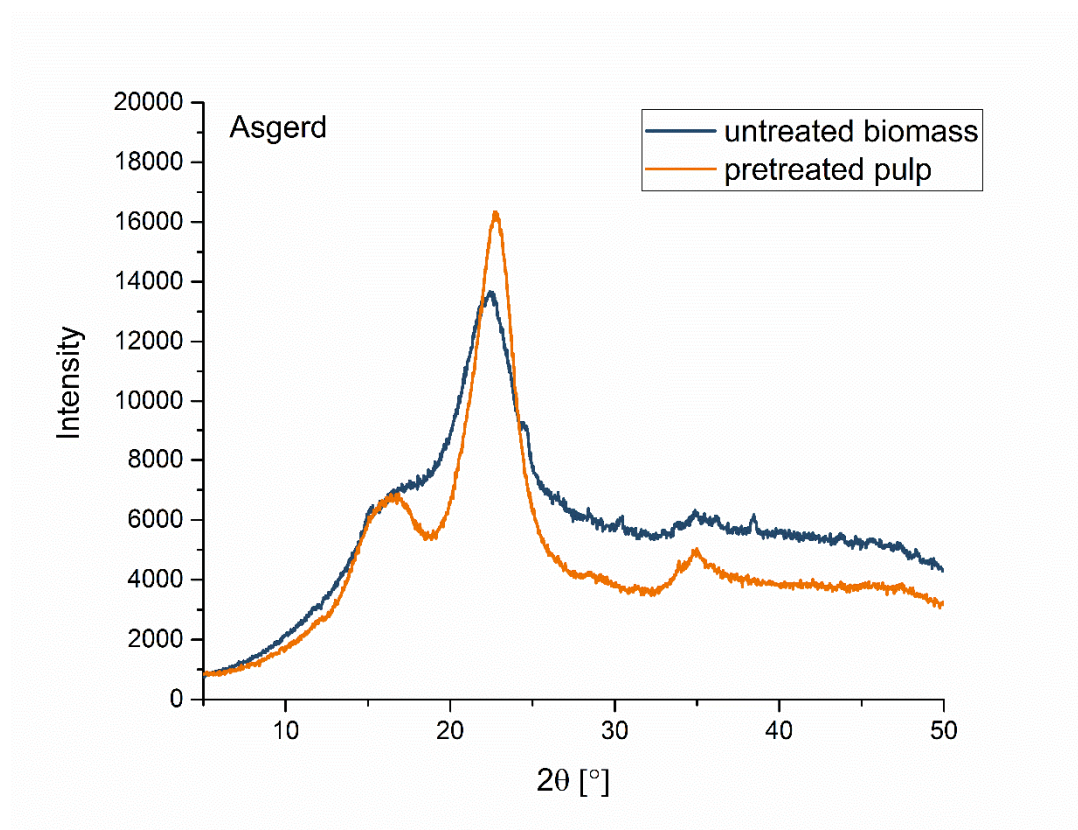


Figure A-41. XRD diffractogram of raw biomass and pulp after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

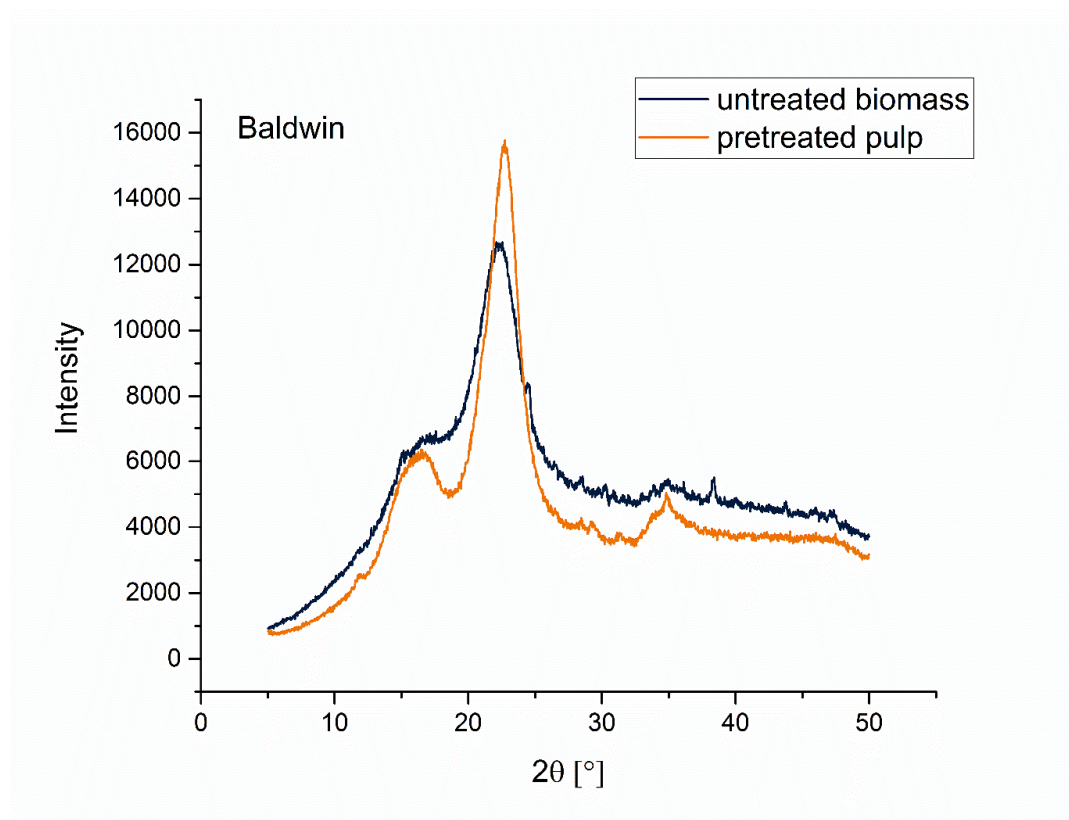


Figure A-42. XRD diffractogram of raw biomass and pulp after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

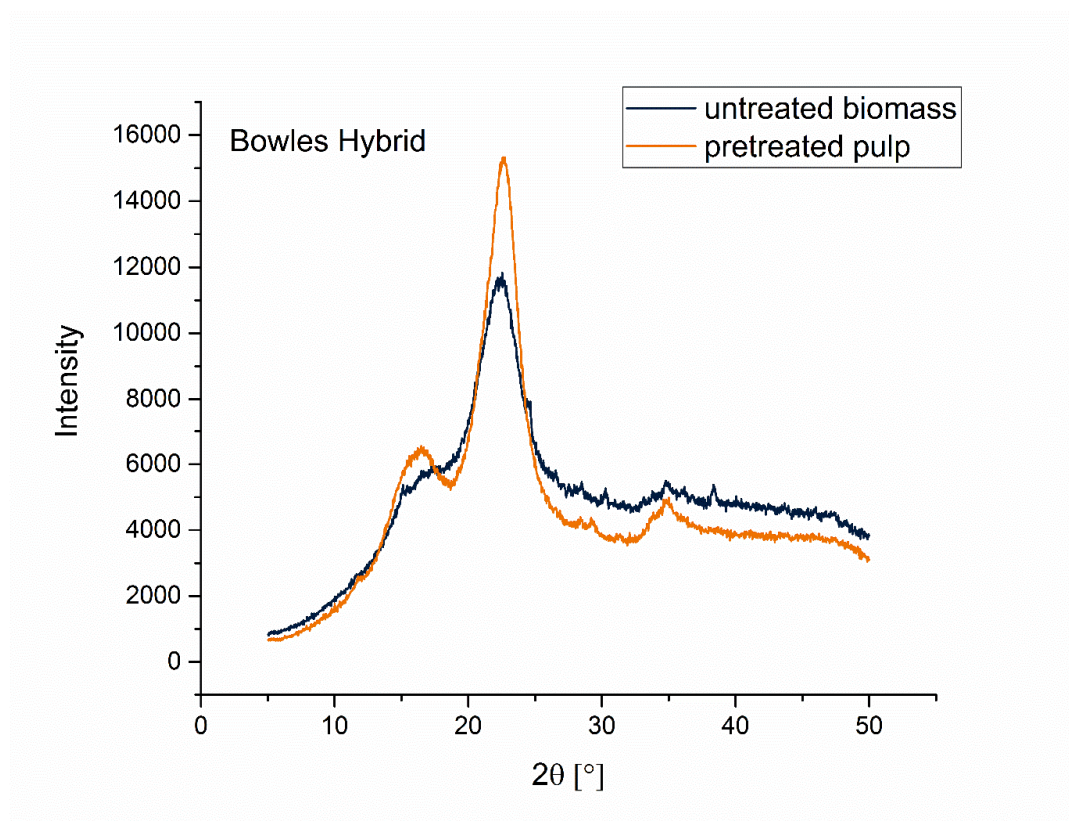


Figure A-43. XRD diffractogram of raw biomass and pulp after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

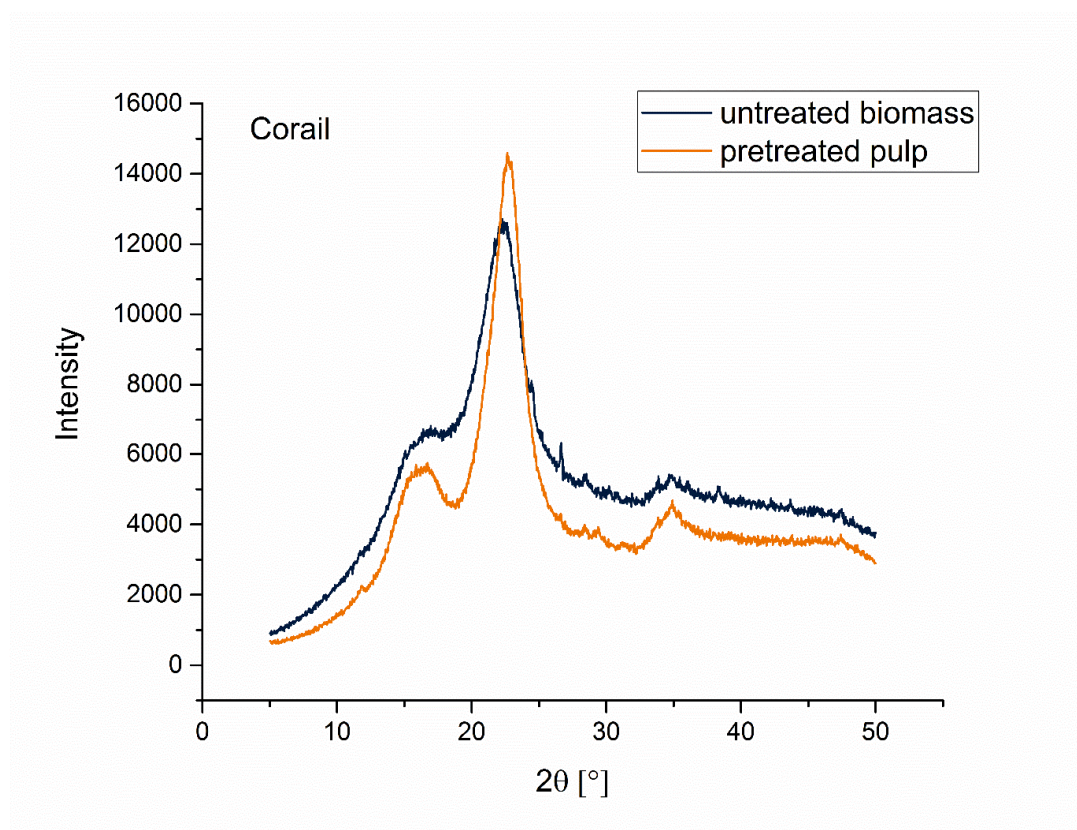


Figure A-44. XRD diffractogram of raw biomass and pulp after pretreatment with $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$.

