

Towards an Economically Viable Ionic Liquids Based Biorefinery: Lignocellulose Fractionation and Value-Added Products from Lignin

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Declaration of Originality

The entirety of the work described in this thesis was carried out at Imperial College London between October 2013 and October 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.

F. J. V. Gschwend, A. Brandt, C. L. Chambon, W.-C. Tu, L. Weigand and J. P. Hallett, Pretreatment of lignocellulosic biomass with low-cost ionic liquids, *Journal of Visualized Experiments*, 2016.

F. J. V. Gschwend, A. Brandt-Talbot, C. L. Chambon and J. P. Hallett, Ultra-Low Cost Ionic Liquids for the Delignification of Biomass, In: *Ionic Liquids: Current State and Future Directions*, ACS Symposium Series Vol 1250, Chapter 9, pp 209-223, Mark B. Shiflet and Aaron M. Scurto, Eds., 2017.

C. L. Chambon, T. Y. Mkhize, P. Reddy, A. Brandt-Talbot, N. Deenadayalu, P. S. Fennell and J. P. Hallett, Pretreatment of South African sugarcane bagasse using a low-cost ionic liquid: Comparison of whole and depithed bagasse and fibrous and pith fractions, *Biotechnology for Biofuels*, 2017 (submitted)

Abstract

Lignocellulosic biomass has the potential to partially displace fossil fuels in the production of energy, materials, chemicals and transportation fuels. In order to realize this potential, cost-effective and low-energy fractionation of biomass into different product streams is necessary.

This work uses low-cost protic ionic liquids to deconstruct and fractionate lignocellulosic biomass, with a focus on agricultural residues, energy crops and woody biomass. It aims to demonstrate process intensification and scale-up leading to potential cost reductions. Firstly, the use of triethylammonium hydrogen sulfate was investigated as an alternative to expensive, thermally unstable aprotic ionic liquids for pretreatment of high-ash and high-lignin agroresidues. Pretreatment of sugarcane bagasse, rice husk and rice straw was shown to give up to 90% glucose release under mild conditions. 100-fold scale-up of IonoSolv pretreatment from the 10 mL to the 1 L-scale was performed, enabling evaluation of key parameters expected to impact process economics, including solids loading, feedstock particle size and stirring speed. It was shown that excellent performance could be maintained using a biomass to solvent ratio of 1:2 g/g. Use of larger particle sizes up to 10 mm offered significant potential to reduce comminution energy.

Attention was then turned to utilization of the lignin stream derived from IonoSolv biorefining. Fine-tuning of the process severity (temperature, time, acidity) enabled control of lignin molecular weight and functionality. Fast pyrolysis of mildly treated lignins gave the highest bio-oil yields with maximal energy recovery in the liquid product. Fractional precipitation produced lignin fractions with narrow polydispersity and low molecular weight of 1000 g mol^{-1} with yields of up to 14 wt%. Potential applications of lignin were assessed based on HSQC NMR, ^{31}P NMR, gel permeation chromatography and thermal analysis. Lignin condensation and re-precipitation were identified as the most important parameters affecting both glucose yields and valorization potential of lignin.

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Glossary

[Emim]	1-ethyl-3-methylimidazolium
[Bmim]	1-butyl-3-methylimidazolium
[DMBA]	<i>N,N</i> -dimethylbutylammonium
[Hbim]	<i>N</i> -butylimidazolium
[Hmim]	<i>N</i> -methylimidazolium
[TEA]	triethylammonium
aq.	aqueous
AFEX	ammonia fibre expansion
AIL	acid insoluble lignin (Klason lignin)
AIR	acid insoluble residue
APIL	aprotic ionic liquid
ASL	acid soluble lignin
BTX	benzene, toluene, xylene
CEL	cellulolytic enzyme lignin
CHP	combined heat and power
CSTR	continuous stirred tank reactor
DA	dilute acid
daf	dry ash-free
d.b.	dry basis
DES	deep eutectic solvent
DMSO	dimethylsulfoxide
DSC	differential scanning calorimetry
DTA	differential thermal analysis
EA	elemental analysis
EMAL	enzymatic mild acidolysis lignin
FA	ferulic acid
G	guaiacyl
GC	gas chromatography
GHG	greenhouse gases
GPC	gel permeation chromatography
H	<i>p</i> -hydroxyphenyl
HHV	higher heating value
HMBC	heteronuclear multiple bond coherence

HMF	5-hydroxymethylfurfural
HPLC	high performance liquid chromatography
HSQC	heteronuclear single quantum coherence
ICP-OES	inductively coupled plasma optical emission spectroscopy
IL	ionic liquid
IR	infrared spectroscopy
LCA	life cycle analysis
LCC	lignin-carbohydrate complex
M	molar concentration
MALDI-TOF	matrix-assisted laser desorption/ionization
MESP	minimum ethanol selling price
M_n	number average molecular weight
MS	mass spectrometry
MW	molecular weight
M_w	weight average molecular weight
NMP	1-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance spectroscopy
NREL	National Renewable Energy Laboratory
ODW	oven-dried weight
PCA	p-coumaric acid
PTFE	polytetrafluoroethene (Teflon TM)
PDI	polydispersity index
PFR	plug flow reactor
PIL	protic ionic liquid
ppm	parts per million
PS	polystyrene
PSD	particle size distribution
Py-GC-MS	pyrolysis-gas chromatography-mass spectrometry
RI	refractive index
R&D	research and development
S	syringyl
SE	steam explosion
SEC	size exclusion chromatography
SRS	sugar recovery standard
T	temperature

T_{onset}	temperature of onset of thermal decomposition
TGA	thermogravimetric analysis
UV	ultraviolet spectroscopy
wt%	weight percent
α	Kamlet-Taft acidity
β	Kamlet-Taft basicity
δ	chemical shift (ppm)
ϵ	molar extinction coefficient
π^*	Kamlet-Taft polarizability
1G	first generation
2G	second generation

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Introduction

With global efforts to combat climate change, countries are seeking alternative sources of energy, fuels, materials and chemicals to displace petroleum and minimize greenhouse gas emissions (GHG). Biomass is projected as having the potential to provide a major part of the projected renewable energy provisions of the future¹. Widely available, (multi)-annually renewable and abundant, lignocellulosic biomass can be used as a platform to produce sustainable liquid fuels, chemicals and materials that are considered carbon-neutral as they have a nearly closed carbon cycle^{1,2}. This means they do not necessarily contribute to the net release of carbon dioxide to the atmosphere. Biomass holds immense potential to displace fossil energy and petrochemicals, yet only 12% of the global energy supply currently derives from biomass³.

It is important that energy production does not conflict with food production or cause land use change^{4,5}, and this is a critical consideration in the development of sustainable energy production. The “food versus fuel” conflict can be avoided by using sustainable non-food feedstocks that are currently underutilized or present a waste disposal problem, including waste generated in agriculture, forestry and other industries, or by using dedicated fast-growing energy crops that achieve high biomass yields and are grown on marginal land with minimal inputs.

The route towards economical biorefining entails two principal challenges: firstly, to develop a low-energy, cost-effective process to deconstruct biomass to separate polysaccharides and lignin; and secondly, to achieve holistic recovery and valorization of all biomass components. Lignocellulose is recalcitrant to chemical and physical attack and requires a pretreatment step so that useful biopolymers can be isolated⁶. This pretreatment step is the most expensive single step in biorefining, representing up to 20% of total capital and operating costs, principally in the form of energy and solvent costs⁷. Lignin is a major contributor to biomass recalcitrance where it reduces accessibility of the substrate and irreversibly binds with hydrolytic enzymes⁸. It is also the precursor for toxic phenolic fermentation inhibitors that are formed during conventional acid pretreatment steps⁹. Therefore, separation of lignin from polysaccharides during pretreatment as well as its conversion into high-value products is thought to greatly improve the economics of biomass conversion processes. The recently developed ionoSolv pretreatment process uses inexpensive and thermally stable ionic liquids (ILs) to separate highly digestible cellulose and lignin streams suitable for upgrading into fuels, materials and chemicals^{6,10,11}. The least expensive known IL¹² is triethylammonium hydrogen sulfate, which can be prepared at a cost of \$1.24 kg⁻¹, comparing favorably with commonly utilized organic solvents and offering significant potential for industrial-scale application¹¹. A primary objective of this study is to investigate its thermal stability and recoverability and to benchmark its effectiveness for pretreatment

of abundant, low-cost feedstocks. Scale-up from the laboratory to the sub-pilot scale and process intensification are crucial to identify bottlenecks and the effect of heat and mass transfer on scaled-up biomass fractionation.

A second challenge for effective bio-refining lies in lignin valorization. Lignin is the only large-scale renewable source of aromatic compounds and makes up 10–40% of biomass weight¹³, yet it is currently an under-exploited resource^{14–16}. To date, the utility of lignin is limited: the residual lignin extracted from biomass is mainly combusted for steam generation, and only a small fraction is used to produce chemicals such as vanillin or lubricants¹⁶. The inherent complexity and heterogeneity of lignins, both in terms of its structure and composition, make it very difficult to develop a conversion technology that can process a wide range of feedstocks cost-effectively¹⁷. IL pretreatment has been shown to isolate high-purity lignins with low sulfur content, suitable for upgrading to fuels or high-value aromatic chemicals^{18,19}. Moreover, lignin functionality and molecular weight can be controlled depending on pretreatment process conditions and isolation process, enabling the tuning of lignin properties and structural variations to facilitate its conversion. A key aspect of this work is the investigation of lignin thermochemical conversion to bio-oils, char and gas *via* fast pyrolysis. Fast pyrolysis is an advanced thermal degradation process carried out in the absence of oxygen with carefully controlled parameters to give high yields of liquids, up to ~50 wt%²⁰ on a dry lignin feed basis²¹. The high oxygen content in bio-oils derived from pyrolyzing biomass has been reported to present problems in their applicability, including high viscosity values, low heating values, corrosiveness and a tendency to polymerise^{22,23}. Lignins are deoxygenated during pretreatment, making them more suitable feedstocks for pyrolysis to produce valuable fuel additives^{13,24–27}. A key research question lies in the link between lignin structural variations and the resulting bio-oil yield, quality, composition and stability. A further comparison is to be made between lignin and the parent biomass as feedstocks for bio-oil production.

The co-utilization of carbohydrates to produce biomaterials and biofuels with lignin upgrading to aromatic chemicals is vital to the realization of profitable lignocellulose biorefineries²⁸. Meeting these dual goals – the cost-effective pretreatment and co-utilization of all lignocellulose components including lignin – would increase biorefinery profitability over 6-fold²⁸, though this remains to be verified by techno-economic and life cycle analyses. These targets set the focus for this research thesis, which aims to improve the economic health of the lignocellulosic biorefinery.

Part I. Background

This section is intended to give a general overview of the theory and background of the use of ionic liquids for biorefining and lignin valorization. A concise review of the literature and previous findings on specific topics is also found at the beginning of each chapter in Part II (Results and Discussion).

1. Ionic Liquids

1.1. General

Ionic liquids (ILs) are salts that are in the liquid state at, or close to, room temperature⁶. Typically composed of an asymmetric organic cation, such as quarternized aromatic or aliphatic ammonium ions, combined with inorganic or organic anions, the chemical composition of an IL can be modified to tune its properties²⁹. Figure I-1 shows a representative selection of common cations and anions.

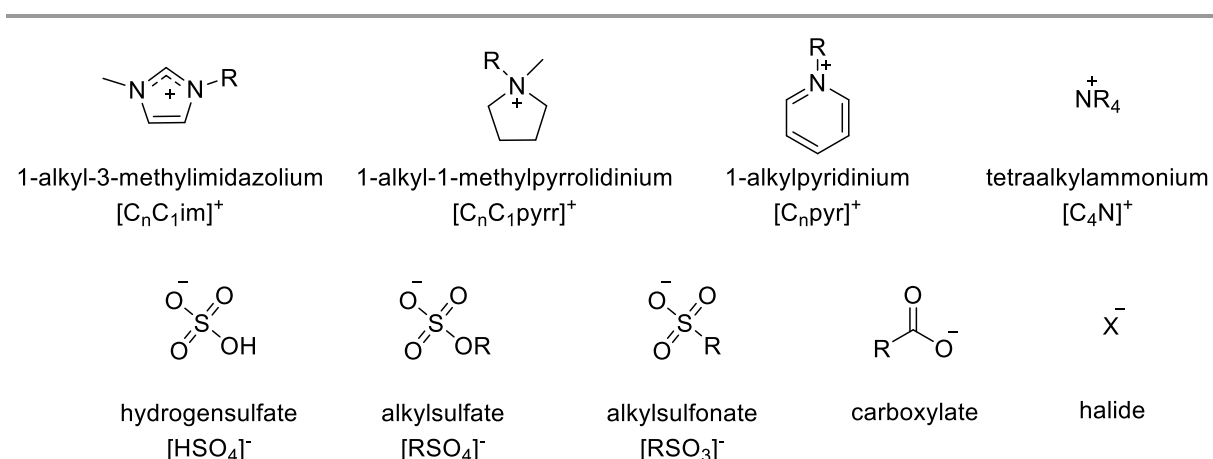


Figure I-1. Representative cations and anions used for biorefining applications. Where R is a hydrogen atom, proton transfer may occur to give a protic ionic liquid.

Ionic liquids have been the subject of intense study since the early 1990s and understandings of their application in areas such as electrochemistry³⁰, carbon dioxide capture^{31,32}, chemicals synthesis³³, biocatalysis^{34,35} and catalysis^{36–39} have increased greatly since then. In the research context, ILs have found two major uses in green chemistry, namely as solvents to replace conventional volatile organic solvents^{35,39–41} and in electrochemistry⁴², for instance as electrolytes for batteries³⁰. In the last 15 years, since Swatloski and co-workers' renowned paper⁴³ on the cellulose-dissolving power of certain ILs, they have also become widespread as solvents for the processing of biomass^{6,44–46}. Ionic liquids have also found a number of industrial applications. Perhaps the most successful example of an industrial process using ILs is the BASIL™ process introduced by BASF in 2002, which uses 1-

methylimidazolium chloride as an acid scavenger and for the production of alkoxyphenylphosphines to increase the space-time yield by a factor of 86,000³⁶. The reaction is currently carried out at a multi-ton scale, demonstrating that handling and recycling large quantities of ILs is practical in an industrial setting.

1.2. Physicochemical properties

Some simple physical properties of ionic liquids render them attractive as solvents. First and foremost, they are excellent solvents for a wide range of both inorganic and organic materials^{41,43}. ILs also possess low or negligible vapor pressure⁴⁷, high thermal stability and relatively low viscosity⁴⁸ at temperatures over 100 °C, which has led to them being considered as versatile substitutes for conventional organic solvents. The solvation abilities of ILs are often attributed to their polarity, which is best understood at the molecular-microscopic level⁴⁹. A multitude of solvent-solute interactions are possible, notably dipolarity, Coulombic interactions, hydrogen bonding and Lewis acidity⁴⁹. The currently accepted definition of polarity is that it is the sum of all possible specific and non-specific intermolecular interactions between solvent and solute⁵⁰. In practice, the high polarity of ILs means that they can dissolve and stabilize dipolar or charged solutes⁴¹. The polarities of ILs can be empirically determined using solvatochromic probe dyes, such as the Kamlet-Taft parameters⁵¹ for dipolarity/polarizability, π^* , the hydrogen bond donating ability, α , and the hydrogen bond accepting ability, β . Although small differences were seen in the π^* values of mono- and quaternary ammonium ILs, the difference in their properties is largely determined by the ability of the salt to act as a hydrogen bond donor/acceptor, i.e. on α and β ⁴¹. Reichardt⁵¹ found that primary and secondary ammonium-based PILs had similar polarities to water, while tertiary ammonium-based PILs had slightly lower polarities. The polarity of *N*-alkylimidazolium-based ILs was higher when combined with stronger acids⁵².

The acidity of ILs is vital as different reactions require specific acidities for optimum efficiency. The relative acidity of ILs can be quantified by various techniques. Where the same cation is used, the relative acidity can be determined from the pK_a value or basicity of the anion⁵². A more sophisticated measure is the Hammett acidity (H_0) scale, a well-understood means to measure acidity in non-aqueous solutions⁵³. The Hammett acidity scale is an effective extension of the pH scale below 0 down to -12 for highly acidic solutions and H_0 values of neat ILs can be determined following reaction with Hammett dyes by UV-vis spectroscopy⁵⁴. Measuring H_0 in aqueous IL solutions is often problematic due to their high viscosity or solid state, peak overlapping in UV and water contamination⁵⁵. To overcome these issues, a common practice is to dissolve the IL in a molecular solvent at different concentrations and then extrapolate H_0 for the neat solution from this data⁵⁶. The relationship between H_0 , density and acid-base ratio of protic $[HSO_4]$ -ILs is reported elsewhere⁵⁷.

Electrochemical applications of ILs rely on their wide electrochemical window, defined as the potential range between the reduction potential of the cation and the oxidation potential of the anion⁵⁸. In other words, the electrochemical window is the voltage range between which the IL is inert. It is linked to the hydrophobicity of the IL and hence depends on the structure of both the cation and anion⁵⁸. Their wide electrochemical window, typically over 4 V, makes ILs a promising electrolyte for battery and power applications⁵⁹. High ionic conductivity has been observed in alkylammonium PILs, making them applicable for fuel cell devices and thermal transfer fluids⁶⁰.

ILs are considered to be Newtonian fluids⁶¹ and are generally more viscous than most common molecular solvents. IL viscosities at room temperature range from around 10 to over 500 mPa·s; by way of comparison, the viscosities of water, ethylene glycol and glycerol are 1, 16.1 and 934 mPa·s, respectively⁶¹. Viscosities are influenced by the nature of both the cation and the anion⁵². Increase in alkyl chain length or number of cations for quaternary ammonium salts tends to make ILs more viscous, due to an increase in van der Waals attractions and hydrogen bonds⁵⁹. ILs viscosities have unusually strong temperature dependence, decreasing to viscosities similar to water upon heating⁶². Additives, such as organic solvents, can also dramatically decrease the viscosity, while chloride impurities have been reported to have the opposite effect⁶³. Equally, the viscosity can be greatly impacted by the presence of water⁶⁴; for instance, the viscosity of [Bmim][BF₄] decreased by 50% upon absorption of 2 wt% of water⁶¹. Wide discrepancies in the measured viscosities of ILs in the literature can therefore be attributed to the presence of impurities, inconsistent water concentrations and fluctuations in temperature, which are regrettably underreported^{52,63}. Detailed data for physical properties of alkylammonium ILs can be found elsewhere⁶².

1.3. Toxicity

In the 1990s and early 2000s, imidazolium ILs were widely studied due to their “remarkable” properties⁶⁵ for applications ranging from electrochemistry⁶⁶ to catalysis^{67,68} and as reaction media^{69,70}, notably for biomass processing^{43,71–73}. Many are now known to be toxic⁷⁴, and earlier claims of their chemical and thermal stabilities have since been more carefully scrutinized, showing these claims to be applicable only to certain classes of ILs^{65,75}. A second potential concern with imidazole-derived ILs is the expense and source of these solvents, which are either of limited stability or very expensive or environmentally unfriendly to synthesize⁷⁶. Due to their synthesis from petroleum-derived feedstocks⁷⁷, the impact of many established ILs is likely to be quite high when their entire life cycle is taken into account.

By the mid-2000s, although many ILs had important industrial and commercial applications, the environmental fate and toxicity of many ILs was not known⁷⁸. Their widely touted stability led to

concerns on the potential for ILs to accumulate in the environment⁷⁸. More recently, there has been a focus on biocompatible^{79–81} and biodegradable^{82–84} ILs to avoid environmental toxicity. This has prompted interesting new research directions, such as the work of Gathergood *et al.*^{82–84} to incorporate structural modifications to render alkylimidazolium ILs biodegradable. Recent research has also examined the synthesis of bio-based ILs, such as from amino acids, carbohydrates, lignin and other renewable sources⁷⁷. One salient development is the use of amino-acid based ILs, notably incorporating the cholinium cation, $[(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OH}]^+$, and anions such as chloride, alkanoates and lactate⁸¹. However, cholinium ILs are thermally unstable under conditions used for biomass processing and likely to be very expensive⁸⁵. Thus, improvements in IL design are still necessary to develop stable, low-cost ILs that can be synthesized from bio-based feedstocks at industrial scale⁷⁷.

Recently, alkylammonium-cation-based protic ionic liquids have spurred research interest due to their relatively low cost, low toxicity and high ionic conductivity at room temperature⁶⁰. These ILs have been found to be less toxic than aprotic imidazolium-based ILs⁵² and several alkylammonium ILs have also been shown to be biocompatible⁸⁶. Nevertheless, manufacture of most IL components remains dependent on petroleum and natural gas feedstocks, though it is possible to synthesize alkylammonium ILs from bio-based materials⁷⁷. Deep eutectic solvents (DES) are a subset of ionic solvents that are composed of two or more organic compounds that have a final melting point much lower than the individual components⁸⁷. DES are very easily synthesized by mixing two solids, one containing a hydrogen bond donor (such as ethylene glycol) and the other a hydrogen bond acceptor (typically quaternary ammonium salts such as choline chloride), without the need for separation steps⁸⁸. First synthesized by Abbott⁸⁹ in 2004, they have similar physical properties to traditional ILs, can be derived from natural and renewable components and have low cytotoxicity⁹⁰. Though the emergence of biocompatible ionic solvents is a promising development, safe disposal of ILs for their environmentally sustainable large-scale use still needs to be convincingly and broadly demonstrated⁹¹.

1.4. Protic ionic liquids

In all discussions of industrial-scale applications of ILs, the cost of the IL solvent will be a critical point. In general, ILs are expensive to synthesize relative to common organic solvents such as toluene, acetone and ethanol, even when manufactured at industrial scale¹¹. Many ILs require numerous and complex synthesis steps and owing to their non-volatile character and low melting points they are not easy to isolate and purify⁹². One way to reduce the cost for multi-ton IL production is through the use of protic ILs, as in the BASF BASIL[™] process and indeed all large-scale applications of ILs to date³⁶. Protic ILs (PILs) are synthesized through simple neutralization of a Brønsted acid and a Brønsted base, in dry or aqueous conditions, to yield a PIL that does not require purification⁵² (Figure I-2). Detailed properties and applications of PILs were reviewed elsewhere⁵².



Figure I-2. Protic ionic liquid formation through proton transfer from a Brønsted acid to a Brønsted base.

PILs are in equilibrium with their parent acid and base, which leads to distinguishing features between PILs and APILs. PILs have a proton available for hydrogen bonding and usually non-negligible vapor pressure⁵², which can lead to boiling rather than decomposition upon heating by reversing the proton transfer to form the more volatile neutral acid or base species⁵². The volatility can be indicated by the pK_a values of the parent acid and base, with a larger difference suggesting a higher degree of proton transfer and hence higher boiling point^{52,62}. When the pK_a difference crosses a certain threshold ($\Delta pK_a > 10$)⁹³, the boiling point becomes inaccessible because chemical decomposition (i.e. pyrolysis) occurs first⁹⁴. PILs formed by proton transfers from strong acids to strong bases behave much like aprotic ionic liquids (APILs)⁶². On the other hand, PILs with less complete proton transfers can be evaporated under reduced pressure and thereby purified or separated, leading to the term “distillable” ionic liquids⁴⁷. The ability to vary the molar ratio of acid and base used for IL synthesis is another attractive feature of PILs, enabling manipulation of their hydrogen-bonding capacity, reactivity, solvent properties and catalytic activity⁵².

Without a doubt, the greatest advantage of PILs over APILs is their lower cost, as PILs can be designed to be based on ions of very low cost, such as alkylamines and mineral acids. Hallett *et al.*¹² recently estimated the cost of triethylammonium hydrogensulfate ([TEA][HSO₄]) to be as low as \$1.24 kg⁻¹, comparing favorably with common organic solvents and offering significant potential for industrial application. In contrast, the most widely studied candidate IL for biorefining to date is 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]), an APIL with bulk cost⁹⁵ estimated at over \$50 kg⁻¹. The costs of PILs are dominated by their material costs⁵² and, since mineral acids are generally very cheap, depend mostly on the cost of the organic base involved. Due to the vastly reduced number of synthetic steps (only seven for [TEA][HSO₄] compared to at least 30 for [Emim][OAc]) compared to APILs, bulk manufacturing of PILs is also bound to have lower E-factor and environmental impact due to a reduction in waste by-products, solvent losses and energy usage during synthesis¹¹. Recently, less expensive [HSO₄]-based PILs have gained significant research attention, notably for large-scale applications such as biomass processing^{11,96,97}.

1.5. Stability and recyclability

Biomass processing using ILs is typically carried out at elevated temperatures (120–170 °C) for extended periods of time (up to 24 h)^{96,98}. Degradation of the IL would reduce process efficiency, and the build-up of decomposition products can be a hazard, particularly at large scale⁷⁵. Moreover, the large contribution of IL solvent to the process operating costs dictates that a high rate (>99%) of IL

recycling is needed if the process is to be financially sustainable⁹⁵. An understanding of the long-term thermal stability, including knowledge of the upper operational temperature limit and precise mechanisms that are occurring, is thus crucial. Hygroscopicity is another important feature, as the uptake of water can lead to a change in the physical and chemical behavior of the ionic liquid⁹². It is very difficult to compare the stability of different ILs in a general way. In practice, it is important to know under which conditions (e.g. temperature) and environment (e.g. water content) a given IL is stable and, most essential for all industrial application, for how long it is stable.

PILs generally undergo decomposition upon heating above the onset of decomposition temperature, which is generally understood to be the upper limit for their use⁵². In the absence of moisture, many ILs exhibit excellent thermal and chemical stability up to around 400 °C, depending on the structure of the salt^{99–102}. However, PILs are in equilibrium with their constituent acid and base, resulting in non-negligible vapor pressure. Boiling off of the neutral acid or base species may occur at temperatures as low as 120 °C⁵². As a result, the composition of the PILs (notably the acid:base ratio) may change upon heating, leading to the involuntary formation of non-stoichiometric mixtures and to the generation of thermal decomposition products.

Thermogravimetric analysis (TGA) has been used extensively to study the thermal stability of ILs^{75,103,104}. Usually, the thermal stability of ILs is characterized by T_{onset} , which is obtained from the intersection of the baseline weight and the tangent of the weight dependence on the temperature curve as decomposition occurs during a linear ramping experiment. However, T_{onset} values are strongly subject to set-up (e.g. heating rate, gas flow and pan material) and must be interpreted with caution as some degradation processes do not result in weight loss^{102,105}. Some ILs have been found to degrade at a temperature significantly lower than the onset temperature and so T_{onset} values are generally considered to overestimate the thermal stability of ILs¹⁰³. Therefore, some authors have proposed a more practical parameter, $T_{x/z}$, where x represents a given decomposition extent (e.g. 1% weight loss) in z length of time (e.g. 10 hours)¹⁰³. MacFarlane and co-workers determined the $T_{0.01/10}$ (the temperature at which exactly 1% weight loss occurs in 10 hours) for several imidazolium pyrrolidinium and phosphonium ILs, and found them to be ~110 °C lower compared to the T_{onset} temperatures¹⁰⁶. However, even this parameter does not necessarily account for long-term stability of ILs at high temperatures; thus, isothermal experiments are useful to record weight loss over a sustained period (e.g. over 100 hours) at the process temperature⁷⁵. TGA experiments to investigate IL stability are ideally combined with IL titration¹⁰⁵, NMR¹⁰¹ and highly sensitive mass spectrometry⁷⁵ to fully understand the chemistry occurring before the thermal degradation temperature and to distinguish vaporization from decomposition in protic ILs¹⁰⁷.

The thermal stability of ILs is mostly influenced by their structural composition, decreasing with higher hydrophilicity and increasing with larger anion size¹⁰⁸. Besides the structural constitution, the presence of impurities (e.g. water) also influences thermal stability¹⁰⁸. Generally, imidazolium salts are more thermally stable than alkylammonium salts, though most PILs based on these cations had T_{onset} temperatures stated as $>200\text{ }^{\circ}\text{C}$, with most close to $300\text{ }^{\circ}\text{C}$ ¹⁰⁷. Stability of imidazolium-based ILs depends on the anion, with carboxylate anions having lower stability due to their highly basic character, enabling S_N2 attack of the cation¹⁰⁹. We therefore see a trend of greater instability in the order of increasing basicity/nucleophilicity of the anion. The popular IL for biomass processing, [Emim][OAc], was found to have T_{onset} value around $\sim 215\text{ }^{\circ}\text{C}$ ⁷⁵; however this has been shown to be an overestimate of its maximal operating temperature. Clough *et al.*⁷⁵ conducted isothermal analysis of [Emim][OAc] at various temperatures (Figure I-3), showing severe degradation after only a few hours at $120\text{ }^{\circ}\text{C}$ and over 50% degradation after 3 hours at $170\text{ }^{\circ}\text{C}$, corresponding to $T_{0.01/10}$ of $102\text{ }^{\circ}\text{C}$. This temperature is below the operating temperature of IL-based procedures, showing conclusively that [Emim][OAc] is not suitable for economical biomass processing⁷⁵.

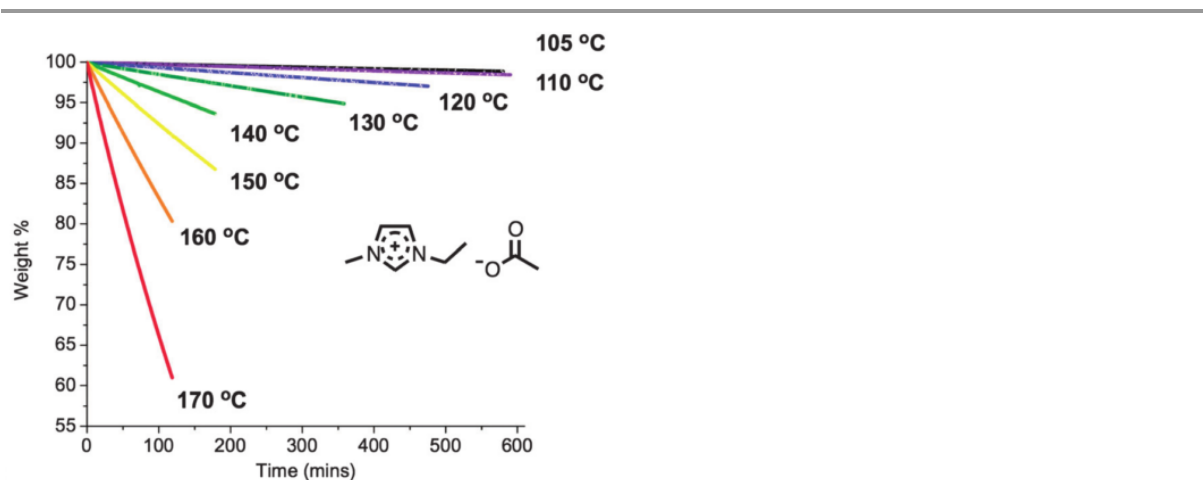


Figure I-3. Isothermal TGA analysis of [Emim][OAc], reproduced from Clough *et al.*⁷⁵.

Tertiary ammonium ILs are reported to degrade by the same S_N2 mechanism as imidazolium salts¹¹⁰ as well as by Hofmann elimination reactions, forming amine by-products by dealkylation or transalkylation^{48,111}. However, these reactions are likely to be very slow in alkylammonium ILs with the $[\text{HSO}_4]^-$ anion as it is a poor nucleophile. Moreover, the presence of low concentrations of degradation products is not expected to lead to a reduction in performance since di- and mono-alkylated ammonium ILs have also performed well as processing solvents¹¹. PILs with inorganic anions have been reported to be among the most stable.⁵² Of primary interest in this study are $[\text{HSO}_4]^-$ -based PILs such as triethylammonium hydrogen sulfate ($[\text{TEA}][\text{HSO}_4]$), dimethyl-butylammonium hydrogen sulfate ($[\text{DMBA}][\text{HSO}_4]$) and 1-methylimidazolium hydrogensulfate ($[\text{Hmim}][\text{HSO}_4]$), which are reported to be thermally stable with T_{onset} values of $300\text{--}320\text{ }^{\circ}\text{C}$ ¹¹². TGA has shown that decomposition of these ILs

occurs at far higher temperatures than [Emim][OAc]¹¹, suggesting their long-term use in a continuous process would be feasible.

1.6. Summary

- Ionic liquids exhibit many favorable properties as solvents for biomass processing, including high polarity, thermal and chemical stability, low volatility and low viscosity at temperatures >100 °C.
- Protic ionic liquids are a low-cost alternative to expensive aprotic ionic liquids and can be synthesized at costs of ~\$1/kg, i.e. at price parity with conventional organic solvents.
- Low-cost protic alkylammonium hydrogen sulfate ionic liquids are strong candidates for use in large-scale biomass processing owing to their low cost, low toxicity and high thermal stability.

2. Biorefinery

A critical concept in biomass utilization is that of the “biorefinery”, defined by NREL⁵ as a “facility that integrates biomass conversion processes and equipment to produce fuels, power and chemicals from biomass”. A biorefinery is analogous to a petroleum refinery which produces multiple fuels and products from crude oil. Biorefining embraces a wide range of technologies able to separate biomass resources (wood, grasses, crops...) into their building blocks (carbohydrates, proteins, triglycerides...) which can be converted to value-added products, biofuels and renewable chemicals. The biorefinery fits into a larger vision for a sustainable bio-based economy.

The field of biorefining has, to a great extent, been driven by the desire to produce more cost-effective transportation fuels¹¹³, made more urgent by climate change and the concept of “unburnable carbon”, meaning that one-third of oil reserves, half of gas reserves and 80% of coal reserves should remain unused in order to meet the maximal atmospheric warming target of 2 °C¹¹⁴. However, not only the transport sector relies on oil (60% of demand); the majority of chemical products are produced from oil refining, such as chemicals and plastics (4% of demand)¹¹³. Thus, the bioeconomy has the potential to displace a large fraction of fossil resources as feedstocks for the energy, chemicals and materials sectors. At the political level, the bioeconomy has received much support, notably in the EU, US and in Brazil, due to its potential for job and value creation, energy security, rural development and positive environmental impact¹¹⁵. For instance, the European Commission has estimated that the bioeconomy already employs 22 million people in the EU¹¹⁶. Today, there is no need to emphasize the importance of the move to the bio-based economy as a key component of decarbonization, combined with energy efficiency, behavioral changes, expansion of public transport and cycling schemes, electrification of transport and introduction of new fuels¹¹⁷. However, there is still a need for R&D in cost-effective biorefining technologies, developing markets for new bio-based products, and pushing policymakers to consider long-term sustainability when developing bio-based value chains.

2.1. 1G and 2G biorefineries

Plant biomass, over 90% of which is lignocellulose, is the largest terrestrial renewable carbon source on Earth, though it currently accounts for only 12% of global energy³. However, the selection of appropriate feedstocks for biorefining is critically dependent on geography, scale and intended use to ensure it is truly sustainable. First generation (1G) biorefineries are based on food, i.e. sugar, starch, vegetable oil or animal fats using established technologies¹¹⁸. The main advantage of 1G feedstocks is the high sugar or oil content of the raw materials and their easy, cost-effective conversion into biofuel (see Figure I-4). The most common 1G biofuels are bioethanol, biodiesel and starch-derived biogas, mainly produced from sugarcane, sugar beet, corn and wheat¹¹⁸. Global bioethanol production was around 115 billion liters as of 2015, led by the USA (60%), Brazil (25%) and the EU (5%)¹¹⁹. Corn is the primary feedstock used in the USA, while sugarcane is the favorite in Brazil; in the EU, bioethanol is derived mainly from sugar beet and starch crops¹¹³. Biodiesel is another major 1G biofuel, which is worth mentioning despite relatively low production volumes (<30 billion liters in 2016)¹²⁰ as the EU mandate on biodiesel production has had devastating environmental consequences. Biodiesel is produced from oil-based crops such as rapeseed but also from palm oil, the latter grown on peatland that has led to extensive, often illegal, deforestation in Indonesia, Malaysia and other countries¹²¹.

This brings us to the topic of the negative environmental and societal impacts of biofuels. Several life cycle assessments (LCA) have evaluated the impacts and other environmental aspects of first generation biofuels, concluding that most, but not all, first generation biofuel substituting fossil fuels led to increased *negative* impacts^{122–124}. This is largely due to increases in acidification and eutrophication as well as soil carbon losses¹²⁵ due to competition with food supplies and agricultural land use¹²⁶. 1G biofuels therefore raise serious concerns regarding their sustainability; these limitations are expected to be partially overcome by developing so-called second generation biofuels.

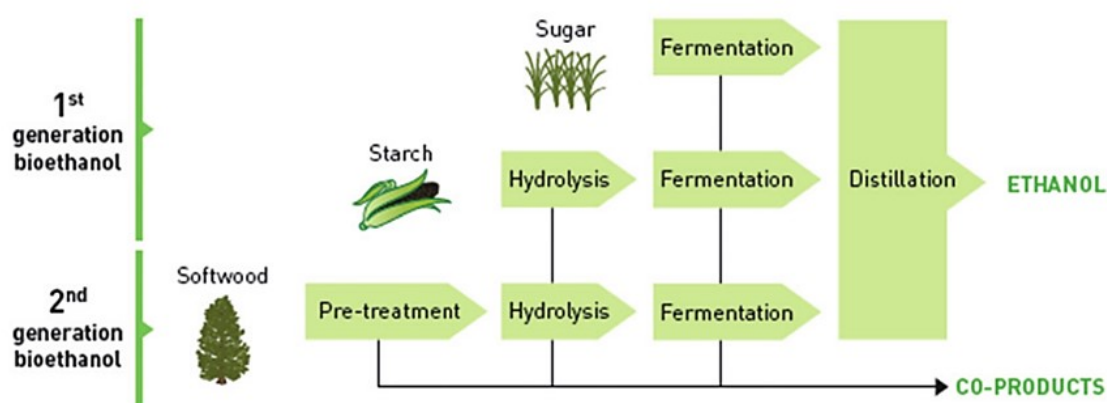


Figure I-4. Biorefinery schematic showing the major bioethanol production steps from 1G and 2G feedstocks.⁶

Second generation (2G) biofuels can be produced from a variety of non-edible crops. These include lignocellulosic materials, such as residues from agriculture, forestry and industry, as well as certain plants. Contrarily to 1G biofuels, where the utilized fraction (fruit, seeds and grains) represents only a small portion of biomass weight, 2G biofuels can use the whole crop for energy production¹¹³. LCA of 2G biofuels have shown that they offer advantages in terms of land-use efficiencies and environmental performance compared to 1G biofuels^{4,127,128}. Some authors estimate that over 10–50 billion tons of lignocellulosic biomass could be harvested globally per year, mostly from agricultural residues¹²⁹. If we assume that 400 liters of bioethanol can be produced per ton of lignocellulose¹²⁹, then 4,000–20,000 billion liters per year of ethanol could be produced, which could replace 2–10 times current global petrol consumption of 1,300 billion liters per year¹³⁰ (assuming E85, a mixture of 85% ethanol and 15% gasoline by volume, is used¹³¹). Simulations by Strapasson *et al.*¹²⁸ have shown that production of lignocellulosic biomass for energy could lead to GHG emissions as low as $-21 \text{ GtCO}_{2\text{eq}} \text{ year}^{-1}$, which would represent a significant source of negative emissions, provided the bioeconomy is developed in conjunction with food security and forest conservation. The use of perennial biomass feedstocks provides added benefits of building up soil carbon levels, reducing fertilizer requirements to limit N_2O emissions and having greater resistance to drought than annual biomass crops¹²⁴.

Lignocellulosic materials are widespread, relatively cheap and easily available¹; however, their utilization is more challenging owing to the higher recalcitrance (i.e. resistance to degradation) of 2G feedstocks, requiring pretreatment prior to their conversion, which entails additional energy and materials (notably enzyme) costs¹¹⁹. Thus, the commercialization of the lignocellulosic biorefinery is currently limited due to technological constraints and high processing costs, notably in the pretreatment step¹¹⁹. There are, however, examples of commercial lignocellulosic biorefineries, notably Borregaard's integrated biorefinery in Norway, a pulp mill that has evolved to produce cellulosic ethanol, lignosulfonates and other lignin bioproducts such as vanillin⁹¹. In 2014, GranBio announced startup of the first cellulosic biorefinery in Brazil. A number of commercial bioethanol plants have opened very recently, such as Beta Renewables' commercial plant in Italy¹³² and Abengoa's and Poet-DSM's plants in the USA^{133,134}. These biorefineries all use agricultural crop residues (such as stalks, straw and leaves) and energy crops that do not compete with food grain.

2.2. Feedstocks

2G feedstocks comprise residues (agricultural, forestry and municipal solid waste), timber and hazardous wood wastes (hardwood and softwood) and energy crops (such as *Miscanthus*, switchgrass and poplar). These can be broadly classified as grasses (also known as herbaceous biomass), softwoods and hardwoods. Algae and seaweeds are another class of non-edible biomass that could be used for "third generation" biorefining¹¹³, and are outside the scope of this work. We focus here on biomass

resources that have moisture contents upon harvesting below ~70% , thereby excluding certain streams such as sewage sludge, manure, food residues and other wet feedstocks which are more suited for generation of biogas through anaerobic digestion¹¹³. Other streams, such as organic municipal solid waste, may be contaminated with heavy metals and are not considered here.

Selection of appropriate feedstocks for biorefining is highly geography-dependent, as the low energy density of biomass dictates that it cannot be transported over long distances¹³⁵. Some promising feedstocks of interest in this work and their characteristics are summarized in Table I-1.

Table I-1. Potential of some lignocellulosic biomasses as bioethanol feedstocks.^{119,136–140}

Biomass	Type/Geography	Major influential features
Miscanthus (<i>Miscanthus × giganteus</i>)	Grass Temperate	• Perennial crop with high yields (10–40 t ha⁻¹ y⁻¹ d.b.) • Low maintenance requirements • Low fertilizer and pesticide requirements • High accumulation of carbon in the soil • High cellulose content (38–40%)
Willow (<i>Salix</i> spp.)	Hardwood Temperate	• Low ash content compared to crop residues • Can be used to extract heavy metals for soil remediation • Flexible harvesting time facilitates year-round supply • High biomass yield (12–30 t ha⁻¹ y⁻¹ d.b.) • Wide range of genetic diversity enabling an increase in yields through breeding and hybridization
Pine (<i>Pinus sylvestris</i>)	Softwood Temperate	• Low ash content compared to crop residues • High density of biomass for more economical transport • Largely unexploited and underutilized biomass source • Wide adaptability and relatively high biomass yields
Sugarcane bagasse (<i>Saccharum officinarum</i>)	Grass Tropical	• High biomass yield (60–80 t ha⁻¹ y⁻¹ d.b. for entire crop) • Global production of 260 million ty⁻¹ • Low lignin and ash content • High efficiency for accumulation of solar energy • Established sugarcane industry for centralized conversion
Rice straw	Herbaceous Tropical	• High cellulose content (42–48%) • Agricultural residue valorization is more eco-friendly than currently practiced crop burning • Short harvest rotation period (~3–6 months) • Widespread, immensely abundant (>800 million ty⁻¹) • Minimizes deforestation and land use

Fast-growing plant species have proven to be serious candidates as energy crops, falling into two categories of either fast-growing woody species such as willow or poplar, or perennial rhizomatous grasses such as *Miscanthus* and switchgrass¹⁴¹. *Miscanthus* has been cultivated in Europe since the 1930s and can produce annual yields ranging from 10–40 t dry matter ha⁻¹ y⁻¹, where the hybrid *Miscanthus × giganteus* was selected for its apparent high productivity¹⁴¹. The advantage of perennial crops compared to trees is that they can be established more quickly and produce annual harvests

with low moisture content¹⁴¹. Indeed, Smith *et al.*¹⁴² have identified energy crops as the agricultural land use change with the greatest potential for carbon mitigation in Europe. Perennial grasses are also easy to grow and harvest, and reportedly have the potential to meet 50–70% of bioethanol feedstock needs¹¹⁹. Short-rotation woody crops, such as willow, have a short harvesting cycle and are another promising high-yield bioenergy crop. Willow has the potential for uptake of heavy metals and other contaminants and is being investigated as a low-cost crop for phytoremediation¹⁴³. Forest biomass is another feedstock type that is largely underutilized in Europe, and is composed of softwoods such as pine and hardwoods such as poplar, but also wood wastes such as sawdust, wood chips, bark thinning residues and contaminated waste wood^{119,144,145}. Sustainable intensive removal of biomass from forests can be achieved by appropriate biomass management, intercropping and careful landscape planning¹⁴⁰. Other feedstocks with enormous global potential include agro-industrial residues such as sugarcane bagasse and other residues from the world's major food crops, notably rice husk, rice straw and wheat straw, though the latter two present some additional challenges due to less established supply chains and high ash content^{146,131,147–149}.

The potential of different lignocellulosic biomass types for ethanol production is primarily determined by the efficiency of glucan hydrolysis into fermentable glucose. Glucan is a collective term for D-glucose polymers linked by glycosidic bonds; cellulose makes up the vast majority of glucan present in native biomass. Great variability has been reported in the glucan digestibility of untreated agricultural residues, bioenergy crops and woody biomass¹⁵⁰. Agricultural residues gave relatively high conversion (10–23% with the exception of bagasse at 47%), followed by energy crops (3–19%) and woody biomass (~7%).

2.3. Lignocellulose

The processing efficiency of lignocellulosic biomass depends on its chemical composition. Biomass is a composite material composed of three biopolymers: cellulose, hemicellulose and lignin, which make up ca. 90% of its dry matter⁶. The three polymers are assembled in a highly functionalized matrix, shown schematically in Figure I-5. Linear carbohydrate chains aggregated within micro-fibrils are surrounded by hemicellulose, which is held together by cross-links with lignin polymers⁶. In native biomass, cellulose and hemicellulose are joined by covalent and hydrogen bonds and further linked to lignin, forming a complex matrix that is highly recalcitrant to depolymerization¹⁵¹.

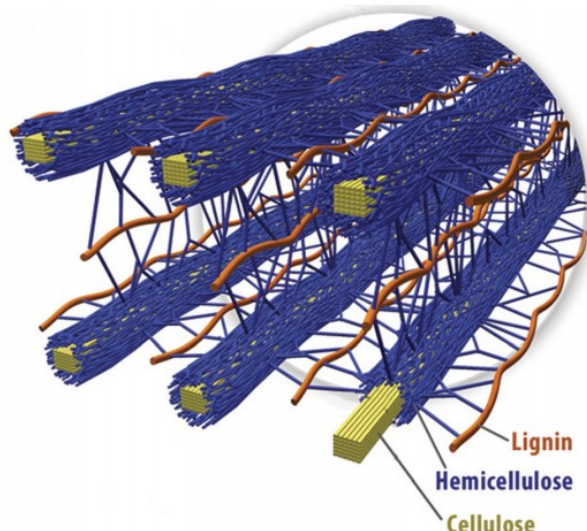


Figure I-5. Spatial arrangement of cellulose, hemicellulose and lignin in the cell walls of lignocellulosic biomass. Reproduced from ref.⁶

Table I-2. Composition of different lignocellulosic substrates on a dry matter basis (wt%). Data from ref. ^{119,152}.

Plant type	Cellulose	Hemicellulose	Lignin
<i>Miscanthus</i> (grass)	38–40	18–24	24–25
Willow (hardwood)	20–25	45–50	20–25
Pine (softwood)	27–30	35–40	25–30

In general, recalcitrance of biomass is associated with a degree of lignification, available surface area for enzyme degradation, protection of carbohydrates by lignin and structural heterogeneity of cell-wall constituents, such as microfibrils and matrix polymers¹⁵³. The structure and composition of these three components determine the digestibility of lignocellulose and its subsequent conversion during physical, chemical and biological treatments. Lignocellulosic biomass contains up to 70% carbohydrates by weight, with the exact proportions depending on the biomass type (grass, hardwood or softwood), species, cell type and developmental stage (see Table I-2).

Cellulose

Cellulose is a linear polysaccharide made up of linear chains of glucose (C_6) units joined together by β -1 $\rightarrow$ 4-glycosidic bonds, with average molecular weight of about 100,000 Da¹¹⁹. Due to their linearity, cellulose strands are able to pack into crystalline microfibrils, held together by strong hydrogen bonds through cross-linking of various hydroxyl groups and Van der Waals interactions¹¹⁹. This aggregation of cellulose fibers provides a crystalline structure that is inert and inaccessible to chemical reagents. Consequently, it is very difficult to hydrolyze cellulose fibers by acids or enzymes⁶. Owing to its high molecular weight, structural rigidity and strong intermolecular forces between cellulose strands, cellulose is water-insoluble⁶. When liberated from the matrix, cellulose may be hydrolyzed to release monomeric glucose units from which bioethanol can be produced.

Hemicellulose

Hemicellulose is a group of hexose (C₆) and pentose (C₅) polysaccharides making up a heteropolymer with average molecular weight of nearly 30,000 Da¹¹⁹. It consists of short, linear and highly branched chains of different monomers including hexoses (glucose, galactose and mannose) and pentoses (xylose and arabinose)⁶. The backbone of hemicellulose is principally made up of xylan (composed of xylose and arabinose), found in all lignocellulose types, while softwoods are typically rich in glucomannan (made up of mannose and glucose)¹¹⁹. Hemicellulose chains may also contain sugar acids, known as uronic acids, which remain associated with cellulose in the plant cell wall⁶. These bind hemicellulose non-covalently to the surface of cellulose fibrils, acting as an amorphous matrix material holding the stiff cellulose fibrils in place. Hemicellulose also contains functionalities such as acetyl and methyl groups, which are thought to enhance the affinity of hemicelluloses to lignin, thus aiding the cohesion between the three major polymers in lignocellulose⁶. Due to their lower degree of polymerization and crystallinity, hemicelluloses are easily hydrolyzed under relatively mild (especially acidic) conditions. However, processing is made more difficult due to the presence of lignin-carbohydrate complexes (LCC), by which hemicellulose forms covalent cross-links with lignin, notably by ether, ester or glycosidic bonds^{154,155}.

Lignin

Lignin is a highly branched polyaromatic polymer present in the plant cell wall, contributing approximately 10–30% of biomass weight and ~40% of its energy content¹⁴. It is a highly heterogeneous, high molecular weight polyphenolic compound that acts as the ‘glue’ holding carbohydrate polymers together⁶. Lignin is water-insoluble, thereby adding to water resistance and serving as a structural support⁶. It is resilient to chemical and physicothermal attack and acts as a shield for the polysaccharides¹⁵¹. Lignin is biosynthesized by radical polymerization of three monomers¹⁵⁶: coniferyl alcohol, sinapyl alcohol, and *p*-coumaryl alcohol. When incorporated into the lignin polymer, the subunits are identified by their aromatic ring structure and become known as guaiacyl (G), syringyl (S) and *p*-hydroxyphenyl (H) subunits, respectively (Figure I-6, bottom row). Lignin subunits are linked by a wide range of C–O and C–C bonds, with about 50% made up of the common β -O-4' aryl ether linkage¹⁵⁷ (Figure I-6, row 1 and 2)¹⁵⁸. Other C–O and C–C linkages are also present, but to a lesser degree. As a result of the radical polymerization of three monomers, lignins are heterogeneous and random with regard to the degree of polymerization, branching, and monomer composition and sequence¹⁵⁶.

Lignin is often identified as one of the major obstacles to the energy-efficient fractionation of lignocellulosic biomass, as gauged by biomass enzymatic hydrolyzability¹⁵⁹. Not only does native lignin prevent access of polysaccharide hydrolases to their substrates, but modified lignin adhering to the

pulp has been shown to cause unproductive binding of hydrolases¹⁶⁰. This increases the enzyme loadings needed and prevents efficient enzyme recycling, thereby increasing the costs of biomass fractionation¹⁶¹. Furthermore, lignin can be a source of compounds that inhibit enzymes and fermentative organisms, including vanillin and syringyl aldehyde¹⁶². Therefore, most chemical deconstruction methods target lignin removal by fragmentation and solvation^{163–166}. Lignin is present in different amounts in various biomass species, generally being most abundant in softwoods (30–60 wt%) followed by hardwoods (30–55 wt%), whereas grasses (10–30 wt%) and agricultural residues (3–15 wt%) contain lower amounts of lignin¹¹⁹. Lignin content can also vary according to the plant tissue^{167,168}, age and growth conditions¹⁶⁹.