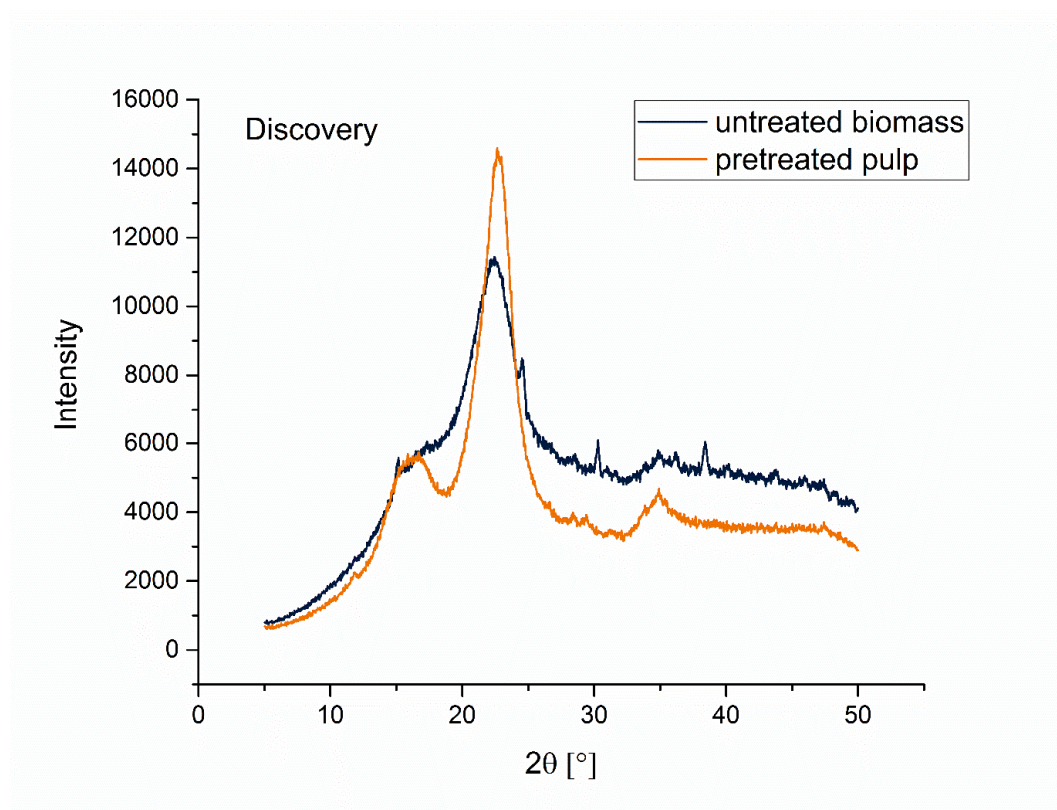


Figure A-45. XRD diffractogram of raw biomass and pulp after pretreatment with $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$.

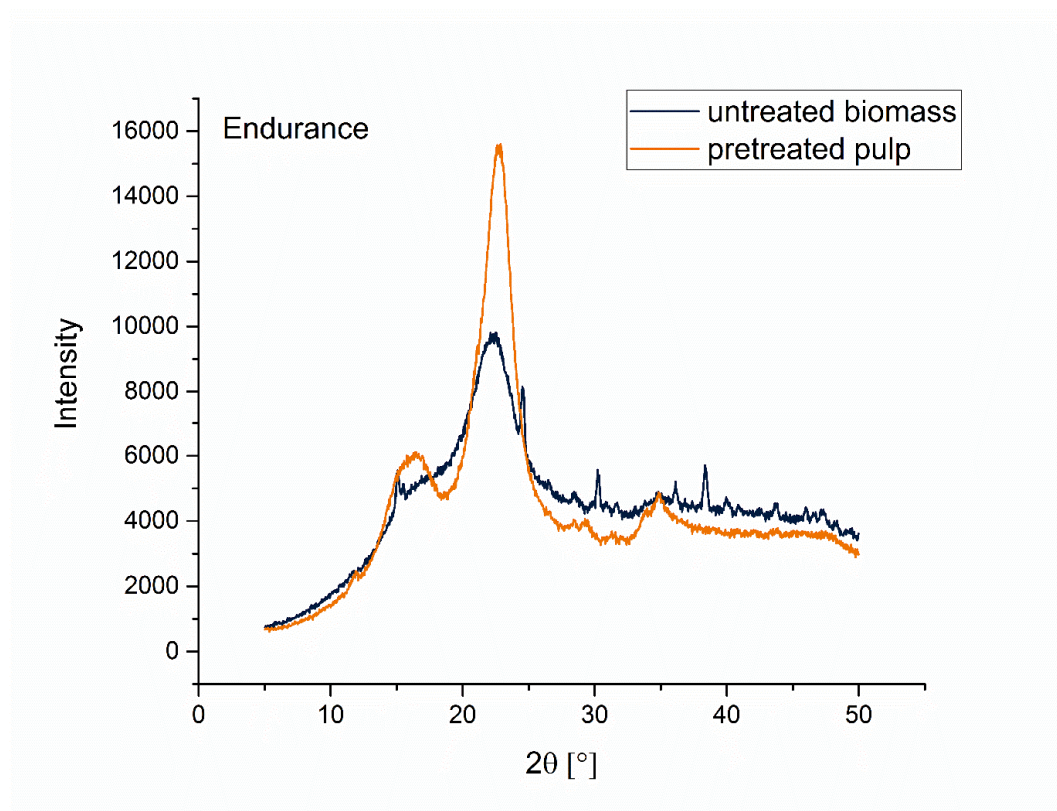


Figure A-46. XRD diffractogram of raw biomass and pulp after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

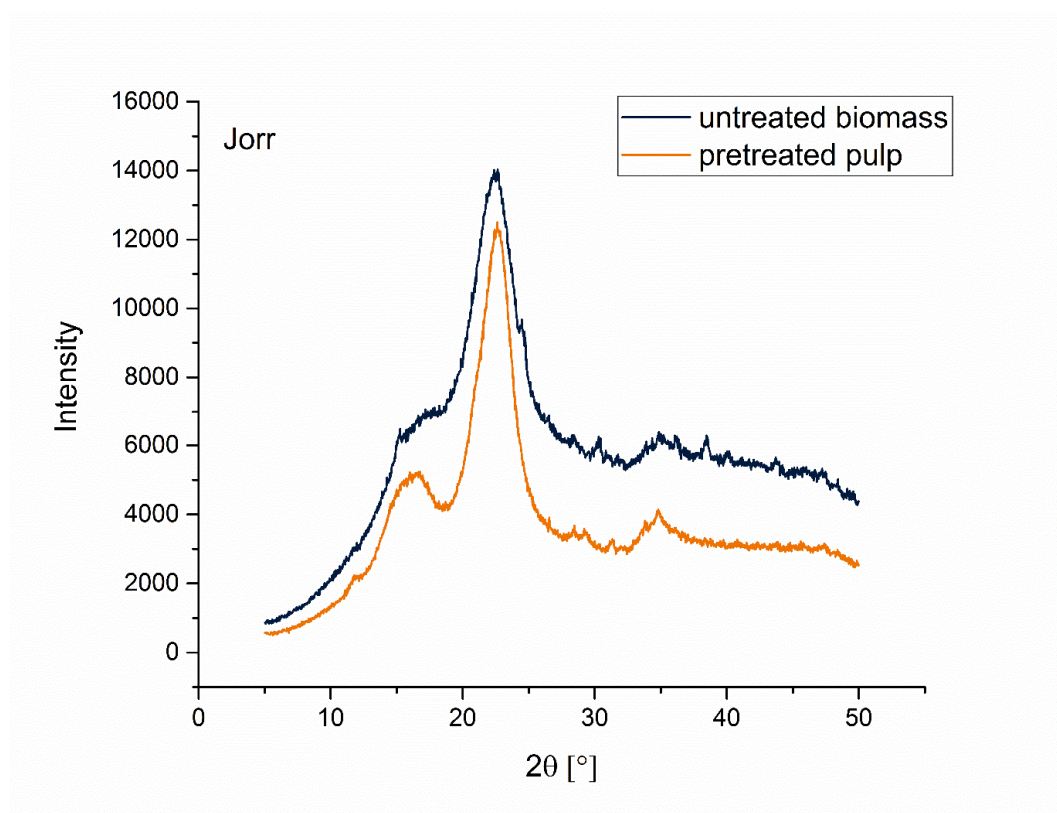


Figure A-47. XRD diffractogram of raw biomass and pulp after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

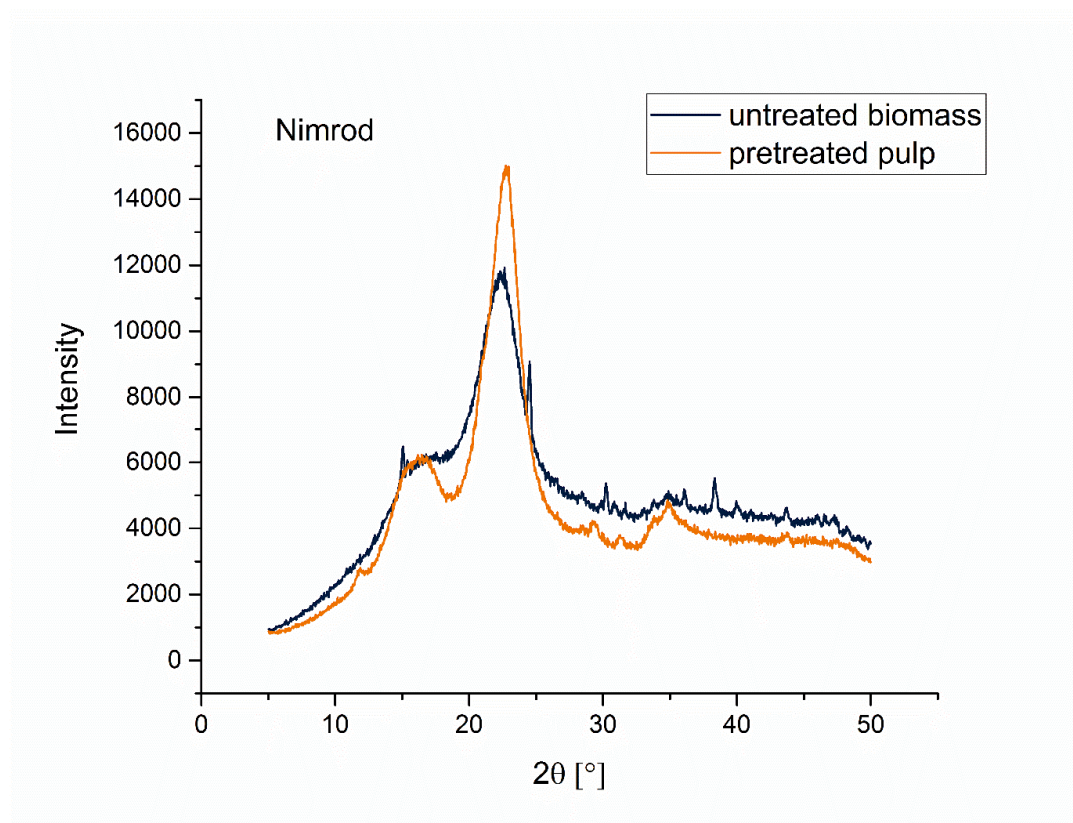


Figure A-48. XRD diffractogram of raw biomass and pulp after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

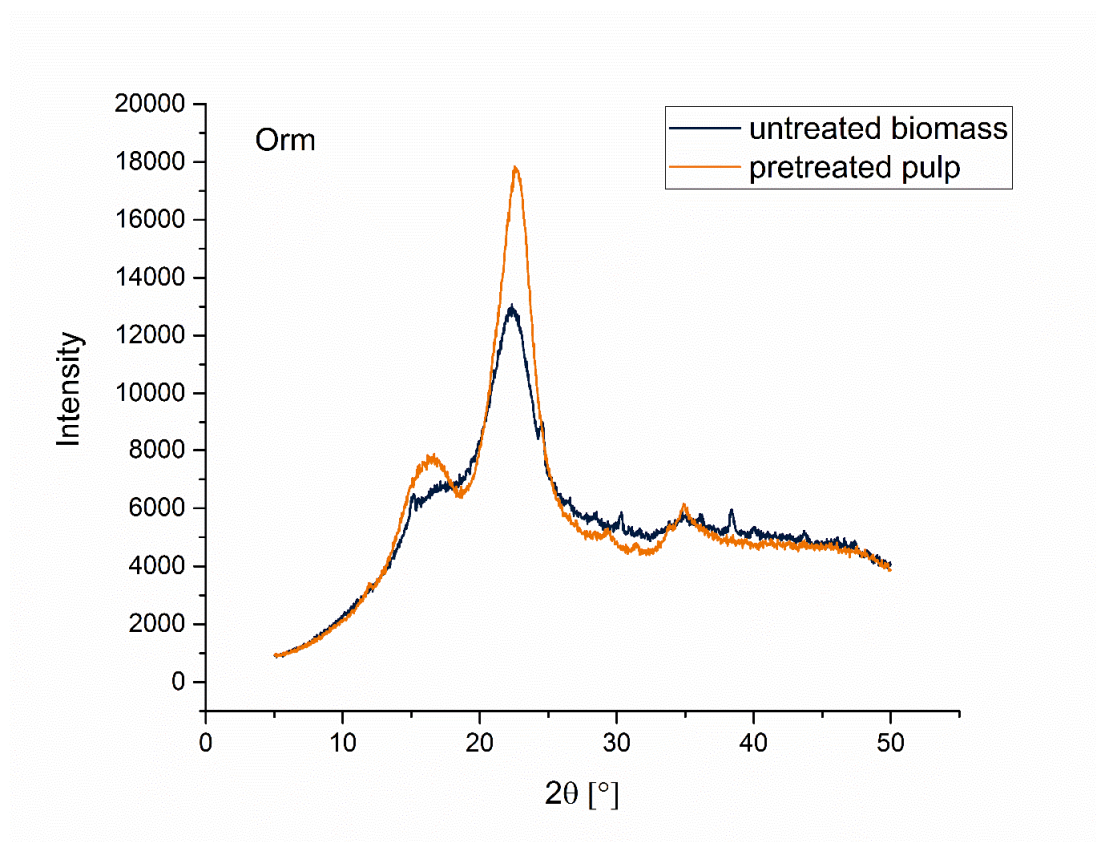


Figure A-49. XRD diffractogram of raw biomass and pulp after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

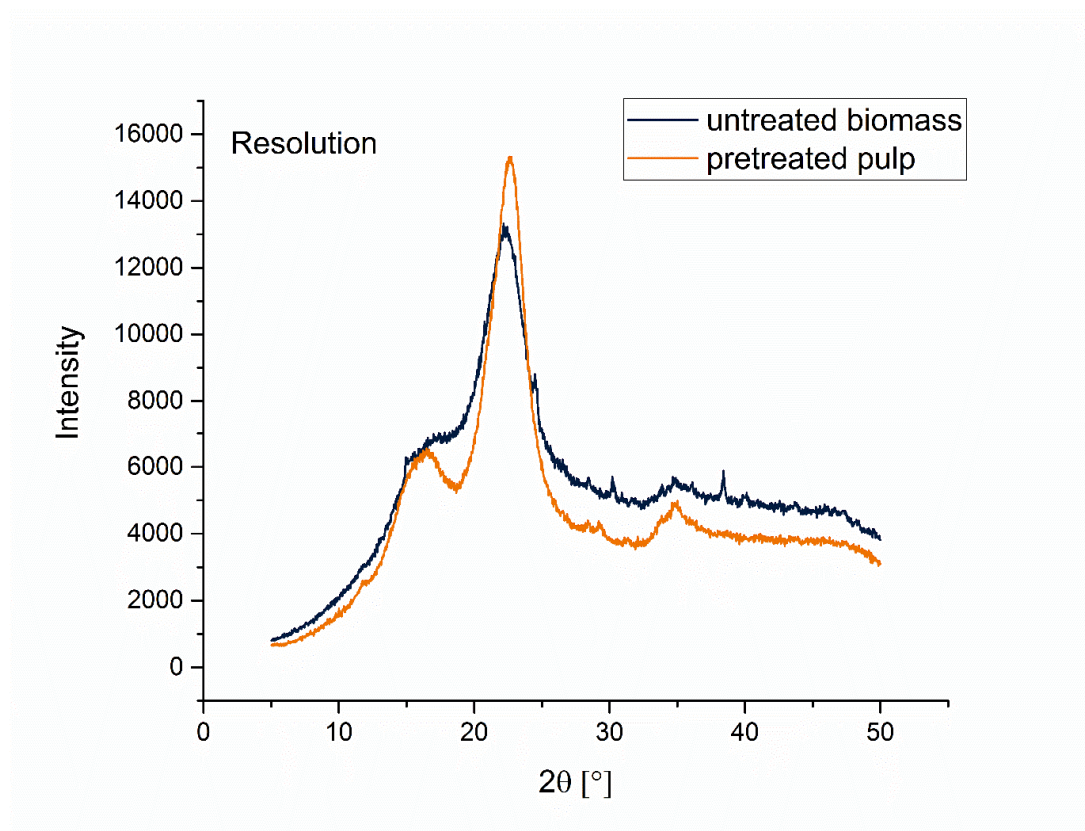


Figure A-50. XRD diffractogram of raw biomass and pulp after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

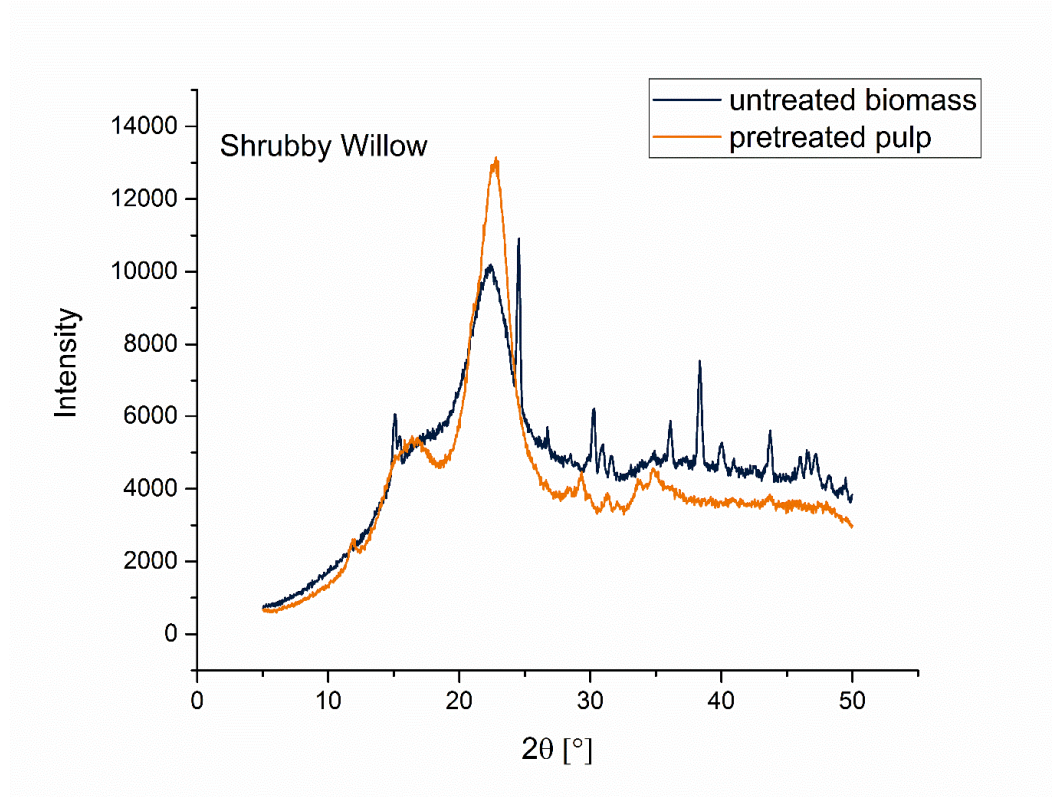


Figure A-51. XRD diffractogram of raw biomass and pulp after pretreatment with $[N_{2220}][HSO_4]_{80\%}$.

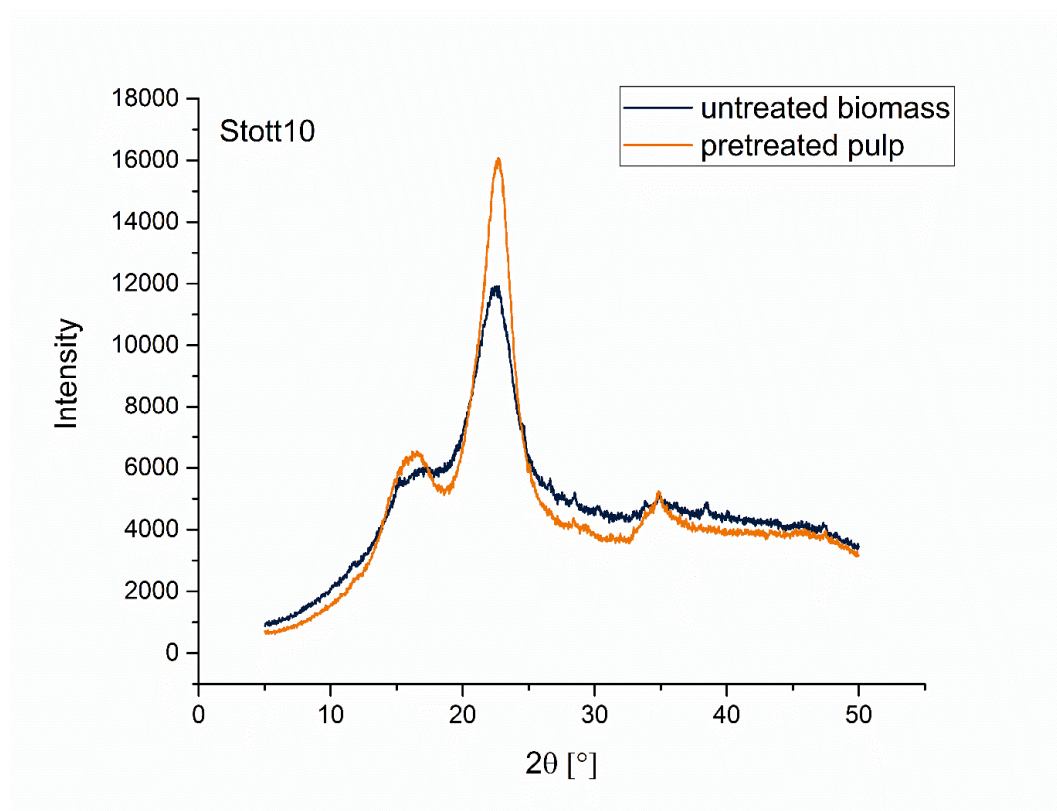


Figure A-52. XRD diffractogram of raw biomass and pulp after pretreatment with $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$.