While high lignin content is usually a barrier to cost-effective biomass conversion to biofuels, lignin properties are also known to directly influence recalcitrance¹⁵⁹. Herbaceous biomass contains a higher abundance of readily hydrolyzable β -O-4' units (up to 95% of interunit linkages) compared to hardwoods and softwoods which contain 60 and 50% respectively¹⁵⁶. The relative abundance of H, S and G lignin has been documented to affect biomass digestibility^{98,170}. Softwood consists almost exclusively of G-units while hardwoods and grasses contain a large number of S-units and grasses contain all three⁶. C–C cross-linkages are readily formed between the C₅ positions of G-units during lignin biosynthesis as well as delignification¹⁷¹ (see structures C, E and E' in Figure I-6); such condensed structures are not readily hydrolyzed, substantially decreasing the rate of delignification for softwoods¹⁷². Several studies found that at equal lignin content, the syringyl:guaiacyl (S/G) ratio in *untreated* biomass was negatively correlated to enzymatic hydrolysis yields^{173,174}. However, the opposite effect has also been reported¹⁷⁰; given these conflicting results, it is suspected that cell wall degradability is not uniquely related to monomeric lignin composition but more precisely to specific structure¹⁵⁹. By contrast, in *pretreated* biomass, enhanced hydrolyzability has been reported for pulps with high S/G ratio, due to the combined effects of lignin depolymerization and delignification⁹⁸. S-lignin features higher levels of labile β -O-4' ether linkages which are readily cleavable during pretreatment, producing small fragments which could facilitate lignin removal¹⁵⁹. The ease of separating lignin from hydrolyzable cellulose fibers also depends on lignin solubility¹⁷². A high proportion of aliphatic hydroxyl groups increases hydrophilicity and hence solubility of lignin in acidic and basic solvents¹⁵⁹. Some biomass lignins may also contain significant amounts of ferulic acid (FA) and *p*-coumaric acid (PCA) units (see Figure I-6) which are involved in cross-coupling with lignin monomers and formation of LCCs¹⁷⁵. The presence of this cross-linking in the cell wall binds lignin to polysaccharides, resulting in increased recalcitrance¹⁵⁹. Though softwoods contain only traces of FA and PCA¹⁷⁵, more direct aryl-ether linkages are present between lignin and hemicellulose¹⁷⁶. Therefore, rather than lignin content *per se* affecting recalcitrance, it has been suggested that the

integration of lignin and polysaccharides within the cell wall prevents selective separation of biomass component, playing a larger role in contributing to biomass recalcitrance¹⁷⁷. The influence of H-lignin on biomass recalcitrance has been less investigated, though several studies have revealed that high H-lignin in grasses exhibited reduced biomass recalcitrance; speculative reasons for this influence include reduced molecular weight of H-rich lignins¹⁵⁹. Much research has attempted to genetically engineer the content and monomer composition of lignins in native biomass to produce new plant species with reduced recalcitrance¹⁷⁸⁻¹⁸¹; however, this falls out of scope of the work in this thesis, which concentrates on naturally-occurring lignin structures and their impact on biomass deconstruction.

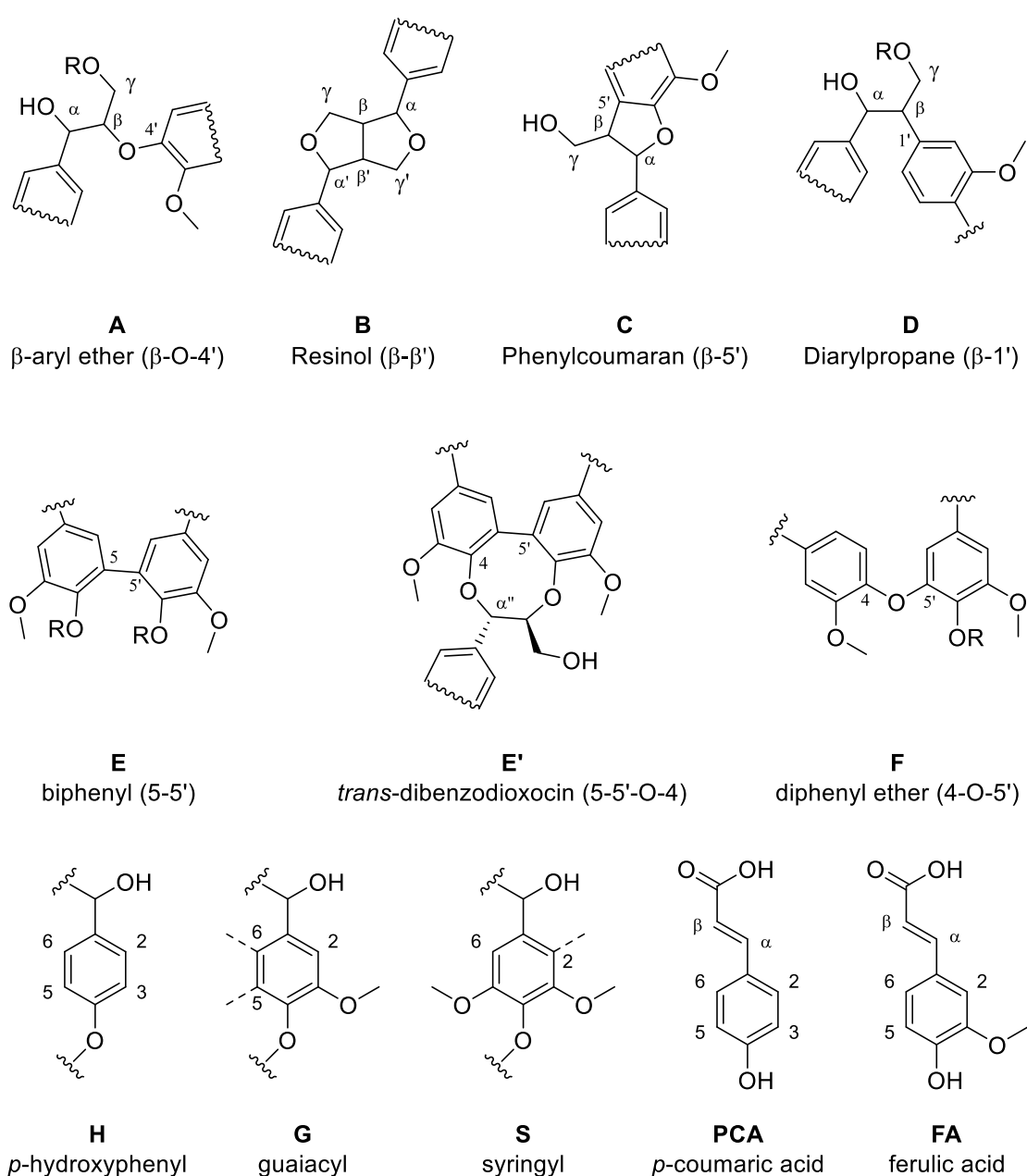


Figure I-6. Common lignin subunits and linkages. Reproduced from Gschwend⁹⁷.

Lignin characterization

Quantitative analysis of the heterogeneous lignin polymer is notoriously challenging, due to poor resolution, low detectability and signal overlap using many techniques¹⁶. A family of techniques has been developed to characterize the structure, chemical composition, polymeric and thermal properties of native lignin extracts and isolated lignins. These are introduced briefly below.

The lignin content of native biomass can be determined gravimetrically using the Klason method¹⁸², consisting of acid hydrolysis to extract acid-insoluble material from biomass, which is subsequently incinerated to obtain the Klason lignin yield. The acid-soluble lignin content can be measured by UV-vis spectrometry of the acidic solution. The same method has also been applied to lignin itself¹⁸³ together with sugar residue determination by high performance liquid chromatography (HPLC) to determine lignin purity, i.e. residual ash and carbohydrate content. Much work has attempted to probe the structure of lignin within plant biomass to understand the changes undergone during biomass processing; only limited probing is possible *in situ*, so lignins are generally isolated prior to structural characterization. The general approach to isolate “native” lignin is by ball-milling to free it from the cell wall, then solvent extraction followed by purification. A commonly used extraction method is use of aqueous dioxane (96%) treatment on ball-milled wood to obtain milled wood lignin (MWL)¹⁸⁴, while other methods employ enzymatic degradation to remove the majority of carbohydrates prior to solvent extraction with aqueous dioxane, resulting in cellulolytic enzyme lignin (CEL)¹⁸⁵. CEL has been found to be structurally similar to MWL, but is produced in higher yield and hence is thought to be more representative of the total lignin in wood than MWL¹⁸⁵. In 2003, a novel procedure using the combination of enzymatic treatment and mild acidolysis was proposed by Wu and Argyropoulos¹⁸⁶. The enzymatic mild acidolysis lignins (EMAL) produced are reported to be most representative of native lignins, offering better purity and higher yields than the previous methods due to the ability of mild acidolysis to cleave remaining lignin-carbohydrate bonds following enzyme treatment¹⁸⁷. Although the above-mentioned methods have been widely used to study the structure of lignins before and after treatment, it is evident that lignin undergoes some modification during ball-milling and extraction¹⁸⁷ in degrading β -O-4' structures and causing some condensation reactions¹⁸⁵.

There are several destructive wet chemistry techniques available to probe the major chemical functionalities of isolated lignins, such as thioacidolysis^{188,189} or nitrobenzene oxidation¹⁹⁰ to determine the lignin monomer composition, permanganate oxidation¹⁹¹ for the degree of condensation, derivatization followed by reductive cleavage (DFRC)¹⁹² for the β -O-4' ether bond content, concentrated acid hydrolysis¹⁹³ for the methoxyl group content, and aqueous and non-aqueous potentiometric titrations¹⁸³ to determine phenolic and carboxylic groups. Other destructive techniques include pyrolysis–gas chromatography–mass spectrometry (Py-GC-MS)^{194–200},

thermogravimetric analysis (TGA)^{201–204} and elemental analysis^{96,199,205} (EA) used for analysis of chemical composition. Analytical Py-GC-MS is a favored technique to assess the monomer ratio¹⁹⁶; however, conclusions regarding the original lignin structure are deduced from the analysis of degradation products, which is more prone to error and ambiguity¹⁹⁹. EA can be used to determine the presence of impurities such as residual solvents as well as to estimate the fuel value²⁰⁶ (i.e. higher heating value or HHV) based on the CHNO content²⁰⁷. Non-destructive methods consist of various spectroscopic methods, including NMR^{208–212}, IR²⁰⁵ and UV²⁰⁵ spectrometry, size exclusion chromatography (SEC)^{213–215} and gel permeation chromatography (GPC)^{216,217}.

Whereas lengthy chemical procedures are very precise for one or two specific functional groups or structural moieties, they cannot give a complete picture of the entire lignin structure²⁰⁸. The development of solution- and solid-state NMR techniques has enormously facilitated elucidation of the structural aspects of complex lignin polymers. Solution-state NMR is unparalleled among other techniques for its high resolution and comprehensive nature²⁰⁸, enabling simultaneous analysis of monomer composition, subunit linkages and functionalization²⁰⁹; some of these methods can be quantitative²¹⁸. Acetylating lignin is frequently used to improve solubility and hence resolution for analysis of isolated lignins¹⁵¹. One-dimensional (1D) proton NMR (¹H NMR) of acetylated lignin was used in the past to determine the quantity of different hydroxyl groups, though is limited by spectral overlap and irregularity of chemical environments surrounding different lignins, making the spectra difficult to accurately interpret²⁰⁹. 1D ¹³C NMR is frequently used to reveal the presence of aryl ethers and condensed and uncondensed aromatic and aliphatic carbons, though it suffers from low sensitivity and signal-to-noise ratio and requires long acquisition times, especially for quantitative measurements. “Inverse detection” techniques can greatly increase the resolution of spectra, while also allowing faster acquisition of multidimensional NMR spectra²⁰⁹. ³¹P NMR, whereby lignins are reacted with a phosphitylating agent combined with an internal standard to produce quantitative spectra, has been widely applied to quantify the aliphatic hydroxyl, phenolic hydroxyl and carboxyl groups in lignin and its derivatives^{219,220}. Similarly, ¹⁹F spectroscopy can be used to quantitatively detect carbonyl groups using trifluoromethylation^{210,221}. Solid-state NMR techniques, on the other hand, are primarily used to analyze the molecular structure of lignin in the plant cell wall, keeping the 3D lignocellulosic matrix intact¹⁵¹. Recent work has employed solid-state ¹³C NMR to confirm the presence of humins and other carbohydrate residues in lignins²²².

2D techniques, involving the correlation of signals from two different nuclei, can allow key resonances in lignin structure to be sufficiently resolved to allow substantive interpretation^{211,223,224}. Heteronuclear single quantum coherence (HSQC) and multiple coherence (HMBC) methods help overcome the problem of overlap, detecting C-H_x groups so that aliphatic and aromatic functionalities

within the lignin structure can be distinguished^{225,226}. HSQC spectra can be divided into an aliphatic oxygenated region, which can be used to estimate the relative abundance of the different interunit linkages present, while the aromatic region allows estimation of the respective monomer ratios, including the S/G ratio²²⁷. Changes in lignin structure following treatment can be semi-quantitatively assessed by comparing signal intensities of cross-peak contours of a particular signal or signals among different spectra²²⁴. Semi-quantitative methods require maintaining the same sample concentration, acquisition and processing parameters across different samples and have been reported to be accurate to within 10% when following the fate of a particular entity during reaction or treatment of a given lignin¹⁷⁵. 2D HSQC NMR has attracted significant attention due to its ability to resolve the structural features and transformations of isolated lignins, such as MWL, CEL and EMAL^{200,228–230}. In addition, lignin isolated from pretreated biomass can be compared with native lignin structures to understand the impact of pretreatment^{96–98,200,215}. The full possibilities of lignin analysis by NMR spectroscopy were reviewed by Ralph and Landucci²²⁴.

An important structural property of lignin is its molecular size, as the molecular mass distribution partly governs its reactivity for valorization and physicochemical properties¹⁸⁸. Vapor pressure osmometry²³¹, light scattering²³², mass spectrometry²³³, ultracentrifugation²³⁴ and GPC²³⁵ are among the various instruments available, compared in detail by Tolbert *et al.*²³⁶. GPC methods now tend to dominate^{235,236}, so will be discussed in further detail. In GPC, polymers are dissolved in an eluent and introduced to a porous gel column, where they are separated on the basis of their hydrodynamic volume²³⁶. The elution volume is coupled with a polymer standard to determine relative molecular size and weight²³⁶. Determination of the concentration of substances eluted from the column is commonly achieved by UV or refractive index (RI) detection, which is assumed to be independent of the molar mass¹⁸⁸. A universally applicable molar mass distribution protocol for a wide range of lignins has not yet been developed, as the polymer properties recorded depend strongly on the experimental set-up (e.g. stationary and mobile phase, detector type) and calibration standard used²³⁵. Separation of polymers by GPC should ideally be solely controlled by the size-exclusion principle; however, secondary separation effects, notably adsorption, ion exclusion and molecular shape, strongly interfere with the obtained results, leading to great inter-laboratory deviations^{183,214,237}. Incomplete solubility is an important source of error, so acetylation in tetrahydrofuran or 1-methyl-2-pyrrolidone (NMP) is frequently used²³⁵. Nevertheless, GPC techniques can be applied to compare *relative* molecular mass distributions within the same experimental set-up. Relative comparison is made between the molar mass distribution of the lignins and narrow dispersed polystyrenes^{188,207,238}. However, for *absolute* molecular weight determination of the number- and weight-average molecular weights, M_n and M_w , and the polydispersity (PDI), most lignins are too polydisperse to enable

comparison with polystyrene¹⁸⁸. Recently, various methods have become available to obtain high resolution for more accurate definition of lignin polymeric properties, such as the use of high-resolution GPC¹⁸³, calibration standards more representative of lignin structure (e.g. pullulan)²¹⁶, or MALDI-TOF-MS for narrow dispersed lignin fractions with low molecular mass¹⁸⁸. It has become recognized that MALDI-TOF-MS is suitable for molecular weight determination of low-polydispersity polymers (<1.2), while GPC is more appropriate for polymers of greater polydispersities that are typically isolated from pretreatment²³⁶. The polymer chain length of native lignin is difficult to measure, as lignin is inevitably fragmented during extraction¹²³. M_w values of 30,000–78,000 (PDI 4–9) have been reported for EMAL isolated from softwood lignin while 3,000–13,000 (PDI 1.5–3) and 1,500–5,000 (PDI 2–4) were reported for hardwoods and grasses, respectively²³⁶.

Thermal analysis is a crucial property for lignin valorization and is usually carried out by TGA or differential scanning calorimetry (DSC)²¹². Upon heating, lignin undergoes softening through a glass transition overlapped with depolymerization, followed by solidification by cross-linking reactions to form a char residue²³⁹. A wide temperature range of 200–500 °C is typically observed for lignin degradation owing to its structural heterogeneity. TGA is typically employed to determine the temperature of onset of decomposition, T_{onset} , the maximum decomposition rate, DTA_{max} , based on the first differential of the weight loss against temperature curve, and the amount of volatile matter and non-volatile solid residue (i.e. char and ash) obtained upon heating up to 600 °C⁶. DSC is the most widely accepted method for determining polymeric properties such as the glass transition temperature T_g , crystallization temperature T_c and melting temperature T_m . Temperature stabilities, notably T_g , must be known for lignin fractions especially if the prospective application lies in manufacture of carbon and graphite fibers or other high-performance polymers²⁴⁰. As T_g is an indirect measure of crystallinity and the degree of crosslinking, lignin T_g will depend on the molecular weight and chemical functionalization¹²³. Baumberger *et al.*²⁴¹ showed that T_g was closely related to the degree of polymerization of lignin, where T_g increased with the degree of condensation (C–C linkages). Heating above the lignin T_g during biomass processing for short periods of time has also been shown to significantly increase delignification; this was attributed to improved separation of lignin from carbohydrate²⁴². T_g of native lignin has been estimated as 90–105 °C for softwood and 65–85 °C for hardwood; other sources have estimated T_g of treated lignins to be as high as 130–150 °C, depending on the isolation procedure and source of the lignin^{242,243}.

2.4. Summary

- Biorefining of second-generation lignocellulose feedstocks such as perennial grasses, short rotation woody crops, agricultural residues and wood offers greatest potential for production of renewable, low-carbon fuels, chemicals, materials and energy.

- Lignin is a heterogeneous polyaromatic polymer that must be partially removed for biorefining.
- Lignin-carbohydrate linkages and lignin structure contribute most to biomass recalcitrance.
- Among the most powerful techniques for lignin analysis are 2D NMR for chemical structure alterations and gel permeation chromatography for changes in polymer length.

3. Deconstruction of Lignocellulose

A number of physical, chemical, biological and thermal processes (and combinations thereof) have been developed to deconstruct and fractionate lignocellulosic biomass in the context of biorefining^{244–246}. Arising from their use in the paper and pulping industry, the initial focus of most deconstruction processes has been to increase the accessibility of cellulose, in order to liberate sugars, which are a ready substrate for fermentation. As discussed previously, the lignin-hemicellulose shield surrounding cellulose microfibrils is highly resistant to chemical and physical attack and must be disrupted to gain access to the cellulose¹⁵¹. This is accomplished in a process step that is commonly known as pretreatment. The goal of pretreatment is thus to bring the sugar polymers into a form suitable for metabolism by fermenting microorganisms (see Figure I-7). The focus is on overcoming the structural and chemical obstacles limiting carbohydrate release, namely removal or redistribution of lignin to increase the accessible surface area of cellulose while limiting the generation of inhibitory compounds^{247,248}.

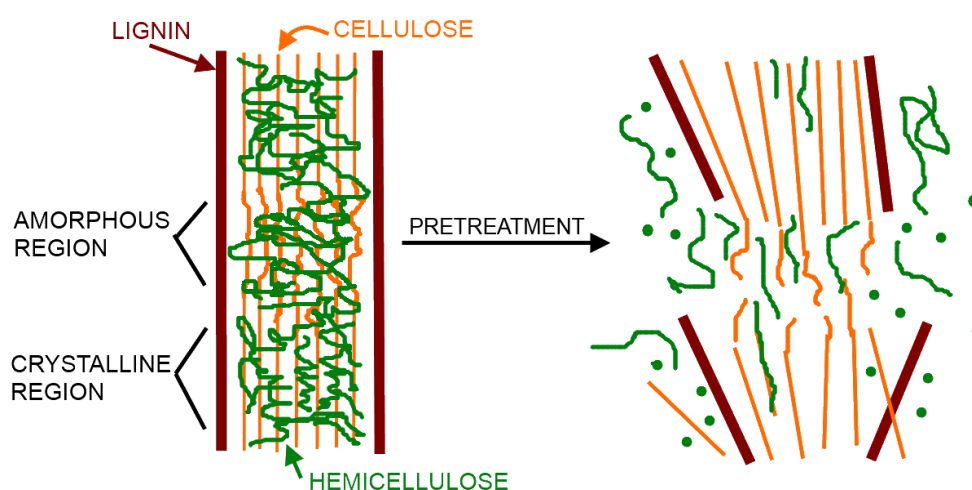


Figure I-7. Diagrammatic representation of the effect of pretreatment on lignocellulose polymers²⁴⁹.

The complexity of 2G lignocellulose compared to 1G (starch/sugar-based) feedstocks requires a number of additional processing steps prior to sugar fermentation: feedstock comminution, deconstruction, conditioning of treated biomass and hydrolyzate and hydrolysis/depolymerization of the polysaccharides⁶. As a whole, pretreatment represents the second largest cost (19–22%) in biofuel

production after the feedstock (30–32%), notably in the form of energy, solvent and enzyme costs^{6,249}. A key reason for high enzyme costs per liter of ethanol is the requirement for high ratios of enzyme to substrate, ~20 mg protein per gram of cellulosic materials, at least one order of magnitude higher than for starch hydrolysis¹⁶¹. Lignocellulose deconstruction is also likely to account for an important fraction of the energy requirement of biomass processing⁶. A second challenge to economic biorefining is to achieve complete utilization of all biomass components, each of which can be converted to products that add value, within a flexible and integrated biorefinery²⁵⁰. The co-utilization of carbohydrates to produce biomaterials and biofuels with lignin upgrading is vital to the realization of profitable 2G biorefineries.

Following pretreatment to overcome the resistance of biomass to degradation, the glycosidic bonds in cellulose and hemicelluloses are hydrolyzed during saccharification, whereby the pulp is treated with enzymes or acids to depolymerize the polysaccharides⁶. Enzymatic hydrolysis is preferred over use of (acidic) chemicals despite lower reaction rates, due to their higher selectivity⁶. Factors affecting cellulose digestibility of lignocellulosic substrates include surface area of cellulose, cellulose crystallinity, lignin and hemicellulose content, particle size and porosity²⁵¹. Low enzymatic digestibility of dried materials compared to wet pulps has been reported for numerous materials such as waste paper²⁵² and air-dried pretreated lignocellulose solids²⁵³. Drying can induce physical changes in fiber structure, such as hornification or pore collapse, that reduce enzyme accessibility to cellulose^{252,254}. Directly hydrolyzing wet pulps (rather than air- or oven-drying them) can eliminate factors such as drying-induced hornification on cellulose saccharification. Next, some of the post-hydrolysis solids are used in the enzyme production step and the remainder can be processed through anaerobic digestion, dried for energy recovery or discharged from the plant²⁵⁵. The liquid hydrolyzate, containing dissolved C₅ and C₆ sugars, is subsequently sent to a fermentation step where microorganisms can convert them to bioethanol or other products²⁵⁶. The fermentation broth is then transferred to a series of distillation columns to recover bioethanol.

3.1. Paper pulping

The most established technologies to deconstruct biomass, namely the Kraft and sulfite processes, were developed within the paper- and pulp-making industries, which are also the main industrial source of lignins^{172,257}. These processes employ strong nucleophiles such as sulfide (S^{2-}) in Kraft pulping and sulfite (HSO_3^-) in sulfite pulping to form water-soluble lignothiols or lignosulfonates, aiding separation of lignin from cellulosic fibers. In Kraft pulping, the dominant process used for lignin chemical modification, biomass is heated in aqueous NaOH and Na₂S at 130–180 °C for several hours to remove up to 80–95% of lignin and part of hemicellulose¹⁷⁷. The lignin- and hemicellulose-containing ‘black liquor’ is concentrated and burned for energy generation. These methods have been

designed from the standpoint of paper production and are optimized for high cellulose yield and fiber strength⁶. The ‘technical’ lignins produced are often highly condensed and contain appreciable amounts of sulfur and ash²¹⁵. In contrast, from the standpoint of biorefining, it is desirable to separate the components, producing sugars that are easily fermented and high purity by-product streams that add value to improve the economic viability of the process²⁵⁸.

3.2. Comparison of pretreatment methods

This section briefly gives an overview of the field of lignocellulose pretreatment, including the respective advantages and disadvantages of different processes (see Figure I-8).

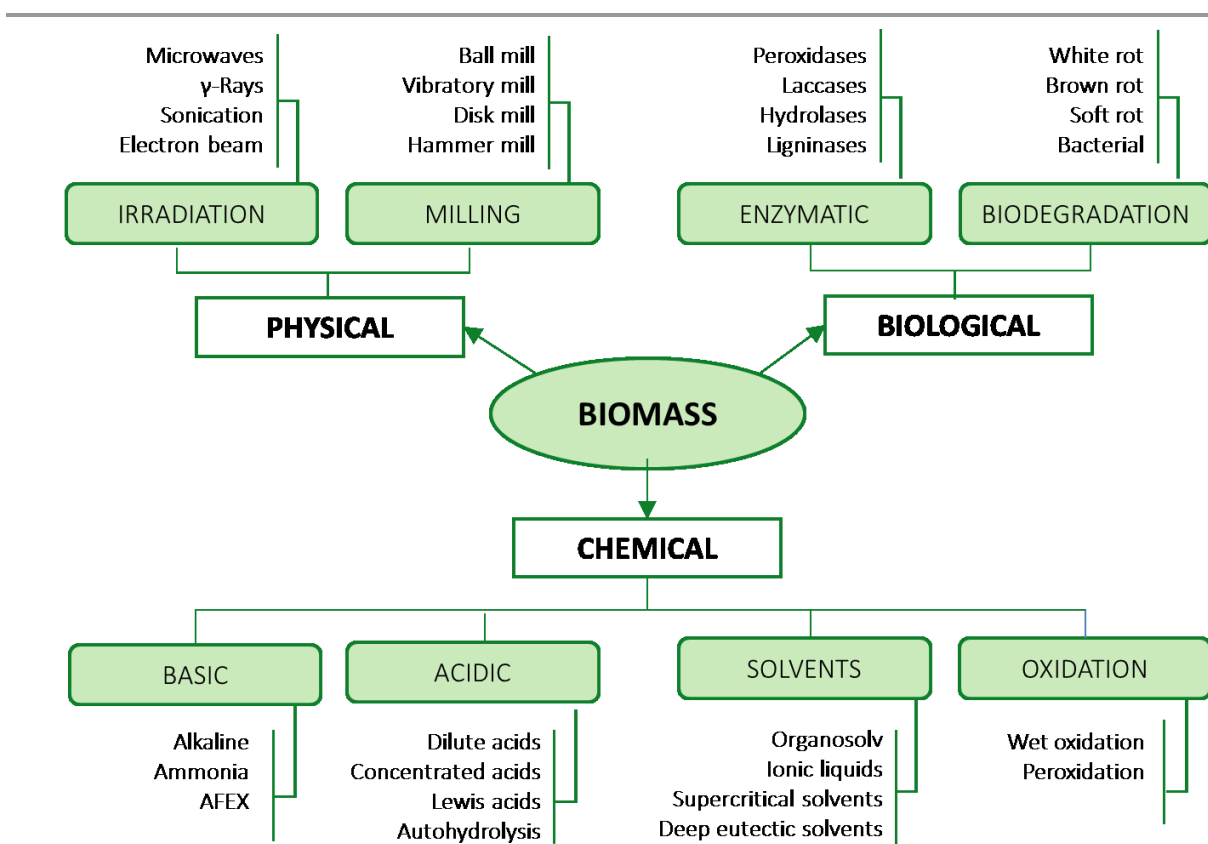


Figure I-8. Physical, chemical and biological pretreatment methods used for biomass deconstruction. Adapted from Silveira *et al.*²⁵⁹

Physical methods, such as mechanical milling, are highly energy intensive and are of limited effectiveness for complete lignin removal²⁶⁰. Use of size reduction as a pretreatment method in itself has been proposed, for instance through hammer milling²⁶¹ or disk milling²⁶² to break biomass into chips or fibers, or ball-milling²⁶³ and vibratory milling²⁶⁴ for pulverization. Milling can increase glucose digestibility by a maximum of ~50%²⁶⁵ by increasing accessible surface area of cellulose²⁶⁶ and decreasing cellulose crystallinity²⁶⁷, though its use as a stand-alone physical pretreatment is uneconomical²⁶⁶. For instance, pretreatment based exclusively on ball-milling has been estimated to require >1500 kWh ton⁻¹ of wood²⁶⁸, or ~30% of the energy content of wood itself. Other physical

methods, such as irradiation with γ -ray²⁶⁹, ultrasound sonication²⁷⁰, electron beam²⁷¹ and microwave heating²⁷², have been shown to enhance the biomass digestibility, typically when combined with other chemical methods; however, high energy radiation techniques lack commercial appeal due to high cost²⁷³. Biological pretreatment employs biomass-degrading microorganisms, including white, brown and soft-rot fungi and bacteria to attack cellulose and/or degrade lignin²⁷³. These methods have the advantages of requiring no chemical inputs, low energy input and very mild conditions; however, they are very slow and space-intensive and therefore unattractive commercially²⁷³.

Thermochemical methods, such as steam explosion^{163,274,275}, dilute acid^{8,276–278} and ammonia fiber expansion (AFEX)^{279–283}, have the primary aim of increasing sugar release rather than delignifying biomass. Steam explosion (SE) applies steam at high temperature and pressure followed by a rapid pressure release, resulting in hydrolysis of glycosidic bonds and cleavage of lignin-hemicellulose bonds¹⁶³. It functions by auto-catalyzed hydrolysis, whereby acetic and other acids released naturally from hemicellulose may catalyze hydrolytic reactions²⁷⁴. Extensive hemicellulose removal and reduction in particle size are thought to increase enzyme accessibility²⁴⁹, improving the digestibility of SE biomass including softwood, though not above around 60% for wood²⁸⁴. Dilute acid (DA) is carried out at 160–220 °C for periods ranging from minutes to seconds²⁴⁹, as acid catalyzes release of oligomers and monosaccharides followed by breakdown of glucose to form 5-hydroxymethylfurfural (HMF) and levulinic acid²⁸⁵ and xylose to furfural and formic acid²⁸⁶ and other degradation products (Figure I-9). DA pretreatment is the most studied process for agricultural biomass²⁴⁹ as it can satisfactorily convert cellulose despite limited delignification. Lignin droplets have been shown to deposit on the cellulose surface during DA pretreatment of lignocellulose^{287,288}, retarding enzymatic digestion of cellulose. DA pretreatment also encounters problems with corrosion that mandates expensive materials of construction²⁴⁹, formation of degradation products such as pseudo-lignin^{160,289} and generation of inhibitory byproducts²⁸⁶ that limit yields and slow down hydrolysis.

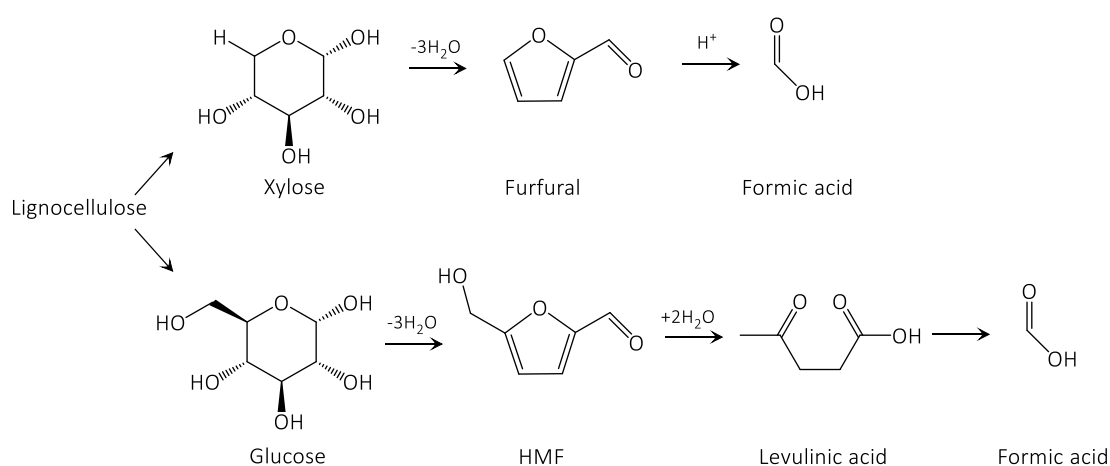


Figure I-9. Proposed conversion pathways of glucose and xylose to degradation products under acidic conditions²⁹⁰.

Alkaline pretreatment methods, typically carried out using lime²⁸¹, NaOH²⁸¹ or ammonia²⁹¹, resemble Kraft paper pulping in that they cause alkaline cleavage of α -aryl ether bonds¹⁷² to remove lignin from the biomass, thus improving the reactivity of the remaining polysaccharides²⁴⁹. These processes utilize comparatively lower temperatures and pressures over a period of several hours or days²⁴⁹. Among these, AFEX, which uses high-pressure ammonia followed by explosive decompression, has had particular success for herbaceous residues, with quantitative sugar yields obtained with low enzyme loadings during subsequent saccharification²⁴⁹. AFEX produces negligible inhibitors and requires no particle size reduction, but it is ineffective for biomass with high lignin content and solvent recovery leads to high operating costs²⁹².

Chemical pretreatments such as Organosolv^{200,293–295}, deep eutectic solvents^{88,296–299} and ionic liquid-based processes^{6,161,300,301} have been widely investigated of late due to their ability to produce a separate lignin stream for additional value generation. Biomass fractionation offers additional benefits including lower enzyme loading during polysaccharide hydrolysis and production of a high-grade lignin for subsequent processing³⁰². Mechanical size reduction is not always required prior to these processes as *in situ* thermochemical size reduction and fiberization may take place²⁸⁴. A highly promising method for treatment of various feedstocks including softwoods is the Organosolv process, by which biomass is pretreated with hot organic or organic–aqueous solvents (e.g. methanol, ethanol or acetone) at 150–200 °C^{303–305}. Organosolv works by delignification with varying degrees of hemicellulose solubilization; acid or alkali catalysts can be added in low concentration (1–2%) to help break the internal lignin and hemicellulose bonds. The Organosolv process yields a highly digestible cellulose fraction, an aqueous hemicellulose stream and lignin precipitate obtained by removal of the volatile solvent from the black liquor by distillation³⁰⁵. The major advantages of Organosolv are the high degree of delignification (~60–95%) and the production of a pure lignin stream with low molecular weight^{164,207}. However, organic solvents are costly and their use requires high-pressure equipment, making the process too costly to attract industrial interest at present and introducing safety hazards due to the possible release of volatiles²⁷³.

ILs are gaining momentum as solvents and catalysts for biomass pretreatment^{7,306–308}. They are a comparatively new pretreatment medium that operate under milder conditions than traditional pretreatment processes, producing less inhibitory compounds and simplifying product separation⁶. ILs are highly efficient at disrupting inter- and intra-molecular hydrogen bonds among cellulose, hemicellulose and lignin⁶. Moreover, some ILs are environmentally benign¹¹, non-volatile and can be recycled and reused with very low losses⁹⁶. While the large-scale use of IL-based processing has been hindered by the high cost of APILs, the design of low-cost PILs has immense potential as an economically viable pretreatment technology⁹⁶. Two distinct approaches to IL processing of

lignocellulosic biomass have emerged to date. The first and more widely studied strategy – termed the Dissolution process – attempts to dissolve the entirety of lignocellulose using cellulose-dissolving ILs¹⁶¹. The second approach chemically disrupts the lignocellulose composite through partial lignin and hemicellulose solubilization⁹⁶, named the *ionoSolv* process, due to its similarity with *Organosolv*.

In the **Dissolution process**, the whole lignocellulosic material is dissolved in the IL and addition of anti-solvent allows re-precipitation of decrystallized cellulose which has high susceptibility to enzymatic hydrolysis⁴⁵. It has been found that the ionic liquid needs to contain anions with high hydrogen-bond basicity (Kamlet-Taft β value > 0.8, e.g. halide, phosphate or carboxylate anions) to fully solubilize cellulose⁶. The Dissolution process is most effective in the absence of moisture (<1%)³⁰⁹, as water can compete for the hydrogen bond-basic sites, reducing the hydrogen bond basicity of the anion and decreasing cellulose solubilization²²². Ionic liquids are hygroscopic and drying them requires heat and vacuum, particularly for strongly hydrogen bond-basic ILs¹⁰. Therefore, extensive drying of the IL and feedstock introduce high energy and cost penalties. Regarding biomass processing applications, the most widely studied candidate IL for biorefining to date is [Emim][OAc], an APIL that has been shown to be both environmentally benign¹¹ and compatible with fermentation organisms³¹⁰. [Emim][OAc] has been shown to be remarkably effective on a variety of feedstocks such as switchgrass, where over nearly 100% glucose yield has been reported within 24 h of hydrolysis¹⁶¹, with ~65% lignin removal and ~85% hemicellulose removal⁶. However, recent studies of its stability have shown considerable thermal degradation at temperatures as low as 100 °C⁷⁵, raising concerns about its suitability for large-scale biomass processing. Nevertheless, these ILs remain a high-performing point of reference^{11,311–313}.

In the **ionoSolv process**, lignin is partially solubilized in the IL while the cellulose portion remains intact, enabling its separation; lignin can then be precipitated from the IL liquor by addition of anti-solvent, such as water. In general, the Kamlet-Taft parameters for ILs suitable for *ionoSolv* pretreatment are high π^* (~1), moderate hydrogen-bond basicity β (0.5–0.7, for instance the MeSO_4^- and HSO_4^- anions) and low hydrogen-bond acidity ($\alpha < 0.5$)¹⁰. This process is conducted in the presence of IL–water mixtures containing 10–40 wt% water, which reduces solvent viscosity and eliminates the need for anhydrous conditions during pretreatment¹⁰. Addition of water was also seen to reduce carbohydrate degradation and pseudo-lignin formation³¹⁴. Under severe conditions (long residence times, high temperatures or high acidity), lignin condensation and pseudo-lignin formation have been shown to inhibit saccharification⁹⁶. As in DA pretreatment, the formation of hemicellulose degradation products has been reported³¹⁵, suggesting milder reaction conditions are preferable. As the pretreatment process of choice in this study, *ionoSolv* pretreatment is discussed in further detail in Section 3.3.

Deep eutectic solvents (DES), a subset of ionic solvents made of biocompatible components, have also shown excellent performance as media for lignocellulose fractionation^{88,316}. DES show similar behavior to ionic liquids, having high lignin solubility and very poor cellulose solubility³¹⁷. Their use has been reported for delignification of herbaceous biomass and softwoods, enabling recovery of lignin precipitate with different types of lignin structures^{316,318}. DES have shown great promise as alternative solvents, despite being limited by high viscosities (> 100 mPa·s at ambient temperature)⁸⁸, which have been tuned by the addition of water³¹⁹. However, their ability to be reused and recycled in a viable process remains to be investigated.

Pretreatment effectiveness

The technical performance of pretreatment technologies is most frequently evaluated by comparing the total fermentable sugar production or glucose release by a saccharification assay following pretreatment³²⁰. Other indicators of successful pretreatment outcomes are gleaned from properties of the cellulose-rich solid, such as cellulose crystallinity and degree of polymerization, lignin removal (i.e. delignification) and structure, and hemicellulose removal, all of which are reported to impact biomass hydrolyzability³²¹. It should be noted that removal of lignin and hemicellulose are not essential for effective pretreatment, as seen in DA and SE pretreatment⁶. Another important indicator, proposed by Zhu and Pan²⁸⁴, is the energy consumed per unit of sugar production. We can define the pretreatment energy efficiency, η , as the unit energy consumption per unit of sugar yield:

$$\frac{1}{\eta} (\text{MJ kg}^{-1} \text{ sugar}) = \frac{\text{Total energy consumption (MJ)}}{\text{Total monomeric sugar yield (kg)}} \quad (\text{Equation 1})$$

Total sugar production and energy efficiency and lignin yield and quality should be evaluated when comparing different pretreatment technologies, process configurations and parameters.

Impact on lignin structure

The effect of industrial pulping and biorefinery methods is to uniquely alter the chemical structure and linkages of lignin, in turn influencing the potential products obtainable from lignin valorization (discussed in Section 4). Technical lignins produced from Kraft and sulfite pulping contain hydrophilic thiol (–SH) and sulfonate (–SO₃) groups (see Figure I-10)³²². Cleavage of ether bonds during pulping processes produces highly reactive intermediates, which may re-combine in condensation reactions to form larger fragments held together by C–C bonds³²³. New functional groups and linkages, such as stilbenes, are also created, as detected by NMR spectroscopy³²². Subsequent chemical derivatization of these lignins has encountered limited industrial success because of their wide molecular weight profiles and the presence of carbohydrate and sulfur impurities¹⁶.

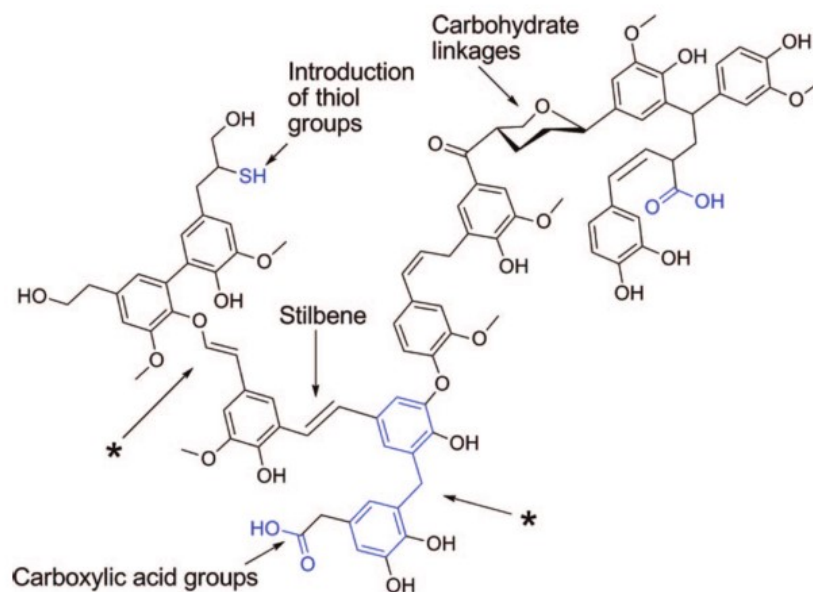


Figure I-10. Model of Kraft softwood lignin depicting key structure features; reproduced from Zakzeski *et al.*³²²

Current research has focused on carbohydrate utilization, with inadequate understanding of the changes to lignin structure following pretreatment. There are two potential streams from which lignin can be valorized in the biorefinery: the pretreatment hydrolyzate (solubilized lignins which can be precipitated) and post-saccharification residue (insoluble lignins)¹⁶⁴. Great variability in lignin solubilization has been reported from different pretreatment processes and intensities, depending on the delignification chemistry¹⁶⁴. While milder methods, such as uncatalyzed SE, cause only minor changes to lignin structure²⁴⁹, harsher conditions used in many acidic or alkaline methods typically lead to lignin depolymerization and repolymerization causing changes in its reactivity and solubility¹⁶³. Acidic methods allowing lignin removal (i.e. Organosolv and IL pretreatment) typically result in cleavage of β -O-4' structures to liberate a free phenolic hydroxyl functionality¹⁶⁴. However, in addition to acidolysis, low pH can also promote inter-lignin condensation reactions, observed by an increase in C-C condensed structures and an increase in molecular weight of lignin²²⁸. Condensation reactions occur when the carbocation participates in electrophilic aromatic substitution involving another aromatic proton¹⁶⁴. Recent publications demonstrated that addition of 2-naphthol^{324,325} or ethanol³²⁶ could act as a carbonium ion scavenger with a positive effect on delignification through inhibition of lignin-lignin recondensation reactions. Many chemical pretreatment processes, such as DA, Organosolv and ILs, suffer from a narrow range of reaction time or temperature for high sugar yields and maximum lignin extractability^{96,199,207}. This effect is attributed to lignin repolymerization reactions taking place simultaneously with depolymerization³²⁷. Among these processes, Organosolv and IL-based processes are unique for producing extractable lignin of a high purity with very low sulfur content²⁰⁷. Lignin properties can also be controlled to a certain extent by selection of degradation conditions (pH, temperature, residence time). For instance, lignin purity, yield, thermal and chemical

properties have been correlated with the pretreatment severity used during Organosolv²⁰⁷ and IL pretreatment^{97,98,199}. A trend of higher lignin purity and more condensed chemical structures has been observed with increasing pretreatment severity¹⁹⁹. The valorization potential of lignin is precluded by condensation under high-severity pretreatment¹⁶⁴, as discussed in Section 4). The effects of pretreatment on lignin structure and the implications for its valorization were reviewed elsewhere¹⁶⁴.

A number of leading pretreatment properties do not lead to significant delignification and so are not capable of isolating a utilizable lignin fraction³²¹. DA, SE and AFEX pretreatments can increase biomass digestibility with limited lignin removal, instead functioning by lignin relocation and redistribution and other changes in lignin structural features, as reviewed by Pu *et al.*³²¹ Of particular interest for acidic pretreatment processes such as IL-based technologies is the observation of lignin migration and accumulation to outer cell walls during acidic pretreatments²⁸⁸. This relocation of lignin is thought to render the majority of cellulose microfibrils accessible, enhancing pretreated biomass digestibility. This effect became more pronounced when the pretreatment temperature was raised above the T_g of lignin ($\sim 130^\circ\text{C}$)³²¹, an effect which is also observed by an acceleration of lignin dissolution during IL pretreatment at higher temperatures²⁴².

3.3. ionoSolv pretreatment

The ionoSolv process, developed in the early 2010s by Brandt, Hallett *et al.*⁷, employs lignin-dissolving ILs to selectively remove lignin and hemicellulose from biomass, leaving behind an insoluble cellulose-rich pulp with high digestibility⁶. This process generally leaves the cellulose portion largely chemically unchanged, although structural modifications have been observed⁶. Despite remaining crystalline, cellulose saccharification rates are improved by a factor of 30 compared to untreated biomass³¹⁴. Moderately hydrogen bond-basic anions such as HSO_4^- , as assessed by the Kamlet-Taft parameter β , were shown to disrupt the hydrogen bonding interactions in the wood matrix (mainly in lignin) without dissolving cellulose, enabling biomass fractionation in the presence of water. Shi *et al.*³²⁸ examined the role of water in pretreatment conducted using IL-water mixtures. Water was found to act as a dual co-solvent, reducing the IL viscosity to enable hydrogen bond attractions between the IL and lignin to assist with lignin removal, and anti-solvent for cellulose, disrupting the hydrogen-bond basicity of the IL anion to reduce its affinity for hydrogen bonding with cellulose. For lignin-dissolving ILs, water addition was found to improve lignin solubilization compared to anhydrous conditions⁷. Pretreatment was also enhanced by use of higher temperatures for shorter reaction times, maximizing the space-time yield⁹⁷, while addition of excess H_2SO_4 shortened pretreatment times at the expense of glucan digestibility⁴⁵.

During ionoSolv pretreatment, lignin is solubilized and re-precipitated upon addition of an anti-solvent, usually water. Sathitsuksanoh *et al.*¹⁷⁵ used SEC measurements to show distinct partitioning of high molecular weight lignin fragments in the lignin precipitate, whilst smaller lignin fragments remained dissolved in the IL. At higher pretreatment temperatures, more high molecular weight material was solubilized in the ionic liquid but depolymerization was not significantly enhanced. Consequently, polydispersity of the recovered lignin was seen to increase with temperature¹⁷⁵. Due to the strong π - π interactions between lignin oligomers and the IL cation, complete extraction of lignin from the liquor is impossible³²². The lignins obtained from ionoSolv pretreatment generally possess high polydispersity (PDI ~4–7) and relatively high molecular weight (M_w ~7,000–18,000). Further research is needed to improve lignin properties, for instance to produce narrow disperse lignin fractions by fractionation of lignin. The ionoSolv process offers several major advantages over other leading pretreatment technologies^{96,300,302}:

- The low-cost ILs employed, principally [HSO₄]-based quaternary ammonium and imidazolium PILs such as [TEA][HSO₄], [DMBA][HSO₄] and [Hbim][HSO₄] could be synthesized at a cost of ~\$1 kg⁻¹ and are thermally stable.
- High delignification (>80%) and quantitative hemicellulose removal during extraction, resulting in excellent digestibility of the recovered cellulose-rich pulp.
- Glucose yields of 70–100% have been obtained by enzymatic saccharification for diverse feedstock types including grasses⁹⁷, hardwoods⁹⁸ and softwoods⁹⁷ and blended feedstocks.
- The IL could be successfully recovered and reused, with >99% IL recovery and minimal effect on efficiency of fractionation upon re-use.
- The ability to isolate lignin and tune its properties through careful optimization of residence time, temperature and pH^{98,199}, with high potential for lignin valorization.

A preliminary economic analysis revealed that pretreatment with these low-cost PILs could result in an economically viable pretreatment process⁹⁶. These findings indicate that the ionoSolv process is suitable for up-scaling and commercialization.

3.4. Process scale-up and techno-economic considerations

Current techno-economic models of lignocellulosic biorefineries assume that the ultimate selling price of the biofuel (the minimum ethanol selling price, or MESP) largely determines its market penetration³²⁹. Multiple cost drivers of economic performance of IL-based pretreatment process have been identified by means of process scale-up trials³³⁰, process flow models³³¹ and techno-economic analyses⁹⁵. Although ionoSolv processing has shown great promise, several challenges remain such as (1) low ratios of biomass to IL solvent, (2) high water consumption during lignin precipitation and

washing, (3) high energy requirement for size reduction prior to pretreatment, (4) challenging biomass rheology to achieve effective mixing and (5) high processing cost for efficient recycling of the IL^{11,95,315,330,331}. Another major challenge, namely valorization of lignin¹⁵², remains to be addressed and is discussed in more detail in Section 4.

Modeling of biomass pretreatment processes is notoriously complex, with many reactions occurring simultaneously. Molecular simulations and structural probing by Langan *et al.*³³² revealed two major processes taking place: aggregation of cellulose fibrils by dehydration and phase separation of cleaved lignin and hemicellulose which dissolve and separate from the cellulose matrix. Other side reactions include lignin-lignin recondensation reactions¹⁶⁷, lignin precipitation on to the fiber surface⁹⁷, pseudo-lignin formation²⁸⁹, degradation of the IL¹¹ and degradation of carbohydrates⁹⁶; these processes can all be accelerated by agitation and by *in situ* size reduction by fiberization, which increases the surface area for ionic liquid contact with biomass. Viell³³³ presented the only known process model for IL pretreatment available in the published literature, using [Emim][OAc] to pretreat beech wood, which allowed identification of process bottlenecks. An analysis of the Organosolv process, given its similarity to ionoSolv, was also used as a reference³³⁴. Major drivers of costs were the water consumption and solvent consumption, both inversely proportional to the biomass loading³³¹. Therefore, increasing the biomass loading appears to be a significant factor to aid the economics of IL-based processes, confirmed by a recent techno-economic analysis by Klein-Marcuschamer *et al.*⁹⁵ A large amount of water is required in the ionoSolv process to dilute the IL and to induce precipitation of lignin³¹⁵. The water ends up in the solvent recovery system, which is the most energy-intensive processing step, requiring ~75% of the heat-integrated energy demand³³³. Major cost savings could also be afforded by reducing the amount of water to be added for lignin precipitation.

An increase in the biomass loading would be expected to introduce heat and mass transfer limitations in the processing of lignocellulosic biomass³³⁵. When pretreatments are carried out at high solids loading (>30 wt/wt%) to improve process efficiency, heat cannot penetrate as quickly and uniformly into viscous IL-biomass slurries. At high solids concentrations, thick and paste-like slurries are obtained³³⁶, with the IL acting less like a solvent and more as a wetting agent or additive. Limited mass transfer within these slurries can decrease the efficiency of lignin and hemicellulose dissolution and cause localized accumulation of lignin during pretreatment, increasing its propensity to redeposit²⁸⁷ or precipitate⁹⁷ onto the cellulose surface. Milling to fine particle sizes can improve some of these mass transfer limitations but adds significant energy costs³³⁷, while mixing can accelerate convective heat transfer³³⁵. To further complicate matters, mixing causes biomass to further break down into smaller particles, while chemical reactions lead to dissolution of lignin and sugars, causing changes in

particle size as well as chemical properties³³⁵. Changes in rheological properties of IL-biomass slurries were examined by Cruz *et al.*³³⁶.

Feedstock particle sizing can impact the economics of cellulosic ethanol commercialization through its effects on energy cost and conversion efficiency²⁶⁶. Size reduction has been estimated to account for up to 60% of the total energy inputs during chemical pretreatment²⁸⁴ and roughly 10% of the MESP³³⁸. Several different strategies have been proposed to decrease the energy requirements of comminution. Brandt *et al.*³³⁹ demonstrated the addition of ionic liquids as lubricants to reduce energy consumption during grinding of pine chips by 80%, equivalent to shaving ~10% off the MESP³³⁸. A more obvious solution is to reduce the amount of grinding needed by using larger particle sizes (i.e. chips or coarsely shredded fibers) during pretreatment. However, intraparticle heat and mass transfer effects are more significant for larger particles³³⁵. These effects delay the heating and reaction of larger particles, rendering pretreatment processes ineffective on biomass particles above a certain threshold particle size²⁷⁶. An altogether different approach was explored by Zhu *et al.*³³⁷, who reported a reduction by 20–80% in the grinding energy of pine wood chips without affecting pulp quality by chemically pretreating pine chips before using a coarse size reduction step. This approach, known as post-chemical pretreatment size reduction, is depicted schematically in Figure I-11. It offers several benefits²⁸⁴: (1) it takes advantage of chemical pretreatment to delignify and soften wood structure to reduce energy consumption in the subsequent size reduction; (2) it avoids the energy-intensive operation of mixing high-consistency pulp when finely ground biomass is used; (3) it minimizes energy requirements of separating fibers from the liquor after pretreatment; and (4) it enables use of a lower liquid to biomass ratio in the case of some processes, as liquid uptake of wood chips is lower than fibers. While critical particle sizes for lignocellulose conversion have been examined for steam explosion³⁴⁰ (~50 mm), dilute acid²⁷⁶ (~3 mm), alkali²⁶⁶ (~2 mm), AFEX³⁴¹ (~0.15 mm) and other pretreatment processes, few studies of the effect of particle size on IL-based pretreatment processes have been published to date^{342,343}.