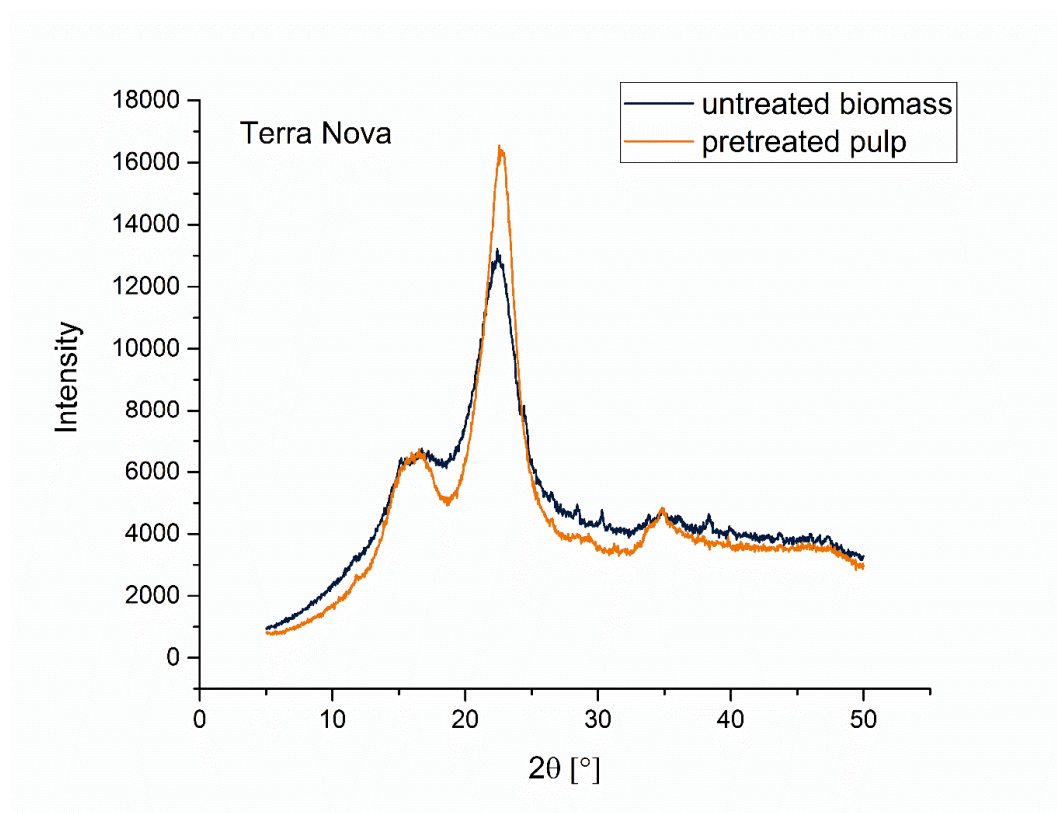


Figure A-53. XRD diffractogram of raw biomass and pulp after pretreatment with $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$.

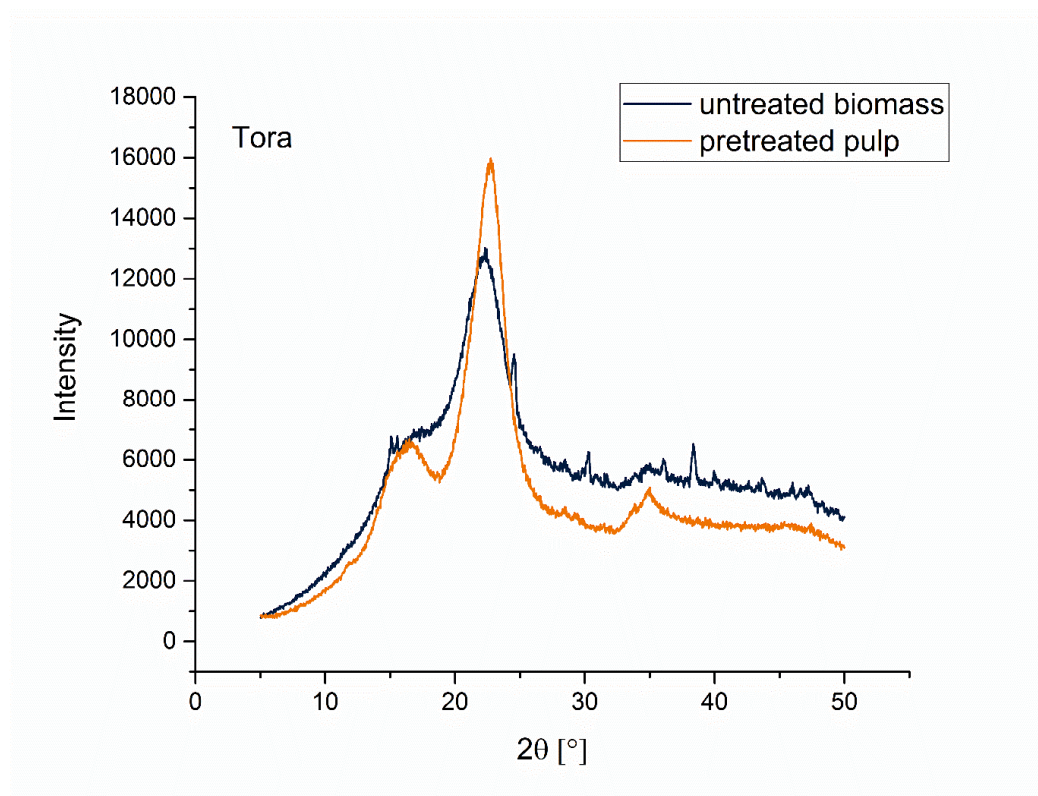


Figure A-54. XRD diffractogram of raw biomass and pulp after pretreatment with $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$.

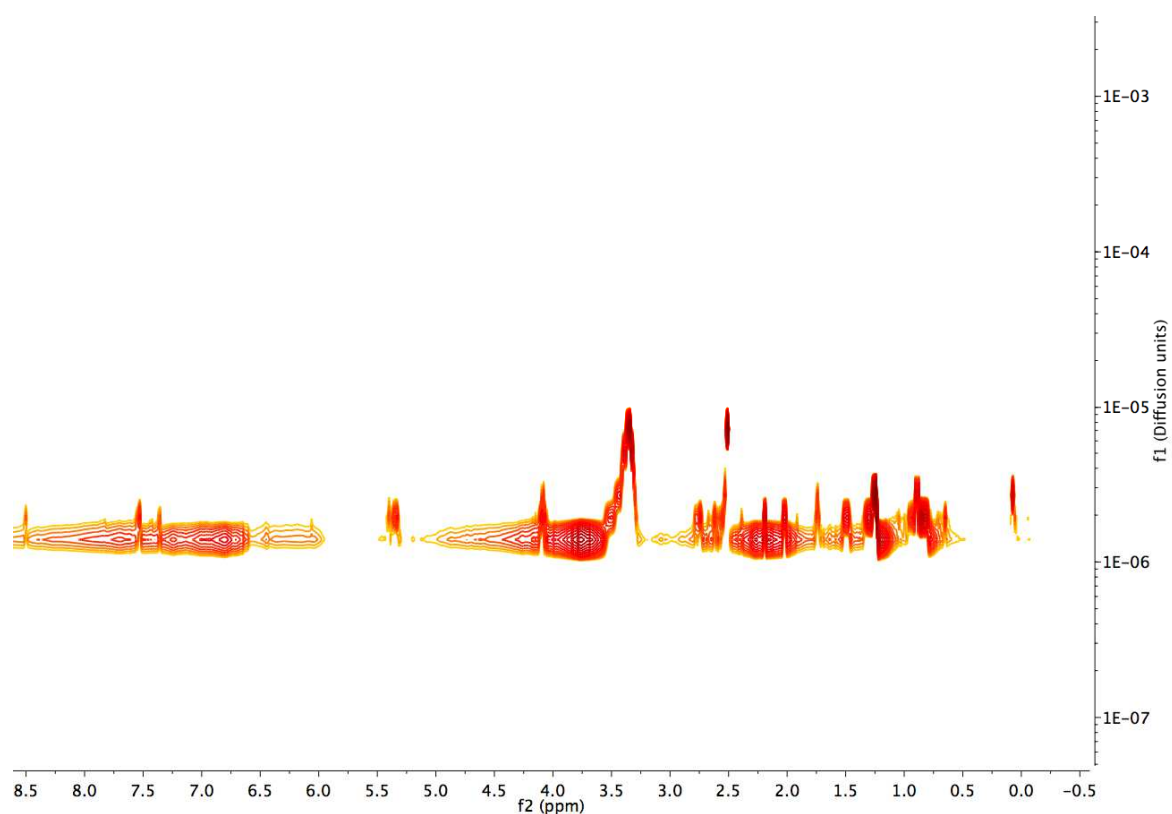


Figure A-55. DOSY spectrum of the product recovered after reaction of lignin and FA (1:1) in $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]_{80\%}$ at 120 °C.

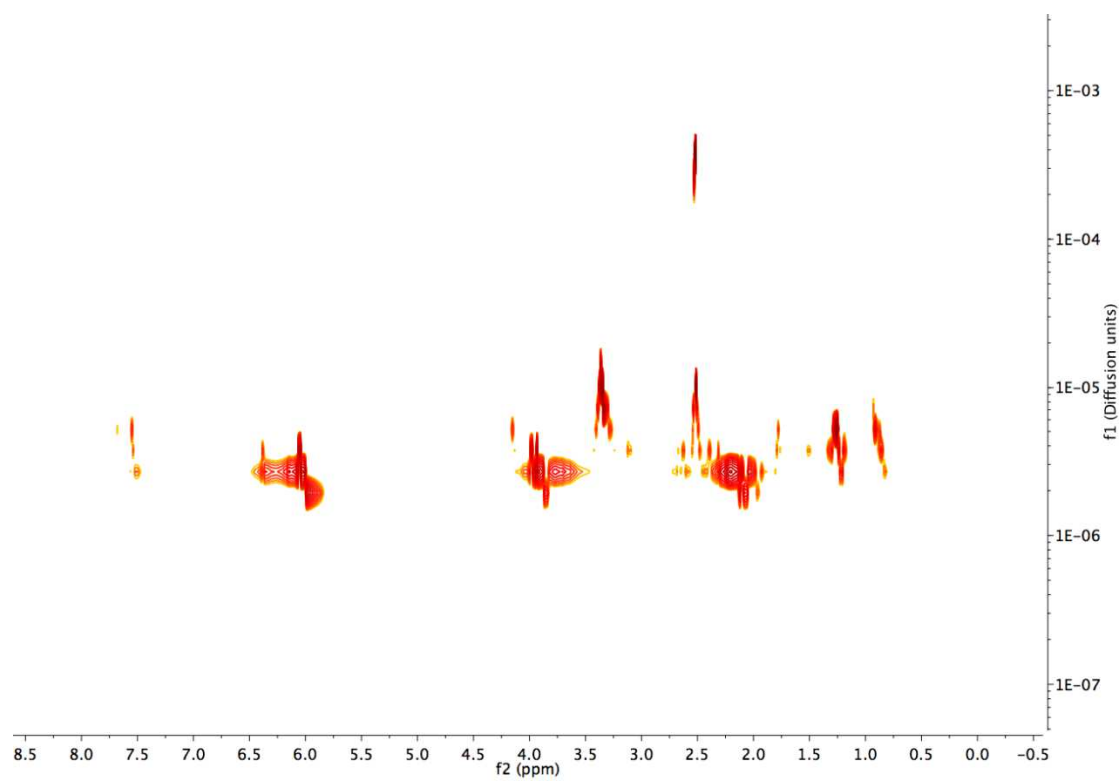


Figure A-56. DOSY spectrum of the product recovered after reaction of lignin and FA (1:4) in $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]_{80\%}$ at 120 °C.