Figure I-11. Schematic diagram showing the approach for mechanical wood size-reduction operation from (a) pre- to (b) post-chemical pretreatment to reduce energy consumption. Reproduced from Zhu *et al.*³⁴⁴

Several process models have also examined the impact of revenues from lignin valorization. Rather than using lignin as a fuel for co-generation, its use to manufacture value-added chemicals or materials could create additional sources of revenue for the biorefinery¹⁵². Potential values of lignin have been estimated as ranging between \$0.2–1 kg⁻¹. Assuming 65% lignin recovery, a techno-economic analysis conducted by Konda *et al.*³³¹ found that lignin valorization could significantly reduce the MESP from around \$4 gal⁻¹ to \$3 gal⁻¹ as the lignin selling price increases. Klein-Marcuschamer *et al.*⁹⁵ saw a sharper decline in the MESP with lignin valorization of ~\$1.50 gal⁻¹ for every extra \$1 kg⁻¹ that was added to the selling price of lignin. Assuming use of low-cost ILs (<\$2.5 kg⁻¹), IL:biomass ratio of 1 and a recycle rate of 99.6%, the MESP could decrease to \$2.5 gal⁻¹ for a lignin selling price of \$1 kg⁻¹ and further to \$0.8 gal⁻¹ for a lignin selling price of \$2 kg⁻¹. At high lignin selling prices (>\$2.5 kg⁻¹), the MESP can be as low as zero. These findings highlight the importance of continued research and development on the lignin valorization front.

3.5. Life cycle considerations

A number of works have employed the life cycle analysis (LCA) methodology to assess the environmental impacts and other socioeconomic opportunities (e.g. job creation potential) of biorefining^{123,125,149}. Most models consider the design of feedstock cropping systems¹²⁵ and biofuel production³⁴⁵ but do not sufficiently cover all aspects of the biofuel supply chain. A multi-scale modeling and optimization framework is needed to integrate different temporal and spatial scales, from the molecular to the ecosystem level³⁴⁶. Moreover, the GHG and energy balances of biofuels have varied widely depending on the choice of methodology, reference system, and assumptions regarding land use change, fuel blend and co-product use, with net lifecycle GHG emissions with respect to gasoline ranging from –112% to +120% for the same ethanol production pathway³⁴⁷.

Nevertheless, some important findings have emerged. LCA of cropping systems have shown that GHG emissions in agriculture are largely determined by the emission of N_2O from fertilizers³⁴⁸. Utilization of agricultural residues, together with winter cover crops, could increase environmental benefits due to reduction of acidification and eutrophication burdens¹²⁵. Research indicates that large biorefineries capable of handling 5,000–10,000 MT of biomass per day are necessary to achieve economic viability³⁴⁹. However, given the highly local nature of biomass supply and transportation, larger biorefineries entail increased transportation and storage costs. Dale *et al.*³⁴⁹ proposed a network of regional biomass processing centers, capable of pretreating and converting biomass to appropriate platform products to form an extended biomass supply chain feeding into a biorefinery. Kim and Dale³²⁹ recently analyzed the effect of feedstock price and biorefinery size on the MESP and global warming impact of cellulosic biofuels. Logistics and local conditions were found to be central to LCA outcomes, notably the feedstock price, which greatly affects the biomass supply chains that will develop. Interestingly, a higher feedstock price was found to incentivize farmers and thus *increased* the number of biomass clusters and biorefineries, increasing the volume of ethanol production; in some geographies, this had the effect of pushing *down* the MESP³²⁹. The various ethanol production technologies have different environmental impacts, with GHG emissions dominated by CO_2 due to electricity generation and fermentation³⁴⁸. As a guideline, the US renewable fuel standard (RFS) set a threshold of 60% well-to-wheel GHG reduction required to qualify as a second generation biofuel³⁵⁰. This target sets our research focus for reducing energy inputs to improve the overall economic performance of the IL-based biorefinery.

3.6. Summary

- Cost-effective, low-energy pretreatment and co-utilization of all lignocellulose components including lignin are the main barriers to economic viability of lignocellulose processing.
- ionoSolv processing with low-cost ILs offers the benefits of feedstock flexibility, high conversion efficiency, water-tolerance and recovery of lignin with tailorable properties.
- Process and economic models indicate the ionoSolv process has potential to be economically viable, making it a good candidate for process scale-up.
- Lower solvent volumes of water used during lignin precipitation and recovery (currently ~75% of heat-integrated energy demand) could afford major cost and energy savings.
- There is a lack of understanding of the effects of biomass loading, particle size and mixing on the conversion yield and energy efficiency of ionic liquid pretreatments.

4. Focus on Lignin

4.1. Lignin applications and valorization

As a 70 million ton per annum³⁵¹ by-product of the paper pulping industries, until recently the primary industrial use of lignin has been as a low-grade fuel for combustion¹⁶. With upcoming focus on biorefineries, research attention has shifted to the use of lignin as a potential source of value-added products, some of which are displayed in Figure I-12. Many of the aforementioned pretreatment processes enable isolation of lignin, as it is insoluble in or can be precipitated from the processing liquor¹⁷⁹. Precipitated lignin is typically more pure, containing fewer carbohydrates and other contaminants, since it is selectively precipitated and therefore more suitable for further applications³⁵². Pretreatment processes can thus produce sulfur-free lignin with greater ability to be derivatized, lower ash content, high purity and low molecular weight^{243,353}. Lignins are notoriously difficult to valorize due to their heterogeneity, especially in a condensed state as this hampers efficient depolymerization and limits reactivity³⁵². Nevertheless, biorefinery lignins are already used in various applications, and new applications are constantly being developed.

A valorization route for isolated lignins that has been intensely investigated is depolymerization to phenolic compounds, which can be used as a platform for fuels and chemicals production^{354–356}. Aromatics are derived from monomeric C₆ fragments from depolymerized lignins. Various methods available to depolymerize lignin using acidic³⁵⁷ or basic³⁵⁸ catalysts were reviewed elsewhere³⁵⁵. In general, oxidative depolymerization³⁵⁹ will result in fine chemicals with a high degree of functionality, such as vanillin, that resemble the lignin structure^{360,361}. Alternatively, reductive depolymerization will produce bulk chemicals *via* extensive removal of functional groups to yield simple aromatic compounds such as phenol, benzene, toluene and xylene (BTX). These platform chemicals can then be reacted in a second step using catalytic techniques to produce chemicals, such as benzyl alcohols or aldehydes^{14,16}. The maximum theoretical obtainable yield of BTX (which represent a US\$100 bn market) from lignin is about 36–42 wt% as lignin contains roughly 60–65 wt% carbon, of which 60–65% is estimated to be in C₆ aromatic rings³⁶².

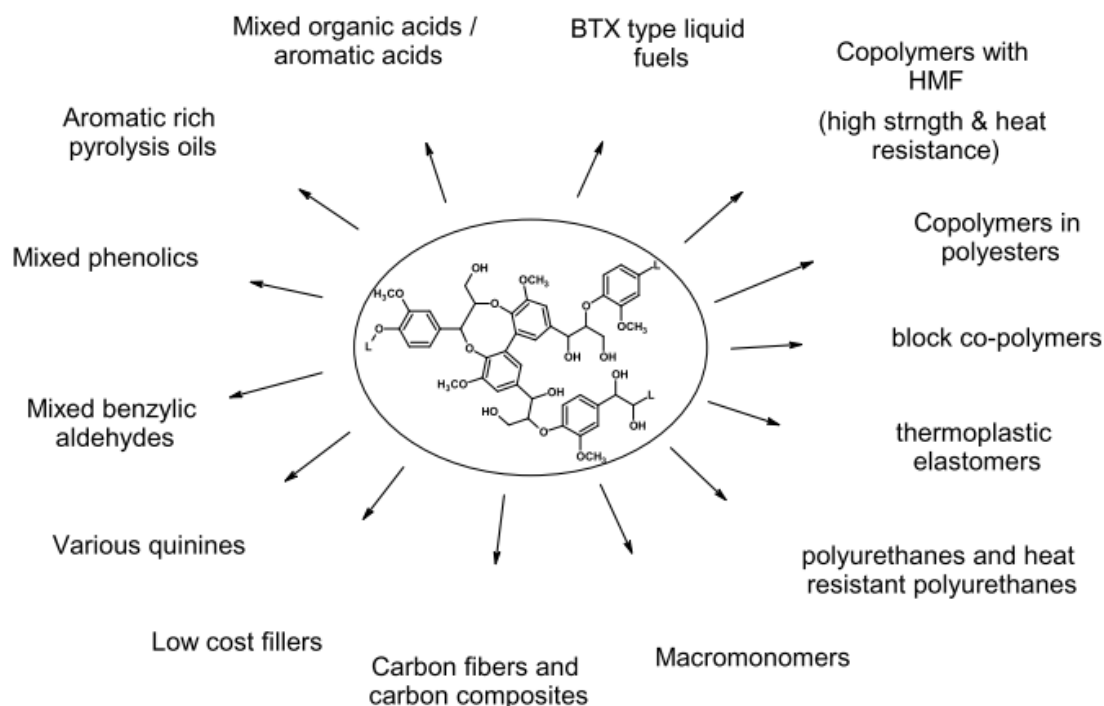


Figure I-12. Various product classes potentially available from lignin valorization¹⁶.

An altogether different approach is lignin conversion by thermochemical means, such as pyrolysis, hydrogenolysis or gasification, to produce a mixture of small molecules which can be further hydrotreated or catalytically upgraded to produce chemicals^{363–365}. Pyrolysis refers to the thermal treatment of lignin in the absence of oxygen, with or without any catalyst, to produce solid char, liquid oil and gases^{355,366}; lignin pyrolysis is discussed in detail in Section 4.2. Hydrogenolysis or hydrocracking involves thermal treatment to induce bond cleavage in the presence of hydrogen, generally at lower temperatures to favor higher yields of liquids including monomeric phenols^{367,368}. Gasification proceeds at higher temperatures with limited presence of oxygen to convert lignin into mainly gases (H_2 , CO , CO_2 and CH_4)³⁶⁴. Barriers to commercialization of these processes include cost-effective separation of multiple products, selective cleavage of C–C and C–O bonds and the ability to meet stringent product purity specifications¹⁶. Thus, a more realistic approach in the medium term, rather than targeting a single commodity chemical from lignin, may be to produce a blended chemical feedstock that can be upgraded via established petrochemical refining technologies¹⁷⁹.

Material applications of lignin, either directly or with slight modifications, have been extensively reported in the literature^{369,370}. Most research has focused on production of polymer modifiers in the form of simple, low-cost adhesives and fillers^{16,370,371}, while more specialized uses such as asphalt binders³⁷², flame retardants³⁷³, sunscreen agents^{374,375}, antioxidants³⁷⁶, antimicrobials³⁵¹ and sorbents^{377,378} have been demonstrated in the laboratory. The use of lignin blends for producing polyurethanes^{360,379}, phenol-formaldehyde resins^{380,381}, epoxies³⁷⁰, hydrogels³⁸² and other

polymers^{353,383} has also been successfully demonstrated, taking advantage of the high phenolic hydroxyl content of biorefinery lignins. Future research should move towards production of high-value additives to improve polymer performance or properties, for applications such as high-strength engineering plastics, heat-resistant polymers, UV-resistant polymers and formaldehyde-free adhesives¹⁶. Another application of lignin is melt-spinning and carbonization to produce glassy carbon and activated carbons³⁸⁴. High-end applications of lignin, such as production of carbon fibers with very high surface areas and pore volumes^{385,386}, are also under investigation. Carbon fibers are very interesting materials and can be used in the production of light-weight vehicles, but their large-scale manufacturing is hampered by high feedstock cost (usually polyacrylonitrile)³⁵². The use of lignin as a feedstock might enable the production of low-cost carbon fibers while reducing dependence on fossil feedstocks. Minimization of reactive C–O bonds, C–C linkages that are chemically less reactive, and unoccupied C₅ sites in high G-content lignins (grasses and softwood) could be favorable properties for high carbon yields and formation of ordered linear fibers^{179,387}. Glass transition properties are also critical to assure low cost and minimal processing prior to fiber spinning; desirable ranges for T_g are between ~90 and 160 °C^{386,388}. Production of a low-cost, consistent lignin stream with desirable chemical properties and controllable T_g will help make these applications a reality.

Another means to improve the scope for lignin valorization is to tailor the isolation methods to optimize lignin properties. Separation and purification of lignin fractions with narrow molecular weight distribution has been investigated by solvent extraction^{389,390}, membrane separation^{391,392} and fractional precipitation methods^{393,394}. For instance, significant progress has been made in lignin carbon fiber production by using Organosolv lignins purified using solvent extraction, shown to produce carbon fibers with mechanical properties of ~50-60% of the target for commercialization³⁸⁸.

4.2. Lignin pyrolysis

Pyrolysis is the thermal decomposition of matter in the absence of oxygen, producing a liquid bio-oil, solid char/ash and various gases. The current state of the art in pyrolysis is called fast pyrolysis or flash pyrolysis to maximize liquid product yields³⁹⁵, achieved by the use of short residence times (<2 s), moderate temperatures (400–650 °C) and rapid quenching of the vapors under inert atmosphere³⁹⁶. Typical conditions are 0.5 seconds at 500 °C and 1 bara using dry feedstock¹⁶. In the past 20 years, fast pyrolysis techniques have been developed for the conversion of whole plant biomass into a liquid (bio-oil) with a view to chemicals production³⁹⁷. These techniques have also been applied to lignins, with many studies carried out involving the pyrolysis of lignin alone^{398–401}, or co-pyrolysis of lignin with other materials, such as polymers⁴⁰². This research has mostly been driven by interest in producing low molecular weight compounds from lignin, notably monophenols^{403,404}.

Mechanisms of lignin thermal decomposition

Early studies of lignin thermal decomposition focused on understanding lignin structure and the mechanisms and kinetics of its degradation using advanced pyrolysis methods combined with separation and detection systems, notably Py-GC-MS and TGA-FTIR^{405–410}. Lignin, being an amorphous, irregular polymer made from three different monomers, has many different bonds capable of undergoing isomerization, dehydration and homolytic cleavage during pyrolysis⁴¹¹. This gives rise to extremely complex chemical reaction mechanisms, which have been studied both via simplified lignin model compounds^{412–416} and using lignin itself^{417–419}. A detailed study of the mechanisms of lignin pyrolysis was presented by Nassar and MacKay⁴²⁰. Lignin decomposes over a wide temperature range of 200–500 °C in an endothermic process that involves physical and chemical transformations. The physical transformations have been analyzed by TGA⁴²¹ and microscopy techniques²³⁹, revealing several key degradation patterns (see Figure I-13). The process begins with the transfer of heat from an external source causing the particle temperature to increase. Next, primary pyrolysis reactions such as dehydration and homolytic cleavage occur, producing gases, liquids, volatiles and char. Heat is transferred from the outside to the inside of the lignin particle through the hot fluids, while cooler portions of the particle may cause some volatiles to condense back into liquids. The condensed compounds may then undergo secondary pyrolysis reactions. Secondary reactions may be controlled by heat transfer within the particle, the residence time of the volatiles and the ability of the particle to expand as gases and volatiles are released⁴¹¹.

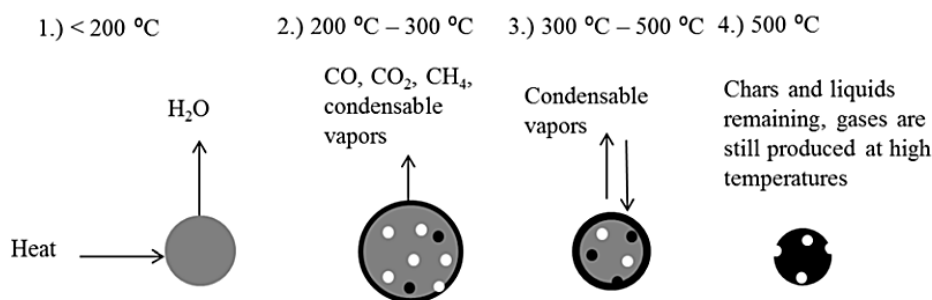


Figure I-13. Schematic of physical processes of lignin pyrolysis, reproduced from Ware *et al.*⁴¹¹. Lignin is gray, gases/voids are white, chars and liquids are black. 1) The particle is heated and moisture is removed. 2) As the particle temperature increases, the particle expands, releasing gases and condensable vapors, and begins forming char. 3) The temperature increases and some vapors condense on the particle as the size of the particle decreases; more vapors, gases and chars are generated. 4) Solid and liquid products remain at the final temperature. Gases will continue to be produced and liquids will further react if the particle maintains the higher temperature or is heated further.

The broad temperature range of lignin decomposition may be attributed to the presence of various functional groups in its structure which have different thermal stabilities⁴²². The aliphatic C–O bonds connecting different aromatic monomers have lower bond dissociation energies than the aromatic C–O and C–C bonds present in the aromatic framework⁴²³. Hence, homolytic cleavage and other reactions of β -aryl ether linkages are the dominant reactions driving the decomposition of lignin

during pyrolysis⁴²⁴. Homolytic cleavage of β -aryl ether linkages results in the formation of highly reactive and unstable free radicals that may further react through rearrangement, electron abstraction or radical-radical interactions to form more stable products⁴²².

Above 500 °C, rearrangement and condensation of stronger bonds of the aromatic rings in the lignin structural units sees the formation of char and more volatiles⁴²². The chemistry is governed by intermolecular and intramolecular aromatic condensation reactions leading to crosslinking and growth to form a char residue⁴²³. The competition between devolatilization and charring reactions can be controlled by the residence time⁴²²: very short residence times result in incomplete depolymerization due to random bond cleavage, resulting in a less homogenous liquid product. On the other hand, longer residence times can cause secondary reactions, reducing yield and adversely affecting bio-oil properties. If the inter-unit linkage abundance is low or if the rate of volatilization of (e.g. highly condensed) lignin fragments through aryl-ether cleavage is not sufficiently high, crosslinking predominates, resulting in char formation. While volatilization is favored by increased temperatures, lignin pyrolysis always gives high amounts of char compared to biomass as a result of its structure⁴²². Table I-2 shows the major bond cleavages taking place during lignin pyrolysis and typical products.

Table I-3. Major bond cleavage mechanisms taking place during lignin pyrolysis and typical products^{423–425}.

Bond cleaved	Mechanism	Products
Aliphatic C–O bond	Pericyclic group transfer elimination; homolytic cleavage; hydrolysis	Unsaturated hydrocarbons, catechols
Aromatic C–O bond	Homolytic ether cleavage	Guaiacols, alkylphenols, phenols, catechols, cresols
Side chain C–C bond	Redox; radical and quinone methide	Phenols, substituted phenols, methylguaiacol, guaiacol, cresols, pyrocatechols
Aromatic C–C bond	Condensation; rearrangements; radical coupling; hydrogen-transfer	Polycyclic aromatic hydrocarbons; char

Products of lignin thermal decomposition

The products of lignin pyrolysis can be classified as non-condensable gases (gaseous hydrocarbons, CO, CO₂ and H₂), volatile liquids (water, methanol, acetone, and acetaldehyde) and condensed liquids (bio-oil containing monophenols and other polysubstituted phenolic compounds). Large quantities of char and high-boiling phenolic compounds are always formed⁴²². The pyrolysis product distribution is strongly dependent on the final pyrolysis temperature and feedstock composition, and to a lesser extent on the heating rate during pyrolysis⁴²⁵. In general, it is advantageous to use shorter residence

times and moderate temperatures (flash pyrolysis) to obtain higher liquid product yields and to decrease char formation³⁹⁵.

At low pyrolysis temperatures below 200 °C, dehydration of lignin forms water. Aryl-ether bonds have low thermal stability and most degradation takes place between 200–400 °C, giving rise to low molecular weight products with unsaturated side chains such as styrene derivatives, eugenol and isoeugenol, coniferaldehyde and sinapaldehyde and *p*-hydroxycinnamic alcohols⁴²². In the temperature range 230–260 °C, the propanoid side chain degrades to form guaiacols, vanillin and vanillic acid⁴²². Acetic acid and non-condensable gases (CO, CO₂ and H₂) are formed by devolatilization at low temperatures followed by CH₄ and then H₂ at higher temperatures. Homolysis of ether linkages between 200–400 °C forms reactive radicals and the β–β' and C–C linkages between aromatic units cleave at 280–350 °C, while the recombination of radicals leads to formation of guaiacyl and syringyl compounds. Liquid pyrolysates usually contain some distribution of aromatic compounds with identities reflecting the monomer distribution and bond types linking them⁴¹¹. Under mild pyrolysis conditions (< 500 °C)^{407,426}, the pyrolysis products generally retain the methoxyl substituents on the phenyl ring, reflecting the relative abundance of the monomers in the lignin feedstock. Therefore, it is possible to measure the S/G ratio in lignin polymers based on pyrolyzate analysis by GC-MS⁴⁰⁵.

The main processes of lignin thermal degradation occur above 400 °C with the complete rearrangement of the phenolic backbone, leading to conversion of 30–50% of lignin weight into char and the release of volatile products⁴²². Above 500 °C, methoxyl groups become more reactive, producing methoxyl homolysis products (catechols and pyrogallols) and methoxyl rearrangement products (cresols and xylenols)^{427,428}. Secondary decomposition reactions take place; for instance, secondary thermal degradation of guaiacol methoxyl groups leads to formation of catechol and its derivatives including *o*-cresol, *o*-hydroxybenzaldehyde and phenol⁴¹⁷. Hosoya *et al.*⁴²⁹ have reported that these secondary reactions were very much influenced by the carbohydrate-derived vapors acting as an H-donor. Highly substituted phenols are selectively formed by cracking of the phenylpropane units. Moreover, combination of radicals results in formation of strong C–C bonds and thus char formation, accompanied by the release of H₂ and CO⁴²². Jegers and Klein⁴¹⁷ have shown that individual product yields exhibit maxima as a function of pyrolysis time. At 400 °C, the maximum yield of guaiacols was reached after 7.5 min, that of the catechols after 15–20 min whereas the phenol yield increased with time. The same reaction pathways were observed for pyrolysis of Kraft pine lignin, milled wood lignin and pine wood lignin. This result indicates that phenol and its derivatives are the only stable pyrolysis products and are favored at long pyrolysis times and high temperatures. After 1 h at 400 °C, the maximum yield of low molecular weight products was 40 wt%⁴¹⁷. The maximum yield of monophenols has been reported as 15 wt%⁴⁰³. Above 550–600 °C, gradual decomposition of lignin

intermediates, homolysis products, char and condensable gases results in the evolution of mainly CO and limited C₁–C₃ hydrocarbons, as shown by several researchers^{427,430}.

The pyrolysis product distribution is strongly influenced by lignin structure and composition, suggesting it may be desirable to tailor lignin isolation conditions to obtain desirable characteristics. Critical characteristics include the biomass type and species^{405,410,431}, β-aryl ether content^{410,424,432}, functional group content⁴³³ and elemental composition and presence of impurities⁴⁰⁷. Additional parameters which have a bearing on the product distribution include the lignin moisture content, purity and presence of contaminants, notably inorganic salts (Na, K, Mg and silicates)^{434–437}. Alkali and other metals originating from the ash content of biomass have the effect of reducing liquid formation in favor of gas and char formation⁴³⁸. The effect of lignin structure on pyrolysis products is the focus of Part II, Chapter 4.

Finally, several studies have concluded that comparison of results obtained for the same lignin samples using different analytical systems can also introduce variations, highlighting the importance of the pyrolysis reactor itself⁴⁰⁵. Fast pyrolysis reactor configurations include fixed bed, fluidized bed, entrained flow, ablative and vacuum systems^{415,439,440}. Fixed bed reactors are a well-understood technology and are simple to construct and operate^{441–443}. These reactor configurations utilize dried, small particle sized feedstocks and an inert heat transfer medium such as sand to allow for efficient heat transfer. Though fluidized beds are more commonly used for biomass pyrolysis, the problems of feeding (due to low melting point of lignins) and the agglomeration of bed materials (which would cause de-fluidization of the bed) can be partially overcome using flash pyrolysis in a fixed-bed reactor⁴⁴⁴. In a fixed bed configuration, the reactor bed is pre-heated to the desired pyrolysis temperature and a gas (typically N₂) is used to carry products through the downdraft reactor. Lignin is then fired in and the products cooled at the reactor exit to condense aerosols into liquids while the char can be collected from the bed after the reaction has elapsed.

Properties of pyrolysis oil and dependence on feedstock

Bio-oil is the liquid product derived from fast pyrolysis of biomass or its constituents. Bio-oils are multi-component mixtures of molecules derived from depolymerization and fragmentation of biomass components³⁹⁶. They are reported to contain over 300 compounds including hundreds of oxygenated molecules; some representative components are displayed in Figure I-14. Owing to myriad components, chemical analysis of pyrolysis oil is somewhat challenging³⁶³, as only ~40 wt% of compounds can be detected by GC-MS due to poor volatility⁴⁴⁵. Spectroscopic techniques such as FTIR⁴⁴⁶ and UV-F⁴⁴⁷ have been used to give limited insights into the whole portions of pyrolysis oil.

More recently, advances in NMR²²⁵ have enabled probing into over 30 different functional groups present in lignin pyrolysis oil to give quantitative results. Nevertheless NMR cannot provide exact structures, making complete characterization of bio-oils impossible³⁶³.

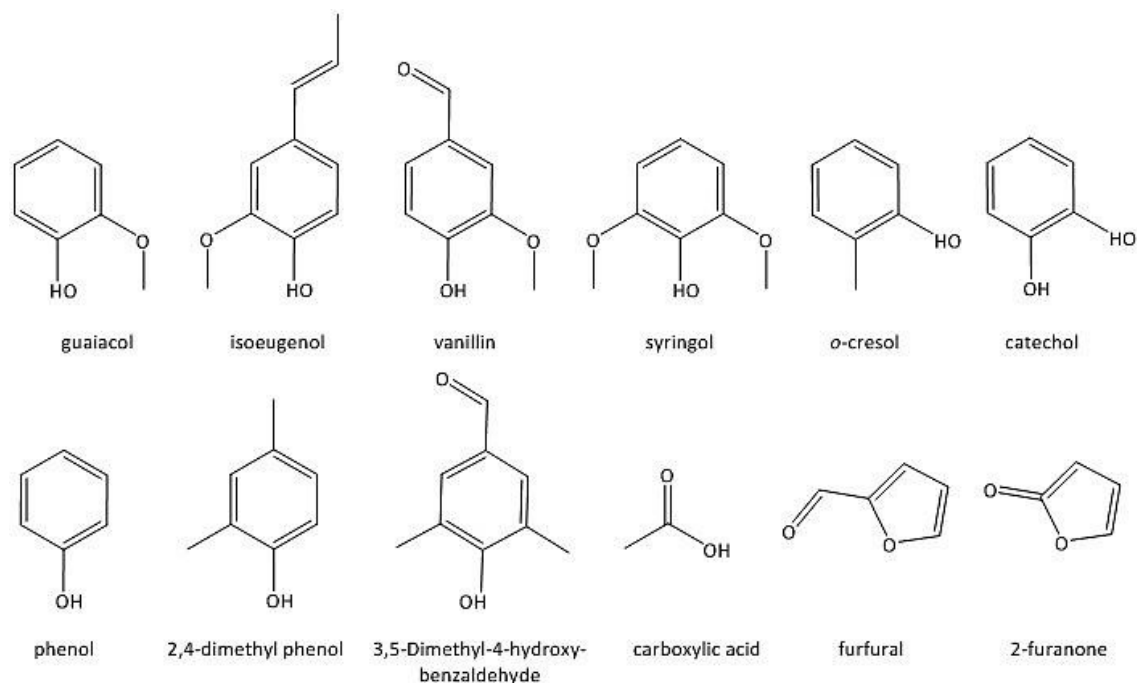


Figure I-14. Representative compounds of lignin and biomass bio-oils^{448,449}.

Pyrolysis oils derived from whole biomass feedstocks typically have water content of approximately 20 wt%, a pH of approximately 3 and higher heating value (HHV) of 18 MJ kg⁻¹; they are not miscible with hydrocarbon fuels²². They are chemically unstable as they contain a range of acidic and other compounds that can react together to produce water^{22,23}; hence, distillation is not practiced and storage and transportation of bio-oil must be carefully considered⁴⁵⁰. However, these bio-oils have a higher volumetric energy density than the dried biomass from which they originated, minimizing transport costs compared to ground biomass feedstocks. The difference in properties between biomass pyrolysis bio-oils and hydrocarbon fuels and biomass pyrolysis oils is illustrated in Table I-4. The root cause of bio-oil instability is believed to be the presence of reactive water-soluble oxygenated compounds, notably organic acids, which could act as catalysts in accelerating the polymerization reactions⁴⁴⁵.

Table I-4. Typical physical properties of biomass bio-oil and heavy fuel oil²².

Property	Biomass bio-oil	Heavy fuel oil
Water content (wt%)	15–30	0.1
pH	2.8–3.8	–
Density (kg/L)	1.05–1.25	0.86
Viscosity 50 °C (cP)	40–100	180
C (wt%)	54–58	83–86
O (wt%)	45–50	<1
H (wt%)	5–7	11–14
S (wt%)	<0.05	<4
N (wt%)	<0.4	<1
Ash (wt%)	<0.2	0.1
H/C	0.9–1.5	1.5–2.0
O/C	0.3–0.5	~0
HHV	16–19	40

Indeed, bio-oil is not a product of thermodynamic equilibrium but is produced with short residence times and rapid cooling or quenching from the pyrolysis temperatures; this produces a liquid condensate that is not at thermodynamic equilibrium at storage temperatures⁴⁵¹. Hence, the chemical composition of bio-oil tends to change during storage⁴⁵². In particular, aldehydes, ketones and acids can react via Aldol condensations during storage and handling to form larger molecules, resulting in an increase in water content and viscosity and a decrease in volatility over time²². This ‘ageing’ is primarily temperature-driven^{22,453} and can be accelerated by exposure to light or oxygen³⁹⁶ and slowed down by addition of polar organic solvents such as methanol or acetone⁴⁵². The following are among the reactions that are thought to take place in bio-oil during ageing⁴⁴⁹:

Organic acids + alcohols → esters + water

Organic acids + olefins → esters

Aldehydes + water → hydrates

Aldehydes + alcohols → hemiacetals + acetals

Aldehydes → oligomers + resins

Aldehydes + phenolics → resins + water

Unsaturated compounds → polyolefins

In previous works to examine the effect of these parameters on the ageing and stabilization of biomass bio-oils, it was demonstrated that the presence of high molecular weight lignin (in the form of pyrolytic lignin) led to a higher rate of viscosity increase in bio-oil⁴⁴⁹. Pyrolyzing high molecular weight (MW) lignins could therefore be expected to produce a less stable bio-oil. However, it is worthy of note that

these experiments examined the effect of pyrolytic lignin oligomers of different MW on *biomass* bio-oil, which is an altogether different mixture of compounds than *lignin* bio-oil and contains significantly higher concentrations of reactive compounds including levoglucosan, acetic acid, hydroxyacetaldehyde and hydroxyacetone derived from carbohydrate degradation⁴¹¹.

Bio-oil derived from lignins has received much less research attention than biomass bio-oils^{397,454}. The liquids produced from lignin fast pyrolysis have been characterized by a limited body of research that was reviewed by Fan *et al.*⁴⁵⁵. Lignin has lower oxygen content (22–32%) than biomass (35–40 wt%), and consequently lignin bio-oils have lower oxygen content (30–50 wt%) than biomass bio-oils (45–50 wt%)^{13,22}. Lignin-derived pyrolysis oils are expected to contain fewer oxygenated compounds have a higher energy density and improved thermal stability compared to biomass pyrolysis oils. Fractionation of biomass and subsequent pyrolysis of pure lignins (which are deoxygenated and have higher carbon content) is therefore desirable. However, lignin pyrolysis entails challenges in itself as it is more difficult to thermally decompose and has a low melting temperature⁴⁵⁶.

As we have seen, most studies of lignin thermochemical conversion have used analytical pyrolysis to investigate the influence of pyrolysis conditions on kinetics and production of monomeric phenols. However, few studies have addressed the development of bench-scale fast pyrolysis techniques for conversion of biorefinery lignins. Even fewer studies have focused on investigating the link between lignin isolation conditions and pyrolysis product distribution and quality. Studies of fast pyrolysis of biorefinery lignins are especially scarce; at the time of writing, only a dozen or so studies had pyrolyzed lignins isolated from steam explosion, Organosolv or ionic liquid processes^{13,111,201,401,457–459}.

Economics of lignin pyrolysis

Despite some success in research, the commercial applications of lignin pyrolysis face considerable challenges. Due to low product selectivity and complex composition, separation processes are costly and tedious⁴²⁵. Another major difficulty lies in continuous processing due to feeding and agglomeration issues¹³. These issues arise from the low melting point of lignin and a tendency to form char. Strategies for overcoming these difficulties include pyrolysis reactor design modifications⁴⁶⁰, catalytic cracking^{461,462}, lignin pretreatment⁴⁶³ to remove reactive functionalities (e.g. methoxyl groups⁴⁶⁴) and high-pressure solvolysis⁴⁰⁴ in the presence of formic acid and alcohol solvents. These methods have all been successfully applied to increase bio-oil yield and to improve lignin quality and fuel value, detected by an increase in the H/C and decrease in the O/C atomic ratios. However, these methods are not sufficient to completely deoxygenate the bio-oil product and achieve complete conversion to aromatics; additionally, they face challenges such as high cost. A number of researchers have claimed that pyrolyzing pure lignins is unfeasible in typical state-of-the-art fast pyrolyzers¹³²⁰, suggesting that catalytic pyrolysis technology is necessary for value addition.

An alternative approach lies in upgrading of raw lignin pyrolysis oils, for instance by catalytic cracking^{449,455} and hydrodeoxygenation (HDO) technologies⁴⁶⁵. Among the former, zeolite cracking has been most extensively studied to promote reactions such as dehydration, decarboxylation and dealkylation; however, the deactivation of zeolites induced by phenolic compounds is the main barrier to this method⁴⁶⁶. HDO in the presence of a catalysts enables oxygen removal in the form of water, significantly improving the quality of raw lignin bio-oil, but relies on expensive noble metal catalysts⁴⁵⁵. Moreover, although HDO catalysts are effective for conversion of phenols into hydrocarbons, large quantities of alkylphenols are generated⁴⁵⁸. In order to obtain high-quality bio-jet fuels from lignin, a series of successive steps for lignin bio-oil upgrading are likely to be needed⁴⁶⁷.

Though fast pyrolysis has been reported as one of the economic ways to produce fuels from solid biomass, very limited studies have examined the economics of lignin pyrolysis^{20,363}. Most pertinently, Obydenkova *et al.*⁴⁶⁸ explored the environmental and economic aspects of fast pyrolytic conversion of lignin obtained from 2G ethanol plants to transport fuels for the marine and automotive markets. Two critical factors were seen to govern fuel viability: first, optimization of logistics was essential, favoring a decentralized processing approach owing to high transport costs of low-energy-dense lignin. Life cycle analysis also showed selection of biomass for CHP could bring the GHG emissions to a level compatible with the 60% target set by the US RFS³⁵⁰; conversely, the process economics were improved using a fossil energy carrier such as natural gas. Low-sulfur heavy marine fuel obtained by fast pyrolysis was seen to offer the best business and environmental case, as such fuels require low fuel cost (\$ GJ⁻¹) rather than high energy density and cetane number and are thus tolerant to higher water content and viscosity. It is worthy to note that the study assumed 33% bio-oil yield based on dry lignin feed; higher oil yield would be highly desirable from an LCA and economic perspective⁴⁶⁸.

4.3. Summary

- The valorization potential of lignin is favored by a low degree of condensation, low level of impurities (ash, sulfur, and carbohydrates) and high presence of chemically labile moieties.
- Adhesives and binders represent short-term products from lignin while polymers, glassy carbon, fuels and chemicals have medium-term potential. Low-cost carbon fiber production from lignin offers immense potential as a high-volume application for biorefinery lignins.
- Thermochemical valorization of lignin by fast pyrolysis represents an economically viable way to produce fuel components.
- Studies of the fast pyrolysis of iSolv lignins are needed to establish the link between pretreatment conditions, lignin properties and bio-oil yield and quality.

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6. Research Gap and Objectives

From the reviewed literature we can see that ionic liquid-based biorefining offers immense potential as a route to cost-competitive biorefining. Notably, the development of low-cost ILs, such as [TEA][HSO₄] and [DMBA][HSO₄], has validated the potential of large-scale commercial use of ILs for pretreatment. This opens up the exciting possibility to produce bioethanol, biofuels, renewable chemicals and materials from widely available agricultural residues, energy crops and other lignocellulosic materials, thereby contributing to climate change mitigation and energy security and stimulating rural development.

To begin to make this a reality, it is desirable to identify an abundant and low-cost biomass resource available with high geographical coverage to enable the development of localized biomass processing centers. Agricultural residues present an immense opportunity for bioethanol production, notably the use of agro-industrial materials such as sugarcane bagasse for which an established supply chain but few competing uses exist. Many of these residues have a high ash content which limits their use in paper and pulpmaking, biogas production or thermochemical valorization, making them suitable multi-million-ton feedstocks for lignocellulosic biorefineries. It is necessary to determine the processing parameters for agroresidues that will maximise carbohydrate recovery, fermentable sugar yields and lignin recovery (i.e. pretreatment efficacy). Second, while the efficacy of use of low-cost ionic liquids has been widely demonstrated for a variety of biomass feedstocks at the bench scale (10 mL), it is necessary to investigate the scale-up and intensification of this process. This entails new challenges such as the use of high biomass loadings, larger particle sizes, effective IL-biomass slurry mixing and optimization of washing and solvent recovery processes. Thirdly, the importance of lignin valorization for the economic health of the biorefinery is frequently emphasized. Biorefinery lignins may be isolated in high purity from lignocellulose using IL pretreatment. Relatively few studies have linked the structural changes occurring in lignin as a result of pretreatment with pyrolysis products of the isolated lignins. This opens up a third area of interest, namely the optimization of lignin properties for production of value-added materials and chemicals. Thermochemical valorization of lignin was also investigated as a shorter-term opportunity to produce blended fuel components. It is also desirable to investigate lignin fractionation to reduce the polydispersity and tailor lignin properties for selected applications. Lignin valorization requires a detailed understanding of changes to lignin chemical structure, composition and thermal properties during processing and fractionation in order to optimize pretreatment conditions and lignin recovery.

Feedstock selection and pre-processing, pretreatment optimization, process intensification and lignin valorization were identified as critical areas of research in order to improve the economics of the ionic

liquid-based biorefinery. These were also key next steps in the commercialization of the ionoSolv process, which is currently underway as Chrysalix Technologies Ltd at Imperial College London.

The objectives of this project were therefore to:

- demonstrate the use of the low-cost ionic liquid [TEA][HSO₄]-water mixtures for pretreatment of widely available high-ash agricultural residues
- optimize pretreatment time and temperature to enable high sugar and lignin recovery
- compare pretreatment outcomes for different sugarcane bagasse residues available from commercial sugarcane processing
- investigate the effect of high biomass solids loading on pretreatment efficacy to increase space-time yields and reduce the costs and energy intensity of ionoSolv processing
- investigate the flexibility of ionoSolv processing to handle larger particle sizes, with a view to reducing the energy requirements of a size reduction step prior to pretreatment
- demonstrate the 100-fold scale-up of the ionoSolv process with the introduction of mixing
- adapt the pulp recovery protocol for larger-scale application to minimize solvent volumes
- examine the link between pretreatment parameters (temperature and residence time) and ionoSolv lignin structure, composition and properties
- investigate the effects of different parameters for fast pyrolysis of ionoSolv lignins on the product distribution and bio-oil quality with a view to production of lignin-derived liquid fuels by thermochemical methods
- conduct an energy and mass balance on lignin fast pyrolysis under the optimum conditions required for high bio-oil yield and quality
- compare the optimum conditions required for lignin thermochemical valorization and fermentable sugar recovery
- investigate fractional lignin precipitation as a means to produce homogenous lignin fractions with narrow polydispersity and controllable functionality and properties

7. Thesis Outline

This thesis is divided into seven sections which are outlined as follows. In Part I, the background to the research topics was presented and the research goals outlined. Part II contains the five experimental results and discussion chapters. Chapter 1 describes the pretreatment of agricultural residues which is then expanded with a detailed look into sugarcane bagasse pretreatment in Chapter 2. Process intensification using the energy crop *Miscanthus* is then explored in Chapter 3, which attempts to optimize solids loadings, particle size and mixing speeds. Chapter 4 examines the link between pretreatment conditions and lignin properties and investigates the fast pyrolysis of ionoSolv lignins. Lignin fractionation by fractional anti-solvent precipitation is then investigated in Chapter 5. The research outcomes and implications for further commercialization of the ionoSolv process are then brought together in Part III, which concludes this PhD thesis.

Part II. Results and Discussion

Chapter 1: Pretreatment of Agricultural Residues

1.1 Introduction

Agricultural residues, such as straws, husks, stalks, stovers, cobs and bagasse, are potentially rich sources of energy because up to 80% of their dry matter consists of polysaccharides¹. These residues are highly abundant, geographically diverse and available at low cost, making them attractive feedstocks for production of renewable ethanol². However, most agricultural residues are not optimally utilized as energy sources as present, since their digestibility is often low³. Current disposal methods include open-field burning or landfilling⁴, resulting in a large amount of GHG emissions, contamination and air pollution with direct consequences for public health and the environment^{4,5}. Given the urgent need for developing scalable solutions to mitigate climate change, research on uses of agro-industrial residues for energy applications deserves serious attention.

Wheat, rice, maize and sugarcane are the four agricultural crops with the largest production as well as area under cultivation⁶. These four crops generate the vast majority of agroresidues available globally, with global resources estimated at 2.4 Gtons of dry matter available annually after discounting ~40% that are currently appropriated³. According to an estimate by Kim and Dale², crop residues could be utilized to produce around 442 GLpa of bioethanol, of which 360 GLpa (>80%) could be produced from rice straw, wheat straw and sugarcane bagasse alone. This amounts to over 3 times current global bioethanol production (115 GLpa)⁷. These feedstocks are most abundant in Asia, which has the highest potential (~60%) for bioethanol production from agricultural residues globally².

Several of these important agricultural crops produce residual wastes that are exceedingly high in ash (up to ~30 wt%)⁸, notably rice straw and rice husk. High ash concentrations are unfavorable for valorization of agricultural residues and the extraction of cellulose. Silica blunts machinery, reduces the digestibility of straw and interferes with processing, forming deposits on the surface of reactors and making combustion more difficult⁹. Using conventional pretreatment processes such as dilute acid (DA) pretreatment, hot water or steam explosion (SE) pretreatment, the ash can react with acids, increasing the amount of chemicals required for processing, notably at high solids loadings¹⁰.

Moreover, such processes are also unable to delignify biomass with significant lignin content (>10 wt%) as is the case for most agricultural residues¹¹. Most recently, development of IL-based biorefining has shown greatest potential for cost-effective pretreatment of agroresidues^{12–17}.

Rice residues and other major agro-industrial byproducts such as wheat straw and sugarcane bagasse are strong potential candidates for our future energy needs. Given their low bulk density, transportation of these residues can be expensive; therefore, efficient processes are needed to transform residues into higher bulk density products, such as bioethanol. This chapter examines the suitability of lignin-dissolving ILs using ionic liquid processing to convert four agricultural residues (rice straw, rice husk, wheat straw and sugarcane bagasse) to give a high yield of fermentable sugars. Successful pretreatment was characterized by high glucose release, biomass delignification and lignin recovery following pretreatment with the low-cost IL triethylammonium hydrogen sulfate ([TEA][HSO₄], henceforth TEA HSO₄). Subsequently isolated lignin fractions were analyzed by HSQC NMR to obtain insights into the structural changes occurring during ionic liquid pretreatment.

1.2 Background

1.2.1 Feedstock availability

Generally, crops are grown for their food value, though usually only a portion of a crop is useful for human and animal consumption. Although some plant residues with commercial value are also harvested, major proportions of crop residues are considered as a waste which have to be disposed of as economically as possible. Disposal by soil incorporation is a widely favored practice as crop residues play an important role in maintaining soil organic matter and moisture, controlling erosion and chemical runoff and ensuring the long-term productivity of the soil¹⁸. In cases where direct soil incorporation is undesirable, too costly or impractical, open-field burning is common, leading to the release of toxic emissions⁵ and particulate matter⁴ with a high associated carbon footprint. This method prevents recovery of the energy content of agricultural residues and produces ash which is usually a poor soil nutrient. In some cases (e.g. sugarcane or rice milling), wastes are burned in pollution-controlled industrial facilities to produce steam which is used for combined heat and power (CHP). However, as a result of the increasing efficiency of industrial boilers, many agro residues are produced in surplus and are vastly underutilized¹⁹. While some of these residues (~40wt% for rice and wheat²⁰) must be left in the soil for soil conservation, as described above, the bulk of the residues are available for industrial use. Using surplus agroresidues as industrial feedstock for fuel ethanol production could meet future energy needs while alleviating concerns regarding land use and competition for food production.

Rice straw and husk are among the most abundant crop residues in the world, particularly in Asia, while wheat straw is the dominant agricultural by-product in Europe. Bagasse is produced in huge

amounts during sugarcane production while straw is generated from wheat farming at a rate of 2–8 dry tons ha⁻¹ annually under modern farming conditions¹⁸. Corn stover is the residue comprising corn stalks, leaves, cobs and husks and is the predominant residue available in North America; however, due to its moderate ash content (3–7%) and low lignin content (<10%)^{21,22}, it can be cost-effectively pretreated using existing technologies such as DA and SE^{23–25} and was therefore not considered in this study. Figure II-1 shows the estimated total quantities of the most abundant agricultural residues available on an annual basis, assuming 40% of crop waste by weight is needed for soil conservation²⁰. The largest quantity of material available for conversion (after harvesting of the rice crop) is rice straw. Every kilogram of milled rice grain produced leaves behind roughly 0.7–1.4 kg of rice straw and 0.30 kg of rice husk during harvesting and milling respectively. This gives an estimated 830 million tons of rice straw per year²⁶ and 200 million tons of rice husk per year globally, with limited parts going to cattle feed and combustion in boilers and the rest as waste. Far behind rice straw, bagasse is the next most abundant waste followed by rice husk, corn stover and wheat straw.

Potential bioethanol production from agroresidues is immense and could potentially multiply current production by a factor of 3 or more. It has been estimated that rice straw could produce 205 GL bioethanol per year, which is the highest among the agroresidues examined, followed by wheat straw (104 GLpa), corn stover (59 GLpa), sugarcane bagasse (51 GLpa) and rice husk (24 GLpa)^{2,27}.

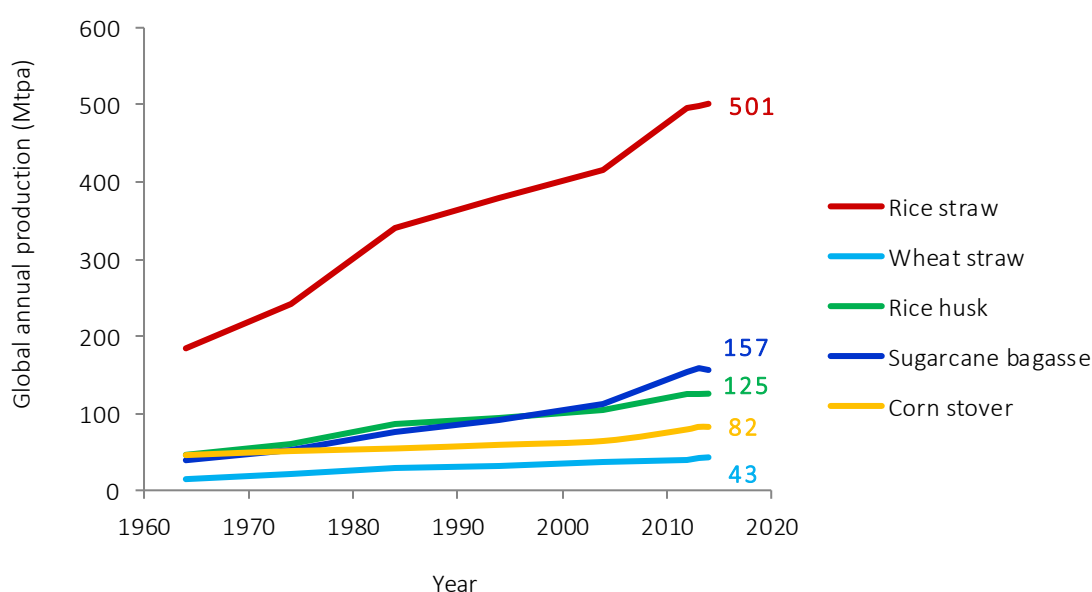


Figure II-1. Estimated total availability of major agricultural residues for bioenergy production on an annual basis. It was assumed that 40 wt% of available residues are ploughed back into soil for soil conservation. Data source: FAOSTAT²⁸. Assumptions: see footnote¹.

¹ Key assumptions: Bagasse: each wet ton produces 260 kg moist bagasse with water content ~50wt%¹⁰¹. Corn stover: 4 tons stover available per acre⁶; Rice husk: 22% of rice paddy weight is rice husk (www.bioenergyconsult.com/rice-straw-as-bioenergy-resource/, last accessed 26/07/2017); Rice straw: rice straw to paddy ratio is on average 4 wt/wt³⁰; Wheat straw: 43% of wheat weight is available as straw (www.hdb.org.uk/projects/Straw.aspx; last accessed 26/07/2017);

1.2.2 Biomass physical properties

The chemical composition of biomass feedstocks is a key factor affecting efficiency of biofuel production during the complex conversion process. The major chemical and fuel properties of four agricultural residues are displayed in Table II-1. It can be observed that all feedstocks are rich in cellulose (36–53 wt%) and hemicellulose (5–28 wt%) and are thus suitable for conversion into bioethanol. It must be noted that the structural composition of lignocellulosic feedstocks is highly variable, depending on genetic and environmental influences. Wheat straw and rice straw appear to have lower lignin content (17–18 wt%), while rice husk and sugarcane bagasse have higher lignin content (20–21%) and are therefore expected to be more recalcitrant to conversion.

The ash contents of agricultural residues are significantly higher than those of wood (< 1.7 wt%)⁸ and most coals (4.5–8.3 wt%, though occasionally up to 40 wt%). Rice straw and husk have particularly high ash contents up to 20 wt% and 23 wt%, respectively. Consequently, these residues have particularly poor fuel value (14–17 MJ kg⁻¹), though rice husk possesses the advantages of uniform size and easy procurement; in contrast, sugarcane bagasse and wheat straw have higher calorific value (up to 19 MJ kg⁻¹) given the smaller contribution of inorganics to their composition. Carbon content can be seen to increase monotonically with successively lower ash content. Compared to woody biomass, agricultural wastes contain a higher proportion of fixed carbon and a lower proportion of volatile matter⁸. This is associated with production of a higher quantity of char and ash during thermal degradation, leading to fouling and slagging problems upon combustion²⁹.

Table II-1. Physical properties of selected abundant high-ash agricultural residues.

Biomass Feedstock	HHV (MJ kg ⁻¹)	Proximate analysis (wt% d.b.) ^a			Chemical composition (wt% d.b.) ^a		Sources
		Fixed carbon	Volatile matter	Ash	Holocellulose ^b	Lignin	
Rice straw	14.1-15.5	14.5-16.2	65.5-71.6	13-23	53	17	8,16,30,31
Rice husk	13.4-16.5	11.1-16.2	63.5-67.0	11-20	58	20	16,30,32,33, this study
Sugarcane bagasse	14.2-18.6	7.0-11.9	70.9-86.3	1-22	71	21	8,16,34, this study
Wheat straw	17.0-18.9	11.7-23.5	63.0-80.6	4-14	77	18	8,16,35,36

^a Dry basis; ^b Holocellulose = cellulose + hemicellulose.

1.2.3 Ash content

Ash represents the mineral content of biomass that depends on the soil and environmental conditions; for instance, tropical biomass species typically need more mineral elements to support their growth³⁷. All of the selected feedstocks in this study possess comparatively high ash content. However, a wide variation in the ash content and distribution of inorganic species can be observed between them (Table II-2). Silica (SiO₂) and potassium (K₂O) are the main ash-forming species for all of the agricultural

residues, which has implications for fuel and combustion properties, unless these are removed during pre-processing. In rice straw and rice husk, the ash consists essentially of SiO_2 (75–95 wt%) which is derived from amorphous silica present in the cellular structure, while SiO_2 makes up roughly half of wheat straw and sugarcane bagasse. The high silica content of untreated rice residues is well known to wear out the components in processing machines, such as conveyors or grinders, and hamper digestibility for livestock⁴. Rice residues have relatively low alkali content (Na_2O and K_2O comprise <15% of total ash) whereas wheat straw has >25% alkali content. This high content of ash, alkali and potassium causes agglomeration, fouling and melting in the components of standard combustors or boilers^{9,35}. The type of inorganic components is also likely to have an effect on the selected method of bioconversion. Studies indicate that 70–90% of the potassium present is either water-soluble or ion exchangeable³⁵. Inorganics are typically only partially acid-soluble, with the exception of CaO , Fe_2O_3 , SO_3 and P_2O_5 which are up to 34% soluble⁹. The very high ash content plus alkali content in rice straw, for instance, could represent a more serious problem during processing as it is more susceptible to react with chemical reagents used for pretreatment. The first step of many bioconversion processes is therefore a physical or chemical de-ashing method^{38–40}.

Table II-2. Major elements of ash in rice straw, rice husk, wheat straw and sugarcane bagasse.

	SiO_2	Al_2O_3	TiO_2	Fe_2O_3	CaO	MgO	Na_2O	K_2O	SO_3	P_2O_5	LOI	Ref.
Rice straw ash	74.7-82.6	1.0-1.1	0.1	0.9-1	3-3.3	1.7-1.8	0.3-1.0	6.3-12.3	0.9-1.2	1.4-1.7	n.d.	30,31
Rice husk ash	72.1-93.2	0.2-0.4	0.1	0.2	0.4-1.1	0.4-0.5	0.5-1.2	0.7-3.8	1.8	0.1-0.3	2-24	41,42
Sugarcane bagasse ash	50.8	3.6	0.3	6.6	8.9	4.5	7.3	1.6	1.3	2.9	12	this study
Wheat straw ash	55.3	n.d.	n.d.	n.d.	6.1	1.1	1.7	25.6	n.d.	n.d.	n.d.	31

1.2.4 Current uses

Among the four agro-residues investigated in this study, several are already utilized for animal feed², domestic fuel⁴³ and paper-making⁴⁴. The utilization fraction is difficult to estimate, as it depends strongly on feedstock type and geographic region; however it is clear that most agricultural wastes are under-utilized^{3,20}. Only sugarcane bagasse and rice husks have established industrial applications in the generation of CHP in grain, sugar and ethanol mills^{27,43,45}. With recent improvements in boiler efficiencies, it is estimated that over 50% of generated bagasse is currently unutilized⁴³. Rice husk and straw are considered as extremely low-value by-products of rice milling as they have almost no digestible protein and are not suitable for animal fodder; moreover, their ash and lignin contents are too high to be economical materials for making paper products⁴. Typically about 50% of the husk

produced in rice milling is burnt to produce steam to drive the mechanical milling equipment, while the rest is disposed of by burning the husks in heaps near the mills²⁷. Options for disposal of rice straw are limited by its low bulk density, slow degradation in the soil, harboring of rice stem diseases and high mineral content¹⁸. Consequently, most rice straw is disposed of by open-field burning, a common practice all over the world, resulting in a large amount of GHG emissions, air pollution and affecting human health⁴³. With the development of recent technologies, rice residues can be processed and managed using better practices. Rice huskash has been frequently used as an additive in manufacture of durable concrete⁴², production of activated carbon and silica production⁴¹ or electricity generation²⁹ and the raw husk has been utilized for mushroom cultivation²⁰. Although the technology of using rice husk is well-established in many Asian countries, rice straw is rarely used as a source of renewable energy²⁶. The principal reason is the easy procurement of rice husk at rice mills, whereas collection of rice straw is laborious and its availability is limited to harvest time. Improvements to rice straw collection logistics could be made by baling but the high cost of equipment makes this uneconomical to most rice farmers³¹. Thus, technologies for rice straw utilization for energy must be particularly economical to compensate for the high costs of collection of this distributed but extremely abundant resource.

Thermochemical conversion of agricultural residues has also been explored^{29,32,33,46–48}. Agricultural residues are generally very poor fuels for combustion, as alkali components in the ash creates serious fouling and slagging problems in conventional boilers⁹. Alkali metals and silicate components also lead to operating problems, such as ash melting and agglomeration, during pyrolysis and gasification^{49,50}. For fast pyrolysis, in comparison to wood chips, char yields from agricultural residues are roughly double, with correspondingly lower bio-oil yields⁴⁷. This has been linked to both the inorganic content of the biomass, which can catalyze char-forming reactions, as well as the chemical composition and physical properties of the biomass, which affects both biomass devolatilization and char conversion⁵¹. Economical fast pyrolysis of high ash feedstocks is unlikely unless mechanisms to limit the impact of the ash constituents are implemented. For instance, washing biomass with acid or water in order to reduce the ash content is frequently used prior to pyrolysis of agricultural residues³⁸. However, washing biomass will increase its moisture content, introducing an energy penalty for drying to reduce the moisture level to an acceptable level prior to pyrolysis^{38,52,53}. Chemical and biological processes such as biogas production have also been explored; however rice crop residues were found to be poor substrates for anaerobic digestion due to their high lignin content and poor C–N ratio⁵⁴.

1.2.5 Pretreatment of lignin- and ash-heavy agroresidues

All feedstocks considered in this study are rich in cellulose and hemicellulose (see Table II-1) and thus suitable for conversion into bioethanol. However, the presence of high ash and silica content is problematic for many conventional bioethanol production processes, such as DA, with sugar yields

typically limited to 50–60% or less⁵⁵. Moreover, use of agricultural residues is hindered by their high moisture content (30–50 wt% just after harvest)^{14,56}, low bulk density⁵⁷ and low energy density⁵⁸.

The most promising technologies to date for pretreatment of ash- and lignin-heavy agricultural residues, in order of increasing success, are alkali^{59,60}, Organosolv^{61,62} and ILs^{14,16,63,64}. Due to their high lignin content, numerous studies have examined pretreatment of rice straw and sugarcane bagasse using alkali and ammonia^{26,65}. Alkali pretreatment of chopped rice straw with 2% NaOH at a biomass to solvent ratio of 1:5 g/g at 85 °C for 1 h achieved 36% delignification⁶⁵; however, at higher solids loadings more representative of industrial biorefining, delignification reached 23.1% but sugar yields were limited to ~50%⁵⁹. Optimized conditions for sugarcane bagasse pretreatment using KOH solution have achieved over 91% release of available glucose⁶⁶. Alkaline methods were less effective for rice husk, releasing up to 71% of glucose following combined microwave and alkali pretreatment of rice husk⁶⁷. The main effect of alkaline pretreatment on lignocellulose is lignin removal by disrupting the lignin-carbohydrate complex, thus increasing the porosity of biomass⁶⁸. Due to its lower lignin and ash content, steam explosion is probably the most suitable method for pretreating wheat straw¹⁸. Yoon *et al.*⁶⁰ compared the performance of alkali, acid and IL pretreatment of bagasse. It was found that alkali pretreatment (92.3% glucose release) outperformed IL pretreatment (69.5%), most likely due to effective delignification by alkali combined with a lower solids loading used in the alkali treatment process. A comparison of the energetic requirements found that pretreatment with [Emim][OAc] consumed over 30% less energy than alkali pretreatment, making it a less costly option.

Organosolv pretreatment is another high-potential alternative for conversion of agricultural residues to bioethanol⁶¹. Sugar yields for ethanol Organosolv were found to be low compared to alkali pretreatment, though no reports of Organosolv pretreatment of rice husk were available at the time of writing. Mesa *et al.*⁶⁹ applied ethanol Organosolv on sugarcane bagasse and found that under optimal conditions (1.25% wt/wt H₂SO₄ in aq. ethanol for 60 min at 175 °C) it gave only ~30% glucose yields. Similarly, glucose yields for ethanol Organosolv pretreatment of rice straw were limited to 46%⁶². However, more promising results were obtained for mixtures of alcohols and CO₂, which gave delignification extents up to 95%⁷⁰ though sugar yields were not reported. Organosolv processing seems to have been most successful for wheat straw, where Huijgen *et al.*⁶¹ reported the use of acetone Organosolv process and achieved 79% delignification and 87% glucose yields.

Recent studies have shown that ionic liquids can be used to dissolve cellulose from ash-rich feedstocks including bagasse, corn stalks and wheat straw. Of these, the expensive APIL [Emim][OAc] has been shown to release over 90% of glucose from bagasse pretreated at 160 °C for 3 h using a biomass to solvent ratio of 1:6.67 g/g¹⁵ and over 95% of glucose at 150 °C for 90 min from straw at a biomass to solvent ratio of 1:20 g/g¹¹. Another study demonstrated use of cellulose-dissolving IL [Bmim][Cl] to

pretreat corn stalk, rice straw and bagasse, obtaining 66%, 74% and 68% glucose yields respectively⁷¹. Only one previous study¹⁶ has attempted rice husk pretreatment using ILs; however, sugar yields were limited to 40% obtained using [Emim][OAc]. It should be noted that these ionic liquids are not only prohibitively expensive ($>US\$50\text{ kg}^{-1}$ compared with $<\$1.5\text{ kg}^{-1}$ for the IL used in this study^{72,73}) but also thermally unstable, limiting their recyclability and hence the economic viability of lignocellulose processing using cellulose-dissolving ILs. Moreover, these ILs work optimally under completely dry conditions and have reduced performance in the presence of water⁷³. However, most agricultural residues have moisture contents up to 50 wt% immediately after harvesting and drying prior to pretreatment would introduce a significant energy expense. Moving away from expensive cellulose-dissolving ILs, Hou *et al.*^{14,74} successfully pretreated rice straw using cholinium amino acid IL [Ch][Lys], obtaining sugar yields of 84% and 42% for glucose and xylose, respectively, after pretreatment at 90 °C for 5 h and demonstrating five successful recycles. Again, use of cholinium ILs is limited by their relatively high cost and low thermal stability⁷⁵.

Another challenge for processing of agricultural residues with a view to bioethanol production is the first step, namely mechanical size reduction. The power input for mechanical comminution of agricultural materials depends on the initial and final particle sizes, moisture content and on the type of waste being handled⁵⁷. Previous studies have found that energy consumption for straws is roughly 2.3–3 times higher than for corn stover, using a hammer mill and final size of 1.6 mm^{57,76}. Mani *et al.*⁵⁷ note that the energy consumption of grinding for agricultural residues is proportional to their ash content. Other disadvantages of mechanical grinding of these materials is abrasion and damage of processing tools and potential introduction of inhibiting effects if particles are ground to excessively small sizes⁷⁷.

Beyond the challenges examined that are unique to agricultural residues (high ash and lignin content, high moisture content in raw feedstocks), several other key parameters necessary to achieve cost-effective pretreatment of lignocellulose were compared for some of the more established pretreatment technologies. These are summarized in Table II-3. Conventional pretreatment methods are limited by their inability to delignify biomass at high solids loadings, while cellulose-dissolving (Dissolution) ILs suffer from the drawback that they work optimally under dry conditions. Only Organosolv pretreatment appears to emerge as a competing technology to ionoSolv pretreatment, though this relies on successful scale-up of the process.

Table II-3. Comparison of different pretreatment technologies for pretreatment of agricultural residues.

	Dilute acid	Steam explosion	AFEX	Alkaline	Organosolv	IL dissolution	IL amino acid	IL ionicSolv
Ash tolerant	no	no	yes	yes	yes	yes	yes	yes
Water tolerant (>50% moisture)	yes	yes	yes	yes	yes	no	yes	yes
High lignin content (>15%)	no	a bit	a bit	a bit	yes	yes	yes	yes
High solids loadings (>20%)	no	no	no	no	yes	yes	yes	yes
Sensitivity to particle size	H	M	L	H	L	M	L	L
Thermal stability of solvent	-	-	-	-	-	L	L	H
Chemical and recovery cost	L	-	H	M	H	H	H	L to M
Lignin recoverability	-	-	-	M	H	M	H	H
Inhibitor concentration	H	H	L	L	M	L	L	L
Energy requirement	M	M	M	M	M	M	H	L
Safety (solvent toxicity, volatility)	H	M	L	M	L	H	H	H

1.2.6 Summary

- Agricultural residues are challenging feedstocks due to their high ash and lignin contents.
- Pretreatment processes for agroresidues must be energy-efficient, enable use of wet feedstocks at high solids loadings and not require excessive grinding prior to pretreatment.
- Lignin-rich feedstocks are best pretreated using ionic liquids or alkaline solvents, while steam explosion and Organosolv are best for low-lignin wheat straw.
- Ionic liquids have emerged as one of the most promising technologies for pretreatment of agricultural residues, achieving glucose yields of over 90%.
- The use of moisture-tolerant, thermally stable and recyclable ionic liquids for agricultural residues has not been investigated to date.

1.3 Experimental

1.3.1 Materials and method

Rice husk (*Oryza sativa*) was collected in Bahraich district, Uttar Pradesh, India in May 2015. Rice straw (*Oryza sativa*) was kindly provided by the Institute of Chemical Technology, Mumbai, after being harvested in Tirunveli district, Tamil Nadu, India in late 2015. Wheat straw (*Triticum aestivum*) was obtained from Glasgow, UK in 2015. Depithed sugarcane bagasse (*Saccharum officinarum*) was sourced from a paper and pulp mill in Kwazulu-Natal province, South Africa. It was subjected to an industrial depithing process. All feedstocks were washed to remove adhering inorganic debris and air-dried at room temperature in order to prevent microbial degradation during shipping. Following this, the feedstocks were ground and sieved (0.18–0.85 mm, US mesh scale -20/+80) and each stored air dry in sealed plastic bags at ambient temperature. All other materials and reagents were obtained from VWR International or Sigma-Aldrich, unless stated otherwise, and used as received. The minimum purity of triethylamine was $\geq 99\%$ and sulfuric acid was obtained from Sigma Aldrich as a 5M solution. The Karl-Fisher titrator used in this work was a V20 volumetric Titrator (Mettler-Toledo) and the analytical balance was a Sartorius CPA 1003 S balance (accuracy ± 0.001 g).

1.3.2 Synthesis of [TEA][HSO₄]

The protic ionic liquid [TEA][HSO₄] was synthesized according to the standard operating procedure of our laboratory in ~ 150 g batches. Triethylamine (75.9 g, 750 mmol) was cooled with an ice bath in a round-bottom flask. Under stirring, an equimolar amount of 150 mL of 5 M sulfuric acid (750 mmol) was added dropwise. The water was removed using a rotary evaporator (Büchi) to reduce the water content to below 20 wt%. The ionic liquid was recovered as transparent solution and the water content adjusted to 20 wt% by volumetric Karl-Fischer titration. ¹H NMR spectra were recorded on a Bruker 400 MHz spectrometer to verify IL purity. Chemical shifts (δ) are reported in ppm.

¹H NMR: δ H (400 MHz, DMSO-d₆)/ppm: 3.39 (s (br), HSO₄⁻, N-H⁺), 3.10 (q, J = 7.3 Hz, 6H, N-CH₂), 1.20 (t, J=7.3 Hz, 9H, N-CH₂-CH₃). ¹³C NMR: δ C (101 MHz, DMSO-d₆)/ppm: 46.21 (N-CH₂), 9.15 (N-CH₂-CH₃).

1.3.3 Fractionation of biomass

IonoSolv lignins were isolated using a pretreatment method carried out using a standard operating procedure of our laboratory⁷⁸ in triplicates. A biomass to solvent ratio of 1:10 g/g was used on an oven-dried basis. Before pretreatment, an ionic liquid/water mixture was prepared by adjusting the water content of [TEA][HSO₄] solution to 20 wt%, which was thoroughly mixed to obtain a colorless, homogenous, viscous solution that was subsequently used as the pretreatment solvent for all experiments. The solvent mixture was used to pretreat depithed sugarcane bagasse, wheat straw, rice straw or rice husk as feedstock. Pretreatment experiments on each feedstock were conducted in two

sets of triplicates, with one set of pulps that were air-dried after the Soxhlet step and one set that were stored wet to avoid hornification.

Biomass was placed in a wide-mouthed 15 mL pressure-resistant glass tube with screw-on Teflon cap and O-ring seal (Ace Glass, USA) resistant to ~10 bara. The solvent mixture was added to the biomass and the tubes were sealed and incubated without stirring in a preheated convection oven (ThermoScientific HeraTherm) under the desired conditions between 150 and 170 °C for a time between 30 and 180 minutes. After the pretreatment, the samples were cooled to room temperature and washed with ethanol (40 mL of ethanol per g of biomass) four times. The cellulose-rich material (i.e. pulp) was separated by centrifugation (3000 rpm for 50 min or 2000xg). The supernatant was decanted and the washing step repeated three more times. The pulp was Soxhlet extracted with ethanol (150 mL, 24 h) in cellulose thimbles and the extractives combined with the previous ethanol washes. The thimbles were then left on the bench overnight to dry. 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 added to the pretreatment tube. The air-dried pulp was then subjected to saccharification and compositional analysis.

For experiments where pulps were saccharified without air-drying in order to avoid hornification, the procedure was followed unchanged until the Soxhlet extraction step. The pulp-containing thimbles were removed from the Soxhlet adapter after completion of the extraction and put in 50 mL Falcon tubes which were immediately filled with DI water. The pulp was left in the thimble inside the Falcon tube for at least an hour. The thimble was then taken out of the Falcon tube and the pulp transferred back to the Falcon tube using a spatula. The tubes were centrifuged (3000 rpm, 50 min or 2000xg) and the supernatant decanted; the washing step was repeated once more in DI water. The wet pulp was weighed, its moisture content determined immediately and saccharification started the following day.

For lignin recovery, the four ethanol washes and Soxhlet extractives were combined and the ionic liquid/ethanol mixture was concentrated by evaporating the ethanol to leave a dried ionic liquid/lignin mixture. Lignin was precipitated by addition of deionized water as an anti-solvent to the original IL mixture quantity (with water : IL mixture ratio of 3.0 g/g). Lignin was allowed to precipitate for one hour and then isolated by centrifugation as above and washed two more times as before. Washed lignin (the solid precipitate) was dried *in vacuo* at 45 °C overnight and weighed to determine the lignin yield. The ionic liquid/water mixtures were discarded.

1.3.4 Isolation of enzymatic mild acidolysis lignin (EMAL)

EMAL was isolated from depithed sugarcane bagasse following the three-stage protocol for isolation of “native” lignin developed by Wu and Argyropoulos⁷⁹. First, depithed sugarcane bagasse was ground

to less than 0.75 mm (SM200 cutting mill, Retsch, Germany). It was then acetone-extracted for 48 h and dried in a vacuum oven at 45 °C. Approximately 60 g of dry extracted biomass was subjected to planetary ball milling (PM400, Retsch, Germany) for 14 days at a rotation frequency of 150 rpm. It was ground in four 250 mL ball mill jars in the presence of four stainless steel balls which occupied ~50% of the active jar volume, using toluene as a grinding aid. The biomass was then allowed to dry in a fume cupboard overnight until the toluene had evaporated. Following ball-milling, enzymatic mild acid hydrolysis was performed in two separate stages. In the first stage, the ball-milled biomass was treated with cellulase enzymes (Ctec2, Novozymes, Denmark) in the amount of 190 mg protein per g biomass. The enzymatic hydrolysis was carried out at 50 °C for 48 h at 3% consistency in the presence of 2% Tween 20 in 0.1 M citrate buffer (pH ~ 4.75). The slurry was stirred in a 2 L jacketed borosilicate glass pressure vessel (polyclave, Büchiglasuster, Switzerland) that was agitated using an anchor impeller at 120 rpm. The process temperature was controlled using an oil recirculator equipped with a thermostat (Unistat 405, Huber, Germany). After 48 h had elapsed, the slurry was centrifuged at 3000 rpm for 15 min with a high-speed centrifuge (Megastar 3.0, VWR, UK). The insoluble materials were re-suspended in a fresh batch of enzyme/citrate mixture for another 48 h at 50 °C under the same reaction conditions. After the enzymatic hydrolysis, the insoluble materials were again collected by centrifugation and washed twice with acidified (pH 2.0) deionized water to remove soluble sugars. Residual proteins on the surface of the insoluble solids were washed twice with 6 M guanidine hydrochloride followed by freeze drying for 72 h to obtain a crude lignin sample.

In the second stage, the crude lignin was finely ground in a pestle and mortar and subjected to mild acid hydrolysis using an azeotrope of dioxane-water (96 : 4 v/v) containing 0.01 M HCl at 87 °C under a nitrogen atmosphere. Butyl hydroxytoluene (BHT) was added as a radical scavenger and stabilizer to limit the formation of explosive peroxides. The crude lignin was suspended in the dioxane-water azeotrope with a solid-to-liquid ratio of 1:20 (g/mL) and refluxed for 2 h. After the reaction, the suspension was centrifuged and the supernatant was carefully withdrawn. The solid residue was washed with fresh dioxane-water until the supernatant was clear. The combined supernatants were then neutralized with sodium bicarbonate and then added drop-wise into 8 L acidified water (pH 2.0). The precipitated lignin was allowed to equilibrate with the aqueous phase overnight and was recovered by centrifugation and washed twice with deionized water. The solid was then washed once with hexane to dissolve any extractives and residual BHT and the purified solid was freeze-dried. The final yield of EMAL recovered through the entire procedure was 3 wt% based on dry bagasse starting material, and that based on the acid-insoluble lignin content of bagasse was 15 wt%.

1.3.5 Compositional analysis

Compositional analysis was carried out according to the NREL protocol 'Determination of Structural Carbohydrates and Lignin in Biomass'⁸⁰. All biomass samples, untreated or pretreated, were Soxhlet

extracted for 24 hours with ethanol and vacuum-dried for 48 h prior to compositional analysis. 300±5 mg of air-dry biomass, pulp or lignin was accurately weighed into a pressure tube and the weight recorded. Sulfuric acid (3 mL, 72%) was added, the samples stirred with a Teflon stir rod and the pressure tubes placed into a preheated water bath at 30 °C. The samples were stirred every 15 min for one hour; they were then diluted with 84 mL distilled water and the lids sealed. The samples were autoclaved (Sanyo Labo Autoclave ML5 3020 U) for 1 h at 121 °C and left to cool for 1.5 hours. The samples were then filtered through filtering ceramic crucibles of a known weight. The filtrate was filled in two 50 mL centrifuge tubes and the remaining black solid washed with distilled water. The crucibles were placed into a convection oven (VWR Venti-Line 115) at 105 °C for 24±2 h. They were taken out and placed in a desiccator for 10 min before they were weighed and the weight recorded. They were then placed into a muffle oven (Nabertherm + controller P 330) and ashed to constant weight at 575 °C. The crucible weight after ashing was recorded. The content of acid insoluble lignin (AIL) was determined according to (Equation 2). The content of one of the Falcon tubes was used for the determination of acid soluble lignin content (ASL) by UV analysis at 240 nm (Equation 3) (Perkin Elmer Lambda 650 UV/Vis spectrometer).

$$\%AIL = \frac{Weight_{crucible+AIR} - Weight_{crucible+ash}}{ODW_{sample}} \cdot 100 \quad (\text{Equation 2})$$

$$\%ASL = \frac{A}{l \cdot \epsilon \cdot c} \cdot 100 = \frac{A \cdot V_{filtrate}}{l \cdot \epsilon \cdot ODW_{sample}} \cdot 100 \quad (\text{Equation 3})$$

where $Weight_{crucibles+AIR}$ is the weight of the oven-dried crucibles plus the acid insoluble residue, $Weight_{crucibles+ash}$ is the weight of the crucibles after ashing to constant temperature at 575 °C, A is the absorbance at 240 nm, 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, ODW_{sample} is the oven-dried weight of the sample in mg and $V_{filtrate}$ is the volume of the filtrate in mL and is equal to 86.73 mL.

Calcium carbonate was added to the contents of the other Falcon tube until the pH reached 5. The liquid was passed through a 0.2 µm PTFE syringe filter and subsequently submitted to HPLC analysis (Shimadzu, Aminex HPX-87P from Bio-Rad, 300 × 7.8 mm, purified water as mobile phase at 0.6 mL/min, column temperature 85 °C) for the determination of total sugar content. Calibration standards with concentrations of 0.1, 1, 2 and 4 mg/mL of each of glucose, xylose, mannose, arabinose and galactose were used. Sugar recovery standards were made as 10 mL aqueous solutions close to the expected sugar concentration of the samples and transferred to pressure tubes. Sulfuric acid (278 µL, 72%) was added, the pressure tube closed and autoclaved and the sugar content determined as described above. The sugar recovery standard (SRS) coefficient was determined according to (Equation 4 and the sugar content of the analyzed sample using (Equation 5:

$$SRS = \frac{c_{HPLC} \times V}{initial\ weight} \quad (\text{Equation 4})$$

$$\%Sugar = \frac{c_{HPLC} \times V \times corr_{anhydro}}{SRC \times ODW_{sample}} \cdot 100 \quad (\text{Equation 5})$$

where c_{HPLC} is the sugar concentration detected by HPLC, 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), initial weight is the mass of the sugars weighed in, $corr_{anhydro}$ is the correction for the mass change during hydrolysis of polymeric sugars (0.90 for C_6 sugars glucose, galactose and mannose and 0.88 for C_5 sugars xylose and arabinose) and ODW is the oven-dried weight of the sample in mg.

The AIL and ASL contents were combined and reported as the total lignin content. Compositional analysis was conducted in triplicates on untreated biomass and in duplicates on the air-dried pulps.

As will be shown repeatedly throughout this thesis, pulp compositional analysis was typically used to enable detection of cellulose, hemicelluloses and residual lignin, though the latter cannot be distinguished from reprecipitated lignin and pseudo-lignins. The pulp composition was used to calculate the glucan recovery, delignification and hemicellulose removal; these were used together with the lignin yield and sugar yield from enzymatic saccharification as the key indicators of pretreatment performance, shown schematically in Figure II-2.

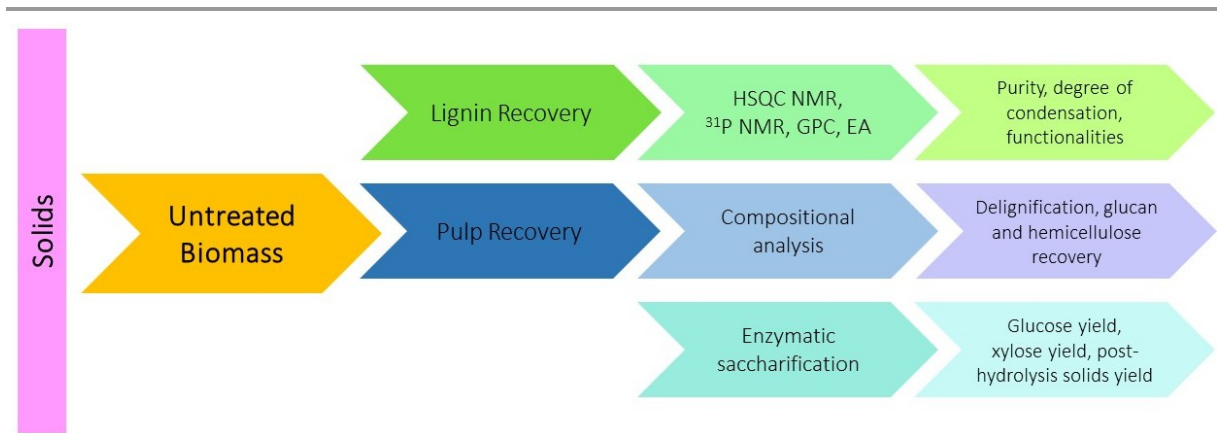


Figure II-2. Component recovery following ionoSolv pretreatment, key indicators and analytical techniques used.

The delignification was calculated using (Equation 6):

$$Delign. = \frac{Lignin_{untreated} - (Lignin_{pulp} \times Yield_{pulp})}{Lignin_{untreated}} \quad (\text{Equation 6})$$

where $Lignin_{untreated}$ is the lignin content in untreated sugarcane bagasse, $Lignin_{pulp}$ is the lignin content in the pulp and $Yield_{pulp}$ is the oven-dried yield of pulp.

Glucan recovery in the recovered solids, i.e. the glucan detected during pulp analysis compared to glucan detected during analysis of native biomass, was calculated using (Equation 7):

$$\text{Glucan recovery} = \frac{\text{Gluc}_{\text{untreated}} - (\text{Gluc}_{\text{pulp}} \times \text{Yield}_{\text{pulp}})}{\text{Gluc}_{\text{untreated}}}$$

(Equation 7)

whereby $\text{Gluc}_{\text{untreated}}$ is the glucan content of untreated sugarcane bagasse, $\text{Gluc}_{\text{pulp}}$ is the glucan content in the pulp and $\text{Yield}_{\text{pulp}}$ is the oven-dried yield of pulp. We note that glucan is a collective term given to D-glucose polymers made up of dehydrated glucose units; the majority of glucan in lignocellulosic biomass is in the form of cellulose.

Hemicellulose removal was calculated using (Equation 8):

$$\text{Hemicellulose removal} = \frac{\text{Hem}_{\text{untreated}} - (\text{Hem}_{\text{pulp}} \times \text{Yield}_{\text{pulp}})}{\text{Hem}_{\text{untreated}}}$$

(Equation 8)

where-by $\text{Hem}_{\text{untreated}}$ is the hemicellulose sugar content in untreated sugarcane bagasse, Hem_{pulp} is the hemicellulose content in the pulp and $\text{Yield}_{\text{pulp}}$ is the oven-dried yield of pulp.

1.3.6 Saccharification assay

Saccharification assays were carried out according to NREL protocol ‘Enzymatic saccharification of lignocellulosic biomass’⁸¹ in triplicate using Cellic® CTec2 enzyme blend obtained from Novozymes (Denmark). This is an analytical enzyme mixture containing cellulases, β -glucosidases, and hemicellulase, for the application of degrading cellulose to fermentable sugars.

For wet samples, moisture contents were determined immediately before saccharification. 100 ± 5 mg of wet or dry biomass (on an oven-dried weight basis) was placed into a Sterilin tube and the weight recorded. Three blanks were run with 100 μ L of purified water in order to correct for sugar residues present in the enzyme solutions. The water contained in the biomass sample (calculated using its moisture content and the total sample mass) was subtracted from 1.5 mL. The difference was added as water using a pipette. A total of 8.4 mL of solution consisting of 5 mL 1 M sodium citrate buffer at pH 4.8, 40 μ L tetracycline antibiotic solution (10 mg/mL in 70% ethanol), 30 μ L cycloheximide antibiotic solution (10 mg/mL in purified water), 3.38–3.41 mL purified water and 20–50 μ L of Ctec2 enzyme mixture were added, the tubes closed and placed into an Stuart Orbital Incubator (S1500) at 50 °C and 250 rpm for 7 days.

The respective enzyme and water mixtures used for less recalcitrant samples (sugarcane bagasse, rice straw and wheat straw) were 20 μ L enzymes and 3.41 mL water per sample, while for more

recalcitrant samples (rice husk) the amounts used were 50 μ L and 3.38 mL respectively. The total liquid volume added to each saccharification sample was therefore kept constant at 9.90 mL.

Saccharification end point samples were obtained by filtering 1 mL of the saccharification mixture through a PTFE syringe filters. Samples were analyzed on a Shimadzu HPLC system with refractive index (RI) detector and an Aminex HPX-87P column (BioRad, 300 x 7.8 mm) with purified water as mobile phase (0.6 mL/min). The column temperature was 85 °C and acquisition time was 40 min. 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. Glucose yields were reported as a percentage of the total glucan content found in untreated biomass (as determined by compositional analysis). Glucose and xylose yields were calculated as a function of saccharification residence time t according to (Equation 9 and (Equation 10, respectively:

$$\%Glucose\ yield(t) = \frac{c_{HPLC,t} \times V \times 0.9 \times Y_{pulp}}{G_0 \times ODW_{sample}} \times 100$$

(Equation 9)

$$\%Xylose\ yield(t) = \frac{c_{HPLC,t} \times V \times 0.88 \times Y_{pulp}}{G_0 \times ODW_{sample}} \times 100$$

(Equation 10)

where c_{HPLC} 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; ~40–70% for pretreated pulps) G_0 is the glucan content (g/g) in untreated biomass determined by compositional analysis, and ODW_{sample} is the oven-dried weight of the biomass sample before saccharification in mg.

1.3.7 Ash content determination

Ash samples were obtained from all four feedstocks (~0.5 g) by ashing at 575 °C to remove any organic material using a ramping program in a muffle furnace (Nabertherm + controller P 330). The heating program was as follows: heating from room temperature to 105 °C; hold at 105 °C for 12 min to remove moisture; heating to 250 °C at 10 °C/min; hold at 250 °C for 30 min; heating to 575 °C at 10 °C/min; hold at 575 °C for 3 hours; cool to 105 °C. Ash contents were determined in triplicates.

1.3.8 Elemental analysis

CHNS analysis of untreated air-dried biomass (sugarcane bagasse, wheat straw, rice straw and rice husk) 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.

1.3.9 HSQC NMR spectroscopy of lignins

For HSQC analysis of precipitated lignin, ca. 20 mg of lignin was dissolved in 0.25 mL of DMSO- d_6 and the solution transferred to a Shigemi tube. HSQC NMRs were recorded on a Bruker 600 MHz spectrometer (pulse sequence hsqcetgpsi2, spectral width of 10 ppm in F2 (^1H) with 2048 data points and 160 ppm in F1 (^{13}C) with 256 data points, 16 scans and 1 s interscan delay). HSQC experiments were recorded in monoplicate for bagasse EMAL as well as precipitated lignins isolated under the optimized conditions for each of the following four feedstocks: sugarcane bagasse (170 °C, 45 min); rice husk (170 °C, 45 min); rice straw (170 °C, 30 min); wheat straw (170 °C, 30 min). HSQC experiments were conducted in monoplicate. Full spectra may be found in the Appendix.

1.3.10 Error calculations

In this thesis, all experimental errors were presented as one standard deviation calculated from triplicate measurements (or duplicate measurements at a minimum, in the cases of certain techniques or limited samples, as indicated in the methodology). The error bars shown in all Figures and Tables and reported in the text refer to one standard deviation. A sample calculation may be found in the Appendix.

1.4 Results and Discussion

In this study, four of the most abundant (>80 Mtpa) lignocellulosic feedstocks available annually as crop waste were pretreated to produce highly digestible pulps with a view to bioethanol production. The materials investigated were rice straw, rice husk, wheat straw and depithed sugarcane bagasse, all of which were prepared by washing, grinding to a standard size fraction (0.18–0.85 mm) and stored air-dry (5–8 wt% moisture). All pretreatments used the low-cost ionic liquid TEA HSO₄, which can be produced at industrial scale at cost parity to many organic solvents (~\$1.24/kg). Prior to pretreatment, the elemental and chemical composition of the feedstocks was determined. The rice husk pretreatment time course was carried out by Angela Chen (PhD student).

1.4.1 Feedstock characteristics

The chemical composition of the four feedstocks investigated is presented in Table II-4. Clearly, the agroresidues have lignocellulosic compositions, but vary in terms of their polysaccharide, lignin and ash content. Rice husk (27%) and sugarcane bagasse (24%) were observed to have the highest lignin content while wheat straw (22%) and rice straw (18%) were less lignin-rich. These values are even higher than those found in the literature, illustrating the geographical variability in composition. All feedstocks contained between 70–72% holocellulose (hemicellulose and cellulose) except rice husk, which had a lower polysaccharide content of 62% on account of its high ash and high lignin content.

The ash contents of the materials were compared using two different methods: first, an ashing process was conducted by temperature ramping up to 575 °C in a muffle furnace to obtain the total inorganic content; ash content was also determined via the two-step acid hydrolysis and ashing method used for compositional analysis. In both cases, the inorganic species are expected to be present as a mixture of metal oxides and metal salts. Total inorganic content ranged between 1 wt% for bagasse and 13 wt% for rice straw.

Table II-4. Elemental and chemical composition of agroresidues investigated in this study, as determined by CHNS analysis (reported in wt% d.b.) and compositional analysis (wt% d.b.).

	C	H	N	S	O ^a	Extractives (%)	Hemi-cellulose (%)	Cellulose (%)	Lignin (%)	Acid-insoluble Ash (%)	Total Ash at 575 °C (%)
Rice husk	41.2	4.7	0.8	0.5	52.8	0.4	17.8	44.4	26.8	10.6	10.7
Sugarcane bagasse	48.3	5.3	0	0.7	45.7	2.6	29.1	43.5	24.1	0.7	0.7
Wheat straw	48.0	5.2	0.8	<0.1	46.1	4.3	29.4	43.5	21.5	1.4	3.7
Rice straw	41.8	4.9	1.1	<0.1	52.2	4.6	25.8	43.9	18.1	7.6	12.7

^a Calculated by difference (O = 100 – C – H – N – S, wt %).

Ash contents determined by acid hydrolysis were lower (0.7–10.6 wt%) than from ashing in oxygen, as acid hydrolysis measures only water- and acid-insoluble inorganic species whereas ashing measures all inorganic species. The straws showed greater discrepancies between the acid-insoluble and total ash contents, possibly because of higher alkali species content which are acid-soluble⁹. The ash content obtained for all feedstocks was lower than reported in literature (see Table II-1); however, inorganic matter is not only dependent on plant environment but also on time of harvest and feedstock preparation (for instance, washing could have removed debris/pith from the biomass surface hence reducing the ash content detected)⁸². Elemental composition of the biomass materials showed that materials with higher ash contents had correspondingly lower carbon contents, another factor in the low energy densities of rice residues⁴.

1.4.2 Optimization of rice husk pretreatment

As the feedstock with the highest lignin content, pretreatment of rice husk was investigated to enable identification of optimal conditions, with respect to glucose released from wet (non air-dried) pulps. Figure II-3 shows the key pretreatment parameters investigated, namely the glucose yields, lignin precipitate recovered and the calculated delignification, glucan recovery and hemicellulose removal from compositional analysis for pretreated pulps for time courses run at 150 °C and 170 °C. Glucose yields are shown as a % of the glucan content of untreated biomass (theoretical maximum).

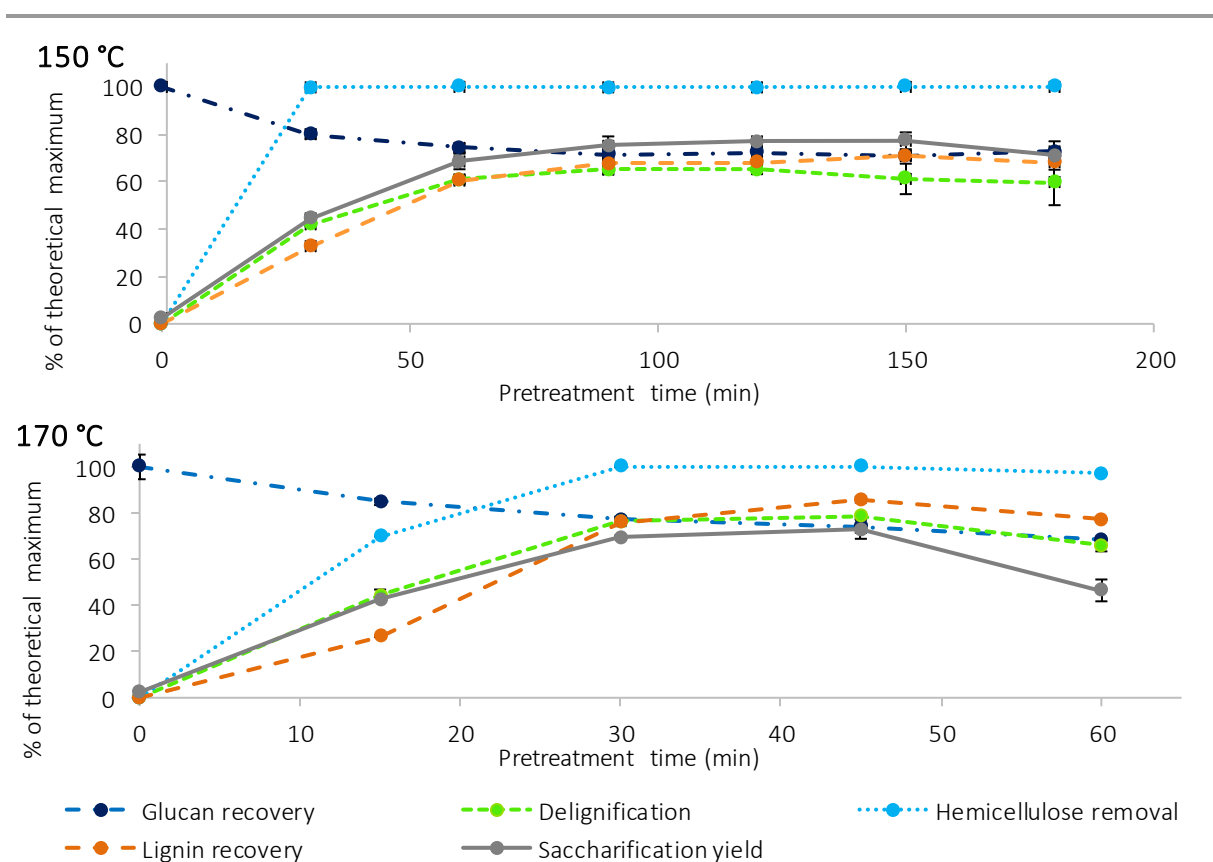


Figure II-3. Saccharification yields for wet pulps, glucan recovery, lignin and hemicellulose removal and lignin precipitate after pretreatment of rice husk at (a) 150 °C and (b) 170 °C with [TEA][HSO₄] at a final water content of 20 wt% with a biomass to solvent ratio of 1:10 g/g. Errors calculated as one standard deviation across triplicates.

The time courses shown mark the changes in pulp composition and lignin extracted during the initial stages of pretreatment until the glucose release was observed to peak. This occurred after 120 min at 150 °C and 45 min at 170 °C, where sugar yields reached 77 and 73%, respectively. Glucan was seen to degrade comparatively quickly even at 150 °C, suggesting this may be one of the limiting factors for glucose release, as discussed below (section 1.4.4). The optimum condition for rice husk pretreatment was selected as 170 °C for 45 min since a shorter pretreatment time at higher temperature is desirable to reduce reactor volumes and hence capital cost. A strong relationship between delignification and saccharification was observed (see Figure S0-1), as reported previously for *Miscanthus*⁸³ and pine⁸⁴. After peak saccharification and delignification, the detected lignin in the pulp started to increase again. This has been attributed to both condensation reactions within lignin fragments producing new high molecular weight structures which are insoluble in the IL, as well as the formation of pseudo-lignin, an insoluble polymer resulting from reactions of carbohydrate degradation products with depolymerized lignin, generating condensation products that are indistinguishable from lignin in the conventional Klason lignin analysis⁸⁵. Formation of the former is strongly suspected under conditions where the lignin recovery exceeds the measured delignification, as reported previously by Gschwend *et al.*^{83,84} Lignin recovery exceeded delignification shortly before peak saccharification; thereafter, the formation of pseudo-lignin exerted a negative effect on saccharification yields and kinetics, making the cellulose substrate less accessible⁸⁶.

170 °C was thus selected as the optimal pretreatment temperature for rice husk to maximize space-time yields, which could potentially translate to lower capital cost due to smaller reactor volumes.

1.4.3 Effect of pulp drying on saccharification yields

In an industrial process, the cellulose pulp is expected to be subjected to enzymatic hydrolysis immediately after its recovery as a wet solid, rather than undergoing an energy-intensive air-drying step. Moreover, considerable evidence exists that air-drying induces hornification, whereby drying or air removal leads to the irreversible shrinkage and collapse of pore structures within the cell wall of lignocellulose pulps, reducing enzyme accessibility to cellulose and enzymatic hydrolysis yields⁸⁷. An investigation was conducted to investigate the effect of air-drying on sugar release from pretreated rice husk pulps. Figure II-4 shows the saccharification yields for air-dried pulps and for wet pulps which did not undergo drying-induced hornification. **Clearly, the omission of an air-drying step can be seen to have a significant positive influence on saccharification yields**, which increased by a factor 1.0–2.1. Very similar findings were obtained by Gschwend⁸⁴ whereby sugar release increased by a factor of 1.6 for pine pretreated at 170 °C for 30 min using the same solids loading (1:10 g/g).

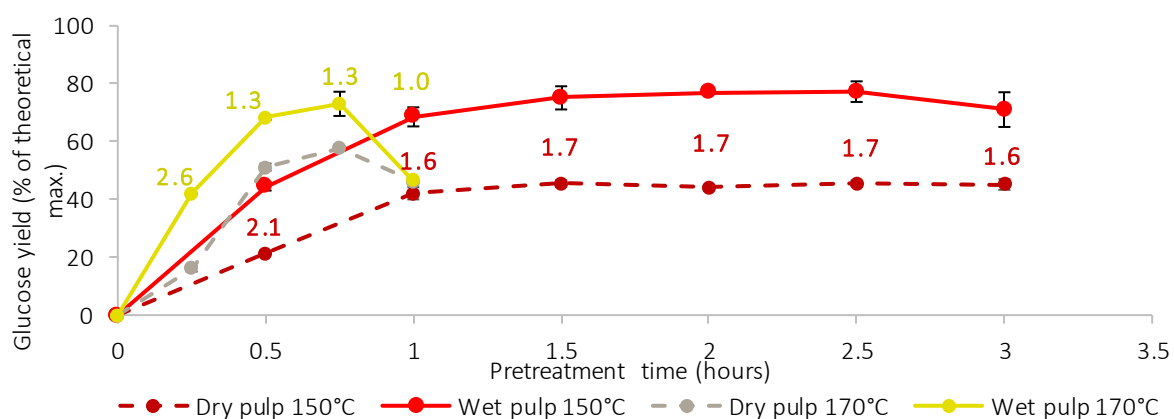


Figure II-4. Comparison of saccharification yields for air-dried (dashed line) and wet (continuous line) pulps for rice husk pretreated under various conditions. Inset values show factor of increase in glucose yield for wet vs dry pulps.

The strongest difference was seen in less pretreated pulps, where sugar yields increased by a factor of two or more. As pretreatment progressed beyond peak saccharification, the effect of hornification seems to have become less significant, especially under the most severe conditions where air-dried and wet pulps gave the same sugar yields (e.g. at 170 °C and 60 min). If hemicelluloses are not present in the fiber wall, the severity of hornification can be increased⁸⁸; however, hemicellulose removal was nearly quantitative in most pulps compared (see Figure II-3). **We therefore attribute the apparent decreasing effect of hornification to other structural changes caused by the process of pretreatment, such as lignin re-precipitation onto the pulp and pseudo-lignin formation, which are known to reduce enzymatic digestibility even in wet pulps^{83,89,90}.** Based on these findings, pulps in the remainder of the study were not air-dried (see Figure S0-3).

1.4.4 Comparison of various agricultural residues

Having successfully demonstrated the use of ionoSolv pretreatment to improve the enzymatic digestibility of rice husk, similar pretreatment conditions were applied to other high-ash and high-lignin feedstocks. Optimal pretreatment conditions for rice straw, rice husk, wheat straw and sugarcane bagasse at 170 °C were identified and the same indicators of pre treatment efficacy were then compared for the four feedstocks. It should be noted that without pretreatment these materials are highly recalcitrant, as seen from the untreated biomass hydrolysis yields (Figure S0-1).

Table II-5. Summary of maximal glucose yields obtained after ionoSolv pretreatment of agroresidues at 170 °C.

Feedstock	Optimal pretreatment condition	Saccharification yield with air drying	Saccharification yield without air drying	Increase in saccharification yield ^a
Rice husk	170 °C, 45 min	58.8±1.5%	73.0±4.3%	30.4
Sugarcane bagasse	170 °C, 45 min	78.4±2.1%	89.4±5.5%	8.1
Wheat straw	170 °C, 30 min	80.5±0.8%	86.5±1.9%	5.6
Rice straw	170 °C, 30 min	68.3±0.5%	88.8±0.2%	3.4

^a Calculated as ratio of wet pulp sugar yield compared to sugar yield for untreated biomass.

At 170 °C, it was found that the optimal sugar release for agroresidues ranged between 30–45 min, with more prolonged treatment (45 min) required for rice husk and bagasse. The latter also had the highest lignin contents in the feedstock (see Table II-4), which can limit enzymatic digestibility⁹¹. More facile deconstruction of straws can also be linked to the less dense, less lignified and more porous structure of their cell walls, giving higher sugar yields even for untreated biomass. Lignin composition may also play a role, for instance the ratio of S/G functionalities in the cell wall, as some studies have reported that S-lignin (which is less prone to condensation) led to more easy removal of lignin with concomitant increases in sugar yields⁹². Literature shows that the S/G ratio in native lignin isolated from bagasse (0.9–1.1)⁹³ and rice husk (0.2–0.6) is, on average, lower than in wheat straw (1.0–1.3) and rice straw (0.6–1.3)^{93–95}. However, the effect of S/G ratio is known to be feedstock dependent and lignin subunit composition also varies significantly depending on genetic and environmental factors for a given agroresidue⁹⁴. **The greater recalcitrance of rice husk and bagasse is consistent with their higher lignin contents, dense cell wall structures and lignin compositions.**

Very promising sugar yields were obtained for all four agricultural residues, exceeding 73% obtained for rice husk, with glucose release of nearly 90% obtained for rice straw (89%), wheat straw (87%) and sugarcane bagasse (89%). These findings illustrate that IonoSolv pretreatment can be used to successfully deconstruct agricultural residues with a high lignin and ash content. Saccharification values were seen to increase by a factor of 3–8 compared to untreated biomass, except for rice husk where it increased 30-fold, a reflection of the highly dense and lignified structure of untreated rice husk which gave close to zero sugar yields. Figure II-5 shows lignin and pulp characteristics under optimized pretreatment conditions. As with other lignocellulosic biomass types, there appeared to be a strong relationship between high sugar yields and lignin removal from the biomass (see also Figure S0-1). Delignification was advanced (~80%) for all feedstocks except rice straw (67%), though this does not appear to have limited its enzymatic hydrolysis as its native lignin content was lowest among the feedstocks examined.

Regarding changes in polysaccharides during pretreatment, it can be seen that prolonged treatments led to nearly quantitative hemicellulose removal accompanied by 10–20% glucan loss, as glucan degrades rapidly at 170 °C. Cellulose degradation, rather than delignification, is strongly suspected to be the limiting factor for glucose yields obtained. This was investigated by comparing the glucose yields as a percentage of glucan in the *pulp* (rather than in untreated biomass), shown in Table S0-1 and Figure S0-2 in the Appendix. This analysis showed that glucan that survived the treatment was quantitatively released, suggesting that glucose yields were limited by glucan availability and that further optimization may be possible by adjustment of reaction times. It is worth noting that only 15 min intervals were investigated in this study, yet reactions progress quickly at 170 °C and the actual heating time is not accurately known.

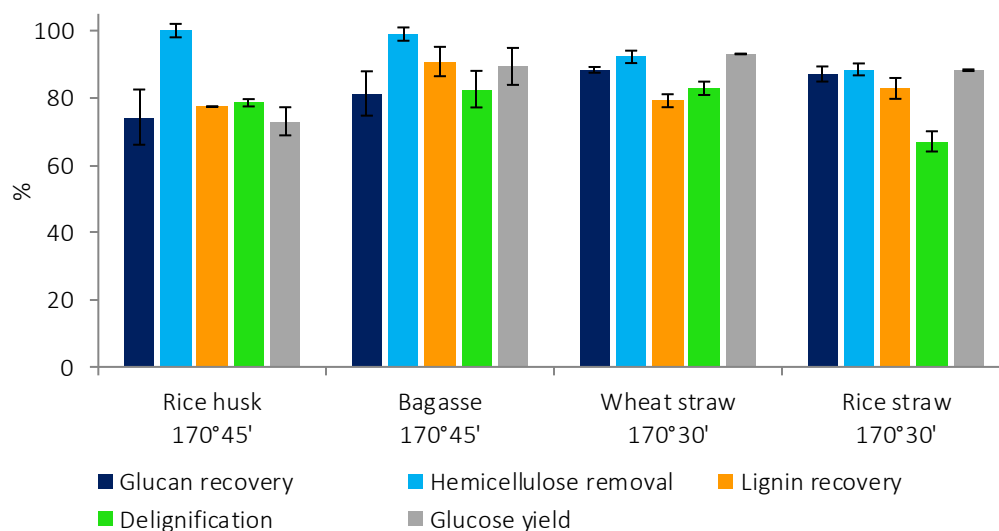


Figure II-5. Key pretreatment indicators for rice husk, sugarcane bagasse, wheat straw and rice straw.