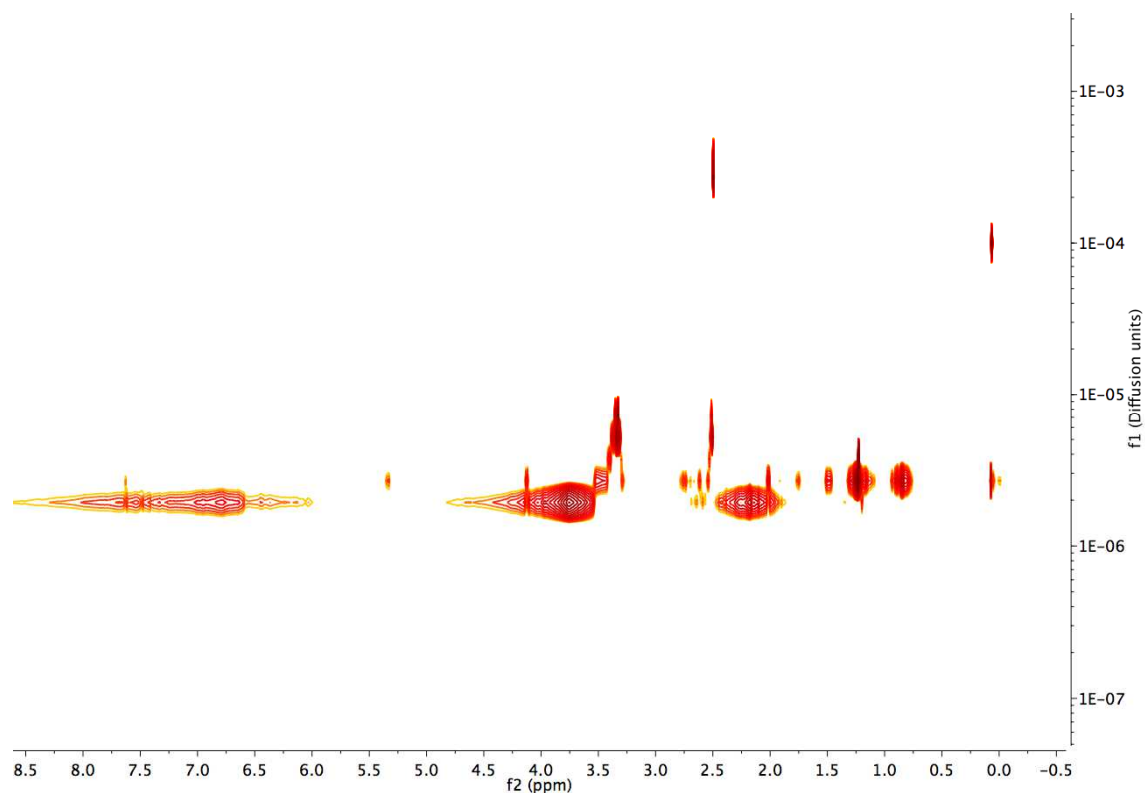


Figure A-57. DOSY spectrum of the product recovered after reaction of lignin and FA (1:1) in $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]_{80\%}$ at 150 °C.

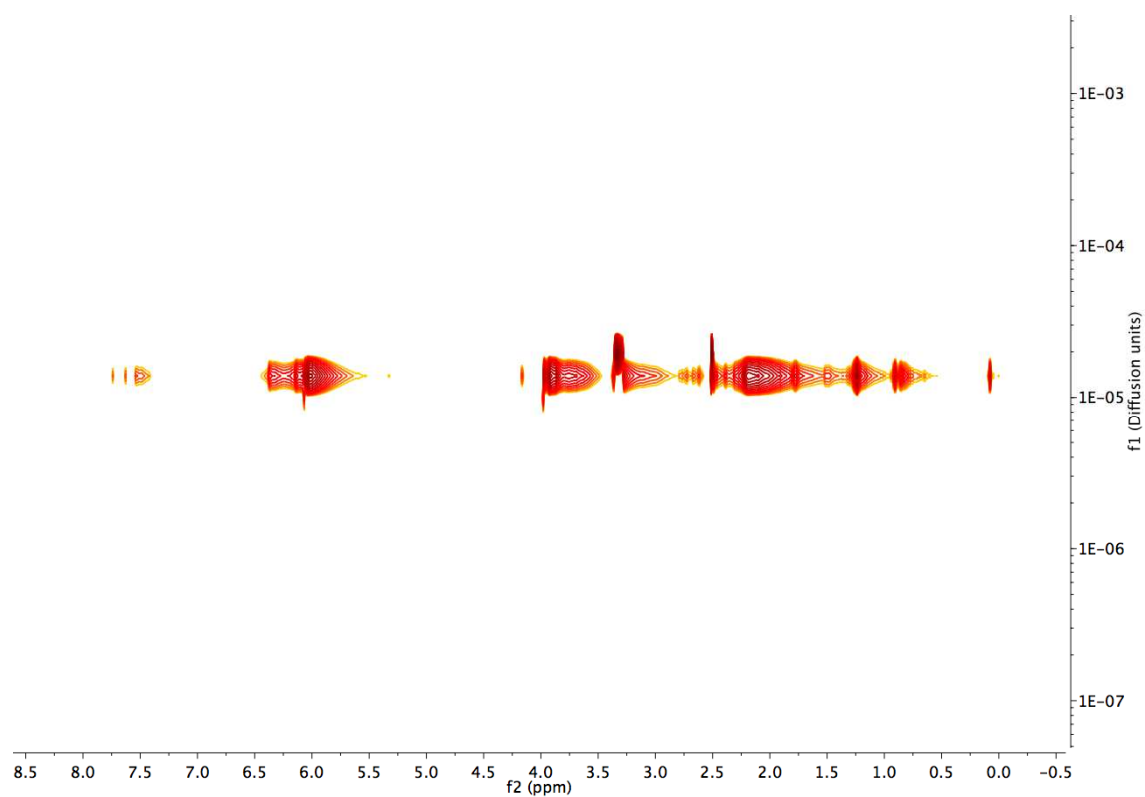


Figure A-58. . DOSY spectrum of the product recovered after reaction of lignin and FA (1:1) in $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]_{80\%}$ at 170 °C.

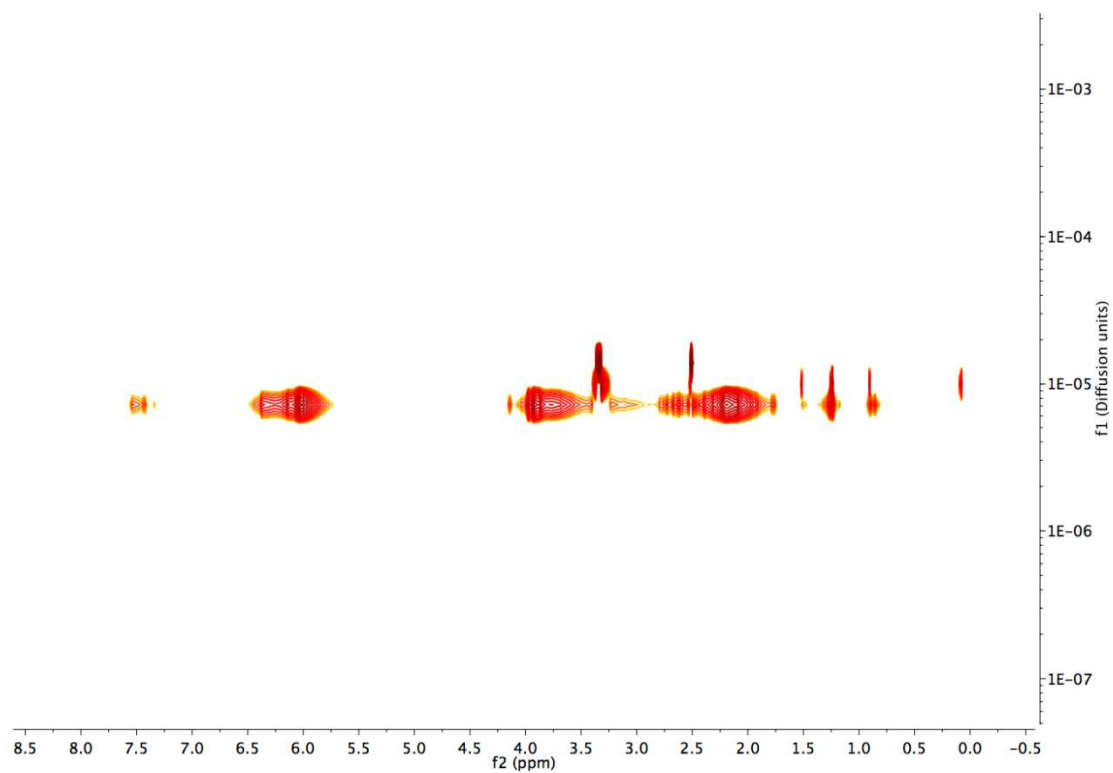


Figure A-59. . DOSY spectrum of the product recovered after reaction of lignin and FA (1:4) in $[\text{C}_4\text{C}_{1}\text{im}][\text{HSO}_4]_{80\%}$ at 170 °C.

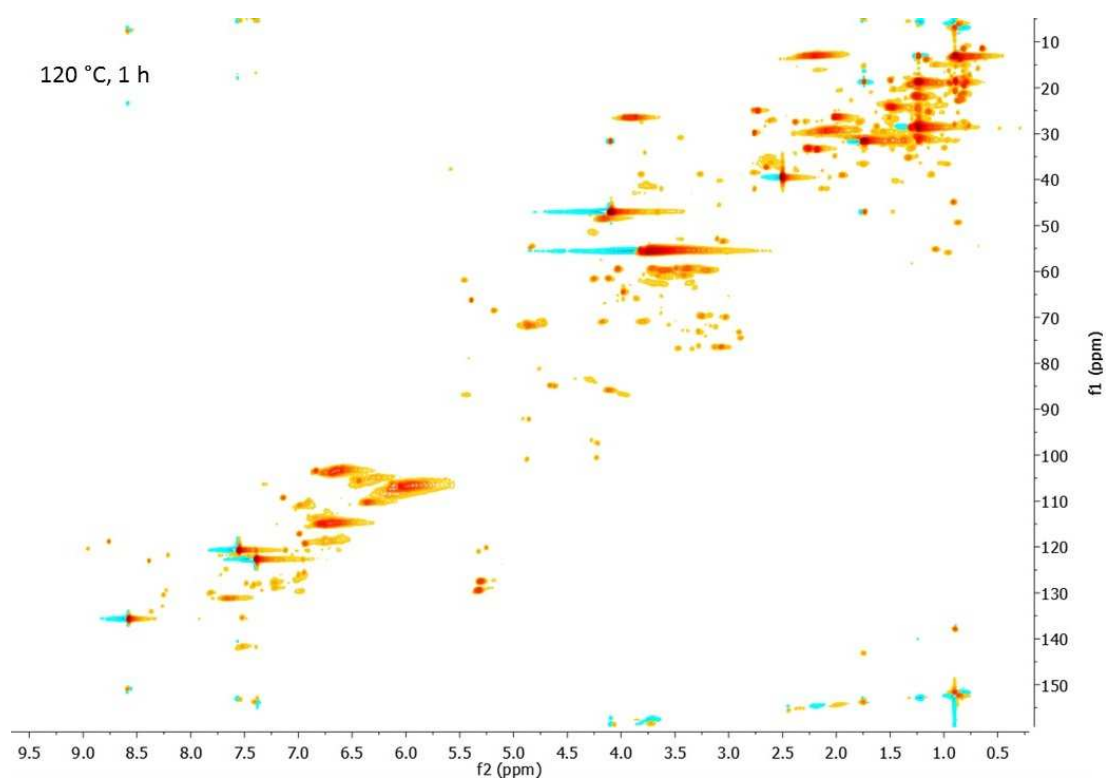


Figure A-60. NMR spectrum of product recovered after *in-situ* modification of lignin with FA during pretreatment of willow Endurance with $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ at 120 °C for 1 hour.