As for the fate of inorganic matter during pretreatment, a comparison of the untreated and pretreated pulps, shown in Figure II-6, reveals that the ash mostly partitioned with the pulp. This was most clearly observed for high-ash rice husk and rice straw where $92 \pm 5\%$ and $84 \pm 8\%$ of ash were recovered in the pulp. It should be noted that chemical analysis reveals only acid-insoluble inorganic matter; most acid-soluble ash components are likely to have dissolved in the acidic IL medium during pretreatment. This inorganic matter, especially silica, could be easily recovered following the enzymatic hydrolysis step in the form of the post-hydrolysis solid. Table S0-1 shows that between 88–100% of the pulp was hydrolyzed for all samples except rice husk (58%) due to its greater proportion of residual lignin. **This suggests that nearly all of the pulp was converted into glucose, indicating the amount of residual post-hydrolysis solids is likely to be desirably low, also enabling facile separation of inorganic matter and simplifying processing.**

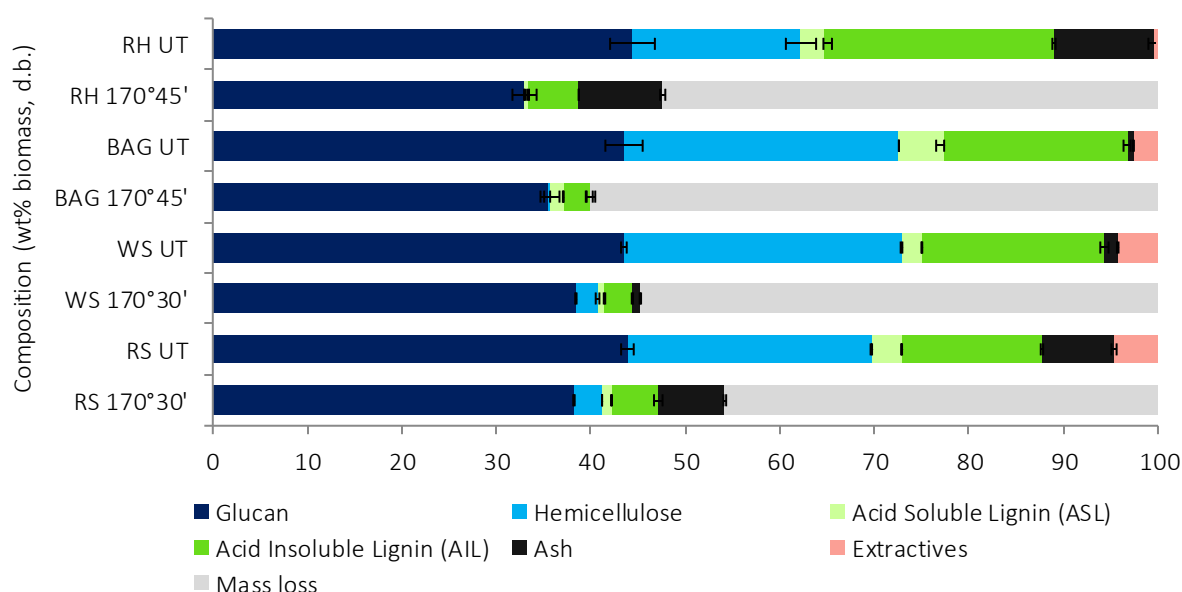
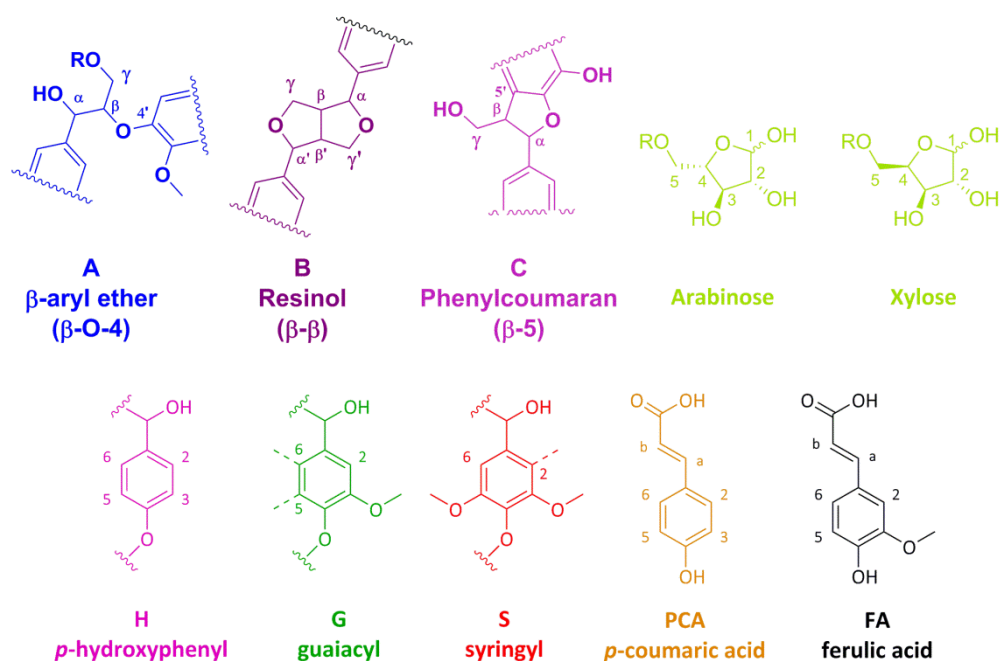


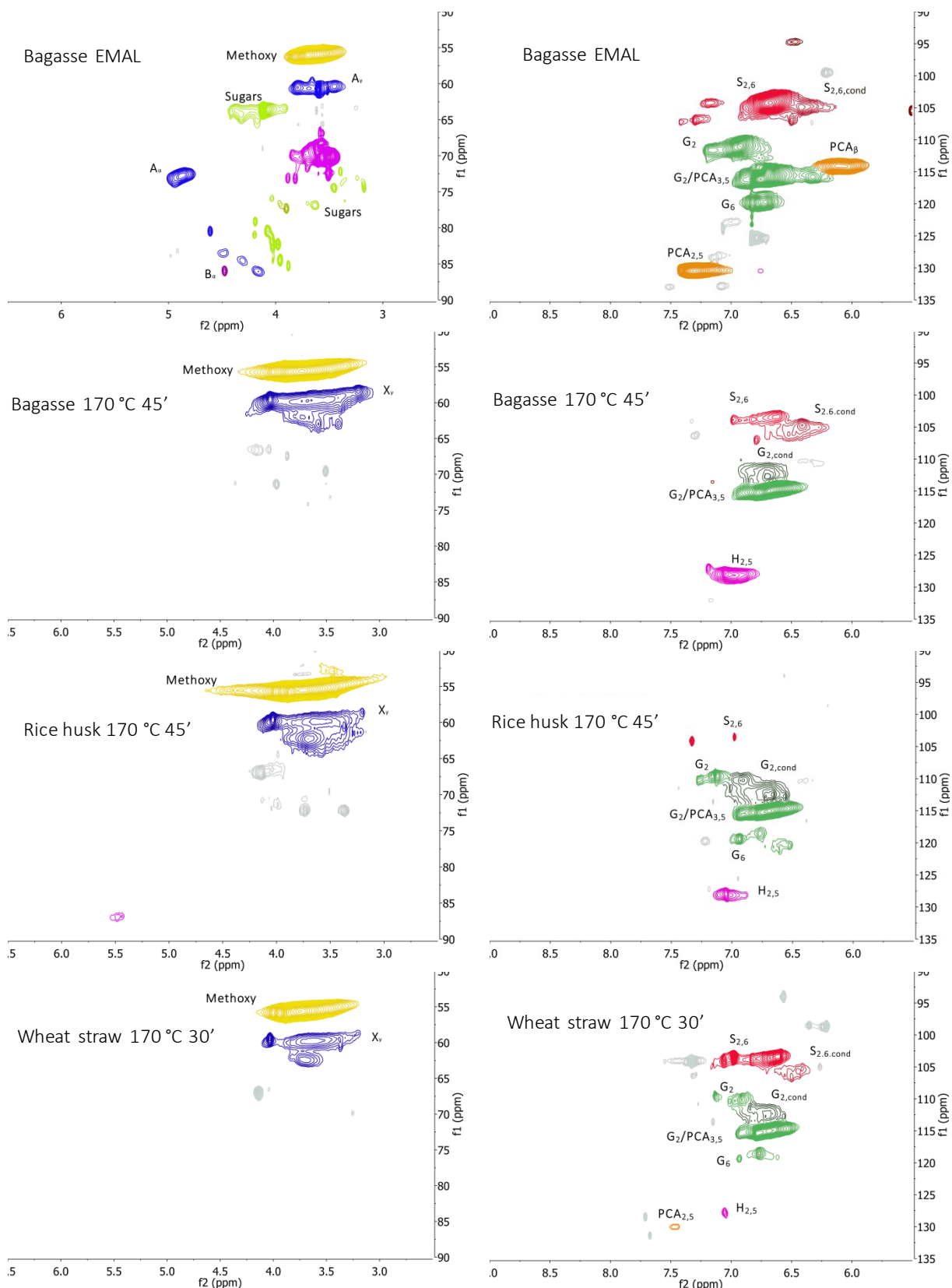
Figure II-6. Chemical composition of untreated and pretreated cellulose-rich pulps isolated from ionic liquid pretreatment of rice husk, sugarcane bagasse, wheat straw and rice straw. UT = untreated.

1.4.5 HSQC NMR spectroscopy of isolated lignins

The previous section showed a strong link between enzymatic glucose release and lignin removal from the pulp. It is known that delignification is achieved through cleavage of phenolic α -O-4' linkages, β -O-4' linkages and C-C linkages in lignin⁹⁶. In order to obtain insights into changes in lignin structure taking place during the pretreatment process, the lignin fractions isolated under the optimized conditions at 170 °C were analyzed using HSQC NMR spectroscopy. Enzymatic mild acidolysis lignin (EMAL), thought to have a structure closely resembling native lignin, was isolated from depithed sugarcane bagasse and used as a reference for untreated bagasse lignin. Figure II-7 shows the spectra for the five lignin fractions analyzed, including both the side chain (δH 6.5 – 2.5; δC 90 – 50) and the aromatic regions (δH 9.0–5.5; δC 135–90).

Observing the side chain region, the most significant difference between bagasse EMAL and the pretreated lignins was the absence of signals corresponding to polysaccharides in the pretreated lignin spectra, reflecting their high purity. Delignification proceeds through cleavage of α -O-4' and β -O-4' linkages, facilitating degradation and dissolution of the lignin into the IL, and these changes can be clearly seen to occur during pretreatment. Signals assigned to the major interunit linkages β -O-4', β -5' and β - β' disappeared after pretreatment, as they were rapidly cleaved or modified in acidic media⁹⁷. In the aromatic region, it was observed that the signals for G₂ and S_{2,6} became less intense with the appearance of condensed S_{2,cond} and G_{2,cond} signals indicating the formation of condensed aromatic units. Resonance of the non-condensed G₂ and S_{2,6} units decreased considerably, showing only mere traces for rice husk lignin.





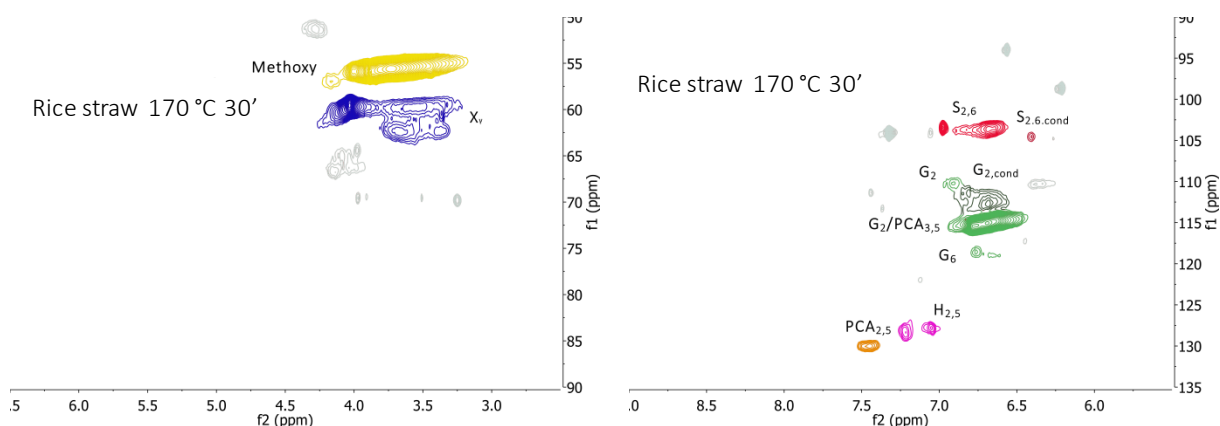


Figure II-7. HSQC NMR spectra of bagasse, rice husk, wheat straw and rice straw lignins after extraction with [TEA][HSO₄] under the optimum conditions for enzymatic hydrolysis with a biomass to solvent ratio of 1:10 g/g and a final water content of 20 wt%; side chain region (left) and aromatic region (right).

A semi-quantitative 2D HSQC NMR technique was used to quantify the changes in the lignin structure. While the pulse sequence used gives only semi-quantitative information, it has been suggested in the literature that the G₂ signal (consisting of the sum of the G₂ and G_{2,cond} signals) can be used as an internal standard in the case of herbaceous biomass. It is thought to be the most stable C–H unit in lignin and therefore representative of the total number of aromatic units in lignin⁹⁸. Signal intensities were compared in Figure II-8. The analysis revealed substantial lignin β-aryl ether cleavage (~90% for bagasse), indicating that nearly all ether linkages were cleaved under the acidic conditions used, before the lignins were released and eventually precipitated from the IL liquor. **Condensation between aromatic rings was observed to be quite extensive**, as evidenced by the decrease of the relative signal intensity of the G₂ and G₆ units and an increase in the G_{2,cond} units, compared to the sum of G₂ and G_{2,cond} signals. Reduced G₆ units are thought to stem from C–C condensation reactions occurring in this position. Additionally, ~87% of PCA units were seen to disappear resulting in ~78% apparent increase in H units, in agreement with previous findings^{97,99}.

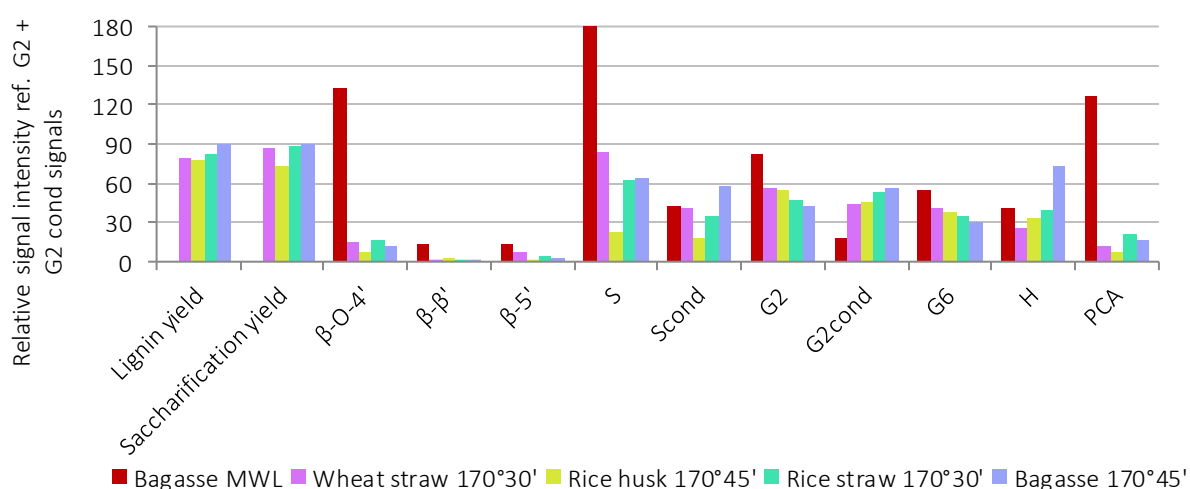


Figure II-8. HSQC NMR signal intensities of different lignin C–H units relative to 100 G₂+G_{2,cond} units, obtained after pretreatment of four feedstocks with [TEA][HSO₄] with 20 wt% water for the optimum pretreatment time for glucose release during enzymatic hydrolysis.

Although the isolated lignins resembled each other with respect to the near-quantitative cleavage of β -aryl ether bonds during the treatment, the extents of condensation did not appear to be mirrored in this. It can be discerned that bagasse ionoSolv lignin appears to be most condensed, evidenced by the lowest $G_2:G_{2cond}$ ratio and lowest G_6 content. Given that bagasse and rice husk were treated under the same conditions (170 °C, 45 min), this suggests that bagasse lignins were more easily extracted from the biomass and underwent more extensive reactions before being precipitated. Interestingly, bagasse also gave the highest lignin recovery (90.8%) which exceeded delignification (82.6%), indicating that reaction time was sufficient to extract lignins from bagasse. These lignins were presumably fully precipitated without a considerable amount of lignin precipitation onto the pulp surface. Another distinctive feature is the much lower S/G ratio observed for rice husk lignin, which could be a factor in its recalcitrance¹⁰⁰. **Overall, the ionoSolv lignins appear to be quite extensively condensed, again suggesting a slight shortening of pretreatment times may be desirable to further optimize glucose yields and lignin characteristics for upgrading.**

1.5 Conclusions and Future Work

In summary, the findings in this chapter show that ionoSolv pretreatment is suitable for the production of highly digestible pulps from a range of agroresidues, including rice husk and rice straw. The low-cost ionic liquid TEA HSO₄ was successfully used to deconstruct four high-ash and high-lignin agroresidues, obtaining sugar yields exceeding 75% and approaching 90% in three cases. Optimum pretreatment conditions were identified (170 °C, 30–45 min) and it was found that more prolonged treatment was needed for feedstocks with more dense, lignified structures. A strong link between residual pulp lignin and saccharification yields was noted. Delignification was seen to proceed *via* extensive cleavage of β -O-4' aryl ether linkages and this was accompanied by condensation reactions in the isolated lignins. The omission of an air-drying step after pulp recovery to avoid drying-induced hornification was seen to have a significant positive influence on saccharification yields. Further optimization of glucose yields and lignin properties may be possible by careful selection of pretreatment time at 170 °C with the introduction of mixing. Though this work has validated the potential use of the ionoSolv process for conversion of abundant agroresidues to bioethanol, further work is required to reduce energy requirements and intensify the process, notably by use of larger particle sizes, higher solids loadings and direct pretreatment of moist raw feedstocks after harvesting to eliminate the need for air-drying of feedstocks.

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Chapter 2: Pretreatment of Sugarcane Bagasse

2.1 Introduction

Sugarcane bagasse (*Saccharum officinarum*) is the fibrous portion of sugarcane from which the juice has been extracted. Bagasse is a major agro-industrial residue produced in most tropical countries as a byproduct of the sugarcane industry¹. It is estimated that over 520 million wet tons (~260 million dry tons) of sugarcane bagasse are produced annually². About 50 wt% of this residue is used in distillery plants as a source of energy³; the remainder is stockpiled⁴. Therefore, due to its low cost, high carbohydrate content and widespread abundance, sugarcane bagasse is an excellent candidate material for bioethanol production¹.

Sugarcane bagasse is an inhomogeneous feedstock with a wide range of particle sizes and varying fiber composition³. Raw bagasse contains ~30 wt% pith, a non-fibrous tissue with higher ash content and poorer fiber morphology⁵, which is problematic for paper manufacture⁶. Pith is separated from the fiber in an industrial depithing operation prior to paper production^{3,7} in an energy-intensive first step used in nearly all bagasse utilization pathways. Commercial depithing is accomplished through dry and wet processes wherein bagasse is mechanically abraded to break the clusters of pith off from the fibrous portion⁸. However, the capital cost and energy requirements of depithing operations are high and depithing can damage fibers, results in loss of material, and creates a waste disposal problem for the large quantities of wet pith produced⁹.

In Chapter 1, ionoSolv pretreatment was successfully demonstrated to produce highly digestible cellulose-rich pulps from various agricultural residues, including sugarcane bagasse. In this section, we

investigated its application for pretreatment of various fractions of whole, industrially depithed and laboratory depithed sugarcane bagasse to investigate the need for depithing.

2.2 Background

2.2.1 Impetus for bagasse valorization

Sugarcane is well-established as a feedstock for 1G bioethanol production from sugarcane juice, leaving behind the fibrous residue known as bagasse¹. Currently, bagasse is used as a fuel for boilers in ethanol distilleries and is also the second most commonly used non-wood fiber material for pulp and paper production¹⁰. However, the pith content in bagasse is problematic for paper pulping, causing foaming, clogging and increased use of chemicals⁸ and reducing the quality of the resulting pulps⁷. Removal of pith is highly expensive and energy-intensive, so that depithing adds appreciably to the cost of bagasse as a raw material^{8,11}. Therefore, it is desirable to find high-volume applications for bagasse that are not adversely affected by the presence of pith in whole bagasse. Expansion of the product portfolio of the sugarcane industry to include bioethanol production from bagasse has the potential to double bioethanol production per hectare without expanding cane fields or threatening food and feed production^{12,13}. Large-scale bagasse processing would also produce lignin, some of which could be valorized, further improving the competitiveness of the sugarcane biorefinery¹⁴.

2.2.2 Depithing

Sugarcane bagasse is an inhomogeneous, low-density biomass containing up to 65 wt% fiber. The pith (30 wt%), made up of low-density cylindrical particles, can be seen in the center of the stem in Figure II-9 while the dense non-fibrous rind makes up the remainder (5 wt%). While fiber and pith have similar chemical composition, they vary in their particle size and aspect ratio. Fiber has high aspect ratio of ~70 while pith particles have aspect ratio of ~5 and are highly hygroscopic and cohesive. The pith fraction can cause significant problems in papermaking and is usually removed by industrial depithing to separate the rind, pith and other fine extraneous material from the exploitable fibrous portion used for paper-making⁷.

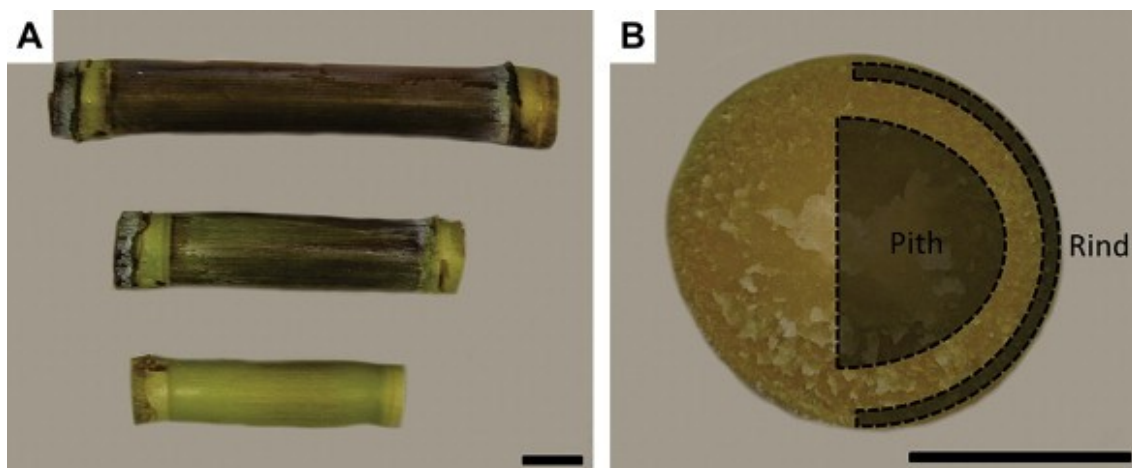


Figure II-9. Longitudinal view of (a) sugarcane stems and (b) cross-section of stems, showing inner pith tissue and outer rind tissue. Reproduced from Cesarino *et al.*¹⁵

In its simplest form, depithing works by mechanical separation on the basis of particle size (dry depithing)³. Since this first method is often insufficient to fully remove the pith, wet methods are applied, whereby pith is separated on the basis of their buoyancy in water⁸. Commercial depithing increases the fiber content of bagasse from 60 to 85% before paper pulping⁵ and has also conventionally been used to reduce the ash content of the material to improve the appearance of paper pulps⁷. Removal of the fine pith particles has also been used to reduce dust formation and spontaneous combustion hazards prior to bagasse stockpiling¹⁶. The resulting effect on bagasse composition was investigated by Andrade and Colodette¹⁷, who separated whole bagasse into depithed bagasse and pith by hammer milling. It was found that pith had higher lignin and ash content and lower sugar content than whole and depithed bagasse. Pith lignin also had slightly higher S/G subunit ratio than depithed bagasse and whole bagasse¹⁷ (1.12, 0.95 and 1.00 respectively), so we might expect it to be less recalcitrant to pretreatment.

Given its high energy intensity and capital cost of depithing equipment^{8,9}, effective use of whole (i.e. non-depithed) material could further improve the economics of sugarcane bagasse biorefining. Few studies have compared whole and depithed bagasse conversion. Sieving bagasse in the laboratory is likely to remove a higher proportion of larger pith than commercial depithing (ca. 40% of total material removed compared with ca. 30% in a commercial depither)¹⁶. Aghcheh *et al.*¹⁸ compared the acid-catalyzed hydrolysis of depithed and whole bagasse on xylose production and subsequent fermentation to xylitol. It was found that depithed bagasse gave a higher hydrolysis rate compared to whole bagasse, which was attributed to its higher hemicellulose content. Depithing also resulted in a higher xylitol yield following fermentation. Strangely, however, the depithed bagasse used had *higher* ash content than the whole bagasse, suggesting these results may not be representative of most depithing processes. Hernández-Salas *et al.*¹⁹ compared the effect of dilute HCl hydrolysis on reducing

sugar yields from depithed bagasse and pith itself. No significant differences were found in the different bagasse materials with respect to their hydrolysis yield under dilute acid hydrolysis. When the hydrolyzates were fermented, no statistically significant difference was detected in the ethanol yields from pith bagasse and depithed bagasse¹⁹.

2.2.3 Pretreatment of bagasse with IL

Cost-effective utilization of bagasse as a feedstock for bioethanol production relies on the development of pretreatment technologies able to handle the high moisture content (50 wt%)³, ash content⁵ and heterogeneous fiber morphology present in raw bagasse. Previous studies of bagasse pretreatment with ILs have all faced at least one of several drawbacks that limit their large-scale application, namely low biomass loadings, the use of expensive ILs, thermally unstable ILs, moisture-intolerant ILs, excessive size reduction and limited xylose yields^{20–24}. Notably among these, Hou *et al.*²² employed the amino acid IL [Ch][Lys] to pretreat bagasse without size reduction, though loading was limited to 5 wt%. Glucose yields of 80% and xylose yields of 84% were obtained by enzymatic hydrolysis. However, this IL is thermally unstable under typical pretreatment conditions and is likely to be very expensive to synthesize, limiting its commercial applications²⁵. Bagasse has also been successfully pretreated using imidazolium APIs; for instance, Da Silva *et al.*²⁴ used [Emim][OAc] at 120 °C for 2 h at 5 wt% solid loading to obtain over 90% sugar release within 24h of hydrolysis following pretreatment. The fact that bagasse pith presents no obstacle to IL pretreatment was demonstrated by Wang *et al.*²⁰, who employed aqueous [Bmim]Cl solution containing 30 wt% water and 1 wt% HCl to pretreat pith (0.18–0.42 mm). After pretreatment at 120 °C for 30 min and 10 wt% loading, the digested pulp gave a reducing sugar yield of 95% after 72 h of hydrolysis. However, such studies suffer from the general drawbacks of the Dissolution process, namely the use of expensive and thermally unstable ILs²⁶, limited ability to recover lignin^{20,22} and reduced performance in the presence of water²⁷. In contrast, the IonoSolv process uses IL-water mixtures and is therefore highly moisture-tolerant²⁸. Aqueous TEA HSO₄ is therefore an ideal candidate solvent for pretreatment of wet and ash-heavy sugarcane bagasse at higher solids loadings. Here it was of particular research interest to investigate the elimination of the depithing step and to compare the pretreatment of whole bagasse to industrially depithed and laboratory-depithed bagasse.

2.2.4 Summary

- Industrial depithing of sugarcane bagasse, which removes ~75% of low-density, fine pith particles¹¹, is undesirable due to its high capital and energy costs.
- Sieving bagasse in the laboratory is likely to remove a higher proportion of larger pith than commercial depithing.
- Few studies have compared the pretreatment effectiveness of whole and depithed bagasse.

- The use of thermally stable, low-cost PILs at moderate loadings for deconstruction of various bagasse fractions is of research interest.

2.3 Experimental

2.3.1 Materials and method

All chemicals used were reagent grade from Sigma-Aldrich and used as received, unless otherwise noted. Whole sugarcane bagasse (*Saccharum officinarum*) (WB) was sourced from a local pulp and paper mill in the Kwazulu-Natal province of South Africa. Depithed bagasse (DB) was obtained from the same mill, where the biomass had been subjected to an industrial depithing process, a two-stage mechanical process that removes pith, short fibers and undesirable particulates (e.g. sand) to leave behind industrially depithed bagasse. In a second set of experiments, WB was ground and subjected to a laboratory fractionation process by sieving²⁹ (Figure II-10) to obtain pith bagasse (PB), short fiber bagasse (SFB) and long fiber bagasse (LFB). All feedstocks were washed to remove adhering inorganic debris and air-dried at room temperature prior to shipping to the laboratory. They were ground and sieved (0.18–0.85 mm, US mesh scale -20/+80) and stored air dry in sealed plastic bags at ambient temperature. Moisture content of untreated material was determined following NREL/TP-510-42618³⁰. The moisture contents of untreated bagasse were 6.8% (WB), 5.2% (DB), 8.2% (PB), 6.6% (SFB) and 6.7% (LFB), respectively.

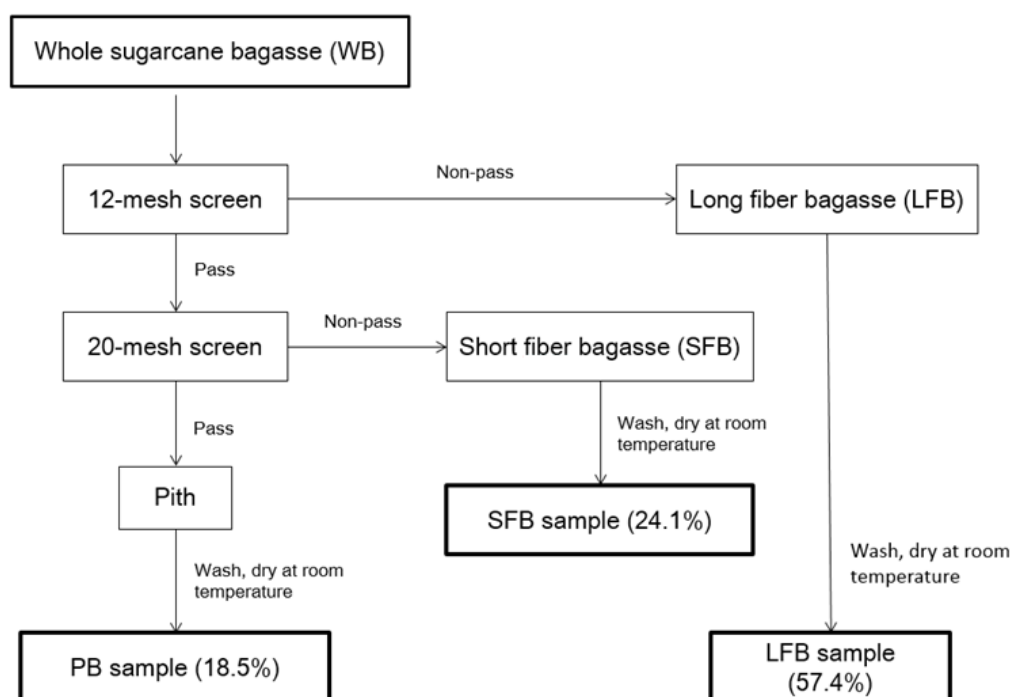


Figure II-10. Schematic representation of laboratory fractionation process used by Chinsamy *et al.* (2004)²⁹.

2.3.2 Ionic liquid synthesis

All pretreatment experiments in this study used a solution of 80 wt% [TEA][HSO₄] in DI water as pretreatment solvent. Experiments conducted at the 10 mL-scale used [TEA][HSO₄] that was manually synthesized in batches of ~150 g following the method described in section 1.3.2.

2.3.3 Biomass fractionation at bench scale

ionoSolv pretreatment was carried out using a standard operating procedure of our laboratory³¹ as previously described in section 1.3.3. A biomass to solvent ratio of 1:10 g/g was used (equivalent to 10 wt% loading) in all experiments. Pretreatment experiments were carried out in triplicates at 120 °C for a residence time ranging between 1 and 24 hours. Four ethanol washes were used followed by a Soxhlet extraction step; the pulps were then recovered by air-drying.

2.3.4 Compositional analysis

Untreated sugarcane bagasse feedstocks were Soxhlet extracted for 24 hours with ethanol and air-dried for 48 h prior to compositional analysis. Compositional analysis of untreated extractives-free biomass was carried out following the NREL protocol 'Determination of Structural Carbohydrates and Lignin in Biomass', NREL/TP-510-42618³⁰ as described in section 1.3.5. Results of all biomass compositional analyses are reported on a dry weight basis. Compositional analysis was conducted in triplicate on untreated extracted biomass feedstocks and Klason analysis was conducted in triplicate on pretreated air-dried pulps. Delignification, glucan recovery and hemicellulose recovery were calculated using (Equation 7), (Equation 8 and (Equation 9, respectively.

2.3.5 Saccharification assay

Saccharification assays were carried out on air-dried untreated and pretreated bagasse samples as described in section 1.3.6. All bagasse samples were saccharified with 20 µL of Novozymes NS22201 experimental enzyme mixture (Novozymes, Denmark) per sample. The hydrolysis time was 7 days. Glucose and xylose yields were calculated based on the glucan and xylan content of untreated biomass as determined by compositional analysis.

2.3.6 Particle size distribution measurements

Particle size distributions of untreated, air-dried long fiber bagasse, short fiber bagasse and pith bagasse were determined for untreated and pretreated pulps using nested sieves. The sample particle size distribution was obtained using a vibratory shaker (Retsch model SM 200, Hann, Germany) equipped with stacked sieves with decreasing pore sizes (850, 710, 500, 300, 212 and 180 µm) by shaking on a vibratory sieve shaker for 20 minutes. The percentage weight of material retained by each sieve was measured, and from this data the log-normal distribution mass median diameter (D_{50}) was calculated, i.e. the particle size that 50% of the sample is smaller than by mass when a log-normal

distribution is fitted to the measurements. It is used herein as a measure of the average particle size of a biomass sample and was calculated by linear interpolation according to (Equation 11:

$$D_{50} = 10^{\left((\log x_2 - \log x_1) \times \left(\frac{50 - y_1}{y_2 - y_1} \right) + \log x_1 \right)}$$

(Equation 11)

where x_1 and x_2 are the pore sizes of the sieves (in mm) which allowed just under and just over 50% of the sample to pass through by weight, respectively, and y_1 and y_2 are the percentages of material passing through the sieves.

Particle size analyses were conducted in triplicate and percentage errors were assigned according to previous analysis for *Miscanthus* pulp particles, which found an average of 1% error for untreated biomass samples. Particle size distributions were plotted as weight percentages of samples passing through different sieve pore sizes (on a log scale) and geometric mean particle sizes D_{50} were compared.

2.3.7 Size exclusion chromatography

The 1-methyl-2-pyrrolidinone (NMP) eluent was obtained from Rathburn Chemicals Ltd (peptide synthesis-grade, UV absorbance cutoff 265 nm). SEC experiments were performed using lignins isolated from depithed bagasse and long fiber bagasse (120 °C, 4 h of pretreatment) using a Mixed-D column (5 µm particle size, 300 mm x 7.5 mm I.D.; Polymer Laboratories, UK) packed with polystyrene/polydivinylbenzene beads. The Mixed-D column was operated with 0.5 mL min⁻¹ NMP as eluent at 80 °C with a Knauer M100 isocratic HPLC pump. A Perkin-Elmer LC 290 variable wavelength UV-absorbance detector was used and routinely set to 270 and 300 nm. The system was calibrated using linear polystyrene standards with M_p values of 580, 970, 2,960, 19,880, 185,400, 523,000 and 5,000,000. Three linear PS standards across with M_p values of 970, 2960 and 19,880 were eluted to verify the calibration prior to each set of SEC experiments. The calibration curve used was $y = 0.4038x + 10.632$, $R^2 = 0.9746$, where $y = \log[\text{molecular weight (Da)}]$ and $t = \text{elution time (min)}$, valid between $t = 11\text{--}19$ min. SEC experiments were performed in triplicate. Polystyrene does not represent the geometry of the highly branched lignin macromolecule, so the molecular weights reported bear the risk of a systemic error; however, the relative trends in molecular weight distribution observed are valid. The ratio between the excluded ($t = 0\text{--}15$ min) and retained ($t = 15\text{--}30$) peak areas, i.e. $A_{E/R}$, was calculated by numerical integration of the UV-absorbance signal versus time in Microsoft Excel.

2.3.8 Wavelength-dispersive X-ray Fluorescence (WD-XRF) analysis of ash

Ash (inorganic material) samples (~1g) were obtained from whole bagasse, depithed bagasse and pith bagasse by heating to 575 °C to remove any organic material in a programmable muffle furnace (Nabertherm Controller P330 LT 5/13). The heating program was as follows: 25 → 105 °C with a heating rate of 20 °C min⁻¹, plateau of 30 min at 105 °C, 105 → 350 °C with a heating rate of 3 °C min⁻¹, plateau of 30 min at 350 °C, 350 → 575 °C with a heating rate of 3 °C min⁻¹, plateau of 1 h at 575 °C, and annealing to room temperature. The ash obtained was a pink/orange powder and its composition was analyzed by quantitative WD-XRF. Sample preparation was by fused bead using an automated FluXana Vulcan system (Fluxana GmbH, Germany) using a standard 10:1 ratio of flux:material. This was carried out by ITRI Innovation Ltd (UK). The fused bead was analyzed using a PANalytical AxiosmAX minerals wavelength dispersive X-ray fluorescence spectrometer (PANalytical, Netherlands) equipped with a 60 kV generator and a 4 kW Rh SST-mAX X-ray tube. Analysis was carried out using Omnia software package for semi-quantitative XRF.

2.3.9 Scanning electron microscopy (SEM)

The morphologies of untreated and pretreated long fiber bagasse and pith bagasse samples were visually observed using scanning electron microscopy (JEOL JSM-5610 LV, JEOL Ltd, Japan). The pulp samples were mounted on a 1 cm² metal sample holder using a carbon black sticker. The sample was then gold-coated in an argon atmosphere using a sputter coating inside an EmiTech K550 (Emitech Ltd, Chelmsford, UK). Representative images of pretreated sugarcane bagasse were acquired with a 20 kV accelerating voltage at magnifications ranging from 50× to 500×.

2.4 Results and Discussion

The aim of the present study was to compare the pretreatment of whole and industrially depithed South African sugarcane bagasse with bagasse separated into three fractions in the laboratory. The low-cost IL TEA HSO₄ in 20 wt% water was used to pretreat five bagasse fractions. This study used washed and dried bagasse to be as close to an industrially relevant scenario as possible, as storage of dried residues for processing later in the season is common in the sugarcane industry. A comparison of industrial and laboratory depithing processes on bagasse pretreatment is assessed *via* Klason analysis, enzymatic saccharification and SEM of the pulps as well as lignin analysis by SEC.

Whole bagasse (WB), industrially depithed bagasse (DB) and laboratory-depithed fractions referred to as long fiber bagasse (LFB), short fiber bagasse (SFB) and pith bagasse (PB) were analyzed by compositional analysis. Compositions are shown in Figure II-11 and descriptions given in Table II-6.

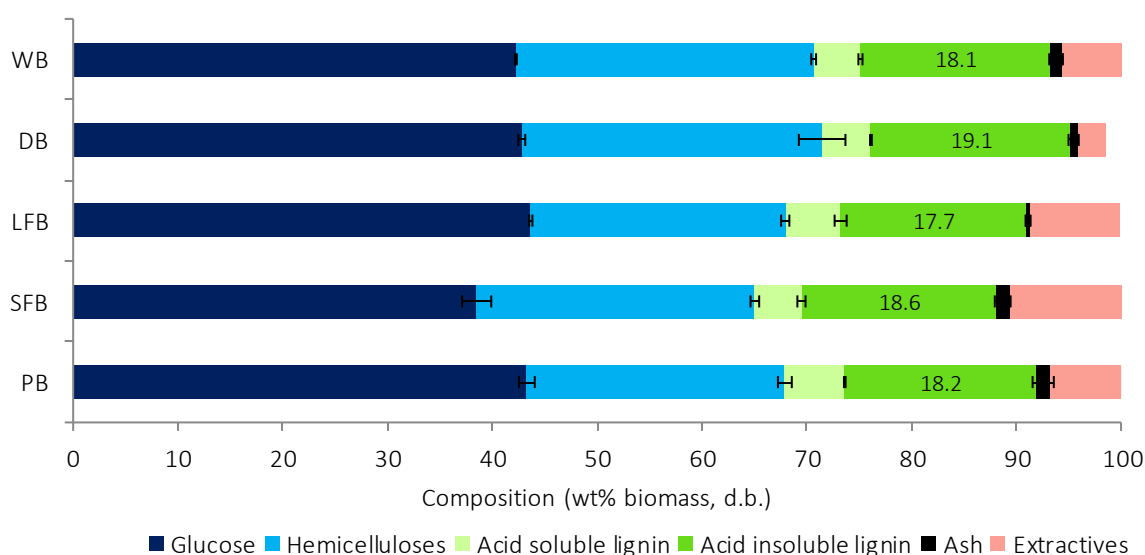


Figure II-11. Composition of untreated bagasse fractions reported as a percentage of unextracted biomass.

Table II-6. Lignocellulosic materials from South African sugarcane bagasse used in this study.

Whole bagasse (WB): Mixture of short fibers from the vascular bundle of sugarcane stalk ('pith') as well as longer fibers from the cortex of sugarcane stalk 'rind'.

Depithed bagasse (DB): High density, fibrous material left over after removal of pith particles by industrial depithing.

Short fiber bagasse (SFB): Short fibers from the cortex of sugarcane stalk 'rind' with moderate length/width ratios; obtained from laboratory depithing process.

Long fiber bagasse (LFB): Longer fibers from the cortex of sugarcane stalk 'rind' with high length/width ratios; obtained from laboratory depithing process.

Pith bagasse (PB): Small, spongy spherical particles from the vascular bundle of sugarcane stalk. Low density, porous material with a higher ash content; obtained from laboratory depithing process.

The five bagasse fractions did not show substantial differences in their chemical composition. All had total lignin contents of 22.6–24.0% (of which ~80% was AIL) and holocellulose (cellulose +

hemicellulose) contents between 65.1–71.5%. Industrial depithing is known to remove only ~75% of pith from whole bagasse¹¹, so the composition of depithed bagasse was not drastically different. We note that laboratory depithed materials did not originate from the same whole bagasse sample, so the extractives contents did not correspond. In spite of the high energy intensity of depithing, the ash content was not significantly reduced after depithing. Pith ($1.4\pm0.0\%$) had the highest ash content, though not significantly higher than whole bagasse ($1.2\pm0.3\%$). However, it is telling that ash content appeared to increase with decreasing size fraction for laboratory depithed bagasse (0.3, 1.2 and 1.4% for LFB, SFB and PB, respectively); this finding is in agreement with literature reports that pith particles contain more ash than fibrous bagasse¹⁷. However, the ash content in the five bagasse fractions analyzed was low compared to unwashed bagasse analyzed by others, who reported ash contents up to 5–6 wt% for whole bagasse⁵ and 10% for pith¹⁷. It is likely that inorganic material (e.g. sand and soil particles) adhering to the bagasse was removed during feedstock washing, reducing the pith and ash (i.e. acid-insoluble inorganics) content detected.

2.4.1 Whole vs depithed bagasse

Pretreatment of whole and industrially depithed bagasse was examined in detail. A pretreatment time course was conducted at 120 °C with residence times ranging from 1 to 24 h. Figure II-12 shows the mass balance of pulp, lignin and components dissolved in IL compared to untreated bagasse. Pulp yields decreased smoothly as treatment progressed, indicating progressive dissolution of lignin and hemicellulose into the IL. The recovery of lignin precipitate also increased with treatment time, except at 24 h where both the lignin yield decreased and the Klason lignin in the pulp increased. The coincidence of these phenomena suggests the formation of highly condensed lignins that became insoluble in the IL liquor or the IL-ethanol wash and instead precipitated onto the pulp surface. This agrees with previous *Miscanthus* studies at high residence time, temperature, acidity or loading^{28,32}.

Key indicators of pretreatment are shown in Figure II-13, noting that delignification was calculated from measurement of acid-insoluble lignin only (it was assumed that acid-soluble lignin had fully dissolved in the acidic IL) so may be slightly overestimated. For whole bagasse, delignification progressed rapidly, reaching 75% after only 4 h of treatment, coinciding with maximum glucose yield ($69\pm1\%$). Depithed material showed very similar trends, except that the pulp was most delignified (81%) after 8 h of treatment where saccharification yields also peaked ($65\pm1\%$). Maximal delignification values obtained for bagasse using TEA HSO₄ solution at 10 wt% loading compared closely to the values obtained for *Miscanthus* under identical pretreatment conditions, where 85% delignification was achieved²⁸; quantitative lignin removal is rarely observed²⁸.

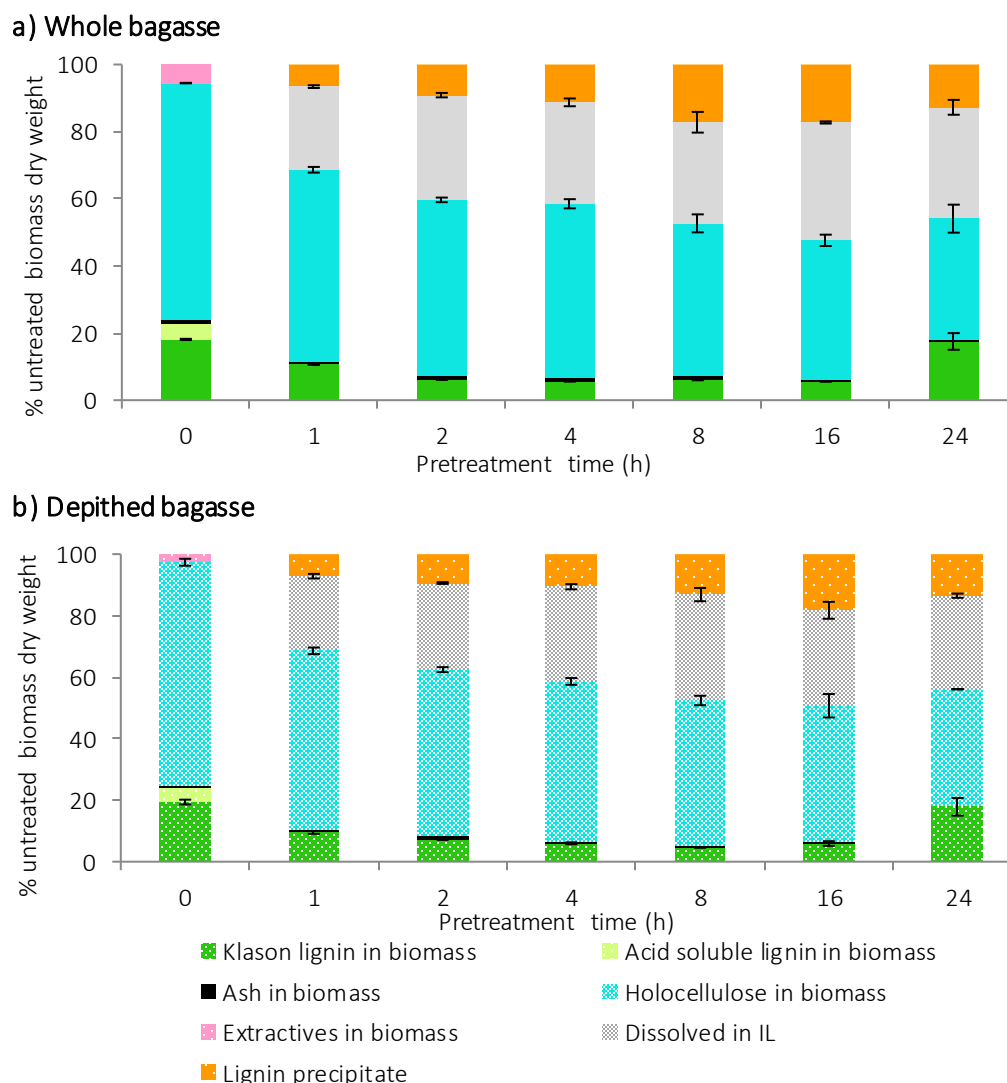


Figure II-12. Mass balance of untreated bagasse and recovered pulp, lignin precipitate and components dissolved in IL as a function of pretreatment time at 120 °C, shown for whole (above) and depithed (below) sugarcane bagasse.

Lignin recovery increased with time and was found to exceed delignification after 16 h, suggesting the formation of pseudo-lignin or re-precipitation of lignin at longer treatment times, as previously observed for *Miscanthus*, rice husk, willow and pine during ionic liquid processing^{28,32–34}. Which of the two is responsible for the apparent decrease in delignification is unclear, as water-insoluble sugar degradation products (pseudo-lignins) and condensed lignins adhering to the pulp are both detected as acid-insoluble lignin by the gravimetric Klason test²⁸. Large errors at 24 h may be attributed to substrate heterogeneity due to local variation in lignin redeposition onto cellulose fibrils (see 2.4.2).

Comparison of the glucose and xylose yields from whole and depithed bagasse (Figure II-14) revealed that depithing did not have a significant effect on improving sugar yields. Under optimal pretreatment conditions (120 °C, 4 h), glucose yields reached 69% and 65% of the theoretical maximum glucan content in untreated biomass, for whole and depithed bagasse respectively. This corresponds to a 5.3- and 8.1-fold increase in glucose yields compared with untreated biomass.

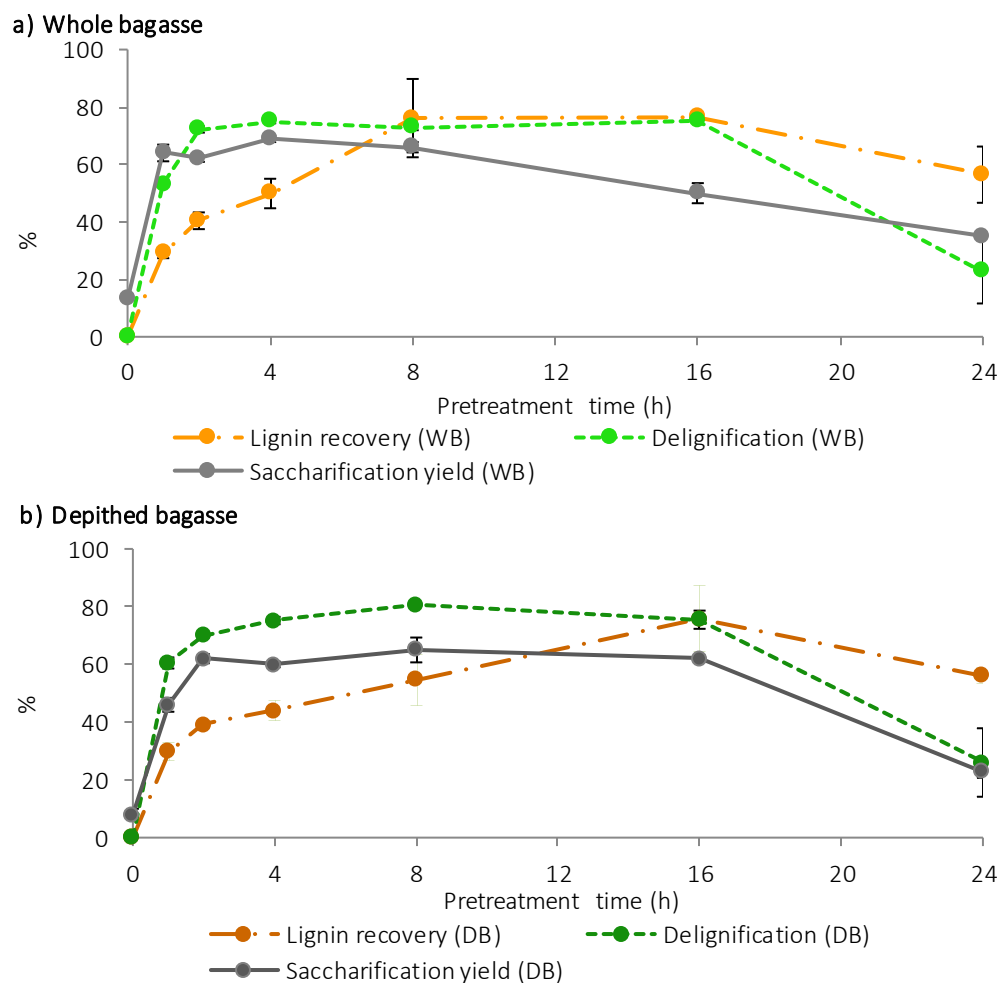


Figure II-13. Key pretreatment indicators for whole (above) and depithed (below) sugarcane bagasse pretreatment at 120 °C using [TEA][HSO₄] with a final water content of 20 wt% and solids loading of 10 wt%. NB. Delignification is based on Klason lignin (i.e. ALL) removal from the pulp only so may be overestimated.

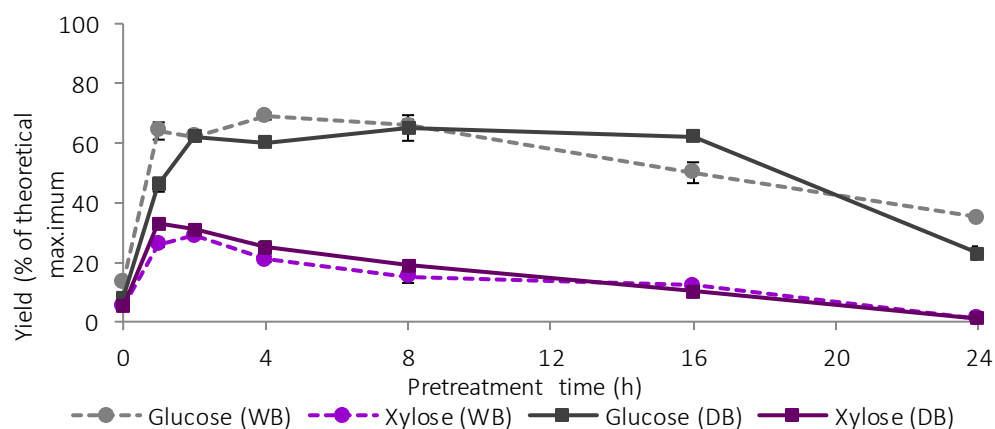


Figure II-14. Comparison of glucose and xylose yields after 7-day enzymatic saccharification for whole (WB) and depithed (DB) sugarcane bagasse pulps following ionoSolv pretreatment at 120 °C.

These results agree with observations of Hernández-Salas *et al.*¹⁹ who saccharified depithed and pith bagasse after acid hydrolysis and fermented the hydrolyzates; they obtained very similar reducing sugar yields and ethanol yields. Given their close to identical chemical compositions and that the

spread in particle sizes was reduced by grinding and sieving to 0.18–0.85 mm before pretreatment, it is unsurprising that whole and depithed bagasse gave similar pretreatment outcomes.

Xylose yields were maximized (~30%) following only 1–2 h of pretreatment for both materials. The fact that glucose and xylose yields are optimized non-simultaneously has also been observed for ionoSolv *Miscanthus* pretreatment at 120 °C²⁸. The enzymatic hydrolysis yields fall short of the 87% glucose and 64% xylose release achieved using the expensive and thermally unstable APIL [Emim][OAc] after 0.5 h of treatment at 120 °C under bone-dry conditions³⁵. We note that the enzymatic hydrolysis yields reported here were for air-dried pulps, whereas in Chapter 1: it was seen that saccharifying wet pulps to avoid hornification could significantly improve glucose yields. **The glucose yields obtained using inexpensive [TEA][HSO₄]-water mixtures were ~75% as high as for [Emim][OAc]; this result is highly promising given that this IL is 40 times less expensive^{27,36}.** Incomplete digestion of the pulp is unlikely to be a waste disposal problem in the sugarcane industry, as the post-hydrolysis solids could be dried and burned to help meet the energy requirements of the mill or ethanol plant.

2.4.2 Laboratory-depithed fractions

Three fractions were obtained by sieving to decreasing size fractions, yielding long fiber bagasse (>1.7 mm), short fiber bagasse (0.85–1.7 mm) and pith bagasse (<0.85 mm); these fractions were then further ground and sieved to 0.18–0.85 mm size fractions. To verify the possibility of interfering particle size effects, the particle size distributions (PSD) of the fractions were obtained by sieving (see Figure II-15). After size reduction, long fiber and pith bagasse had very similar PSD and geometric average particle size D_{50} of ~0.4 mm. Short fiber bagasse appeared to have larger particles on average, with D_{50} of 0.55 mm, suggesting it was someone less extensively ground. The surface area to volume ratio is hence likely to differ for the fractions, though it cannot be derived from this data as the aspect ratios are unknown; both are likely to impact the pretreatment³⁷.

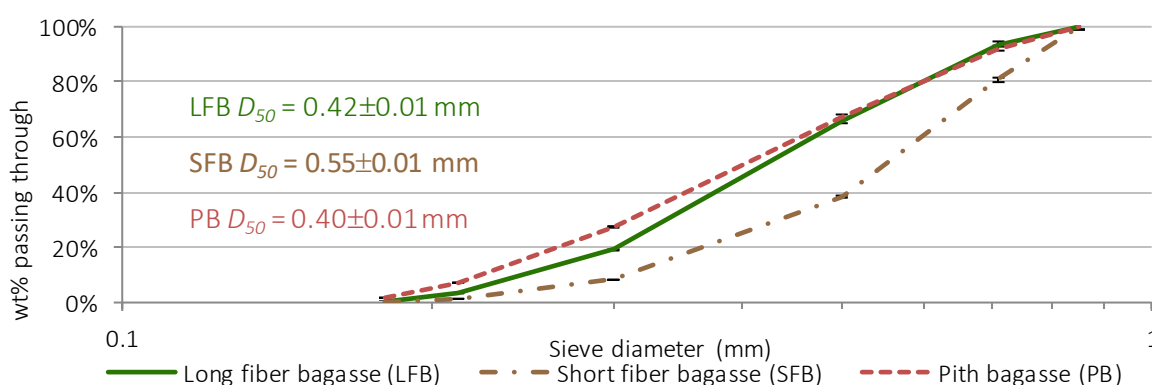


Figure II-15. Particle size distribution of untreated pith, long fiber and short fiber bagasse obtained by sieving within the pretreatment size fraction (0.18–0.85 mm). Inset values show calculated geometric mean particle length D_{50} .

Pretreatment of three bagasse fractions obtained from laboratory depithing process was compared to results from whole and depithed bagasse at the optimum condition (120 °C, 4 h) identified in Section 2.4.1. The mass balance of pulp, lignin and components dissolved in IL compared to untreated biomass for each of the five fractions is shown in Figure II-16. Very similar behavior was observed, whereby between 69–85% of Klason lignin and a larger proportion of hemicellulose were removed from the pulp. Greater dissolution into the IL was seen for laboratory depithed fractions, which also produced more lignin precipitate, suggesting lignin extraction was more extensive.

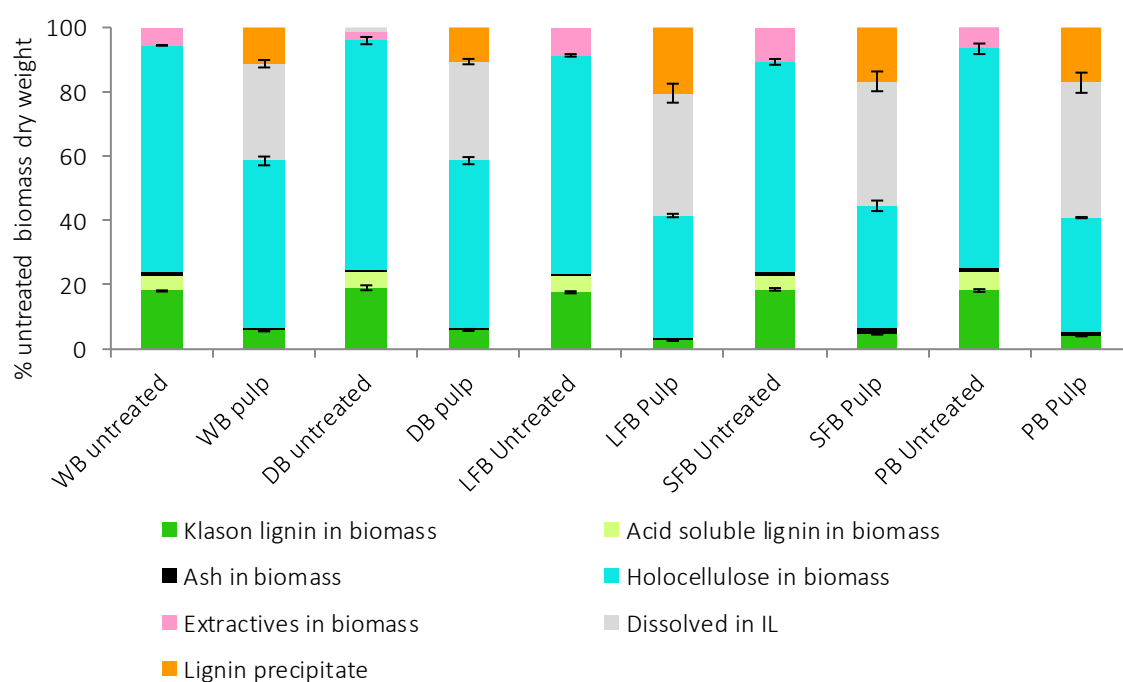


Figure II-16. Mass balance of untreated bagasse and recovered pulp, lignin precipitate and components dissolved in IL after pretreatment at 120 °C for 4 h, shown for whole, industrially depithed and laboratory-depithed fractions.

Figure II-17 shows the pulp and lignin yield, delignification, glucose and xylose yield for all five fractions. Lignin recoveries were 72–89% of total lignin in untreated bagasse, markedly higher than those for whole and industrially depithed bagasse (45–50%). Given the similar compositions of all untreated biomass types (Figure II-11), the difference in pulp yields is attributable to differences in lignin extraction and hemicellulose solubilization, possibly as a result of different lignin characteristics in laboratory depithed bagasse fractions or to slight excess in acidity of the IL mixture used. The data show that high lignin recovery was also accompanied by higher delignification, which approached 90% for laboratory depithed materials. This suggests that a large proportion of lignin extracted into the liquor was recovered as a precipitate. It is noteworthy that long fiber bagasse was most delignified and gave the highest lignin yield among the laboratory depithed fractions; this may be explained by its lower surface area to volume ratio, reducing lignin re-deposition onto the pulp surface, or by its lignins being less soluble in anti-solvent than short fiber and pith bagasse lignins.

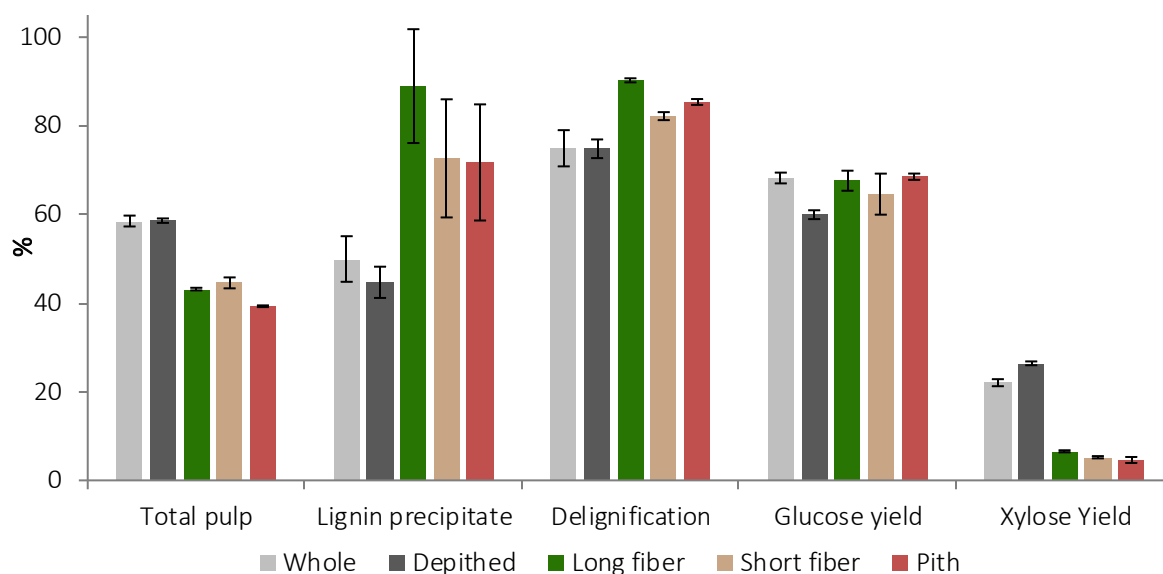


Figure II-17. Pretreatment outcome for whole, depithed and laboratory-depithed fractions of sugarcane bagasse following pretreatment at 120 °C for 4 h, reported as in previous figures. NB. Delignification reported as a % of acid-insoluble lignin remaining in pulp compared to total (acid-soluble + acid-soluble) lignin in untreated biomass.

The results of the enzymatic saccharification assay reveal that glucose yields were very similar for the five fractions, ranging between 60–69%. No enhancement in glucose yield was seen for long fiber bagasse though it was more delignified. Instead, the glucose yields reflect the similar glucan and lignin contents of the starting materials (Figure II-11). Xylose yields were limited to 5% for laboratory depithed materials, which was significantly lower than the yields of 22% and 26% obtained for whole and depithed bagasse, respectively, after 4 h of treatment. It is likely that hemicellulose extraction from laboratory depithed fractions was more rapid during pretreatment, leaving lower hemicellulose sugars available for enzymatic hydrolysis. This is supported by lower pulp yields for laboratory depithed fractions. Another explanation may be lower surface area to volume ratios of these fractions; the possible role of particle size distribution in enhancing hemicellulose removal following laboratory depithing remains to be further investigated. Improved hemicellulose removal may also have facilitated lignin extraction from these materials, though curiously this was not accompanied by higher glucose yield. It is suspected that slight excess acidity in the IL may have accelerated hemicellulose and lignin removal from the pulp for laboratory-depithed fractions; however, this would have a negative impact on glucan recovery in the pulp (not analyzed in this study), limiting sugar yields.

Given the similar chemical compositions of all fractions (Figure II-11), the higher lignin extraction (89%) obtained for long fiber bagasse (see Figure II-17) is likely to be due to differences in its lignin structure. Size exclusion chromatography of the lignins precipitated from depithed bagasse and long fiber bagasse was performed in order to compare their molecular size and tendency to precipitate. Figure II-18 shows the SEC chromatograms, which have a bimodal distribution typical for biomass-derived

compounds in this system, with an excluded peak (short elution times) and retained peak (long elution times) corresponding to large and small molecular mass material. The ratios of the excluded peak area to the retained peak area (A_E/A_R) indicate a larger proportion of high molecular mass material in long fiber bagasse lignin compared to depithed bagasse lignin. The lower solubility of high molecular weight lignin fragments extracted from long fiber bagasse is thus proposed as the major reason for its high lignin precipitate recovery.

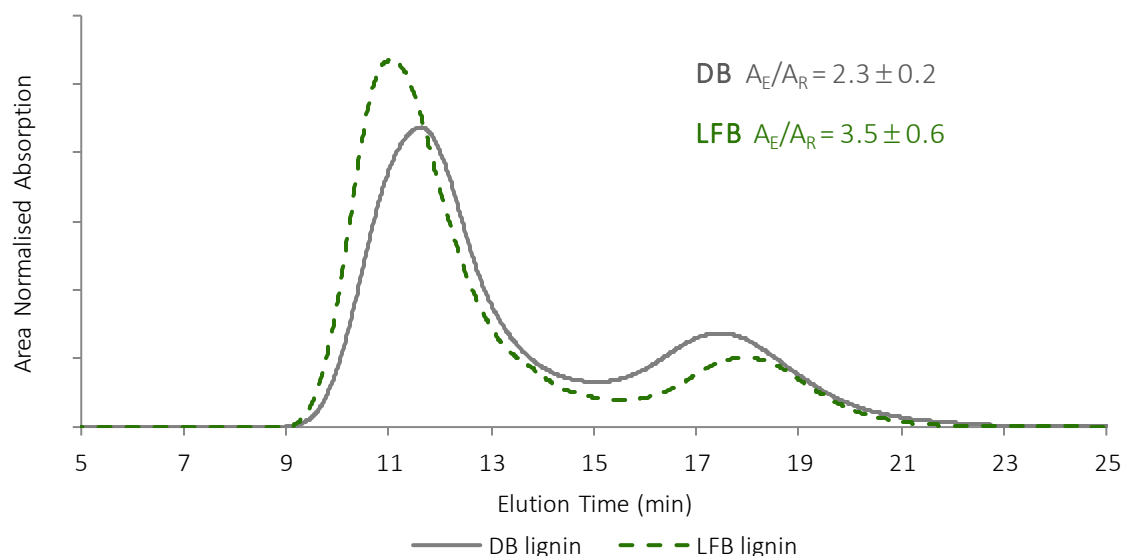


Figure II-18. Area normalized size exclusion chromatograph of ionoSolv lignins isolated from industrially depithed (DB) and long fiber bagasse (LFB) pretreated in $[\text{TEA}][\text{HSO}_4]$ containing 20wt % water at 120 °C for 4 h with 10 wt% solids loading. Mixed-D column, NMP eluent, 300 nm.

To further investigate the hypothesis of lignin deposition onto bagasse, the microstructural features of untreated and pretreated long fiber and pith bagasse were visually observed using scanning electron microscopy (SEM) (see Figure II-19). Untreated pith has an anisotropic, porous structure consisting of $\sim 100 \mu\text{m}$ cavities, known as the lumen, with low aspect ratio and rough surface. This is in agreement with literature observations of spongy pith particles mixed with less abundant cellulose fibers³⁸. After pretreatment, disruption of the lignocellulosic structure occurs, resulting in a collapsed lumen and a lot of debris. In contrast, untreated long fiber bagasse is made up of cellulose fibers of $\sim 12 \mu\text{m}$ width bound by natural polymers including lignin and pectin³⁷. The fibers have a much higher aspect ratio than pith bagasse. Pretreated long fiber bagasse pulp appears to have slightly increased fiber width of ca. $14 \mu\text{m}$, an effect which may be attributed to disruption of hydrogen bonding between the fibers. Both the pith and long fiber bagasse pulps appeared smoother after pretreatment, which could be evidence for lignin deposition on the pulp surface. The deposition of lignin droplets onto the surface of pretreated biomass during *pretreatment* has been reported previously by Selig *et al.*³⁹; however, in this case, droplets were not observed even at magnifications up to 10,000 $\times$. Instead, the surface appeared smooth, suggesting lignin became insoluble in the IL/ethanol mixture and

deposited as an even film during (ethanol) *washing*. This surface layer would retard enzymatic hydrolysis. **The particle size distribution and aspect ratio are suspected to be major factors in bagasse saccharification owing to the greater likelihood of lignin precipitation onto the high surface area to volume particles such as pith bagasse.**

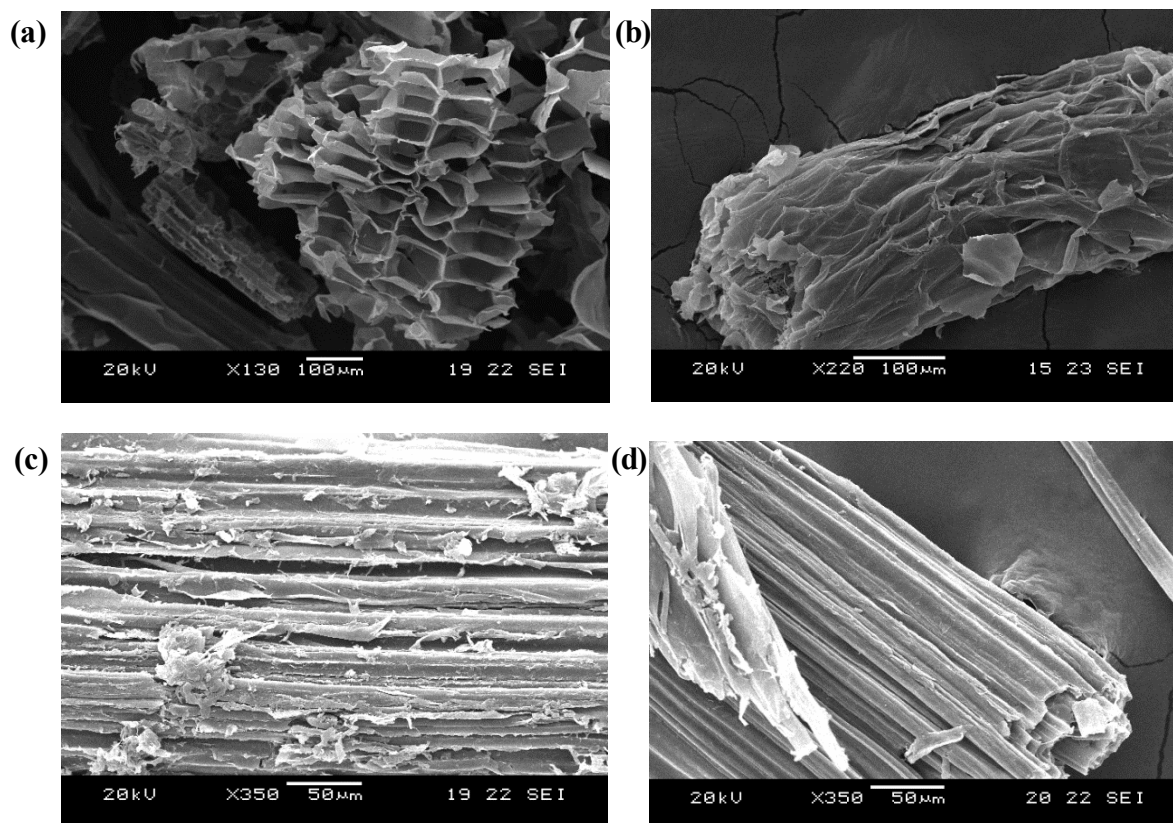


Figure II-19. Scanning electron micrographs of longitudinal view of (a) pith bagasse before pretreatment, (b) pith bagasse after pretreatment, (c) long fiber bagasse before pretreatment and (d) long fiber bagasse after pretreatment. Treatment was conducted in [TEA][HSO₄] containing 20 wt% water at 120 °C and 4h at 10% loading.

Mass balances are crucial when performing techno-economic analyses and carbon tracking. They are also important for holistic utilization of the biomass, as all by-products should be utilized so as to minimize waste generation if the process is to be sustainable. An overall mass balance of the ionoSolv process applied to long fiber bagasse is presented in Figure II-20. This shows that, for every mass unit of bagasse input in the process, we obtain 43% as pulp, 20% as lignin and the remaining 37% remains dissolved in the IL solution. The pulp was then saccharified, whereby ~70% of sugar polymers were released as free sugars (equivalent to ~30% of untreated biomass dry weight saccharified), leaving behind the post-hydrolysis solids that contained roughly 25 wt% lignin. It is anticipated that the post-hydrolysis solids would be dried and burned to contribute to the energy requirements of the sugarcane mill or ethanol plant, the implications of which are discussed further in section 2.4.3.

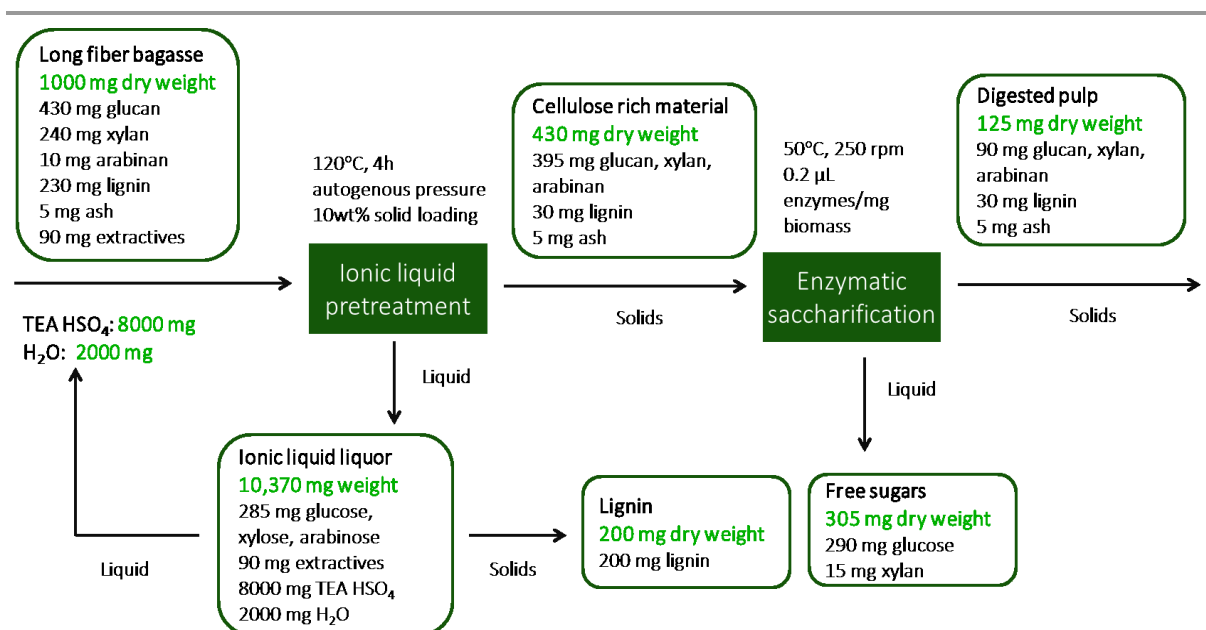


Figure II-20. Mass balance during ionic liquid pretreatment of long fiber bagasse at 120 °C for 4 h in [TEA][HSO₄] and subsequent enzymatic saccharification, assuming 100% ionic liquid recovery.

2.4.3 Ash analysis

At present, about 50% of the bagasse produced in the sugarcane industry is burned, producing significant quantities of fly ash³. It was found that the vast majority of ash partitioned with the pulp, in agreement with findings of Brandt *et al.*²⁸ **The post-hydrolysis solids would therefore be expected to contain most of the inorganic content of bagasse; this solid residue is also a likely fuel source in the paper, sugar or ethanol mill.** The chemical composition of the ash is of interest for bagasse combustion and pyrolysis^{40–42}. Long fiber bagasse, depithed bagasse and pith bagasse were ashed in a muffle furnace at 575 °C to obtain ash which is free of trapped or unburnt carbon. The elemental composition of ash was obtained by wavelength-dispersive X-ray fluorescence (WD-XRF) and is presented in Table II-7. Silica was found to be the main component, accounting for roughly half of depithed and long fiber bagasse ash and over 70% of pith ash by weight. Other major constituents were aluminium, calcium, iron and magnesium oxides; similar findings were reported in the literature⁴³. The high silica component of bagasse pith is well known in the industry and is among the reasons for pith removal from bagasse prior to paper pulping¹⁷. However, depithing does not necessarily improve combustion performance, other than that depithed bagasse retains less water¹⁶.

Table II-7. Composition of depithed bagasse ash, long fiber bagasse ash and pith ash as determined by WD-XRF.

Compound (wt%)	Depithed bagasse (DB) ash	Long fiber bagasse (LFB) ash	Pith (PB) ash	Trace elements (ppm)	Depithed bagasse (DB) ash	Long fiber bagasse (LFB) ash	Pith (PB) ash
SiO ₂	50.8	54.6	70.1	Cu	N/A	136	<60
Al ₂ O ₃	3.6	0.5	1.1	Cr	N/A	185	96
Fe ₂ O ₃	6.6	0.7	1.4	Hf	N/A	<95	<95
Mn ₃ O ₄	0.2	0.3	0.1	Ni	N/A	<60	<60
SO ₃	1.3	2.2	1.8	Pb	N/A	<90	<90
CaO	8.9	11.2	13.1	V	N/A	<60	62
MgO	4.5	10.2	5.2	Zr	N/A	<90	<90
ZnO	0.07	0.08	0.06				
Na ₂ O	7.3	4.4	5.0				
K ₂ O	1.6	9.6	1.00				
P ₂ O ₅	2.9	6.0	1.7				
TiO ₂	0.3	0.09	0.2				
SrO	0.05	0.05	0.05				
BaO	<0.01	0.08	0.09				
LOI ^a	12	N/A	N/A				

^a Loss on ignition.

2.5 Conclusions and Future Work

Depithing is usually considered an essential step prior to bagasse valorization to separate undesirable pith from the exploitable fibrous portion of bagasse, reducing the silica content for paper/pulp-making and the formation of dust during stockpiling. This study compared industrially depithed (obtained from a commercial mechanical process) and laboratory depithed fractions (obtained from an automated mechanical pith-fiber separator at ambient temperature) to investigate the impact of depithing on ionoSolv pretreatment. In spite of the high energy intensity of depithing, the ash content of whole and depithed material was not significantly reduced after depithing. Removal of adhering sand, soil and pith from whole bagasse prior during washing to shipping is suspected to have reduced the ash content relative to unwashed bagasse. Pretreatment in [TEA][HSO₄]-water solutions effectively fractionated whole, depithed, fibrous and pith bagasse into a cellulose-rich pulp and a lignin precipitate. Laboratory depithing produced high purity cellulose pulps with 90% of lignin removed giving 69% glucose release under optimal conditions (120 °C, 4 h). The glucose released was ca. 75% of that obtained for the expensive aprotic IL [Emim][OAc] by others; this result is highly promising given that [TEA][HSO₄] is 40 times less expensive. The results suggest low-cost acidic ionic liquid solutions are promising candidate solvents for large scale processing of raw sugarcane bagasse.

Depithing did not appear to have a significant effect on the chemical composition of bagasse, although depithed materials had varying particle size and aspect ratio. The starkest differences in the pretreatment outcomes of industrially and laboratory depithed material lay in the pulp yield, xylose yields and lignin recoveries. Much higher lignin recoveries (up to 90%) were obtained for laboratory sieved fractions than for whole and depithed bagasse (limited to 50%). However, glucose yields were not very similar, presenting strong evidence that depithing seems to be an unnecessary step prior to bioethanol production from bagasse. The impact of depithing on fermentation remains to be investigated, as depithing may produce different carbohydrate degradation products or impurities that would affect ethanol yields, and the safety implications of storing non-depithed bagasse would need to be taken into account.

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Chapter 3: Process Intensification

3.1 Introduction

Chemical pretreatment is an essential step in the conversion of lignocellulosic materials to biofuels, but is generally found to be the second most expensive conversion step, representing ~20% of conversion costs^{1,2}. Modern biotechnology has enabled significant advances to reduce the costs of bioethanol production from lignocellulosics (notably in enzyme production³, engineering of plant lignin content and composition⁴ and advances in fermentation and hydrolysis technologies⁵). However, there is a need for considerable cost reduction of pretreatment operating and capital costs within the lignocellulosic ethanol biorefinery^{1,6}. A recent techno-economic study of an IL-based biorefining process identified three key focus areas for more cost-effective pretreatment¹: reducing IL cost, reducing IL loading and increasing IL recycling. Use of low-cost PILs with high thermal stability and recyclability has been demonstrated by our research group^{7,8}. This chapter addresses key remaining challenges to enable cost reduction *via* process intensification of IL pretreatment:

- Reducing the IL loading per unit of biomass (i.e. increasing solids loading) during IL pretreatment to reduce the need for reoccurring chemical inputs and minimize reactor size
- Reducing the need for grinding of biomass to small particle sizes (< 1 mm) by investigating the pretreatment efficacy of larger particle sizes (up to 10 mm)
- Improving the pulp washing and recovery protocol to increase saccharification yields
- Optimizing biomass stirring to improve heat and mass transfer while reducing power demands for pretreatment

Pretreatment of *Miscanthus × giganteus* was chosen as the basis for these investigations because of extensive experience with its processing and the high glucose yields obtained in the laboratory^{7,9}.

3.2 Background

3.2.1 Biomass (i.e. solids) loading

Increasing biomass loading relative to IL was found to be among the most critical process parameters to reduce the cost of IL pretreatment^{1,10}. Klein-Marcuschamer *et al.*¹ showed that increasing solids loading could bring several simultaneous advantages, notably lower capital cost, lower electricity consumption and lower working capital required. An increase in the biomass loading from 10 wt% to 50 wt% decreased the equipment cost by ~50% while an increase in the biomass loading from 20 to 50 wt% had the effect of reducing the minimum ethanol selling price (MESP) by over 70%. However, higher biomass concentrations (> 20 wt%) introduce heat and mass transfer limitations, which can be overcome by mixing¹¹. Use of high solids loadings also has implications for the density and viscosity of the slurry, whereby increased power consumption is required to overcome the high yield stresses at these concentrations^{1,12}. The rheological properties of IL-biomass slurries with varying amounts of solids have been studied by Cruz *et al.*¹³, using the lignin- and cellulose-dissolving APIL [Emim][OAc]. At solids loadings >20 wt%, shear thinning behavior and a reduction in viscosity reduced the complex viscosity to levels comparable to low loadings (10 wt%)¹³. However, the rheological properties of ionoSolv slurries (which are only lignin-dissolving) are expected to differ due to the lower proportion of biomass that dissolves in IL and higher amount of insoluble solids present, but have not been studied to date.

A number of recent studies have shown successful application of IL pretreatment at high solids loadings (>20 wt%)^{14,15}. Wu *et al.*¹⁴ pretreated corn stover using [Emim][OAc] at biomass loadings of 5–50 wt%. They found that glucose yields remained roughly constant at ~80% between 5–33 wt%; a further increase in solids loading to 50 wt% decreased glucose yields to ~50%. At loadings ≥ 23 wt%, the IL was only able to wet the surface of the biomass during pretreatment and hence ceased to act as a true solvent¹⁴. This had the effect of reducing lignin extracted from the pulp; however, the IL was nevertheless able to disrupt cellulose crystallinity and so glucose yields were largely unaffected. These results do not apply to ionoSolv pretreatment where *lignin removal* has been found to be the most important factor in increasing cellulose digestibility, while cellulose crystallinity is more or less unchanged^{7,8}. More recently, Cruz *et al.*¹³ demonstrated that high solids loadings up to 50 wt% during switchgrass pretreatment using [Emim][OAc] maintained similar initial saccharification kinetics to experiments at 10 wt% loading. However, the final enzymatic digestibility decreased from quantitative glucose release to ~40% yield upon increasing loading from 10 to 50 wt%. It is worthy to note that dissolution of lignin but not cellulose during ionoSolv treatment introduces the possibility of lignin re-depositing onto the cellulose surface, known to negatively impact saccharification^{16–18}.

3.2.2 Biomass size reduction

Biomass size reduction, broadly referred to as comminution, is a highly energy-intensive operation that is routinely conducted as a first step in many bioconversion processes^{19–21}. Comminution takes biomass from its as-received condition (i.e. baled, logged or coarsely shredded) and produces biomass particles with a smaller size suitable for further transport and processing²². However, size reduction adds practically no value to the raw materials and adds considerably to the total process cost of bioethanol production²⁰. Zhu and Pan²³ estimated that biomass size reduction accounts for up to 60% of the total energy inputs during chemical pretreatment, representing 30% of the thermal energy of ethanol from biomass. This energy intensiveness translates into a higher process cost for bioethanol production, estimated by Hallett as ~10% of the MESP²⁴. Therefore, it is crucial to minimize energy consumption of size reduction to make biorefining more cost-competitive²⁵.

The development of pretreatment and hydrolysis technologies able to convert larger biomass particle sizes is highly desirable to reduce process costs and has been investigated using leading pretreatment technologies^{26–28}. For instance, a major advantage of steam explosion processes is that they are effective on feedstock with large particle sizes, which can reduce the energy required for size reduction¹⁹. Grous *et al.*²⁹ demonstrated that poplar chips (20×10×10 mm) treated by steam explosion could be 90% hydrolyzed in 24 h, as compared with only 15% hydrolysis for untreated chips. Similarly, size reduction of incoming biomass is not needed for liquid hot water pretreatment, as the lignocellulose particles break apart when cooked in water³⁰. In-situ thermochemical size reduction and fiberization also take place during Organosolv processes, reducing the need for size reduction²³. Another pretreatment technology being considered for commercialization is dilute acid pretreatment (DA)³¹. Theoretical critical particle sizes for DA based on diffusion and reaction kinetics constants were found to be in the range of 1–3 mm diameter³². This appeared in agreement with experimental data suggesting maximal particle sizes of 3 mm, beyond which enzymatic digestibility decreased²⁶. Grinding to these sizes would introduce a significant energy penalty for woody materials³³. Hence, the high energy consumption of comminution appears to be a clincher for the economic viability of DA processes, especially for hardwood and softwood conversion^{23,26}. Vidal²⁶ reviewed the maximal feedstock particle size to obtain acceptable enzymatic hydrolysis for widely studied pretreatment processes. They found that dilute acid and alkaline (AFEX, lime and dilute alkali) treatments had small maximal particle sizes (<3 mm), while only liquid hot water (<15 mm) and steam explosion (<50 mm) offered greater flexibility for pretreating larger particle sizes²⁶.

Ionic liquid pretreatment is among the more novel chemical pretreatment technologies that can handle larger feedstock particle sizes^{15,28,34–37}. Several studies have examined the ability of cellulose-dissolving ILs to pretreat biomass of varying particle sizes, as summarized in Table II-8.

Table II-8. Effect of particle size reduction on the enzymatic digestibility of various lignocellulosic materials following IL pretreatment.

Feedstock	IL processing conditions	Particle size range ^a	Glucose yield (% of glucan in untreated biomass)	Reference
Sugarcane bagasse pith	[Ch][Lys]; 5 wt% loading; stirring	5×3×2 mm 30×25×8 mm	82 82	34
Corn stover	[Ch][Lys]; 50 wt% loading; stirred	<2 mm 12.5–75 mm	98 83	15
Wheat straw	[Ch][Tau] ; 5 wt% loading; stirring	<0.15 mm 0.9–2 mm	73 66	35
Cotton stalk	[Emim][OAc] and [Emim][Cl] ; 5 wt% loading; stirring	<0.15 mm 1–2 mm	57 [OAc] ; 49 [Cl] 71 [OAc] ; 33 [Cl]	28
Rice straw	[Emim][OAc]; 5 wt% loading; unstirred	<2 mm 2–5 mm >10 mm	80 78 60	36
Pine, eucalyptus, corn stover and switchgrass	[Emim][OAc] ; 10 wt% loading; stirring	Flour : 25 / 5 mm (grasses / wood) Pelletized : 30 ×6 mm	92 91	37
Corn stover	[Emim][OAc]; 50 wt% loading; stirred	<2 mm 12.5–75 mm	98 83	15
Sugarcane bagasse	[Emim][OAc]	<0.25 mm	-	38
Pine	[Emim][OAc]	<0.125 mm	-	

^a Particle size fractions are indicated either as “opening” (for screen size retaining the fraction) or “average” (for average particle size); in other cases the reported range is used.

Among the cellulose-dissolving ILs (dominated by studies using [Emim][OAc]), increasing particle size above 5 mm was seen to cause glucose yields to decrease for rice straw without the use of stirring³⁶. Bahcegul *et al.*²⁸ noted that the optimum particle size for cotton stalk depended on the IL used. Whereas [Emim]Cl pretreatment efficacy decreased for particle sizes >0.5 mm, treatment with [Emim][OAc] was *least* effective for particle sizes <0.15 mm²⁸. Given rapid dissolution rates in [Emim][OAc], accelerated glucan losses were observed leading to a reduction in glucose yields for smaller particle size fractions, as observed by several researchers^{15,28,36}. Encouraging results were obtained by Papa *et al.*¹⁵ whereby >80% glucose yields were achieved using [Emim][OAc] at 50% solids loading using very coarsely chopped (>12.5 mm) corn stover. A fascinating study by Shi *et al.*³⁷

investigated the pretreatment of densified 6 mm pellets compared to flour of mixed herbaceous and woody biomass feedstocks. Sugar yields from both the mixed flour and pelletized feedstocks reached 90% within 24 h of saccharification³⁷. These findings illustrate the ability of cellulose-dissolving ILs to successfully maintain high enzymatic digestibilities using particle sizes <5 mm; mass transfer limitations were encountered using large particles due to slower penetration and dissolution in IL of biomass with larger particle sizes^{38,39}.

Among lignin-dissolving ILs, a range of cholinium-amino acid based ILs have been tested on herbaceous feedstocks^{15,34,35}. Hou *et al.*³⁴ demonstrated the use of a [Ch][Lys]-water mixture for pretreatment of sugarcane bagasse pith, using maximum particle size dimensions ranging from 5–30 mm. This study was notable for its use of moisture-tolerant and recyclable ILs that function by lignin removal rather than by decreasing cellulose crystallinity, similarly to IonoSolv pretreatment^{40,41}. Delignification was found to decrease from ~45% to ~35% upon increasing particle size from 5 to 30 mm, though the resulting pulps maintained high glucose digestibilities ~80%³⁴. However, limitations included use of low biomass solids loading (5 wt%) and limited thermal stability and high synthesis cost of amino-acid based ILs⁴². Papa *et al.*¹⁵ found similar success using [Ch][Lys] to pretreat corn stover using high solids loadings (50 wt%) and large particle size (>12.5 mm), similar to results obtained for [Emim][OAc] pretreatment. On the whole, it seems that lignin-dissolving ILs had high delignification capacity even at conditions that avoided energy-intensive size reduction (particle sizes <10 mm) and high solids loadings^{15,34}. Papa *et al.*¹⁵ noted that [Ch][Lys] showed greater biomass solubilization and higher delignification compared to pretreatment performed by [Emim][OAc], regardless of particle size. This suggests that due to lower mass transfer limitations imposed by dissolution of lignin but not cellulose during pretreatment, lignin-dissolving ILs may have greater potential for use of larger particle sizes at high biomass loadings.

It is worthy to note that studies examining the effect of particle size on pretreatment efficacy have largely focused on agricultural residues, which have low density and low lignin content and are less energy-intensive to grind than woody biomass^{26,43}. In a study by Li *et al.*³⁸, it was shown that sugarcane bagasse (<0.25 mm particle size) dissolved 3× faster than the same amount of pine, even compared with smaller particle sizes of the latter (<0.125 mm). For agroresidues, Cadoche and López proposed that energy inputs for comminution could be kept below 30 kWh ton⁻¹ of lignocellulosic biomass, providing final particle size is held in the range 3–6 mm²¹. For hardwoods and particularly softwoods, energy requirements for size reduction are up to an order of magnitude larger²³. Among the various strategies proposed, addition of IL as lubricants during grinding^{24,44} and post-chemical pretreatment size reduction^{43,45,46} have been applied to reduce energy inputs of size reduction.

3.2.3 Process scale-up

The IonoSolv process offers several advantages over conventional IL processing techniques, notably in its use of low-cost, thermally stable, moisture-tolerant and recyclable ionic liquids for fractionation of a wide variety of biomass feedstocks, making it well-placed for economic viability at industrial scale⁴¹. Having extensively demonstrated process robustness and flexibility at the bench scale within our research group^{9,41,47}, it is now time to turn our attention to scale-up of IonoSolv processing. In parallel to the process intensification work undertaken in this study, researchers within the Hallett/Fennell groups have recently constructed robust scale-up equipment for reproducible production of high-purity PILs such as TEA HSO₄, the solvent of choice in this study⁴⁸.

Previous progress in scale-up of pretreatment processes has focused on more established physical and chemical processes such as steam explosion, AFEX, dilute acid and alkali pretreatment^{10,49,50}. Among the continuous pretreatment processes under study, screw extrusion (see Figure II-21) offers significant advantages such as short residence time, the use of moderate temperatures to minimize inhibitor formation, use of high solid loadings and facile adaptation to industrial-scale operation^{50,51}. Often combined with chemical pretreatment, extrusion functions by mechanical pulverization and fibrillation of the biomass, which works best for herbaceous biomass which is softer and easier to extrude⁵², though additives are necessary to improve flow characteristics^{50,53}. Da Silva *et al.*⁵³ investigated continuous two-screw extrusion of sugarcane bagasse with added ionic liquid [Emim][OAc] at 25 wt% solid loading at 140 °C for 8 min, giving glucose yields of more than 90% after 24 h of enzymatic saccharification of the pretreated pulps. This result was comparable to glucose yields carried out at 5 wt% bagasse loading at 120 °C for 2 hours in a stirred reactor⁵⁴. Further increase of the solid loading to 50 wt% in the extruder afforded a high glucose yield of 76%⁵³. Batch IL pretreatments were able to out-perform screw extrusion up to a loading of ~35 wt%; conversely, at very high loadings, the extruder equipment gave higher glucose yields (76% vs 40–50%)^{13,14}. However, extrusion has a high mechanical energy input which can resemble that of comminution¹⁰. Serious abrasion of extruders by lignocellulose, especially when combined with acidic ILs, further increases the capital investment¹⁰.

The rheological behavior of the IL-biomass suspension also constitutes a major determinant of the efficiency of the biomass pretreatment process^{13,55}. At high solids loadings (>20 wt%), biomass possesses complex rheological characteristics that affect mixing energy consumption^{13,56,57}. Mixing in conventional stirred tanks becomes too costly and inefficient, requiring alternative reactor geometries. Use of helical ribbon stirred reactors has been proposed for mixing highly viscous slurries, inspired from their use in the petrochemical and paper industries^{10,58}. Helical ribbon reactors have been applied to dilute acid pretreatment at very high solid loading of 70 wt%⁵⁸. Though their use for

IL pretreatment has not been reported to date, helical stirring reactors may offer the potential for high-solids batch pretreatment of ILs to avoid the excessive energy inputs and corrosion problems of screw extruders¹⁰. The configurations of a typical helical ribbon reactor and screw extruder are shown in Figure II-21.

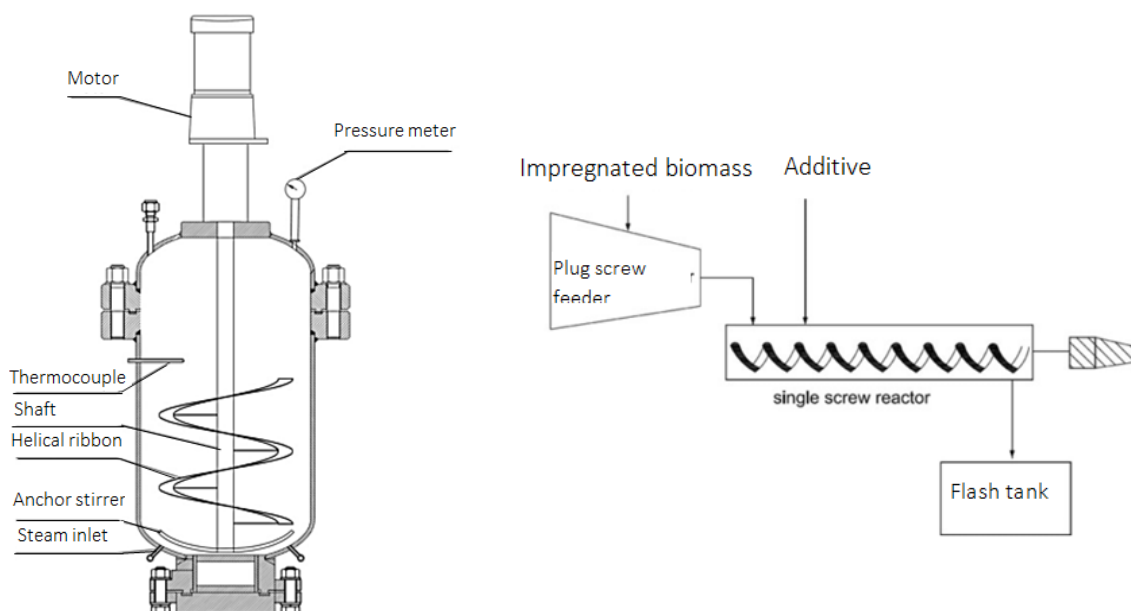


Figure II-21. Schematic diagram of helical ribbon reactor (left) and screw extruder system (right) adapted from Zhang *et al.*¹⁰.

A limited number of studies have been conducted on the scale-up of ionic liquid pretreatment using cellulose-dissolving ILs up to the 6 L-scale⁵⁵. Li *et al.*⁵⁵ demonstrated the scale-up of IL pretreatment of switchgrass using [Emim][OAc]. Upon 600-fold scale-up (6 L vs 0.01 L) using 15 wt% biomass loading, it was found that the IL-based technology could be effectively scaled to larger operations while maintaining high glucose release (95%). The results obtained were consistent with small-scale experiments conducted at 3 wt% loading, with similar pulp recovery, though at the 6 L-scale the pulp was more delignified but contained more hemicelluloses, which was attributed to the effect of loading in preferentially removing lignin rather than structural carbohydrates. The study highlighted the operational challenges of removing residual IL from the recovered pulp, requiring excessive water and water-ethanol washes, as small amounts of IL can act as inhibitors in downstream hydrolysis and fermentation^{55,59,60}. This work was later extended to pretreatment of hardwood and mixed feedstocks (hardwood/grass) at 10 wt% loading by Li *et al.*⁶¹. In the latter study, 30-fold scale-up to 6 L gave less effective lignin and hemicellulose removal due to the combined effects of scale-up, loading effects and pretreatment severity. However, uniform mixing and more effective biomass washing and recovery by centrifugation increased the recovery of structural carbohydrates. Very recently, Li *et al.*⁶² demonstrated scale-up and process integration of acid-assisted IL deconstruction of a blend of municipal solid waste and agricultural residues. Corrosion rates and weight loss of two types of

Hastelloy C276 commercial alloy in contact with pure [Bmim]Cl and [Bmim]Cl-biomass slurry were investigated. Long-term exposure over 6 days of continuous heat treatment at 140 °C showed low weight loss rates attributed to only very minor surface corrosion⁶².

Bench-scale ionoSolv pretreatment of herbaceous biomass (*Miscanthus* and sugarcane bagasse)^{41,63,64}, softwood (pine)⁹ and hardwood (willow)⁴⁷ have been performed within the Hallett group over a wide range of conditions. However, the data generated were obtained at the 10–20 mL level of operation without agitation, which cannot be directly translated to industrially relevant scales. Thus, stirred liter-scale experiments are a necessary intermediate step between the bench and pilot scale to identify operational parameters and potential problems associated with scale-up prior to pilot- and full-scale commercial operations. Process scale-up is therefore a vital step towards commercialization of the ionoSolv process.

Based on the literature and experience of bench-scale ionoSolv processing, four major challenges were anticipated upon 100-fold process scale-up:

- (1) Design of a pretreatment reactor material compatible with strongly acidic and corrosive PILs⁴⁸. Corrosion effects on metals used for reactor construction were unknown at the time of this study. Thus, pressure-resistant glass pretreatment vessels were used for scale-up.
- (2) Achieving efficient mixing of solid with liquid reagents as well as the transfer of heat and mass at high solid loading. Low energy input, satisfactory pretreatment efficiency (minimal reactor volumes) and batch operation were desirable. High solid loading (>20 wt%)¹⁰ leads to poor mixing between bulky solid biomass and a small amount of liquid; however, given the limited stirrer motor power compatible with the glass reactors used, it was not possible to investigate loadings above 20 wt% in this work.
- (3) Material handling and pulp and lignin washing at larger scales. The feasibility and ease of operation of different pulp washing methods (muslin cloth straining, vacuum filtration) requires investigating and comparing to methods used at the bench scale (i.e. centrifugation).
- (4) Minimizing energy input for stirring and size reduction by use of varying stirring speeds and larger particle sizes. Lack of flowability of biomass suspensions at relatively high solids loading precludes energy-efficient mixing¹⁰. The diameter, pitch, thickness and shape of the impeller need to be considered.

3.2.4 Summary

- Increasing solids loading from 10 up to 50 wt% during pretreatment of herbaceous biomass with [Emim][OAc] reduced glucose yields from ~100 to 40–50%.
- Poorer mass transfer using particle sizes >5 mm limited glucose release; lignin-dissolving ILs showed more potential than cellulose-dissolving ILs to decrease size reduction energy needs.
- 600-fold scale-up of [Emim][OAc] pretreatment technology could be effectively scaled to larger operations while maintaining high glucose release (95%).

3.3 Experimental

3.3.1 Materials and method

Miscanthus × giganteus was obtained from Imperial College London Silwood Park campus (Berkshire, UK) in December 2014 and in April 2016. Each set of experiments were conducted with biomass prepared during a single harvesting batch (i.e. batches were not mixed). Different *Miscanthus* size fractions were prepared by manual chopping to cylindrical chips of average size of 24 mm length × 9.9 mm diameter using a band saw. Smaller fractions were prepared using a cutting mill (Retsch SM 2000) and a square sieve opening of 20 mm followed by sieving (Retsch AS 200) to 1–3 mm size fraction. The finest particles were prepared by grinding in the same equipment using a sieve opening of 0.75 mm followed by sieving to 0.18–0.85 mm size fraction. Brown treated pine (*Pinus sylvestris*) was obtained from Travis Perkins (UK); the softwood had previously been pressure-treated with Tanalith E wood preservative. All biomass was air-dried and either manually chopped or comminuted using a cutting mill (Retsch SM 2000) followed by sieving (Retsch AS 200) to the relevant size fraction. Pine chips were prepared by manual chopping using a hand-held saw; medium fractions were prepared using a cutting mill (6 mm sieve opening) followed by sieving to 1–3 mm particle size; fine particles were prepared by grinding (0.75 mm sieve opening) and sieving to 0.18–0.85 μm size fraction. As a standard particle size fraction, unless otherwise indicated, biomass was size-reduced by cutting mill and sieved to 0.18–0.85 μm (-20/+80 US mesh scale) prior to use. All biomass fractions were stored in plastic bags at room temperature in the dark.

3.3.2 Ionic liquid synthesis (scaled-up method)

All pretreatment experiments in this study used a solution of 80 wt% [TEA][HSO₄] in DI water as pretreatment solvent. Experiments conducted at the 10 mL-scale in this study used manually synthesized [TEA][HSO₄], synthesized in batches of ~150 g following the method described in section 1.3.2. The exception is for benchmarking experiments carried out as a direct comparison of scale-up experiments, which used [TEA][HSO₄] synthesized using the scaled-up method below.

Larger quantities of [TEA][HSO₄] were synthesized in a continuous stirred tank reactor (CSTR) designed and built for continuous production of ~1 kg/h protic ionic liquids. It was constructed of corrosion-resistant materials, consisting of a PTFE-lined jacketed vessel (4" nominal bore × 430 mm long, ASME 150, Corrosion Resistant Products Ltd, UK) cooled by a water jacket with PFA-lined Swagelok fittings. The reactor was fitted with stirring control, temperature control and conductivity control to monitor the product quality. Dilute acid and amine were fed continuously into the reactor and reacted to form a solution with a final water content of 20 wt%. The CSTR was used to synthesize a batch of ~18 kg of [TEA][HSO₄] solution which was used for all scale-up and benchmarking experiments.

3.3.3 Ionic liquid pretreatment at bench scale (10 mL)

Standard operating procedure: 10 mL-scale pretreatment

For "bench scale" experiments (10 mL working volume), IonoSolv pretreatment was carried out using a standard operating procedure of our laboratory⁶⁵, as described in section 1.3.3. A biomass to solvent ratio of between 1:2 and 1:50 g/g was used (equivalent to 2–50 wt% loading). For hornification experiments, two sets of triplicates were conducted under identical conditions as described previously: six identical samples were subjected to the same ethanol washing steps until the Soxhlet step, where one set of triplicates was air-dried after the Soxhlet step and one was washed with water and stored wet to avoid drying-induced hornification. The experimental conditions used at the bench scale for different feedstocks were as follows: unstirred 15 mL Ace glass pressure tubes; temperature between 120 and 170 °C for a specified residence time between 0.5 and 6 hours; heating in a circulation oven pre-heated to the desired reaction temperature. Pulp and lignin yields were determined as before. The ionic liquid/water mixtures were either discarded, or in the case where ionic liquid recovery was quantified, they were concentrated by rotary evaporation to remove water and the residual weight and water content used to determine the recovery rate of the IL, R (wt%), given in

(Equation 12:

$$R = \frac{IL_{rec} \times (100 - w_{rec})}{IL_{fresh} \times (100 - w_{fresh})} \times 100 \quad (\text{Equation 12})$$

where IL_{fresh} is the total amount of ionic liquid added to the pretreatment tube and w_{fresh} is its water content while IL_{rec} is the total amount of ionic liquid recovered and w_{rec} is the water content of the recovered ionic liquid.

Biomass loading experiments

For experiments where the biomass loading was increased, *Miscanthus* samples were pretreated with [TEA][HSO₄] at 120 °C for 6 h, using solid loadings of 2, 5, 10, 20, 30, 40 and 50 wt% in unstirred 15 mL pretreatment tubes. 2 wt% biomass loading was achieved by using 25g of IL and 0.5 g of biomass; 5, 10 and 20 wt% loading were achieved using 10 g of IL and 0.5, 1 and 2 g of biomass respectively; 30,

40 and 50 wt% loading were achieved by using 5 g of IL and 1.5, 2 and 2.5 g of biomass, respectively. Pretreatment tubes containing the IL-biomass mixtures were thoroughly vortex-mixed prior to heating, ensuring a thorough soaking and contact of the biomass in IL

Alternative washing protocols

For experiments where pulps were washed with DMSO, the procedure was followed unchanged until after the fourth ethanol wash had been decanted. The pulps were then incubated in 40 mL DMSO overnight, centrifuged as before, the DMSO decanted and the pulps were then Soxhlet extracted with ethanol following the standard procedure.