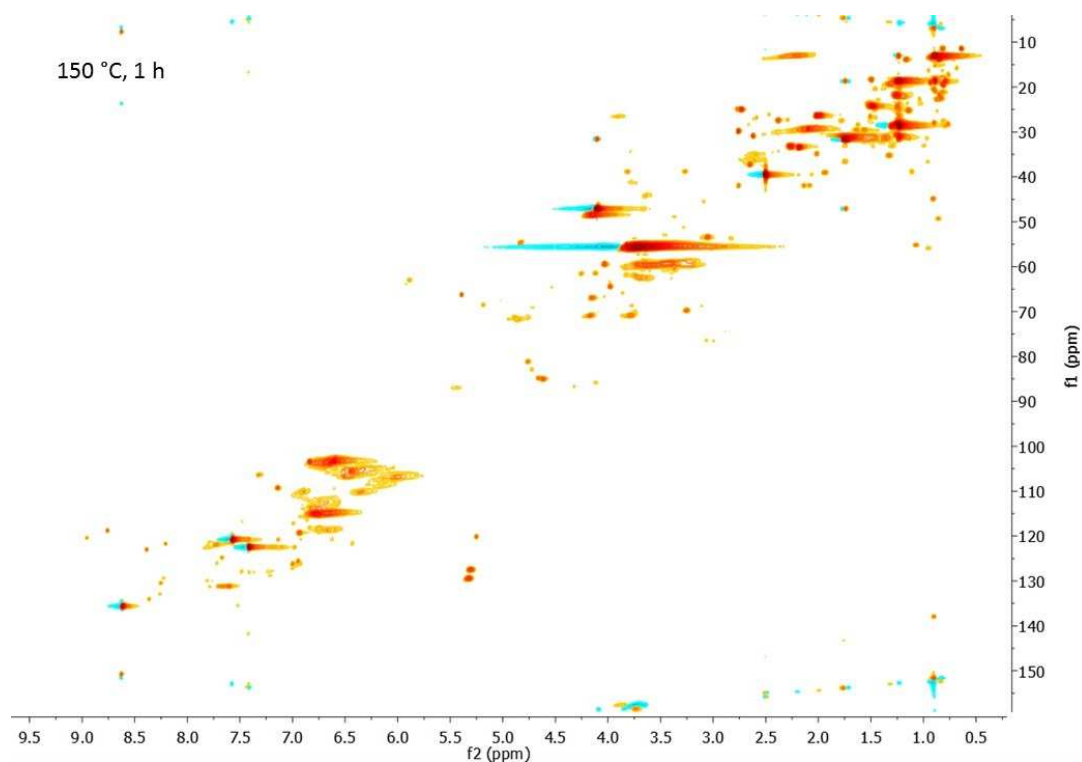


Figure A-61. NMR spectrum of product recovered after *in-situ* modification of lignin with FA during pretreatment of willow Endurance with $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ at 150 °C for 1 hour.

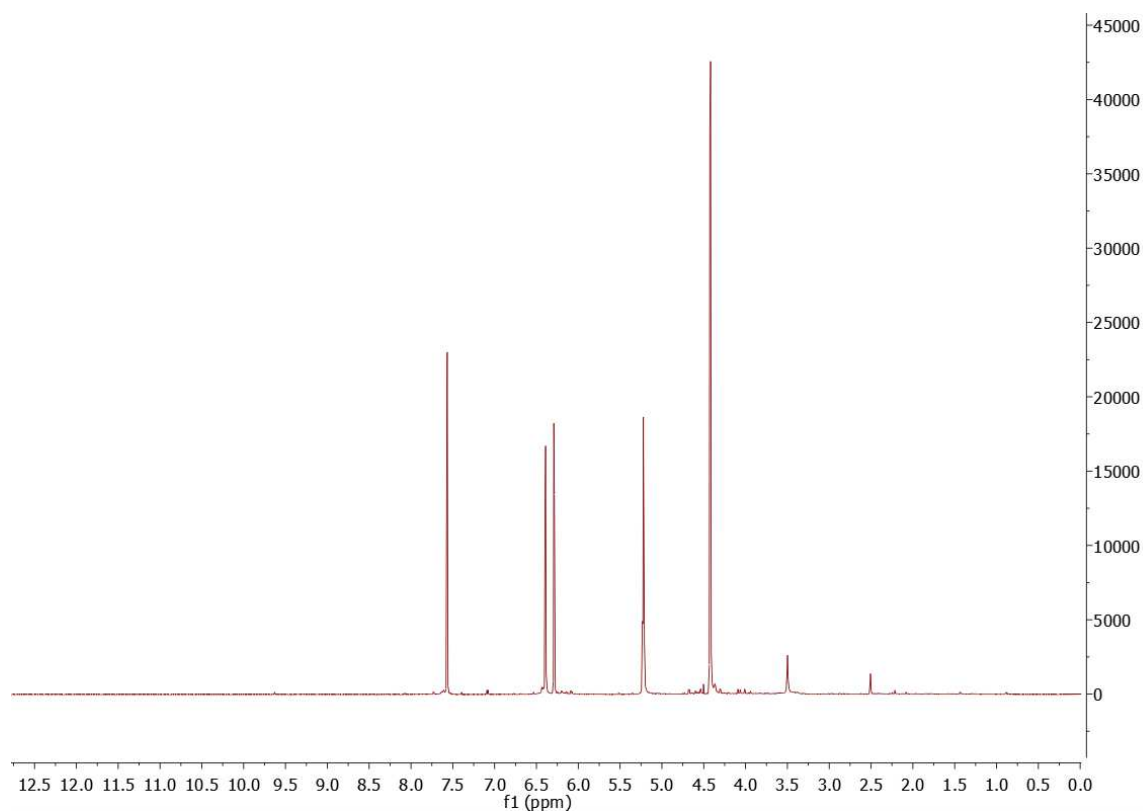


Figure A-62. Proton spectrum of furfuryl alcohol.

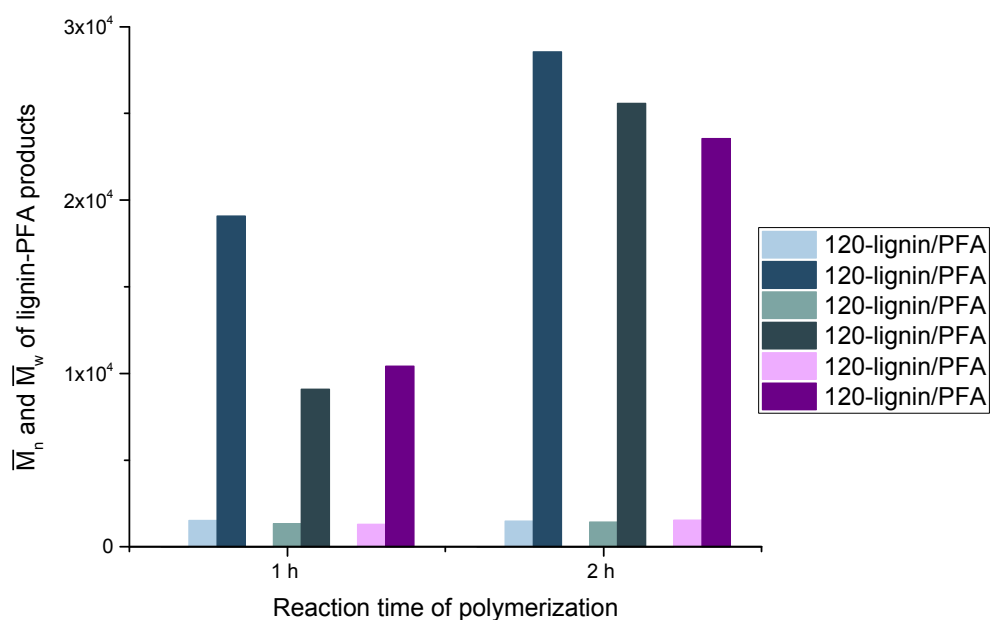


Figure A-63. Number average molecular weight ($\bar{M}_n$) and weight average molecular weight ($\bar{M}_w$) of products recovered after lignin functionalization with FA/PFA in $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ for 1 hour and 2 hours with a lignin:FA ratio of 1:2.

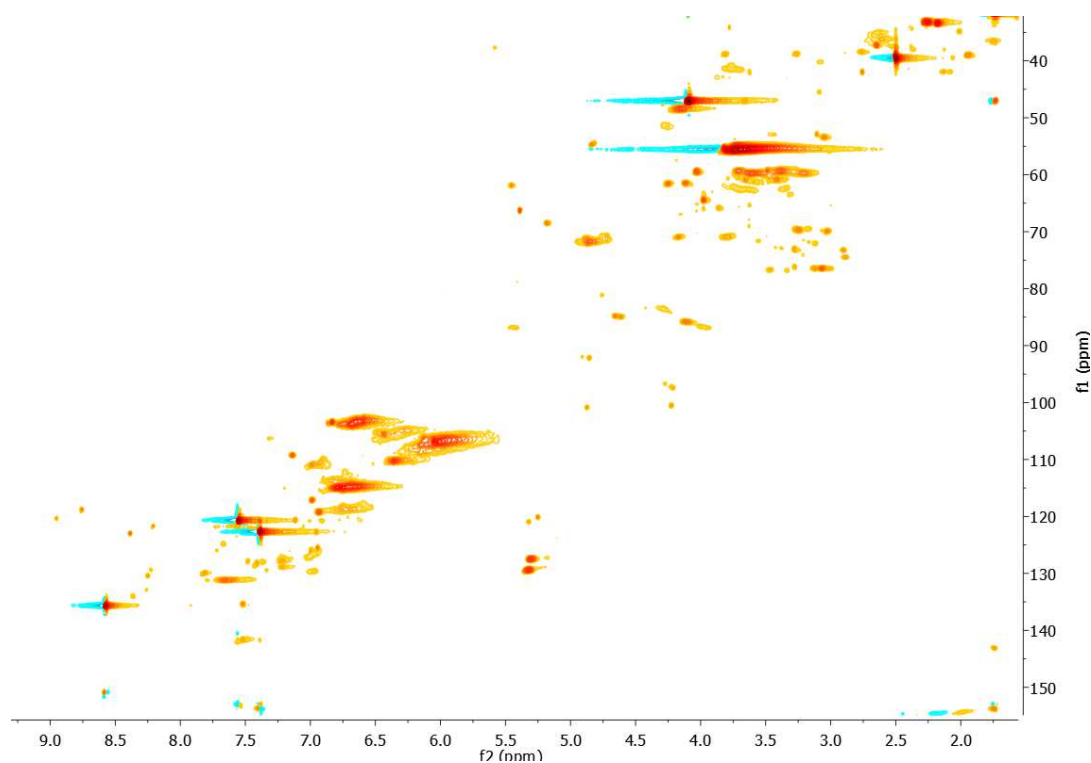


Figure A-64. HSQC NMR spectrum of product recovered after *in-situ* modification of lignin with FA during pretreatment with ionic liquid solution at 120 °C.