For experiments where pulps were saccharified without air-drying in order to avoid hornification, the procedure was followed unchanged until after the Soxhlet extraction step (either with or without a DMSO wash between the fourth ethanol wash and Soxhlet extraction step). The pulp-containing thimbles were removed from the Soxhlet adapter after completion of the Soxhlet extraction and put in 50 mL Falcon tubes which were immediately filled with DI water. The pulp was left in the thimble inside the Falcon tube for at least an hour. The thimble was then taken out of the Falcon tube and the pulp transferred back to the Falcon tube using a spatula. The tubes were centrifuged (3000 rpm, 50 min or 2000xg) and the supernatant decanted; the washing step was repeated once more in DI water. The wet pulp was weighed, its moisture content determined immediately and saccharification started the following day.

Particle size experiments

For experiments involving different particle sizes, three sets of experiments were conducted for three different feedstock-solvent combinations: *Miscanthus*-[TEA][HSO₄] (80 wt% [TEA][HSO₄], 120 °C, 6 h, 20 wt% loading), Pine- [TEA][HSO₄] (80 wt% [TEA][HSO₄], 170 °C, 30 min, 20 wt% loading) and *Miscanthus*-dilute acid (3 wt% H₂SO₄, 120 °C, 1.5 h, 20 wt% loading). The pretreatment protocols followed were as in section 2.3.2 and section 3.3.7 for bench scale ionoSolv and dilute acid pretreatment, respectively.

Three particle size fractions were investigated for *Miscanthus* and pine; preparation of these size fractions was described in section 3.3.1.

Benchmarking experiments

Benchmarking experiments were carried out in 15 mL Ace glass pressure tubes without stirring, using identical pretreatment conditions to those described in the scaled-up experiments (see Table II-9 below). Air-dried *Miscanthus* was combined with the desired quantity of IL (80 wt% [TEA][HSO₄] derived from the same batch as used in scale-up experiments) and placed into a pre-heated convection oven at 120 °C for 6 hours. Subsequently, the pretreatment tubes were cooled and the

pulps were washed by vacuum filtration (Whatman® grade 1, diam. 110 mm) using 1 mL/g IL solvent per wash over 12 washing steps. The pulp was air-dried and weighed before being subjected to saccharification *without* a Soxhlet step. The ethanol-IL washes were concentrated and lignin was recovered as described above. IL recoveries were determined as in section 2.3.3.

3.3.4 Scale-up of ionic liquid pretreatment (1 L)

Scale-up experimental setup

All scale-up reactions were carried out in an 'Ecoclave' laboratory pressure reactor system supplied by Ken Kimble Ltd, UK (see Figure II-22). A 1.5-L jacketed borosilicate glass pressure vessel (Type 2, Büchi AG, Switzerland) with stainless steel 316/316L external fittings and Hastelloy C22 internal components was used for all experiments. The reactor vessels are rated to 6 barg in the laboratory. For safety purposes, the reactor used Swagelok fittings and was equipped with a manometer, proportional relief valve set to 1.7 barg and a bursting disc (3 barg, with lowest rating at 120 °C of 2.7 barg). This pressure is not anticipated to be reached during a reaction of ionic liquid and biomass at 120 °C. Stirring was achieved using a 50 mm anchor agitator (Hastelloy C22) with flat blades assembled axially without a baffle. The agitator shaft was slotted into a high torque magnetic coupling (cyclone 300 bmd, Büchi AG, Switzerland) and a high-torque overhead mechanical stirrer motor (RZR 2021, Heidolph, Germany). The glass pressure vessel contents were heated using an oil jacket containing silicon oil (SiOil M20.195/235.20, Huber, Germany) with process control using a thermostat within the oil recirculator (Unistat 405, Huber, Germany). Reactor contents temperature was monitored using a thermocouple mounted near the reactor core.

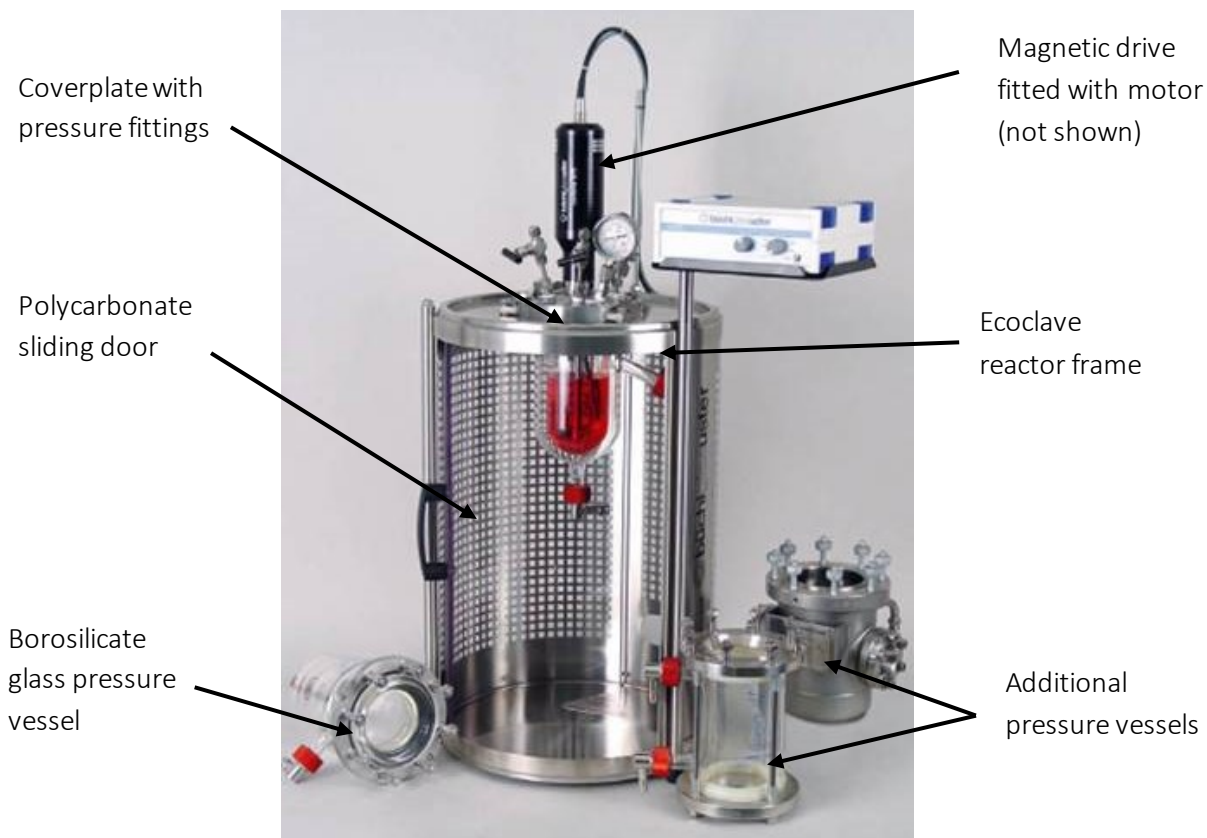


Figure II-22. Ecoclave reactor system used for 1 L-scale scale-up experiments showing key components.

Standard operating procedure: 1 L-scale

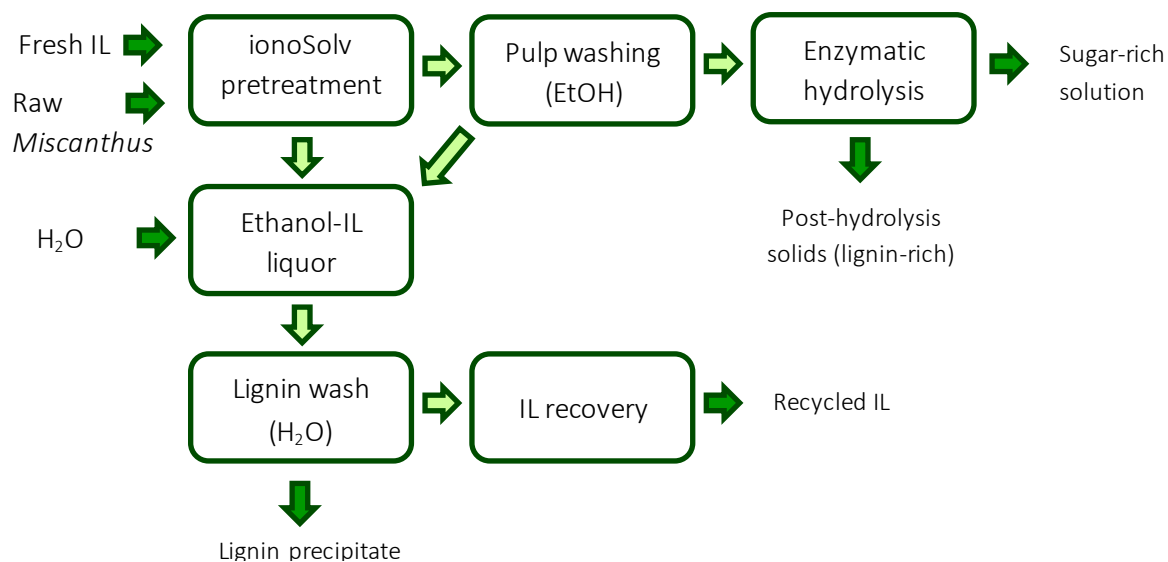


Figure II-23. Scheme for processing *Miscanthus* by scaled-up ionoSolv pretreatment and enzymatic hydrolysis.

Figure II-23 shows the processing steps used in this study. ionoSolv pretreatment was performed in the 1.5-L glass vessel at constant agitation (150 rpm) and indirect heating via an oil jacket. First, the glass reactor was loaded with either 100 g or 200 g bone-dry *Miscanthus* (107 or 214 g air-dried

Miscanthus), for solid loadings of either 10 or 20 wt%, respectively. The vessel was mounted into the reactor frame and the oil jacket heated to 95 °C. The IL was pre-heated separately in a sealed vessel in a circulation oven to 95 °C to shorten the heating time of the IL-biomass mixture. A mass of 1 kg of pre-heated IL (95 °C) was loaded into the reactor via a port in the reactor coverplate and the amount added verified by back-weighing, then the reactor was sealed and wrapped with a layer of Superwool® thermal ceramic insulation. The biomass-IL slurry was then heated to the desired reaction temperature of 120 °C with a stirring speed of 150 rpm (unless otherwise indicated) from the anchor impeller; the heat-up time varied between 30 and 45 min, depending on the viscosity of the slurry. A sample heating curve is shown in the Appendix (Figure S0-4). After 6 h at 120 °C, the reactor was vented and the oil jacket dismantled to allow the reactor to cool to ~60 °C with help of a fan. Cooling times to below 100 °C and below 60 °C were approximately 30 and 90 minutes, respectively. Detailed experimental conditions used for IL pretreatment are summarized in Table II-9. The following experiments were conducted in triplicates: Fine/10wt% loading/450 rpm; Fine/20wt% loading/150 rpm; Medium/10wt% loading/250 rpm; Coarse/10wt% loading/400 rpm; all other experiments (Fine, Medium and Coarse at 10 wt% loading and 150 rpm) were conducted in monuplicate. Errors were calculated as the standard deviation across triplicates. Percentage errors from triplicate experiments at the closest experimental conditions were used in the case of monuplicate experiments.

Table II-9. Pretreatment parameters used for ionoSolv pretreatment scale-up studies and corresponding bench-scale experiments.

Factor	Low	Medium	High
Scale (mL)	10	1000	-
Biomass loading (wt%)	10	20	-
Stirring speed	150	max ^a	-
Particle size	Fine (180 – 850 µm)	Medium (1 – 3 mm)	Coarse (24 mm)

^a The maximum stirring speed that could be achieved for each IL-biomass slurry, i.e. 450 rpm (fine/10 wt% loading), 150 rpm (fine/20 wt% loading), 250 rpm (medium/10 wt% loading) and 400 rpm (coarse/10 wt% loading).

After cooling of the reaction mixture, the pretreated IL-pulp slurry was manually transferred to a 5 L plastic container and completely washed out with 1 L of absolute ethanol; care was taken to wash residual IL off all reactor surfaces and interior parts for mass balance purposes. The slurry was vacuum filtered (Whatman® grade 1, diam. 150 mm) with 1 L of ethanol per wash (i.e. 1 g EtOH/g IL solvent). Each volume of EtOH was allowed to completely filter through the pulp before addition of the next volume. 10 washing steps were conducted in total with each step consisting of 1 L of EtOH (since 1 L of IL-water mixture was used for each scale-up experiment). After washing was complete, the recovered IL and ethanol washes were concentrated by rotary evaporation until all ethanol had been removed. The recovered ethanol was collected and re-used for washing in subsequent washing steps.

to reduce solvent wastage. It was noted that concentrated IL liquors showed signs of lignin precipitation if ethanol-rich washes were added to IL-rich liquors, as pure ethanol is a known precipitant for lignin⁶⁶; therefore, the IL liquors were mixed after concentration i.e. after ethanol removal. When all ethanol had been removed, 4 L of DI water was added to the ~1 L of concentrated IL liquor recovered (i.e. 4 g/g of water to IL solvent mixture) to precipitate the lignin, which was left to equilibrate overnight.

Lignin recovery was carried out by two rounds of centrifugation in four 500 mL centrifuge tubes (Corning, USA) at 3000 rpm for 50 minutes to sediment the precipitated lignin. The lignin solid was then washed four more times, each with 2 L of DI water, until a total of ~10 L of water had been used for precipitation and washing. The supernatants (IL and water) were collected separately and concentrated to determine the IL recovery as given in (Equation 12 above). The recovered water was collected and recycled for lignin precipitation and washing in subsequent experiments to reduce solvent volumes. The recovered solid lignin was dried *in vacuo* at 45 °C for 48 h in the centrifuge tubes before being transferred to pieces of foil and re-dried for 24 h to fully remove any residual water, before determination of the lignin yield.

3.3.5 Ionic liquid solution acidity

The water content of a fresh or recovered IL liquor was determined in triplicate. 0.1 g of ionic liquid (on a dry basis) was added into a 10 mL volumetric flask and mixed with deionized water (up to 10 mL). The solution was poured into a 10 mL Falcon tube and a calibrated pH meter (Mettler Toledo SevenEasy) used to measure the pH. The pH of the solution was converted into a proton concentration using (Equation 13:

$$[H^+]_{IL} = \frac{10^{-pH}}{IL_{conc}} \quad (\text{Equation 13})$$

where $[H^+]_{IL}$ is the calculated proton concentration in the ionic liquid; pH is the measured pH value, IL_{conc} is the ionic liquid concentration in the analyzed sample (here 10 mg mL⁻¹).

3.3.6 Ionic liquid thermal stability

Thermogravimetric analysis of dried fresh ionic liquid (containing <0.5 wt% water as verified by Karl-Fischer titration) was performed on a TA Q500 (TA Instruments, USA) TGA analyzer fitted with a gas switching system, using a platinum pan, with N₂ as the purge gas (20 mL/min). 20±5 mg of ionic liquid was loaded per run; each sample was analyzed in triplicate. The short-term stability of ILs was measured after a 30 min hold period at 80 °C, after which a temperature ramp (10 °C min⁻¹) to 700 °C was performed. For determination of thermal stability, the onset of decomposition temperature

(T_{onset}) was determined. T_{onset} is the intersection of the baseline weight and the tangent of the weight dependence on the temperature curve as decomposition occurs.

3.3.7 Dilute acid pretreatment

Miscanthus samples with the same three size fractions described in section 3.3.1 were placed in pressurized glass pretreatment tubes. A solution of 3 wt% sulfuric acid in DI water (pH ~0.6) was added with a dry solids loading of 1:5 g/g. The sealed glass tubes were then heated in a convection oven at 120 °C for 1.5 hours with three replicates. The acidic slurry was centrifuged and 40 mL H₂O added for washing. After pretreatment, the acidic slurry was transferred to 50 mL Falcon tubes for washing with DI water and separated by centrifugation (3000 rpm for 50 min or 2000xg). The supernatant was decanted and discarded and the washing step with DI water repeated three more times. The recovered solids were weighed to determine the pulp yield.

The combined severity factor (CSF) can be used to compare the pretreatment severity of dilute acid pretreatment processes, and is calculated using (Equation 14⁶⁷:

$$CSF = \log \left(t \times e^{\frac{T-100}{14.75}} \right) - pH \quad (\text{Equation 14})$$

The conditions for dilute acid pretreatment were selected based on results from a previous study by Li *et al.*³¹ applied to switchgrass, which used a CSF of ~2.0 (T of 160 °C, t of 15 min, [H₂SO₄] of 1.2 wt% i.e. pH of 0.93). Instead, we used conditions of T of 120 °C, t of 90 min, [H₂SO₄] of 3 wt% i.e. pH of 0.53) to maintain the same reaction temperature as used for IonoSolv experiments.

3.3.8 Compositional analysis

The compositions of the untreated biomass and pretreated biomass pulps and precipitated lignins obtained from pretreatment were determined as described previously in section 1.3.5. For hornified and non-hornified pulps, only the dried hornified ones were analyzed by compositional analysis and the composition of the non-hornified pulps was assumed to be identical. For pretreated pulps arising from larger particle size experiments (>0.85 mm), the materials were size-reduced using an analytical grinder with stainless steel cutter (Yellow Line A10, IKA Works Inc.) prior to analysis. The samples were ground for up to 30 seconds as this was sufficient to grind the *Miscanthus* and pine chips to a powder that would pass through an 0.85 mm sieve opening. Compositional analysis of pretreated larger particle sizes was conducted after grinding of the pulps to ≤0.85 mm so that all pulps could be fully digested during the acid hydrolysis step of the analytical method. Delignification, glucan recovery and hemicellulose recovery were calculated as described above.

3.3.9 Saccharification assay

Saccharification assays were carried out as described previously in section 2.3.5 for air-dried and wet samples. *Miscanthus* samples were saccharified with 20 μ L of Novozymes per sample while pine samples required higher enzyme concentration of 50 μ L per sample; the purified water added per sample was adjusted to 3.41 and 3.38 mL, respectively, to give a total volume per saccharification sample of 9.90 mL excluding the biomass. Glucose concentrations in the sample were determined after the saccharification period of $t = 7$ days had elapsed (for most cases). Time-point samples were also taken from the saccharification mixture for certain samples at intervals of 0.5, 1, 2, 4, 24, and 72 hours to determine the initial rate of hydrolysis (%glucose released between 0.5 and 2 h of hydrolysis). Glucose yields were reported as a percentage of the total glucan content found in untreated biomass (as determined by compositional analysis). Glucose were calculated as a function of saccharification residence time t according to (Equation 15 below:

$$\%Glucose\ yield\ (t) = \frac{c_{HPLC,t} \times V \times 0.9 \times Y_{pulp}}{G_0 \times ODW_{sample}} \times 100 \quad (\text{Equation 15})$$

where c_{HPLC} 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; ~40–70% for pretreated pulps) G_0 is the glucan content (%) in untreated biomass, and ODW_{sample} is the oven-dried weight of the biomass sample before saccharification in mg.

3.3.10 Particle size distribution measurements

Particle size distributions were determined for untreated and pretreated pulps for all particle size experiments using nested sieves as described in section 2.3.6. Briefly, the sample particle size distribution was obtained using a vibratory shaker equipped with stacked sieves with decreasing pore sizes (5000, 4000, 3150, 2000, 1800, 1400, 1000, 850, 710, 500, 300, 212, 180, 150, 106, 75 and 53 μ m) by shaking on a vibratory sieve shaker for 20 minutes for each sample. The percentage weight of material retained by each sieve was measured and the log-normal distribution mass median diameter (D_{50}) was calculated using (Equation 11 given above. Particle size analyses were conducted in triplicates. Particle size distributions were plotted as weight percentages of samples passing through different sieve pore sizes (on a log scale) and geometric mean diameters D_{50} were compared.

3.3.11 HSQC NMR spectroscopy of lignins

For HSQC experiments of precipitated lignin, ca. 20 mg of lignin was dissolved in 0.25 mL of DMSO- d_6 and the solution transferred to a Shigemi tube, as described in section 1.4.5. HSQC experiments were recorded in monoplicate for *Miscanthus* lignins isolated from selected scale-up experiments.

3.3.12 GPC of lignins

GPC measurements were performed using an Agilent 1260 Infinity instrument equipped with a Viscotek column set (AGuard, A6000M and A3000M). The Agilent 1260 Infinity RID detector was used for detection. HPLC grade DMSO containing LiBr (1 g/L) was used as eluent at a flow rate of 0.4 mL/min at 60 °C. Samples were prepared by dissolving 20 mg lignin in 1 ml eluent and filtering through a 0.2 µm syringe filter. Ten pullulan standards (Agilent calibration kit, $180 < M_p < 780,000$) were used to calibrate the instrument.

3.3.13 Elemental analysis

Representative samples were selected from experiments conducted at 10 wt% loading and at 50 wt% loading. CHNS analysis of untreated air-dried biomass, pulp, lignin and recovered ionic liquid was performed in duplicate by MEDAC Ltd (Chobham, UK). Oxygen content was obtained by difference. Accuracy is $\pm 0.30\%$ absolute. CHNSO compositions and the calculated elemental composition of fresh ionic liquid were presented on a dry basis and used to conduct an elemental mass balance on the system (inputs: biomass and fresh ionic liquid in appropriate ratios; outputs: recovered ionic liquid, pulp and lignin in appropriate ratios) for both 10 wt% loading and 50 wt% loading.

3.4 Results and Discussion

The effect of increasing solids loading and particle sizes for pretreatment of *Miscanthus × giganteus* using the low-cost PIL TEA HSO₄ was first examined at the bench scale in unstirred glass tubes (working volume of 10 mL). The pulp recovery protocol was modified in an attempt to improve saccharification yields at high solids loadings. These results constituted baseline data for comparison with scale-up runs. Subsequently, process intensification was investigated by 100-fold scale-up of the IonoSolv treatment process. The effects of stirring, biomass loading and particle size were investigated in stirred glass pretreatment vessels (working volume of 1 L). Pretreatment efficacy was analyzed by comparing pulp and lignin yields, pulp composition, saccharification yields and kinetics, and lignin characteristics. An energy input assessment was conducted to determine the effectiveness of size reduction and mixing on glucose release following pretreatment.

3.4.1 Effect of biomass loading

Use of high solids loadings is required to improve the economic viability of biorefining processes¹¹³. The vast majority of results reported in the literature on biomass pretreatment to date have been achieved using relatively low biomass loading levels (3 to 10 wt%) with few reports on the impact of high biomass loading (> 20 wt%)^{13,14,55}. We therefore assessed the pretreatment efficacy at loadings ranging from 2 to 50 wt%, corresponding to a solid to liquid ratio of 1:50 to 1:2 g/g, using *Miscanthus*. These experiments were carried out using TEA HSO₄ under non-optimal conditions (120 °C, 6 h) where differences in pretreatment efficacy would be easier to observe.

Lignin and saccharification yields

Figure II-24 shows the pulp, lignin recovery, delignification and saccharification yields as a function of biomass loading. Pulp yields showed a steady increase from 51 to 63% upon increasing the biomass loading from 2 to 50 wt%. Lignin yields concomitantly decreased from quantitative recovery at 2 wt% loading to only half of the theoretical lignin recovery at 50 wt% loading. Delignification similarly reached 86% at low loadings but decreased to 47% at 50% loading. Accordingly, the saccharification yield fell gradually with increasing solids loading, with the highest yields (77%) obtained at 2 wt% loading, from where it decreased to 23% at 50 wt% loading. The decrease in lignin yields and delignification combined with the increase in pulp yields is likely due to precipitation of lignin during the dilution of the IL liquor with ethanol during the pulp washing step. At higher loadings, dissolved lignin that was present at higher concentrations resulted in a greater amount of re-precipitated lignin upon anti-solvent addition. Re-precipitated lignin could not be distinguished from residual lignin (that was not removed during the treatment) using the acid hydrolysis technique applied to analyze biomass composition. The total amount of lignin present during saccharification has been linked to lower saccharification yields⁶⁸. Hence, the re-precipitation of lignin is strongly suspected to have a negative

effect on saccharification yields. Adapting the pulp recovery and washing protocol is thus a possibility to reduce or avoid re-precipitation or to remove re-precipitated lignin from the pulp surface.

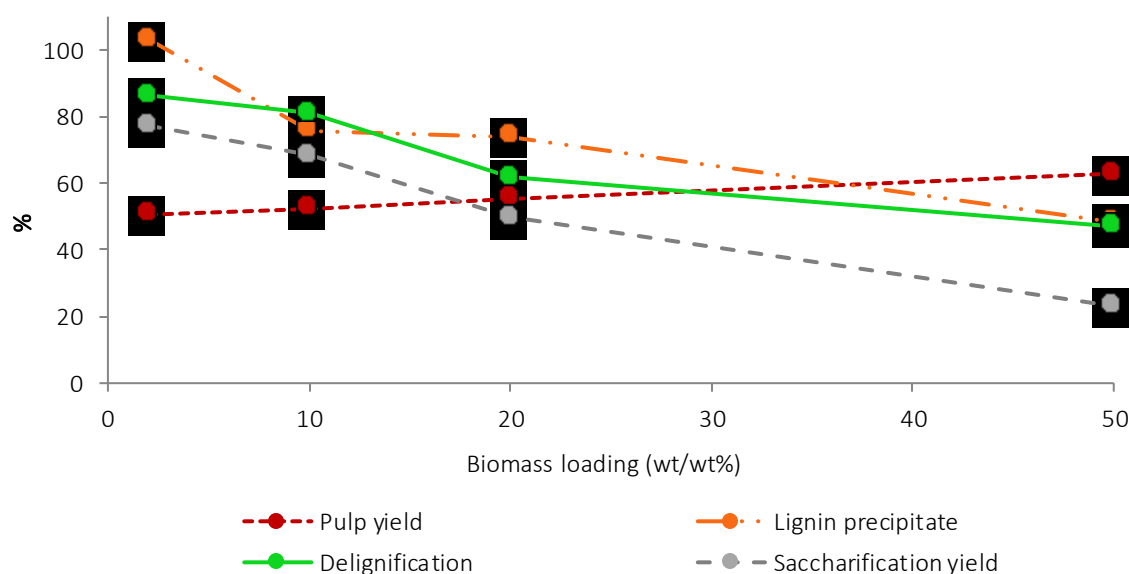


Figure II-24. Pulp, lignin and saccharification yields and pulp delignification after pretreatment of *Miscanthus* with [TEA][HSO₄] with a final water content of 20wt% for 6 h at 120 °C and biomass to solvent ratios ranging from 1:50 to 1:2 g/g, corresponding to loadings from 2 to 50wt%. Errors were calculated as standard deviation across triplicates.

Enzymatic hydrolysis was applied to the pulps produced to gain further insight into the changes occurring upon increasing the biomass to IL ratio. Figure II-25 shows the hydrolysis kinetics over a 7-day period while the initial hydrolysis rate (calculated as the rate of glucose release in the first 2 h of hydrolysis) and final biomass digestibility after 7 days are displayed in Table II-10. The initial rate of hydrolysis was seen to decrease from 6.1 to 1.5 % glucose h⁻¹ when loading increased from 2 to 50 wt%; the hydrolysis rate achieved at 50wt% loading was nonetheless double that of untreated biomass. Decreased initial hydrolysis rates are thought to be related to the enzyme-accessible surface area of biomass^{69,70}, indicating a greater amount of re-precipitated lignin on the pulp surface, which hinders enzyme access to glucose. Final glucose yields after 7 days of enzymatic hydrolysis decreased with biomass loading, attributed to increasing residual lignin content of the pulp¹⁸. As shown in Table II-10, normalizing the glucan yield to the amount of glucan present in the *pulp* (rather than in untreated biomass) at low loadings (2 wt%), pulp glucan was nearly quantitatively hydrolyzed and limited only by glucan recovery following pretreatment. At 10 wt% biomass loading, only 78% of glucan present was successfully hydrolyzed and this fell to merely 27% at 50 wt% loading, presumably due to lignin re-precipitation. Similar results were obtained during [Emim][OAc] pretreatment of switchgrass conducted by Cruz *et al.*¹³, who found that saccharification kinetics and final biomass digestibility were reduced at 50 wt% loading compared to at 10 wt% loading. However, the extent of the negative effect of loading on saccharification was lesser in the Cruz study; this was attributed to

mechanistic differences during pretreatment using cellulose-dissolving ILs, where the possibility of lignin re-precipitation onto the pulp surface is limited.

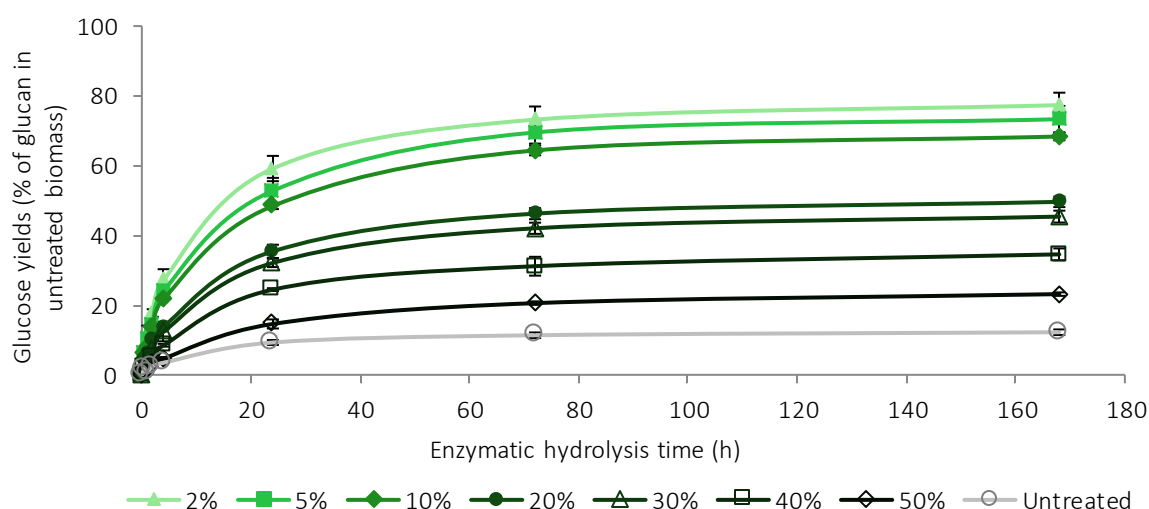


Figure II-25. Effect of biomass loading on enzymatic hydrolysis of pretreated *Miscanthus x giganteus* at 120 °C for 6 hours. Glucose yields shown relative to the amount of glucan in untreated biomass.

Table II-10. Initial hydrolysis rate determined as glucose released after first 2 h of hydrolysis and final glucose release after 7 day enzymatic saccharification experiments for a range of pretreated samples and untreated biomass. Data is expressed as two different percentages of glucose release.

	Initial hydrolysis rate (% h ⁻¹)	7 day glucose yield (wt% glucan in untreated biomass)	7 day glucose yield (wt% glucan in biomass pulp)
Untreated <i>Miscanthus</i>	0.7±0.1	12.2±0.8	12.2±0.8
2% loading	6.1±0.7	77.3±3.6	93.3±5.1
5% loading	5.3±0.1	73.3±3.8	-
10% loading	5.2±0.3	68.3±1.1	78.4±4.3
20% loading	3.7±0.2	49.6±1.5	60.1±1.8
30% loading	2.8±0.2	45.4±1.7	-
40% loading	2.3±0.4	34.5±1.7	-
50% loading	1.5±0.3	23.1±0.5	26.5±0.6

Lignin analysis

The molecular weight (MW) characteristics of the precipitated lignins were analyzed by GPC while HSQC NMR was used to observe changes in interunit linkage and subunit abundance. Semi-quantitative signal intensity integrals observed by HSQC NMR of the recovered lignin (Figure II-26a) did not show stark differences between high loading and low loading experiments. There appears to be a trend towards greater extent of ether cleavage in lignins isolated at high loadings. Signal intensities of the S and G₂ positions appear to have increased, while that of the G₆ position seems to have decreased, indicating condensation reactions in G units. These differences are, however, not very

pronounced. GPC data (see Figure II-26b) showed a general decrease in M_n and M_w with higher loading. Further, the polydispersity index (PDI) of the isolated lignin was found to decrease at higher loadings. The most significant decrease in MW was observed upon an increase in biomass loading from 2 to 5 wt%; thereafter, a gradual decline in MW and PDI was observed. These findings are in agreement with results of Gschwend *et al.*⁹, who compared lignin precipitate characteristics following pretreatment using the PIL [Hbim][HSO₄] on the softwood pine. The explanation for this behavior is believed to lie in the isolation method used to recover lignin, rather than on the effect that higher loading has on the lignin structure. While some protic ILs can dissolve up to 70 wt% lignin⁷¹, making saturation effects an unlikely cause, the addition of ethanol can precipitate larger fragments out of solution during the pulp wash⁶⁶. This effect would become stronger if the lignin was very concentrated. At higher loadings, the cut-off for larger fragments becoming insoluble in ethanol is expected to lie at lower MW than for lower loadings as a result of the increased lignin concentration, resulting in an apparent trend for increased ether cleavage without more pronounced condensation at higher loadings while increasing pulp yield and decreasing lignin recovery. **These results suggest that lignin structure is not significantly altered by use of high solids loadings; however, they suggest the need for an adapted washing protocol that avoids re-precipitation and/or removes re-precipitated lignin from the pulp surface.**

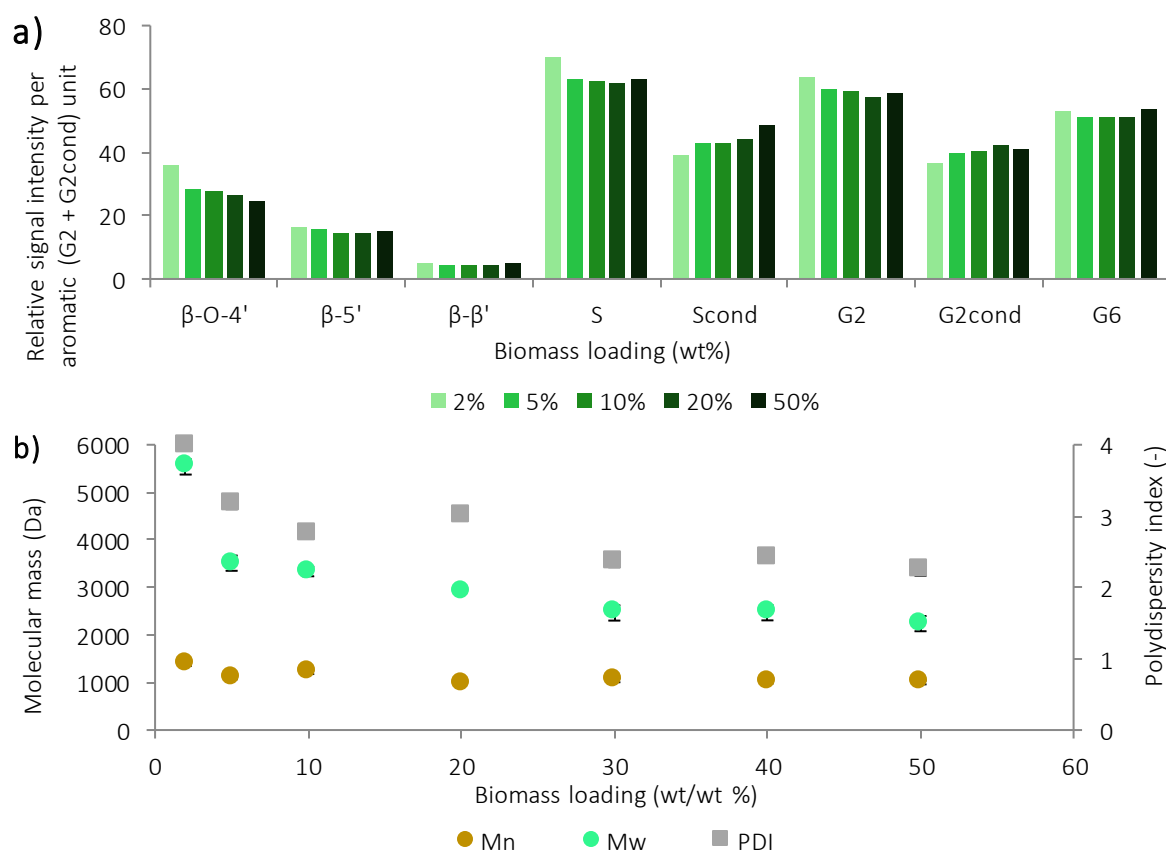


Figure II-26. (a) Signal intensities in HSQC NMR relative to 100 G₂+G_{2,cond} units and (b) molecular markers obtained by DMSO-eluted GPC for lignins recovered after pretreatment with [TEA][HSO₄] as a function of biomass loading.

Ionic liquid recovery

As indicated in the preliminary techno-economic study conducted by Klein-Marcuschamer *et al.*¹, IL recovery must be maximized to favor cost-effectiveness of IL-based biorefining. TEA HSO₄ was demonstrated to be more thermally stable than the APIL [Emim][OAc] used as a reference in said study. Preliminary studies of TEA HSO₄ as part of this study showed it to be thermally stable within the detection limits of ¹H and ¹³C NMR at 150 °C for 100 hours (see Appendix, Figure S0-5 and Figure S0-6). As a result, IL recyclability is expected to be less dependent on IL thermal degradation and instead on IL recovery methods. The IL liquors produced after pretreatment at various biomass loadings were recovered after lignin precipitation, dried and weighed. Using Karl-Fischer titration, the quantity of IL recovered was compared to the quantity of IL used during pretreatment; hence the IL recovery (on a wt/wt dry basis) could be determined. Additionally, the pH values of a 1wt% solution of fresh IL and IL liquors were recorded. The IL recovery and pH are shown in Figure II-27 as a function of loading. While IL recoveries approached 99 wt% for pretreatment at solids loadings of 10 and 20 wt%, an apparent trend of decreasing IL recovery with increasing biomass loading was observed (Figure II-27a). Evidence was previously presented by Brandt *et al.*⁶⁴ that ionic liquid molecules may remain adsorbed or possibly covalently bonded to precipitated lignin. It was therefore initially suspected that decreasing recovery of IL at higher biomass loadings could be due to a larger quantity of IL being trapped or bound within the lignin macromolecule. Evidence to the contrary is presented below; we therefore postulate that the observed “trend” is due to solvent volumes being halved (from 10 mL to 5 mL) for experiments at high loadings compared to low loadings, which was necessary in order to fit large quantities of biomass into the 15 mL tubes used for pretreatment. Moreover, IL recoveries in this setup are likely to be limited by the small quantities of solvent used and should improve upon process scale-up where small losses, such as IL sticking to glassware, would account for a lower proportion of total IL losses. **These findings indicate >98 wt% TEA HSO₄ recovery, which is favorable to IL recycling for continuous ianoSolv processing.**

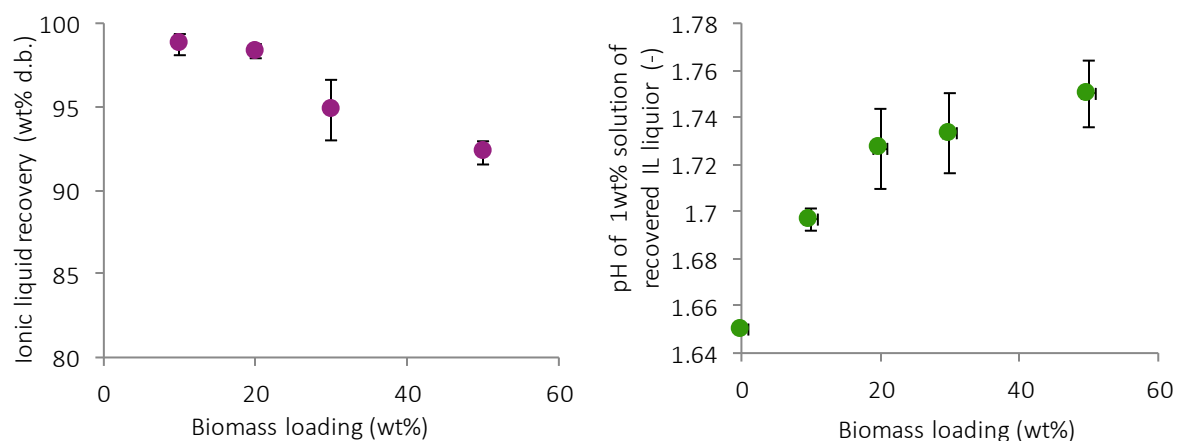


Figure II-27. (a) Ionic liquid recovery in the liquor relative to dry starting material and (b) pH of a 1% solution of IL liquor obtained after pretreatment with [TEA][HSO₄] at biomass loadings from 2 to 50 wt%.

The IL acidity before and after use at various loadings was compared by pH measurements of a 1 wt% aqueous solution of the ionic liquid, which was used to crudely estimate the proton concentration in undiluted IL solutions (see Table S0-2). Whereas the fresh IL had $[H^+]$ of 0.22 mmol/g, recovered ILs had proton concentrations ranging between 0.18–0.20 mmol/g. A trend of decreasing concentration of dissociable protons in the liquor with increasing biomass loading was observed. 11 and 26 mol% protons were lost compared to the fresh IL solution after pretreatment at 10 and 50 wt% loading, respectively⁷. This agrees with previous findings by Brandt *et al.*⁷ which found a 9 mol% decrease in proton concentration after four uses of the IL at 10 wt% loading (equivalent to a single cycle at 40 wt% loading). While this phenomenon is not yet fully understood; it does suggest that the acidity of the IL solution may need to be adjusted during repeated use⁷.

Mass balance

The elemental compositions of the inputs (untreated Miscanthus and fresh IL) and outputs (pretreated pulps, lignin and recovered IL) were used to determine the mass balance of the process at low and high solids loadings. The elemental mass balances of the system for one of the triplicate experiments conducted at 10 wt% and at 50 wt% biomass loading are shown in Table II-11 and the total component mass balances are displayed in Figure II-28. These calculations were prompted by the observation of decreasing IL recovery from 99% to 92% when loading was increased from 10 wt% to 50 wt% (Figure II-27a). If the missing IL portion were trapped in the lignin matrix, it should then be detected in the nitrogen and sulfur contents of the lignins. A detailed elemental balance (Table II-11) shows that nitrogen and sulfur content and total mass in the lignin was actually *lower* at higher loading, with residual IL contents of 4.2 and 3.9 wt% at loadings of 10 and 50 wt%, and did not account for the difference in IL recovery observed. Lower IL recovery was later attributed to the lower total solvent volume used, so minor spillages or other losses accounted for a larger proportion of total mass.

At 10 wt% loading, the mass recovery was fairly good (96 wt%) whilst at 50 wt% loading the recovery decreased to 84 wt%. In each case, roughly 30–40% of biomass weight was lost; this was presumed to be due to evaporation of volatile hemicellulose degradation products from the IL liquor during concentration of the ethanol-IL washes and during removal of the water that had been added during precipitation. Previous work by Gschwend *et al.*^{7,9} found that ~3.8 wt% of biomass weight was lost in the form of volatile hemicellulose degradation products (acetic acid, formic acid, furfural and 5-HMF) during processing at 10 wt% loading. If IL recoveries are set to 100% in the calculations below (see Table II-11), total mass recoveries increase to 96.7 wt% and 89.0 wt% i.e. losses of 3.3 wt% and 11.0 wt% at low and high loadings, respectively. These ~3.3 wt% losses tally well with the 3.8 wt% mass loss observed by Gschwend *et al.*^{7,9} **These findings suggest that the mass losses in the system are due to hemicellulose conversion to volatile degradation products** (if we neglect IL losses due to spillages).

Table II-11. Elemental mass balance of the ionoSolv process conducted at bench scale for biomass loadings of 10 and 50 wt%.

	10 wt% loading		50 wt% loading	
Mass in (g)				
Feedstock	100.31		100.31	
C	49.2		49.2	
H	5.5		5.5	
N	0.1		0.1	
O	0.1		0.1	
S	45.5		45.5	
Fresh ionic liquid (dry)	801.07		160.6	
C	289.7		58.1	
H	68.9		13.8	
N	56.3		11.3	
O	128.9		25.8	
S	257.3		51.6	
TOTAL IN	901.37		260.9	
C	338.9		107.3	
H	74.4		19.3	
N	56.4		11.3	
O	129.0		25.9	
S	302.8		97.1	
Mass out (g)				
	Actual	Corrected ^a	Actual	Corrected ^a
Pulp	48.8	-	57.9	-
C	22.2		28.9	
H	2.5		2.9	
N	0.1		0.1	
O	0.6		0.7	
S	23.5		25.4	
Lignin	21.7	-	13.6	-
C	13.5		8.5	
H	1.3		0.8	
N	0.2		0.1	
O	0.4		0.2	
S	6.4		4.1	
Ionic liquid liquor (dry)	792.6	801.1	147.9	160.6
C	288.1	291.2	54.6	59.3
H	73.2	73.9	13.6	14.8
N	53.6	54.2	9.4	10.2
O	123.4	124.7	21.6	23.5
S	254.3	257.1	48.7	52.9
TOTAL OUT	863.1	871.6	219.5	232.2
C	323.8	326.9	92.0	96.7
H	76.9	77.7	17.4	18.5
N	53.9	54.4	9.5	10.3
O	124.4	125.7	22.5	24.3
S	284.2	286.9	78.2	82.4
Mass recovery (wt%)				
Total	95.8	96.7	84.1	89.0
Losses	4.2	3.3	15.9	11.0
C	95.5	96.5	85.7	90.1
H	103.5	104.5	90.0	96.0
N	95.6	96.6	83.9	91.0
O	96.5	97.5	86.7	93.9
S	93.9	94.8	80.5	84.8

^a Calculations performed assuming 100 wt% IL recoveries (instead of 98.9 and 92.1 wt% for 10 and 50 wt% loading)

The total component mass balance obtained (neglecting IL losses) is displayed in Figure II-28. The results show a larger proportion of the system was lost in the form of volatile hemicellulose degradation products at higher loading. However, the proportion of mass loss was slightly lower at

higher loadings (287 mg/g biomass) than at lower loadings (297 mg/g biomass), suggesting the mass balance was improved at higher biomass loadings.

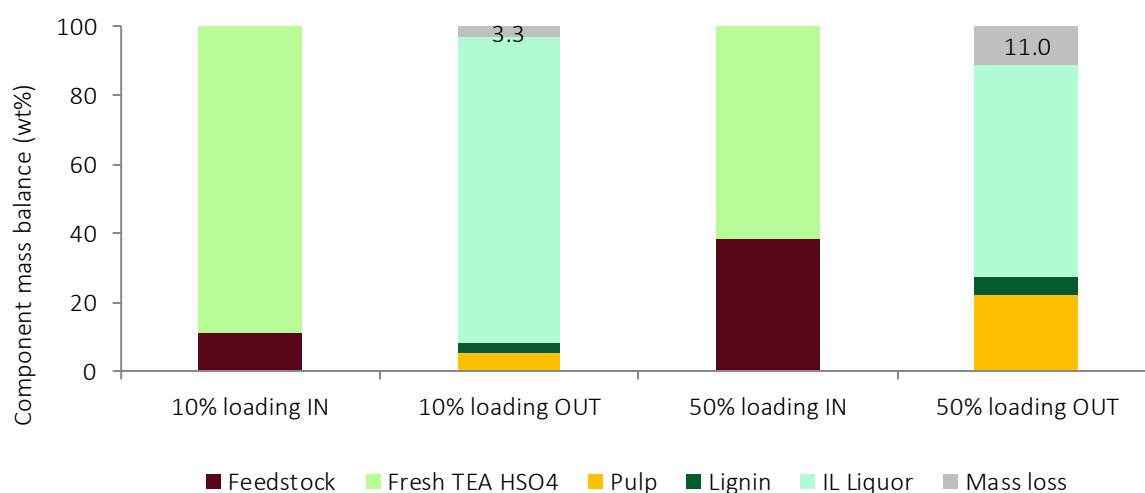


Figure II-28. Total component mass balance for experiments conducted at 10 wt% and 50 wt% biomass loading. Results are shown per 100 g of starting material on a dry basis, assuming no ionic liquid losses.

3.4.2 Effect of pulp washing and hornification

In an attempt to increase the saccharification yields at high biomass loadings, the pulps were washed with DMSO after the four ethanol washes used in the standard pretreatment procedure. DMSO was selected as it is a powerful solvent that is capable of dissolving re-precipitated lignin from the pulp surface. Following incubation in DMSO overnight, the solvent was observed to turn brown, suggesting it contained dissolved lignin. This was also seen by visual observation of the pulps, shown in Figure II-29, which became lighter in color after the DMSO wash, this effect becoming more pronounced for pulps pretreated at higher loadings, signifying that they contained more re-precipitated lignin. Saccharification results, shown in Figure II-30, show that the introduction of a DMSO washing step significantly enhanced enzymatic digestibility of the pulps. DMSO-washed pulps gave between 47–58% glucose yields, i.e. an increase of 28%–140% compared with EtOH-washed pulps. DMSO washing appears to have been more effective for pulps produced at higher biomass loadings where more lignin had been re-precipitated onto the pulp surface. However, it was noted that glucose yields after DMSO washing still decreased with increasing loading, possibly indicating that re-precipitated lignin may not be the only factor limiting pretreatment efficacy at higher loadings, or that DMSO washing alone was insufficient to remove all lignin from the pulp surface. It should be noted that though DMSO is inexpensive (~\$1/kg)⁷² and fairly non-toxic, it is a non-volatile solvent which would be highly energy-intensive to remove from the pulp surface prior to hydrolysis; addition of a DMSO pulp-washing step in an industrial process is therefore undesirable as it could increase the capital and operational costs of the process considerably. Other solvents, such as recycled ionic liquid, are currently being investigated as alternative washing solvents.



Figure II-29. Pretreated *Miscanthus* pulps obtained before and after washing with DMSO for biomass loadings of 20 wt% and 50 wt%.

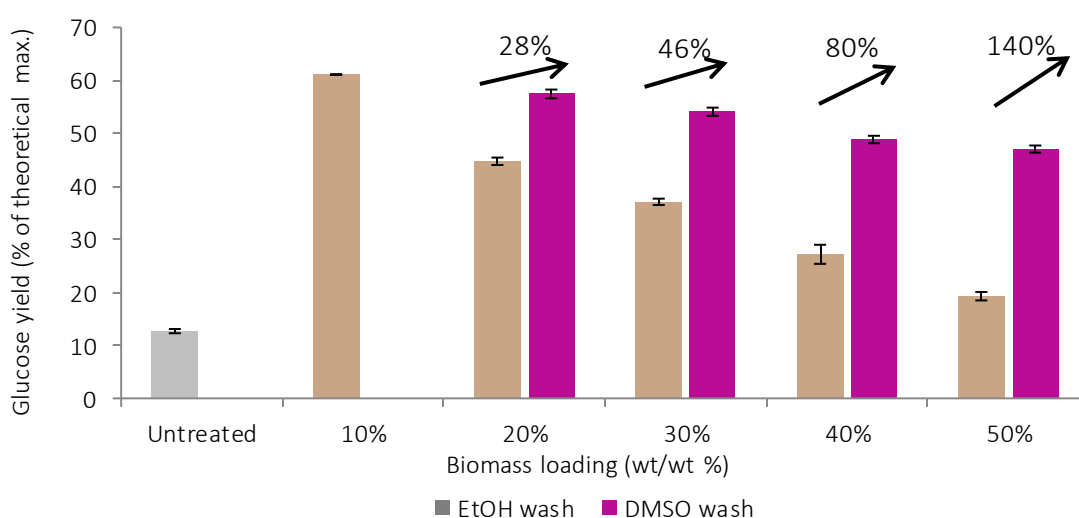


Figure II-30. Glucose yields obtained after four ethanol washes (EtOH wash) and four ethanol washes plus one DMSO wash (DMSO wash) of air-dried pretreated *Miscanthus* pulps at various biomass loadings. Enzymatic hydrolysis was carried out for 7 days and glucose yields are shown relative to the amount of glucan in untreated biomass. Percentage increase following DMSO wash relative to EtOH wash is shown inset.

It is well-known that pulp hornification upon air-drying can reduce enzymatic digestibility⁷³, as seen in Chapter 1:. In an industrial process, pulps would not be air-dried but rather subjected to enzymatic hydrolysis in a wet state. Avoiding hornification is therefore preferable to a DMSO-washing step to increase glucose yields at high solids loading. Pulps pretreated at 50 wt% loading were saccharified without an air-drying step and the influence on glucose yields is shown in Figure II-31. The results indicate that hornification had a greater effect than lignin re-precipitation for air-dried pulps. Introduction of a DMSO washing step increased glucose yields by 50% whereas saccharifying wet pulps increased the yield by 70%. Saccharifying wet pulps after EtOH washing released 66 wt% of glucose, a highly promising result considering the high biomass loading used. Yields were further improved to 74% with addition of a DMSO washing step; however this adds complexity to the process and is therefore unlikely in an industrial scenario. We note that these experiments were conducted at 120 °C for 6 h which is not the optimal pretreatment conditions for glucose release, suggesting further improvements to the yield are possible. However, **these findings indicate that the ionoSolv process could successfully be used to deconstruct *Miscanthus* at biomass loadings up to 50 wt%.**

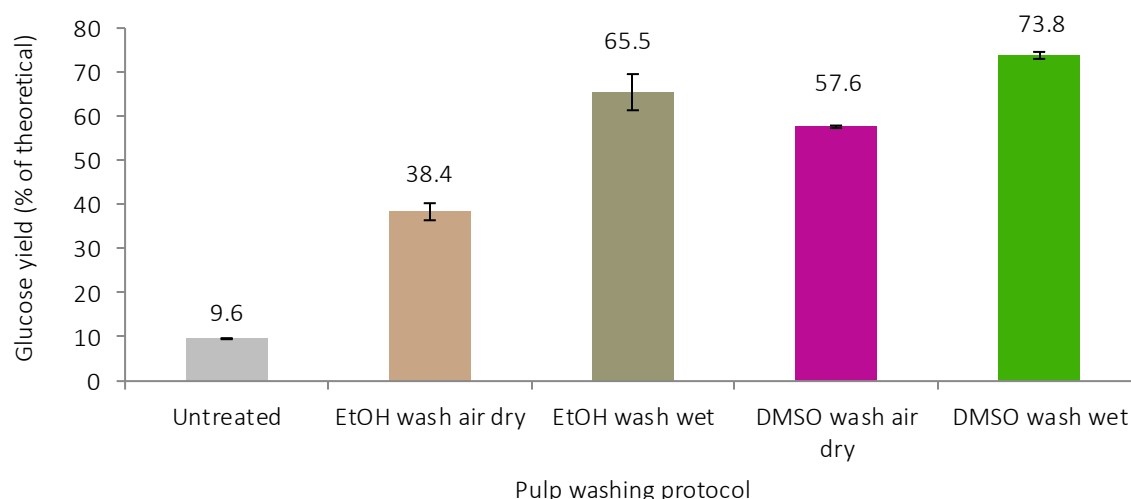


Figure II-31. Glucose yields obtained for air-dried and non-air-dried pulps after both EtOH washing and DMSO washing of pretreated *Miscanthus* pulps at 50 wt% biomass loading. NB. These experiments were conducted for a using a new batch of *Miscanthus* and IL; hence glucose yields for air-dried pulps are different to those presented above. These results are consistent within the batch and were obtained in a single saccharification experiment.