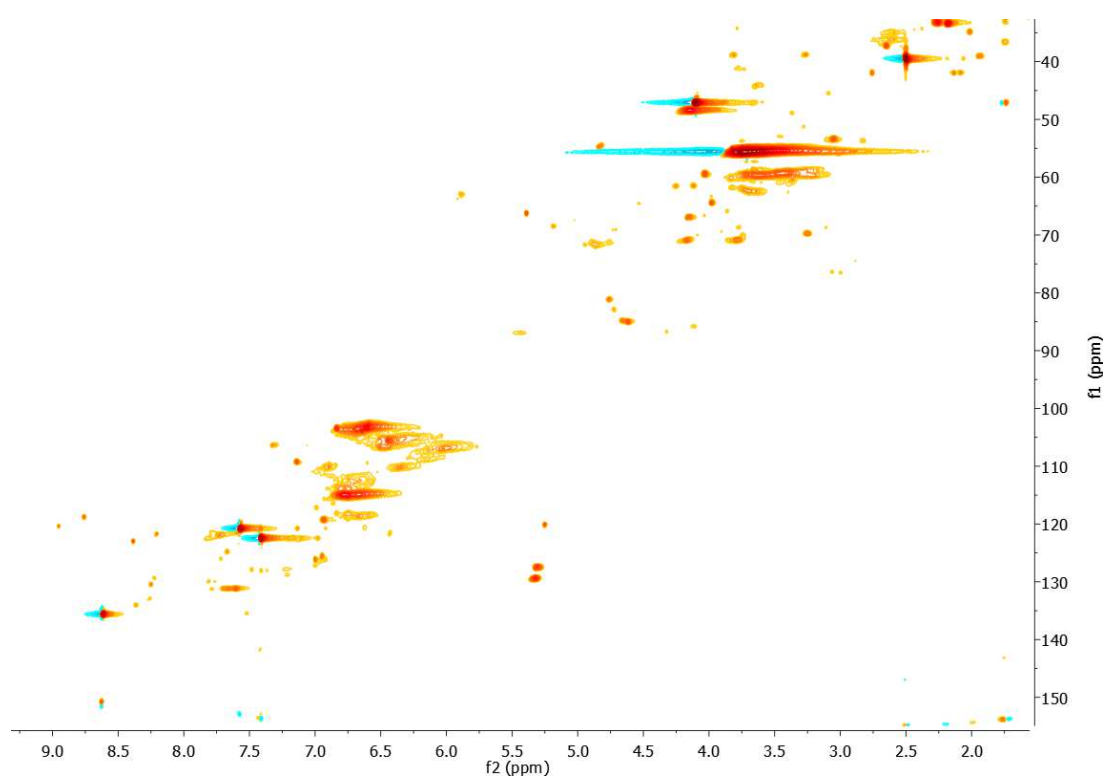


Figure A-65. HSQC NMR spectrum of product recovered after *in-situ* modification of lignin with FA during pretreatment with ionic liquid solution at 150 °C.

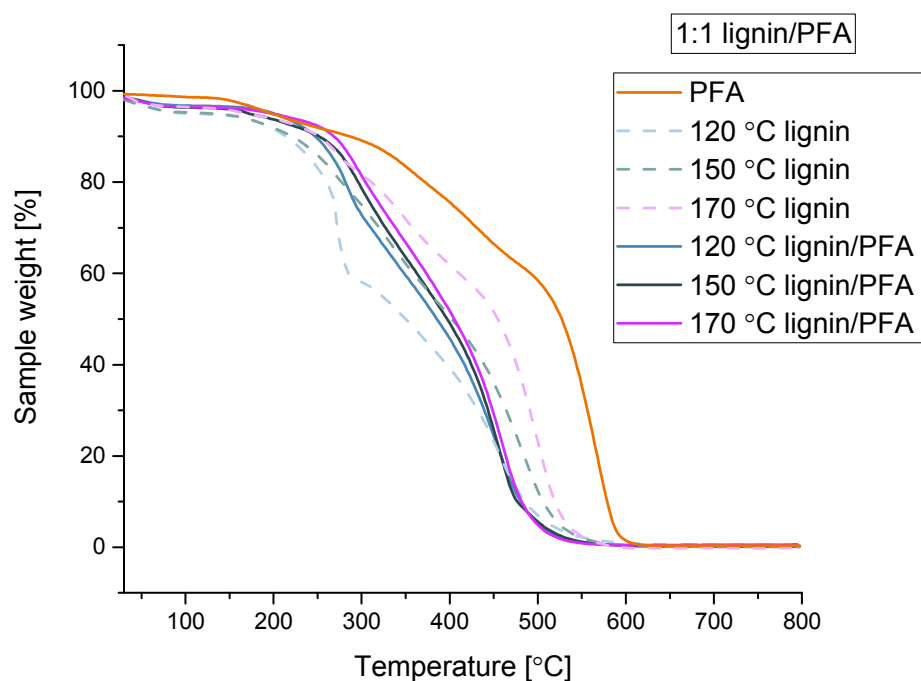


Figure A-66. TGA profile of PFA, lignin extracted at different temperatures using the ionoSolv pretreatment and lignin-PFA copolymers synthesized with $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ using a ratio of 1:1 of the starting materials.

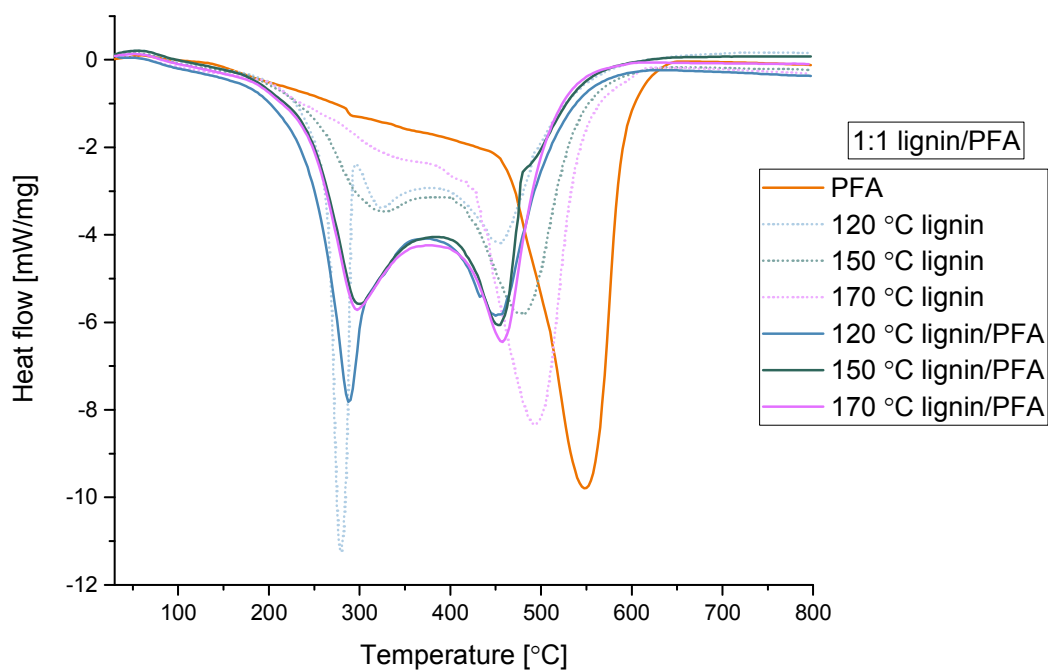


Figure A-67. DSC heat flow profiles of PFA, lignin extracted at different temperatures using the ionoSolv pretreatment and lignin-PFA copolymers synthesized with $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ using a ratio of 1:1 of the starting materials.

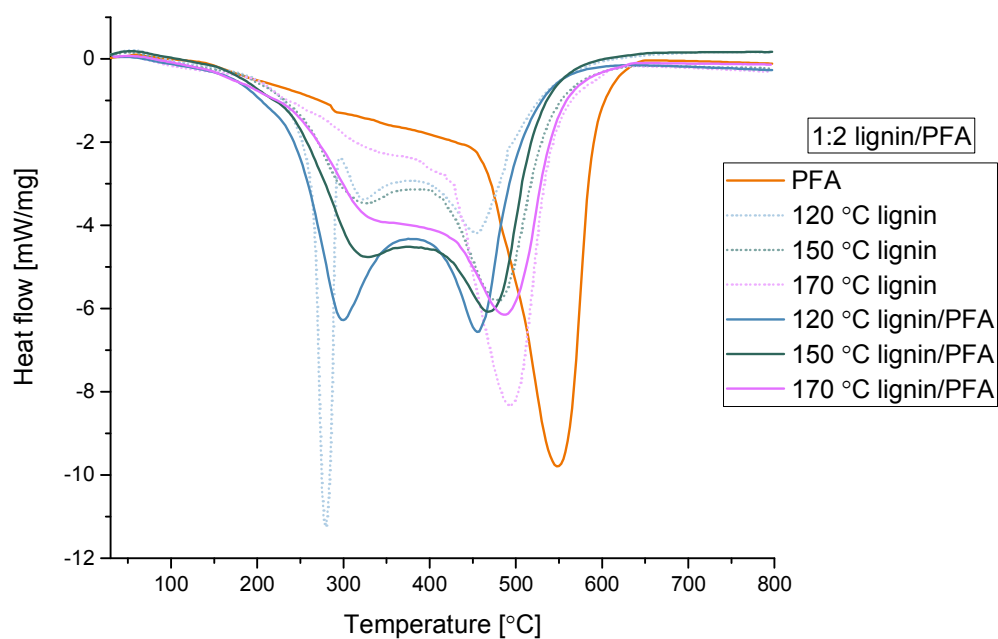


Figure A-68. DSC heat flow profiles of PFA, lignin extracted at different temperatures using the ionoSolv pretreatment and lignin-PFA copolymers synthesized with $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ using a ratio of 1:2 of the starting materials.

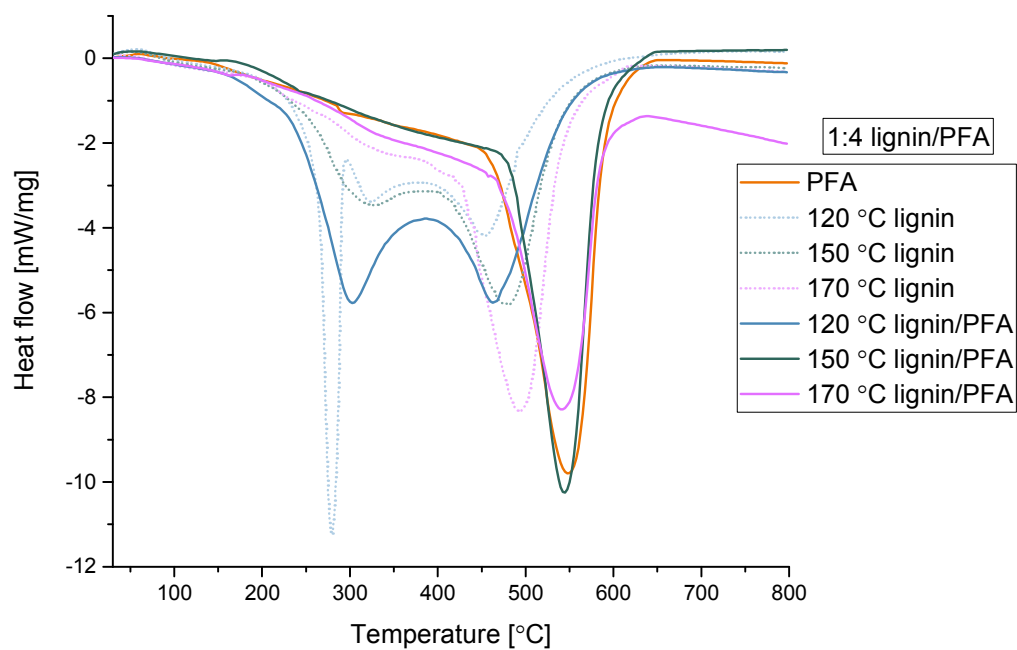


Figure A-69. DSC heat flow profiles of PFA, lignin extracted at different temperatures using the ionoSolv pretreatment and lignin-PFA copolymers synthesized with $[\text{HC}_4\text{im}][\text{HSO}_4]_{80\%}$ using a ratio of 1:4 of the starting materials.

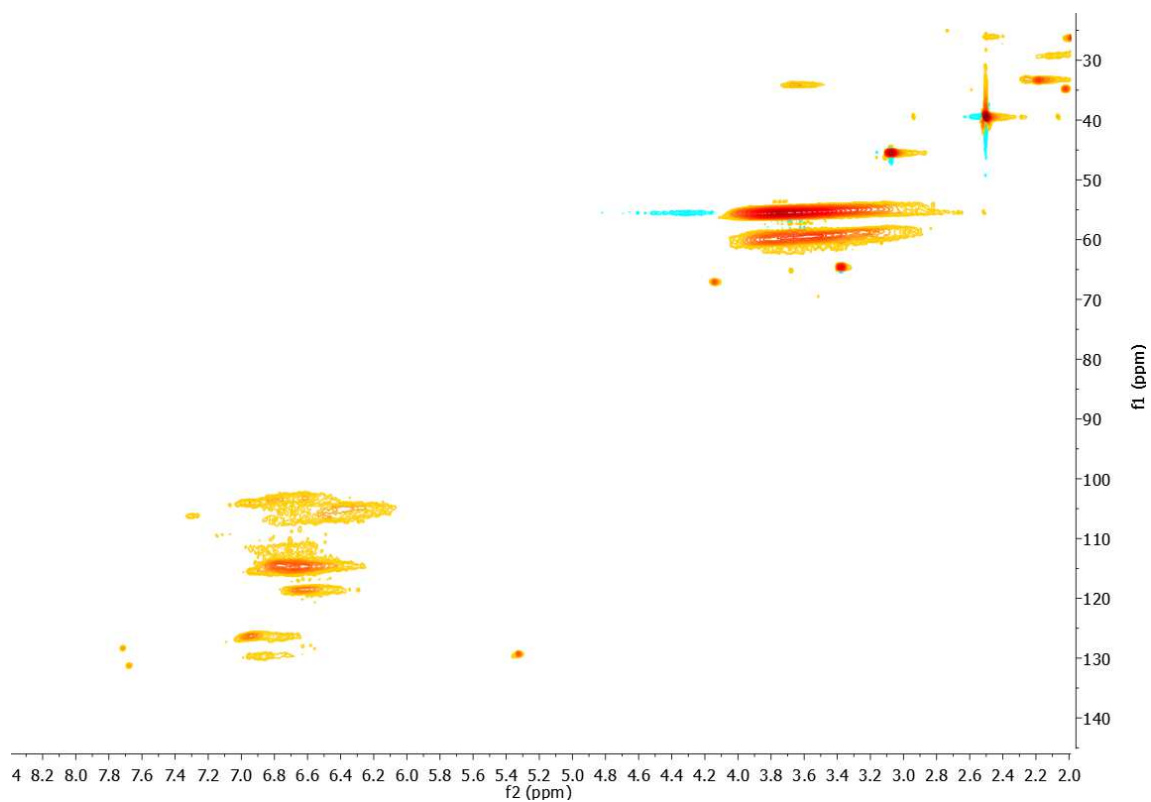


Figure A-70. HSCQ NMR spectrum of lignin isolated after heating in $[\text{N}_{2220}][\text{HSO}_4]_{80\%}$ at 170 °C for 1 hour.

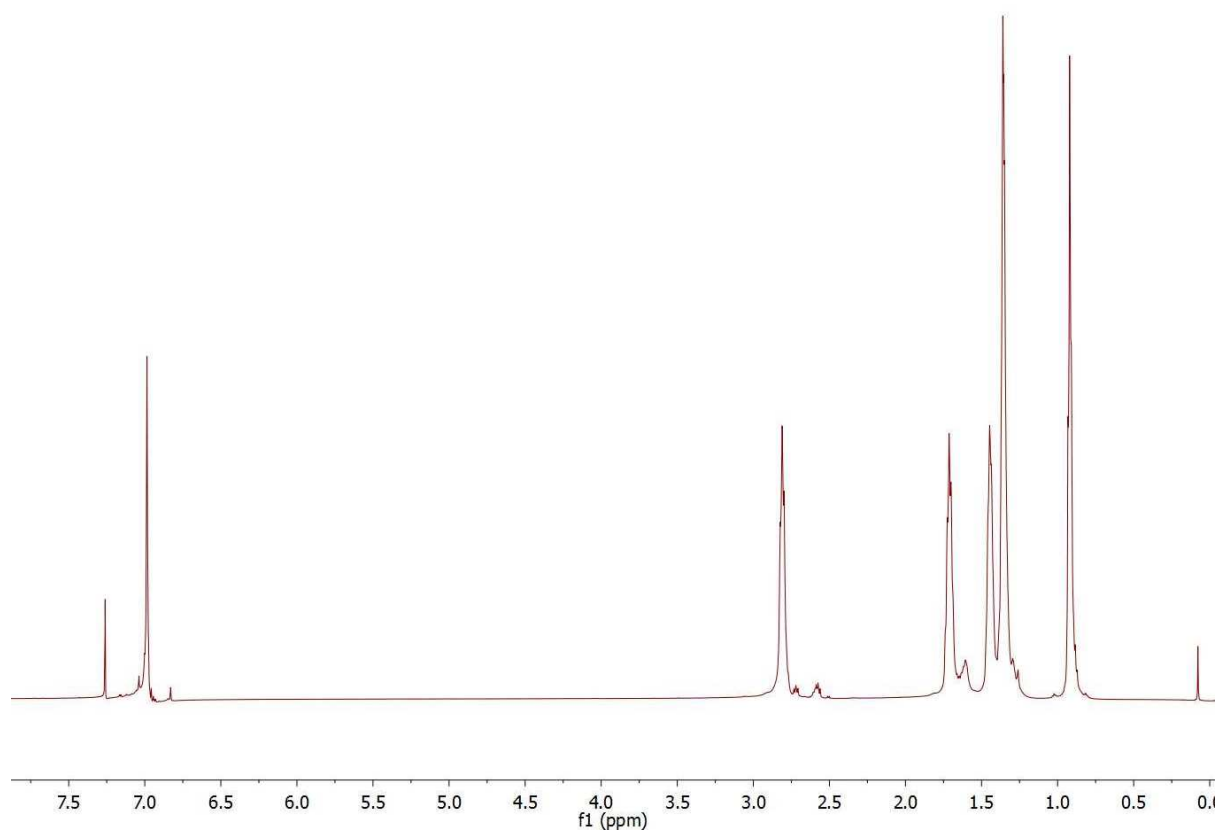


Figure A-71. ^1H NMR spectrum of poly(3-hexylthiophene).

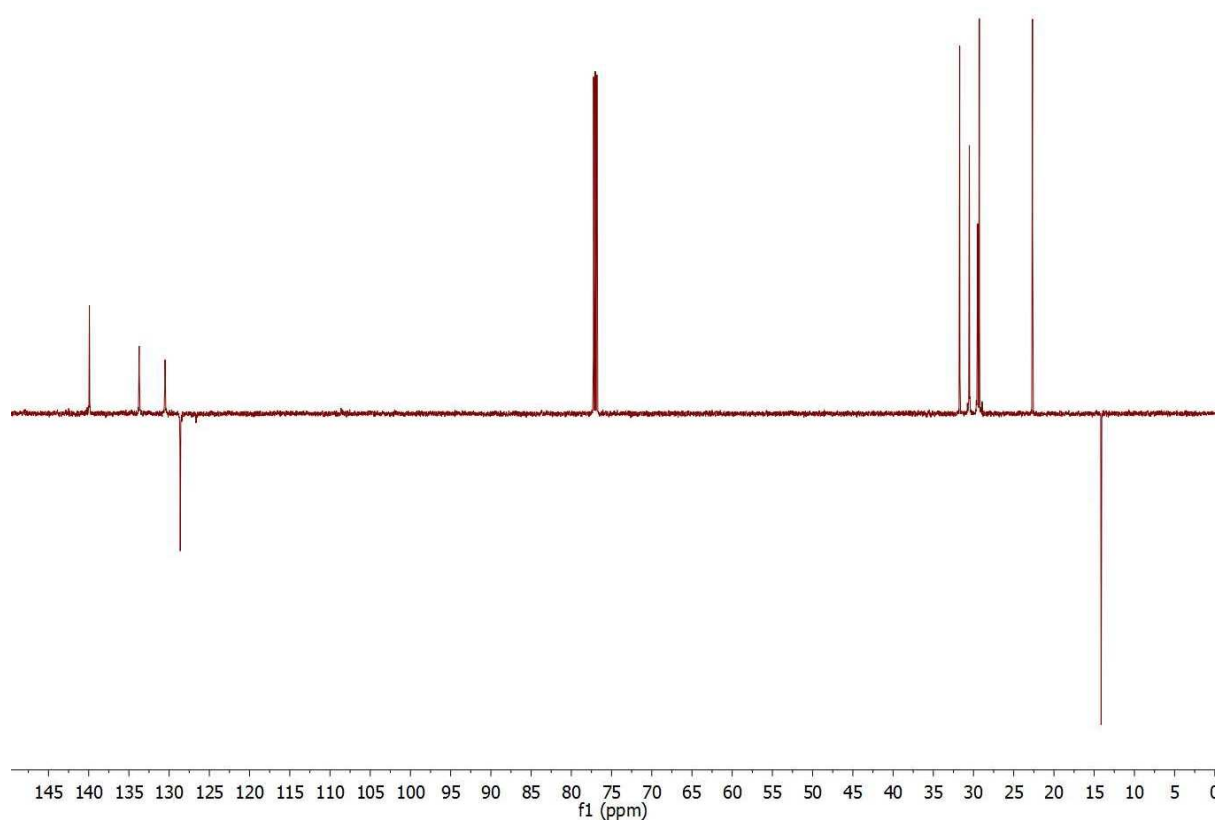


Figure A-72. ^{13}C NMR spectrum of poly(3-hexylthiophene).

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