3.4.3 Effect of particle size

As mechanical size reduction is highly energy-intensive and costly, it is desirable to be able to pretreat larger particle sizes to reduce energy costs during biomass processing²⁵. As a next step in the process intensification of ionoSolv pretreatment, larger particles of the energy crop *Miscanthus × giganteus* and softwood *Pinus sylvestris* were pretreated to investigate the effect of particle size on pretreatment efficacy. Three different particle size fractions (coarse, medium and fine) were pretreated; these are shown summarized in Table II-12 along with estimated energy consumption for producing these size-reduction fractions starting from “whole” biomass. Clearly, the estimated energy consumption of grinding to medium and fine particles (the latter being the standard size fraction used for all previous experiments) is high compared to the total ethanol thermal energy theoretically available from biomass itself (2000 kWh ton⁻¹)²³. Actual energy consumption for comminution is dependent on equipment type, size/capacity, biomass moisture content and final particle size^{74,75}, so the values shown are only indicative of relative energy requirements. Optimization of the particle size, in turn, will depend on the pretreatment technology and biorefinery configuration. However, a useful indication was given by Cadoche and López²¹, who suggested that energy inputs for comminution could be kept below 30 kWh ton⁻¹, providing final particle size is held in the range 0.3–0.6 cm.

The effect of different particle size fractions on pretreatment was evaluated for *Miscanthus* and pine using TEA HSO₄ at 20 wt% biomass loading under previously identified promising conditions for high glucose release using fine biomass particles. Pretreatment of *Miscanthus* with dilute acid, a major competing technology, was also evaluated using the same biomass loading and a combined severity factor of 2 under pretreatment conditions also known to give high glucose release (120 °C for 1.5 h using 3 wt% H₂SO₄) for herbaceous biomass such as *Miscanthus*³¹.

Table II-12. Particle size fractions investigated for pretreatment of *Miscanthus × giganteus* and *Pinus sylvestris* and comminution energy consumptions of air-dried biomass by hammer milling, estimated from the literature^{74–76}.

Particle size fraction	Description (<i>Miscanthus</i>)	Estimated energy consumption for size reduction (kWh ton ⁻¹ d.b.) ^a	Description (Pine)	Estimated energy consumption for size reduction (kWh ton ⁻¹ d.b.)
Coarse	• Cylindrical chips with dimension ~3×1×1 cm • Size reduction by manual chopping	30	• Cuboidal chips with dimension 1.5×1×0.8 cm • Size reduction by manual sawing	90 ^a
Medium	• Fibers with dimension ~3×0.02×0.01 cm • Size reduction by manual chopping	60	• Small chips with dimension ~1×0.03×0.02 cm • Size reduction by cutting mill (10 mm sieve size)	130
Fine	• 0.18–0.85 mm (standard size for previous experiments) • Size reduction by cutting mill (2 mm sieve opening size) and sieving	100	• 0.18–0.85 mm (standard size for previous experiments) • Size reduction by cutting mill (2mm sieve opening size) and sieving	240

^a Production of chips itself has been estimated to consume ~600 kWh ton⁻¹ of dry wood⁴³; however it was assumed that waste wood chips may be readily available from timber mills so is not included in the estimated energy consumptions presented here.

The efficacy of pretreating different particle size fractions was evaluated by comparing the pulp and lignin recoveries, pulp delignification and glucose yields released by saccharification, and particle size distribution (PSD) of the pulps. While the enzyme-accessible surface area of the pulp is of most interest for explaining saccharification yields, its measurement is far more complicated than sieving⁴³, which was used as the preferred method to assess PSD changes in this study. *Glucose yields in this section were determined after grinding/sieving all pretreated pulps to 0.18–0.85 mm.*

ionoSolv pretreatment of Miscanthus

The pretreated *Miscanthus* pulps obtained using coarse (10–12 mm), medium (1–3mm) and fine (0.18–0.85 mm) sizes are shown in Figure II-32. *ionoSolv* pretreatment resulted in a dramatic degree of particle size reduction during pretreatment, with a more visible effect occurring for the coarse chips. It was noted that all pulps resembled each other much more than the starting materials, even though the pretreatment reactions were carried out without stirring.

The particle size of samples is a crucial factor in lignocellulose pretreatment because this parameter directly impacts the contact and diffusion of chemicals into the complex interior of the lignocellulose structure³⁶. Figure II-33 shows key pretreatment efficacy indicators as a function of *Miscanthus* particle size. No significant difference was seen in the glucan recovery values for coarse and medium

particles (all ~ 90%), while a slightly greater degree of glucan loss was noted for fine particles (0.18–0.85 mm). This has been previously observed for rice straw pretreatment³⁶ and suggests the possibility of excess acidity in the IL⁶³, which would have a greater effect for fine particles which have higher volumetric surface area and where most parts of biomass particles are exposed to high proton concentration due to shorter diffusion time into smaller particles. As expected, hemicellulose extraction was more effective for finer particles due to greater sugar accessibility.



Figure II-32. Photographs of (a) untreated biomass and (b) recovered pulps after pretreatment of *Miscanthus* with [TEA][HSO₄] at 120 °C for 6 hours at bench scale using a biomass loading of 20 wt%.

Unlike the cellulose recovery, the delignification showed an interesting trend in that it was greatest (60%) for medium (1–3 mm) particles. Coarse particles (10–12 mm) were less delignified due to poor lignin extraction associated with the lower surface area to volume ratio of larger particles; on the other hand, fine particles (0.18–0.85 mm) appeared to have lower delignification than expected, which was presumed to be due to re-precipitation of lignin onto the large pulp surface area. As a result, saccharification yields were higher (77%) for medium than for fine particles (67%). This suggests that the presence of lignin has a stronger negative effect on glucose yields than the positive effect of hemicellulose removal, as was previously noted for ionicSolv pretreatment⁹. Further evidence for the fact that lignin *extraction* is not poorer for fine particles is that the highest lignin recovery was obtained for fine particles, though delignification was limited due to re-precipitation of lignin onto the pulp surface. We note that these results were obtained for size-reduced pulps, which adds extra energy penalty but may be unnecessary for scale-up studies.

Based on these results, the optimum size fraction for unstirred *Miscanthus* pretreatment appears to be 1–3 mm long particles. This is in line with the proposed particle size range of 2–6 mm recommended by Cadoche and López²¹ to minimize comminution energy.

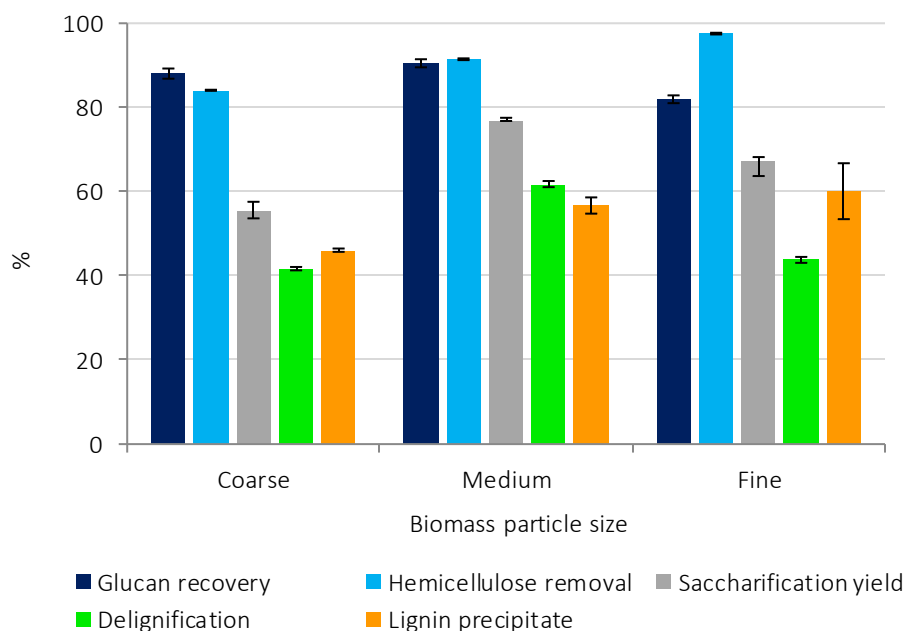


Figure II-33. Effect of particle size of *Miscanthus* on key indicators for ionoSolv pretreatment, after pretreatment with [TEA][HSO₄] at 120 °C for 6 hours at bench scale using a biomass loading of 20 wt%..

ionoSolv pretreatment of pine

Next, the effect of particle size on pretreatment of pine was investigated. Pine possesses a higher density and lignin content (31 vs 21wt%) combined with a low S/G ratio in the lignin compared to *Miscanthus*^{43,77}. These factors are considered to be the reason for its high recalcitrance. The large physical size of woody biomass and strong structure of pine make its size reduction highly energy intensive, which Zhu *et al.* estimated at ca. 10–30% of the bioethanol energy from wood⁴³.



Figure II-34. Photographs of (a) untreated biomass and (b) recovered pulps after pretreatment of pine with [TEA][HSO₄] at 170 °C for 30 min at bench scale using a biomass loading of 20 wt%.

Pine chips (10–15 mm), coarse (1–3 mm) and fine (0.18–0.85 mm) were pretreated at 170 °C for 30 min. From visual observation (see Figure II-34), the extent of deconstruction of pine pulps was considerably less extensive than was seen for *Miscanthus*. This was presumably due to increased recalcitrance of softwoods compared to grasses and also to the pine feedstock used, which is a commercial timber that had been pressure-treated to render it more resistant to chemical and biological attack. Further, while it was attempted to use similar particle size fractions across different feedstocks, it appears pine particles were somewhat larger to begin with than *Miscanthus* particles (see Table II-13), making them more difficult to deconstruct.

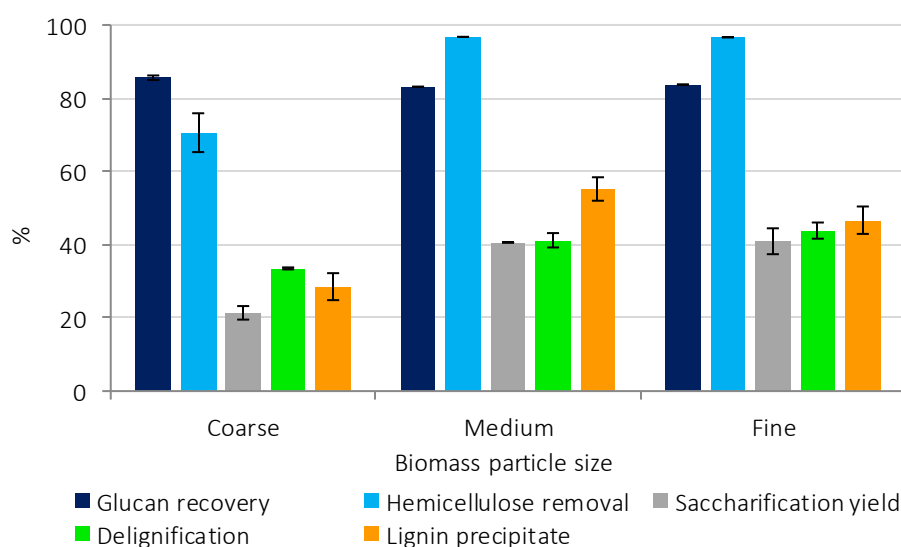


Figure II-35. Effect of particle size of pine on key indicators for ionoSolv pretreatment for pretreatment with [TEA][HSO₄] at 170 °C for 30 min at bench scale using a biomass loading of 20 wt%.

Observing Figure II-35, while glucose recoveries were similar (83–86%) for the different particle sizes investigated, hemicellulose extraction was high except for large pine chips where it was only 70%. In contrast over 84% of hemicellulose was successfully dissolved from all *Miscanthus* pulp fractions analyzed. An increase in pine particle size resulted in poorer delignification; however, lignin precipitate yield was maximal for medium particles (1–3 mm), which can be explained by high lignin extraction combined with limited lignin re-deposition as the surface area of pine chips was moderate. Higher lignin recovery than delignification combined with high hemicellulose extraction for all experiments suggest pseudo-lignin formation, which is not usually observed under these conditions. These observations further add to the suspicion that the ionic liquid batch used contained a slight excess of acid by experimental error; this would have a more pronounced effect for pine experiments conducted at 170 °C than for *Miscanthus* at 120 °C. Glucan digestibility increased in the order of smaller particle size. As observed for *Miscanthus*, a medium size fraction (1–3 mm) appeared to be optimal as saccharification yields were similar to fine particles but larger particles would significantly reduce comminution energy.

These findings suggest that the **pretreatment time may be optimized according to particle size. Larger particle sizes may benefit from longer residence times to extract lignin than small particles, in which lignin removal is faster but lignin re-deposition is also more enhanced.** We would expect the optimum residence time to depend on the lignin removal rate, lignin re-deposition rate, stirring speed, biomass loading (i.e. lignin concentration), IL acidity and temperature. However, this analysis is made highly complex by the fact that the particle size actually decreases during pretreatment as a function of stirring speed, temperature and biomass characteristics, so optimization of the residence time for a given particle size is likely to be empirical.

Comparison of Miscanthus and pine

Chemical pretreatments such as ionicSolv and DA are known to soften biomass structure by removing lignin and to reduce the average particle size compared with the starting materials²³. Researchers such as Zhu *et al.*⁴³ have suggested taking advantage of chemical pretreatments for initial size reduction before conducting post-chemical pretreatment size reduction to produce the fine particles needed for efficient hydrolysis and fermentation. The particle size distribution (PSD) of the pretreated pulps is therefore of interest. The PSD of pretreated *Miscanthus* and pine pulps are shown in Figure II-36 while the corresponding geometric mean particle sizes are found in Table II-13.

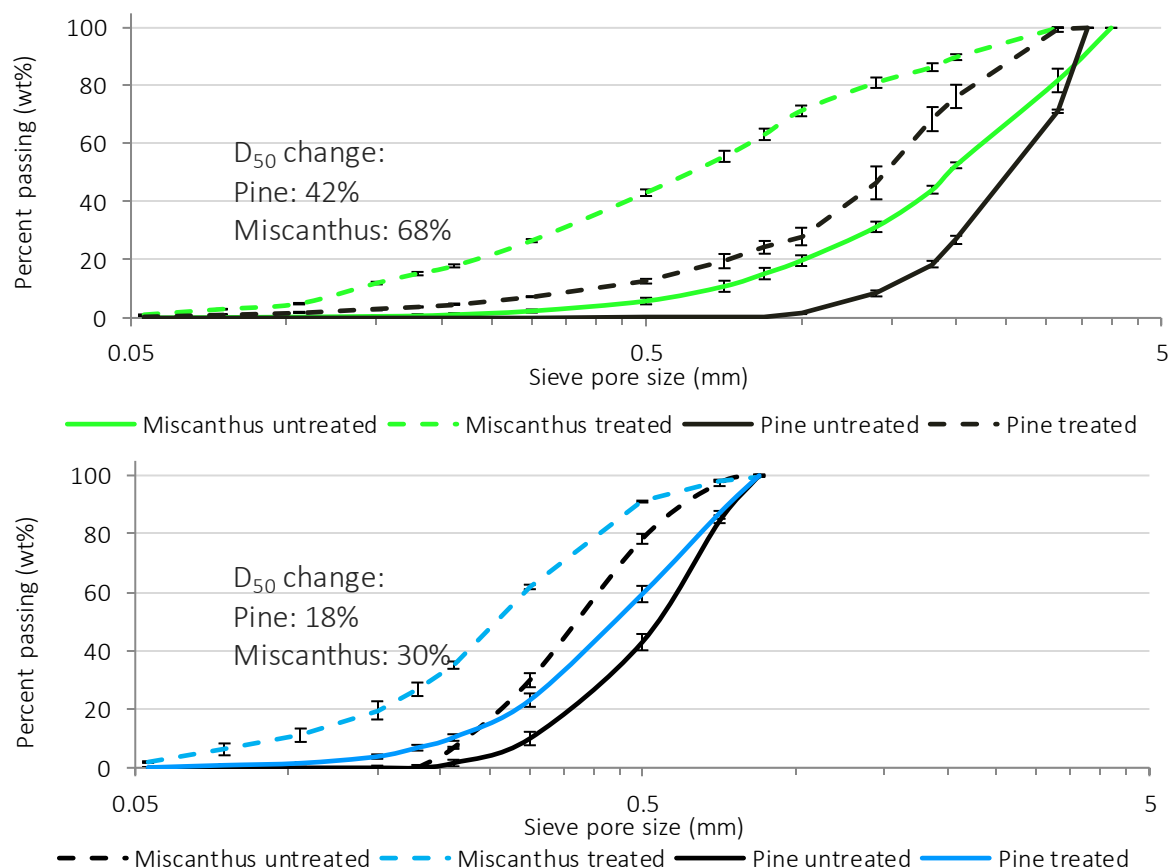


Figure II-36. Particle size distribution plotted as cumulative weight fraction of biomass particles passing through screens of successive sizes. Results are shown for *Miscanthus* and pine medium (above) and fine (below) size fractions before and after ionoSolv pretreatment. Inset values indicate percentage decrease in D_{50} after treatment.

Table II-13. Geometric mean particle size (D_{50}) values obtained for untreated biomass and recovered pulps after ionic liquid and dilute acid pretreatment. Particle size distributions were obtained by sieving. Values in brackets denote the percentage change in D_{50} values of the treated pulp based on the original untreated feedstock.

Geometric mean average diameter D_{50} (mm)	Coarse pulp	Medium pulp	Fine pulp
Miscanthus Untreated	-	1.9±0.1	0.37±0.02
Miscanthus ionoSolv	1.0±0.2 (-)	0.61±0.01 (-68%)	0.26±0.01 (-30%)
Miscanthus DA	-	1.7±0.0 (-11%)	0.4±0.02 (~0%)
Pine Untreated	-	2.5±0.2	0.53±0.01
Pine ionoSolv	-	1.5±0.1 (-42%)	0.44±0.02 (-18%)

Clearly, *Miscanthus* pulps were most size-reduced following pretreatment (30–68% based on the decrease in D_{50} values), as *Miscanthus* can be easily pretreated without the need for considerable size reduction due to its low recalcitrance and low density⁴⁵. Pine pulps also underwent size reduction during chemical pretreatment, though to a lesser degree (18–42%). Size reduction is generally observed following ionoSolv pretreatment as the process alters the chemical composition and physical structure of lignocellulose by partially removing and modifying some cell-wall components (lignin and hemicellulose)⁷⁷. DA, as discussed below, had very limited effect on *Miscanthus* deconstruction (0–

11%) as its function is limited to hemicellulose removal⁷⁸. A stronger size reduction effect was observed for larger particles, though the change in D_{50} for the largest size fraction could not be quantified. Chemical pretreatments appear to be able to size-reduce down to a certain “boundary” in size, which may be linked to the diameter of cellulose microfibril bundles released during hemicellulose and lignin solubilization, as is observed in wood pulps following steam explosion⁷⁹. Fiberization is also reflected in the pulp images for *Miscanthus* which had similar features regardless of initial particle size, characterized by the presence of thin fibers.

Dilute acid pretreatment of Miscanthus

Among the leading pretreatment technologies under commercial development, dilute acid pretreatment (DA), most commonly employing sulfuric acid at concentrations ranging between 1–4 wt%⁸⁰, is generally favored as it is inexpensive, convenient and effective for most herbaceous biomass³¹. In this study, *Miscanthus* particles of different size fractions were pretreated using dilute sulfuric acid (3 wt%) at 120 °C for 1.5 hours; this corresponded to a combined severity factor of ~2. DA led to only a small degree of size reduction with the recovered pulps closely resembling the starting materials (results not shown). This was also evidenced by D_{50} values (Table II-13) which showed very limited size reduction for medium particles and virtually none for fine particles.

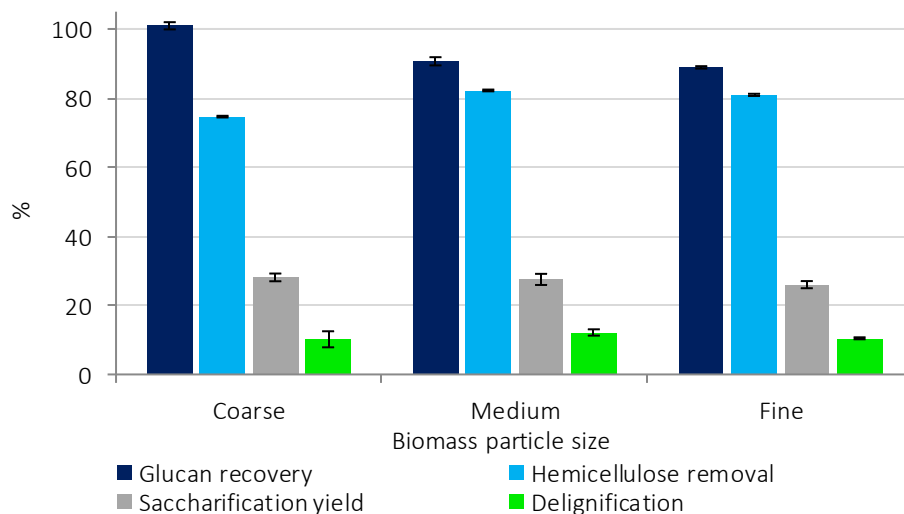


Figure II-37. Effect of particle size on *Miscanthus* key for dilute sulfuric acid pretreatment, after pretreatment with 3 wt% H₂SO₄ at 120 °C for 1.5 hours at bench scale using a biomass loading of 20 wt%.

Based on the key pretreatment indicators shown in Figure II-37, DA removed the majority of hemicellulose and small amounts of lignin, as reported in literature^{78,81}. Hemicellulose removal was slightly improved for smaller size fractions where glucan degradation was also enhanced, reflecting the greater contact with and diffusion of acidic solvent into particles with higher accessible surface area. However, delignification was very low (~10%) for all size fractions, and consequently glucose yields were limited to 28%. This finding is even poorer than findings by Li *et al.*⁷⁸ who reported 48%

digestibility using the same severity factor of 2 when applied to switchgrass, an energy crop with a similar composition to *Miscanthus*. The poor glucan digestibility of DA-treated pulps was attributed to low delignification, which is consistent with the observations of Li *et al.*⁷⁸ Lack of lignin removal resulted in highly limited size reduction of DA-treated pulps, as seen in Figure II-38.

When subjected to ionoSolv pretreatment (dissolution and removal of hemicellulose and lignin), *Miscanthus* exhibited decreased particle size, increased volumetric surface area and decreased lignin content compared to dilute acid pretreatment (hemicellulose removal). **The application of ionoSolv pretreatment for biofuel production appears to offer major advantages over DA, notably the ability to effectively pretreat larger particle sizes.** Furthermore, the high delignification efficiency demonstrated by ILs is promising for recovering lignin as a valuable commercial co-product.

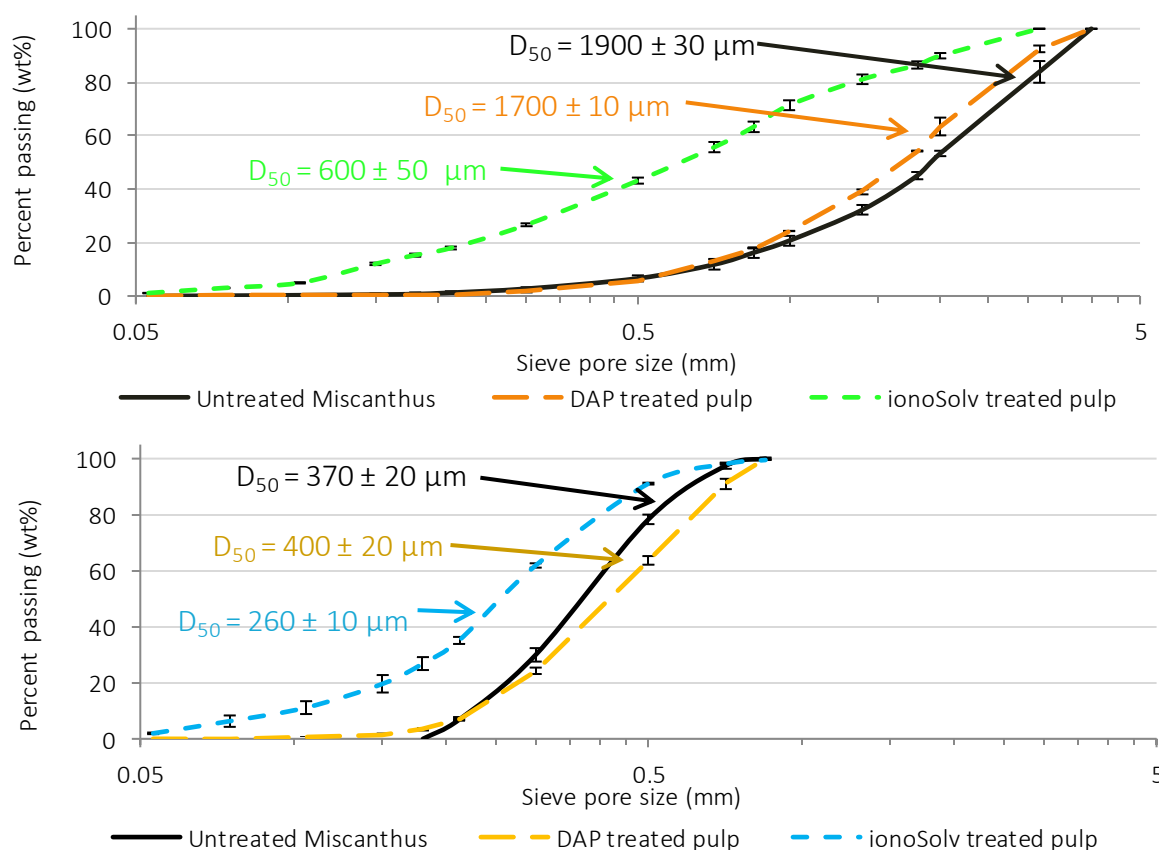


Figure II-38. Particle size distribution plotted as cumulative weight fraction of biomass particles passing through screens of successive sizes. Results are shown for *Miscanthus* treated by ionoSolv and dilute acid for medium (above) and fine (below) size fractions. Inset values indicate geometric mean particle size D_{50} .

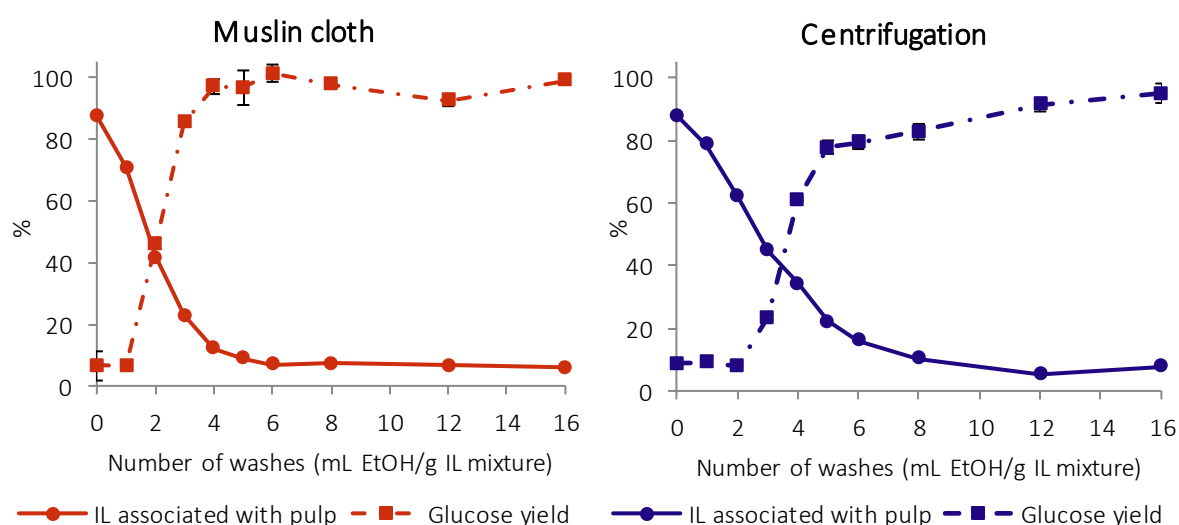
3.4.4 Effect of scale-up

Having successfully demonstrated process intensification at the bench scale by use of high biomass solid loading and larger particle size to minimize size reduction energy inputs, it is now time to turn attention to scale-up of ionoSolv processing. Here, we present the scale-up demonstration of the

ionoSolv deconstruction of energy crop *Miscanthus* by 100-fold, relative to the bench scale (1 vs 0.01-L) at solid loading between 10–20 wt% and using different particle sizes and stirring speeds.

Pulp washing protocol optimization

As highlighted by Li *et al.*⁵⁵ in a study of ionic liquid pretreatment using cellulose-dissolving APIL [Emim][OAc], material handling and pulp washing at larger scales is a critical operation that requires improvement. At the bench scale, the standard operating protocol for ionoSolv pretreatment developed by Gschwend *et al.*⁶⁵ employs four pulp-washing steps of 40 mL of absolute ethanol per 1–2 g of lignocellulose, i.e. approximately 4 mL/g IL mixture per wash or 16 mL/g IL mixture over four washes (at 10 wt% loading). At bench scale, ethanol washing was conducted using centrifugation followed by ethanol Soxhlet extraction in order to achieve good separation, as residual IL adhering to the surface of the pulp may reduce enzyme activity during saccharification, thereby biasing the laboratory-scale analysis⁵⁵. However, at larger scale this becomes impractical due to larger volumes of solvents; therefore, the protocol was adapted for processing of up to ~100 g of pulp at a time. Three methods were investigated for processing at larger scale: namely, muslin cloth straining, centrifugation and vacuum filtration. Each washing step used 1 mL/g ethanol per ionic liquid solvent, up to a total of 16 washes to equal the same total volume of ethanol used at the bench scale. Using multiple washing steps with smaller volumes of ethanol per step is expected to lead to better IL removal from the pulp with lower solvent requirements, as the constant partition coefficient for IL between the wash solvent and pulp is multiplied geometrically over multiple cycles rather than additively over on larger wash steps. To assess this, pretreated IL-pulp was washed using the three methods, the TEA HSO₄ content in the solid fraction was measured to track the efficiency of washing steps, and the pulp samples were subjected to saccharification to evaluate the degree of inhibition by residual IL.



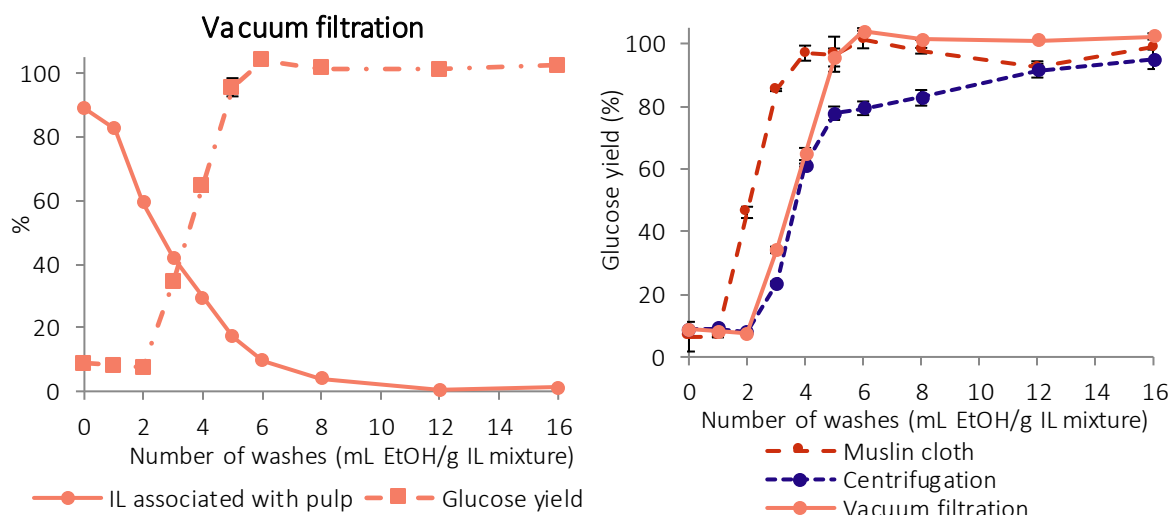


Figure II-39. Amount of IL present and glucose yield obtained from pulp as a function of ethanol washes. Glucose yields shown as a percentage of the maximum glucose yield attained by vacuum filtered pulp after 16 equivalents.

Figure II-39 shows the residual IL content in pulps and corresponding glucose yields upon saccharification of pulps where ethanol washing was conducted with three different methods; images of the pulps obtained after 16 washes are shown in Figure II-40. Straining using a muslin cloth was seen to rapidly reduce IL content initially, giving acceptable digestibility after only 4 washes; however, further washing could not reduce the IL content to below 6%, which could be problematic for downstream processing. Centrifugation required up to 16 washing steps to reduce the IL content to 8% and glucose yields were slow to increase; the pulps obtained appeared clumpy and compressed, which may have limited washing efficacy. Vacuum filtration was seen to be most effective, producing fluffy pulp that may have improved washing efficacy as glucose yields were high after only 6 washes. A steady decrease in IL content to 4% after 8 washes and <0.5% after 12 washes was noted.



Figure II-40. Images of clean pulp after washing using 16 equivalents of ethanol by a) muslin cloth, b) centrifugation and c) vacuum filtration. Miscanthus pulp recovered after pretreatment of *Miscanthus* at 120 °C for 6 hours at 0.5L scale using stirring at 20 wt% loading.

Vacuum filtration with 10 washing steps was therefore deemed sufficient to give high glucose digestibility while offering ~25% ethanol saving compared to the bench scale and eliminating the need for a Soxhlet extraction step prior to pulp saccharification. While this adapted washing protocol

was deemed adequate for scale-up in this study, 10 vacuum filtration steps are highly unlikely to be implemented in an industrial process; instead, filtration as a crude first step followed by a high-efficiency separation process, such as centrifugation, could be more appropriate.

Effect of 100-fold scale-up

Pretreatment of *Miscanthus* at the 1 L-scale was investigated, initially using a fixed stirring speed of 150 rpm under identical pretreatment conditions (120 °C, 6 h). Benchmarks were reported for runs using these conditions without stirring at the 10 mL-scale. 100-fold scale-up showed similar behavior whereby the IL-biomass slurry reacted to produce a dark liquor after the pretreatment reaction (see Figure II-41). Further insights into the effect of scale-up were garnered by comparing (i) pulp and lignin recoveries, (ii) pulp composition and saccharification and (iii) lignin characteristics. The results are presented below for experiments conducted at different biomass loadings (10–20 wt%; >20wt% could not be attempted due to stirrer motor limitations), particle sizes and stirring speeds.

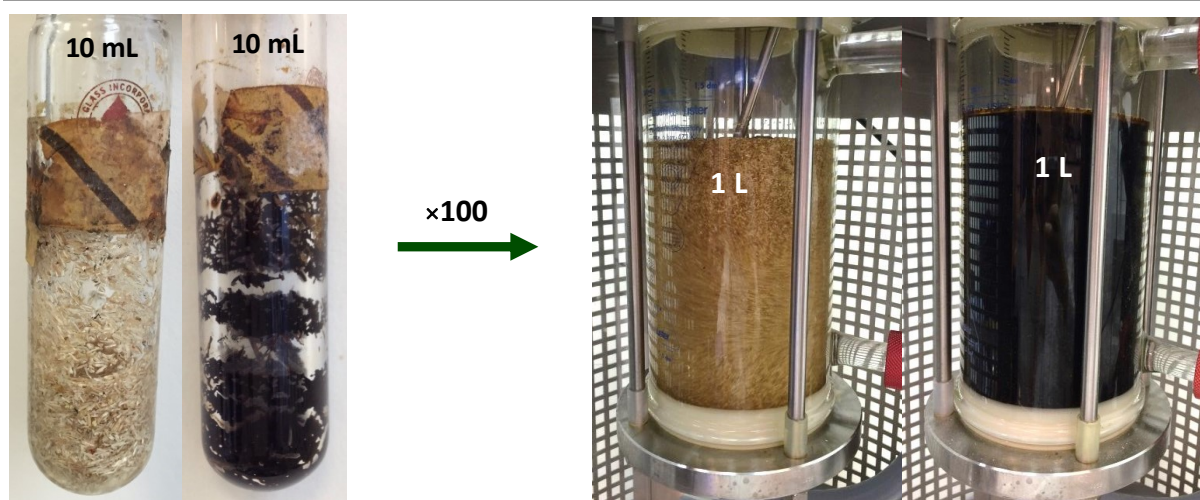


Figure II-41. IL-biomass slurry before/after pretreatment showing 15 mL vessel without stirring used at the 10 mL-scale (left) and 1.5-L glass pressure vessel with stirring used at the 1 L-scale (right). Images not to scale.

Product recoveries and pretreatment effectiveness

Based on the results of 1 L-scale runs for finely ground *Miscanthus* (Figure II-42), 49.7% of the starting material was recovered as cellulose-rich pulp after pretreatment with TEA HSO₄. The pulp recovery is similar to the previous value of 51.6% for *Miscanthus* pretreated at 10 wt% loading at 10 mL-scale. Pulp yield upon scale-up was also slightly lower for 20 wt% loading, with slight differences observed that are likely attributable to the effect of mixing and differences in reaction time at the larger scale. Higher lignin precipitate yields were also seen at the 1 L-scale compared to the 10 mL-scale, suggesting that solubilization of lignin was improved by stirring. Further evidence for this was seen from analysis of the pulp composition, for which key indicators are displayed in Table II-14. For 1 L-scale runs, delignification was slightly higher than at the 10 mL-scale, while hemicellulose removal and glucan recovery were also improved, except at 20 wt% loading where hemicellulose extraction was poorer.

These effects were attributed to improved heat and mass transfer upon the introduction of stirring. Saccharification of the pulps showed improved glucose release for the stirred 1 L runs, which was linked to improved delignification and glucan recovery. Quantitative ionic liquid liquor recovery was obtained upon scale-up due to lower losses at the 1 L-scale.

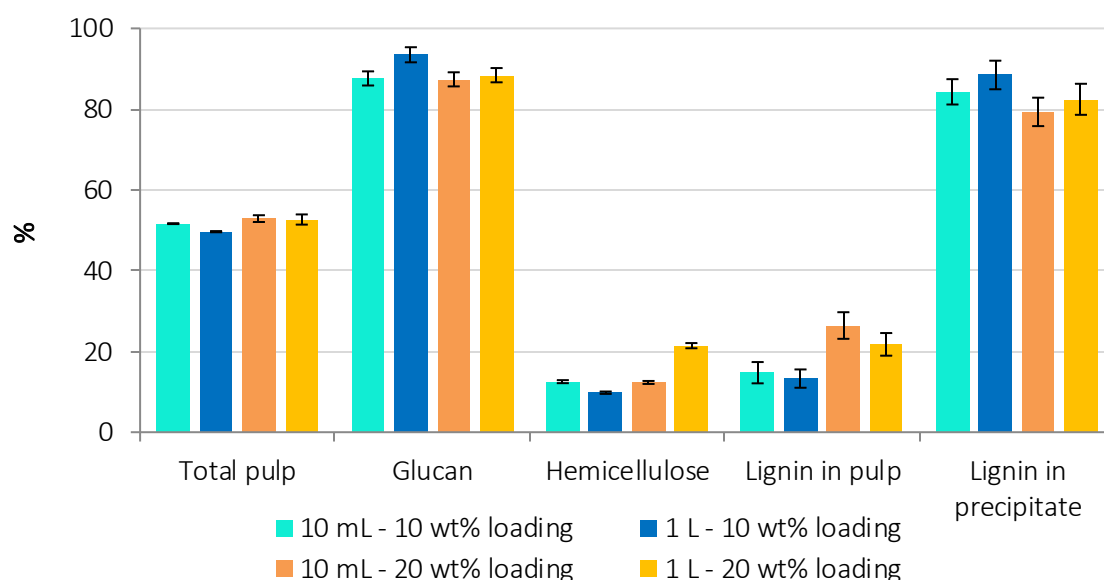


Figure II-42. Comparison of total solids (i.e. pulp), glucan, hemicellulose and lignin recovered in the pulp, and lignin precipitate yield, upon 100-fold scale-up for 10 and 20 wt% solid loading. Results shown as a proportion of component amount in untreated *Miscanthus*.

Table II-14. Comparison of key pretreatment outcomes at 10 mL and 1 L-scale as a function of solid loading.

	Delignification (wt%) ^a	Lignin mass balance (wt%) ^b	Saccharification yield ^c (%)	IL liquor recovery (wt%)
10 mL – 10 wt% loading	85±4	99±6	79.9±0.4	98.7±0.6
1 L – 10 wt% loading	87±2	102±6	79.1±0.5	100.5±0.6
10 mL – 20 wt% loading	74±6	106±7	60.8±3.4	98.3±0.4
1 L – 20 wt% loading	78±3	104±7	63.2±2.7	103.1±0.3

^a Based on feedstock lignin content (AIL + ASL), as determined by compositional analysis of untreated *Miscanthus*.

^b Sum of residual lignin in pulp and isolated lignin yield. ^c Glucose released from pulps without further grinding.

Next, the outcome of pretreatment of different *Miscanthus* feedstock particle sizes was compared for the 10 mL and 1 L-scale. The results (in Figure II-43 and Table II-15) reveal much more pronounced differences in product yields upon scale-up with increasing particle size. Whereas solid recoveries for 10 mL runs increased with 51.6, 61.4 and 69.6% for fine, medium and coarse fractions, at the 1 L-scale the increase in pulp yield was more subtle with 49.7, 51.0 and 59.7% yields obtained. Lower pulp yields were indicative of greater mass loss from the pulp, which can be explained by improved lignin and hemicellulose extraction seen by compositional analysis. Stirred scale-up experiments also gave higher lignin precipitate yields; this effect was again more prominent for larger particle sizes, where the lignin

yield was nearly 2× higher at the 1 L-scale compared to the 10 mL-scale. **These results show that the efficacy of ionoSolv pretreatment was greater upon 100-fold scale-up due to better heat and mass transfer with slurry agitation.** These differences became more prominent for larger particle sizes, as better mass transfer and possible size reduction of *Miscanthus* chips due to the stirrer motion was able to dramatically improve the extent of delignification (69% vs 56%) and lignin precipitate yield (63% vs 32% (Table II-15), resulting in a lignin mass balance close to 100% even for larger particle sizes.

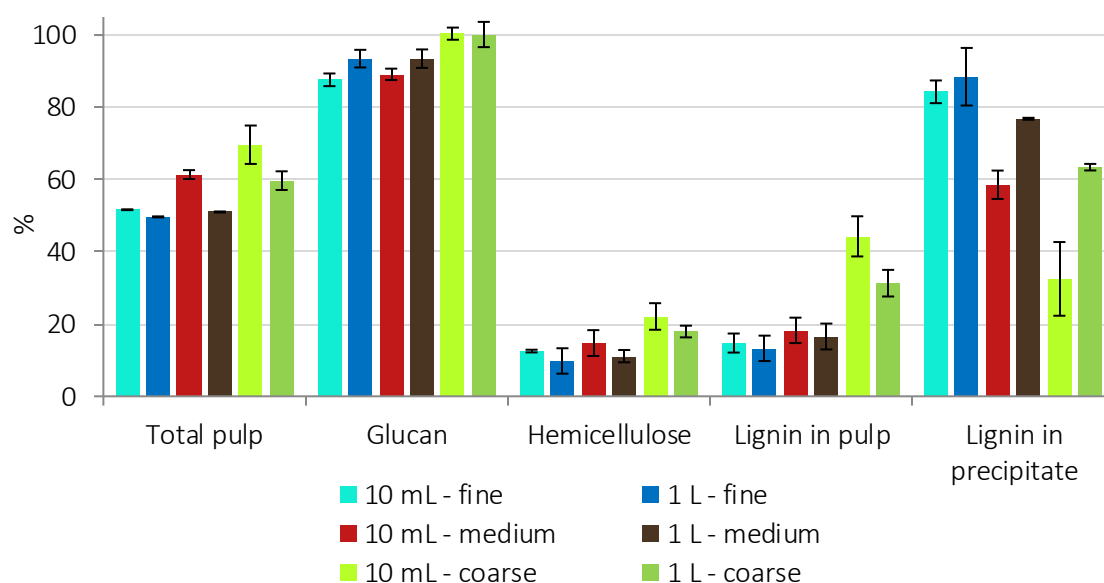


Figure II-43. Comparison of total solids (i.e. pulp), glucan, hemicellulose and lignin recovered in the pulp, and lignin precipitate yield, for fine, medium and coarse particle sizes. Results shown as a proportion of component amount in untreated *Miscanthus*.

The pulps obtained from pretreatment of fine, medium and coarse particle size fractions were subjected to **saccharification without further size reduction**. Whereas glucose yields for fine particles at 1 L-scale compared closely (79%) to results from 10 mL-scale, a slight increase in glucose yield was seen for medium particles at the 1 L-scale (68% vs 66%). Tellingly, saccharification yields improved much more drastically at the 1 L-scale for coarse pulps (59% vs 45%) which was presumed to be due to the effect of improved mass transfer and size reduction of biomass during pretreatment with stirring. High uncertainty (~18% error) in the glucose yield for coarse particles was attributed to particle size heterogeneity of the recovered pulps, which showed a very wide range in PSD. IL liquor recoveries were improved at the 1 L-scale due to larger solvent volumes used; recoveries exceeding 100 wt% is likely due to lignin fragments and other non-volatile extractives remaining in the IL liquor.

Table II-15. Comparison of key pretreatment outcomes at 10 mL and 1 L-scale as a function of particle size.

	Delignification (wt%) ^a	Lignin mass balance (wt%) ^b	Saccharification yield ^c (%)	IL liquor recovery (wt%)
10 mL – fine	85±4	99±6	79.9±0.4	98.7±0.6
1 L – fine	87±2	102±6	79.1±0.5	100.5±0.6
10 mL – medium	82±4	77±7	66.3±6.5	101.6±2.7
1 L – medium	83±4	93±4	68.2±4.8	100.9±0.2
10 mL – coarse	56±6	77±16	45.0±3.6	99.9±0.5
1 L – coarse	69±4	95±5	59.3±10.5	101.8±0.6

^a Based on feedstock lignin content (AIL + ASL), as determined by compositional analysis of untreated *Miscanthus*.
^b Sum of residual lignin in pulp and isolated lignin yield.
^c Glucose yield released from pulps as recovered after ethanol washing (without further size reduction).

Insights from lignin HSQC NMR and GPC

Comparison of the lignin precipitates obtained from stirred scale-up experiments for different biomass loadings and particle sizes afforded further insights into the effects of process intensification at the 1 L-scale with mixing. HSQC NMR results (Figure II-44) revealed differences in lignin ether bond abundance and subunit composition; changes in molecular weight distribution were analyzed by GPC and are summarized in Table II-16.

At the 1 L-scale, the trends observed with increasing loading (Figure II-44) were very similar to those previously reported for 10 mL runs (see Figure II-26a in section 3.4.1). Precipitated lignins isolated at 20 wt% loading showed greater ether cleavage and more condensation as observed by changes in β -aryl ether, G₂ and G_{2,cond} units, respectively. Higher lignin concentrations had the effect of increasing the surface area for lignin re-precipitation onto the pulp, resulting in lower delignification and lignin yield and reducing saccharification yield (from 79 to 66%). However, the extent to which delignification was limited by increasing loading from 10 to 20 wt% was less significant at the 1 L-scale compared to the 10 mL-scale: delignification was only 10% lower while at bench scale a drop of ~25% was seen. Similarly, the effect on glucose yield was less pronounced, resulting in only 17% drop at 1 L-scale compared to 28% at 10 mL-scale. We also note that whereas M_n and M_w decreased with loading at the bench scale, for 1 L runs we observed a slight *increase* in M_w from 4900 to 5000 g/mol and PDI values were higher (4.1) than at bench scale (2.7–3.0). These differences serve to highlight the improvement in ionoSolv processing upon scale-up with stirring. At the 1 L-scale, better mass transfer due to mixing may facilitate extraction and dispersion of lignin fragments in the IL. **With higher loadings i.e. lignin concentrations, there may also be less of a concentration gradient between the pulp surface and contacting IL, which would not only improve extraction but also reduce the level of condensation, thereby reducing re-precipitation. At higher loadings, we would therefore expect stirring to have a larger effect – both in promoting extraction but also possibly in slowing side**

reactions relative to an unstirred reaction. Stirring could thus help to maintain high glucose yields at higher solids loadings, increasing the performance of the process.

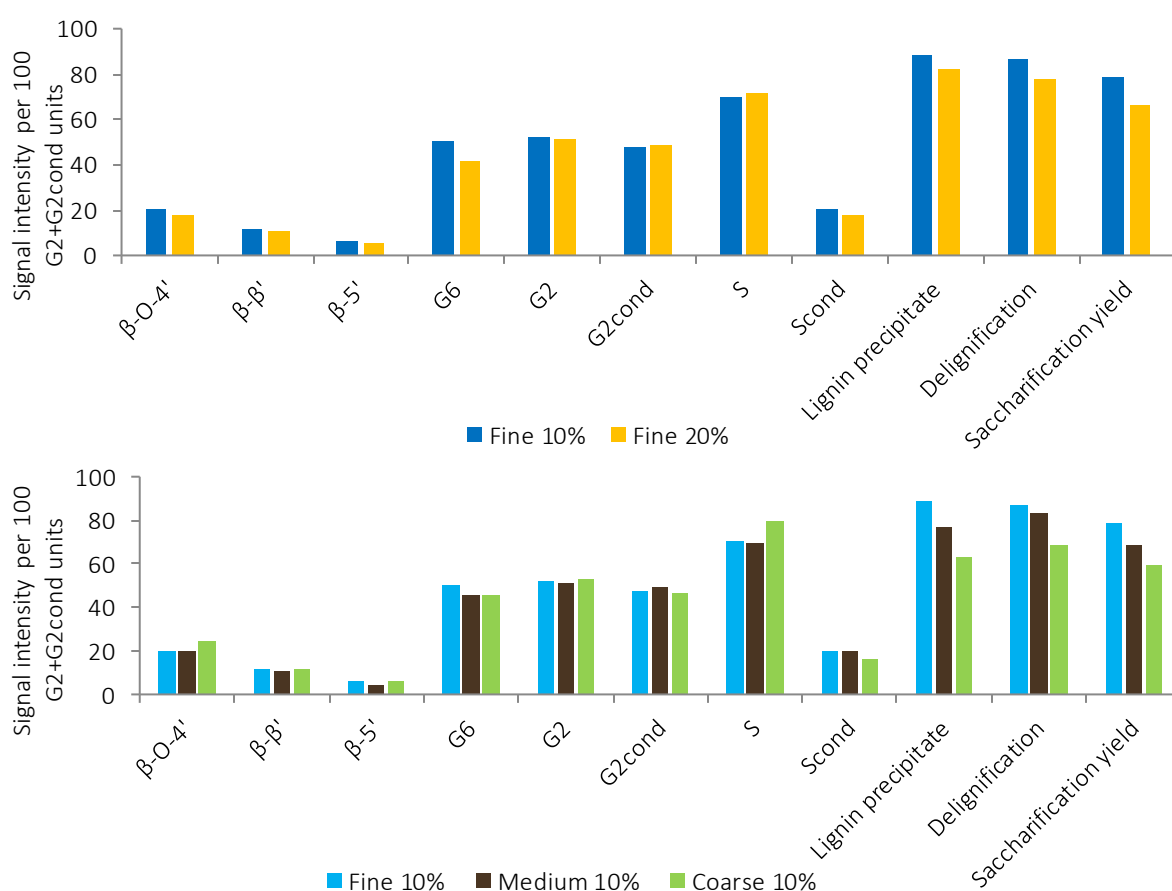


Figure II-44. Relative signal intensities in scale-up lignin precipitates as a function of biomass loading (above) and particle size (below).

Table II-16. Comparison of key pretreatment outcomes and lignin characteristics at the 1 L-scale as a function of solid loading and particle size.

	S/G ratio (-)	Delignifi- cation (wt%)	Lignin yield (wt%)	Saccharifi- cation (%)	M _w (g/mol)	PDI (-)	β -O-4' abundance
Fine – 10%	0.70	87	88.5	79.1	4900	4.1	20.3
Fine – 20%	0.72	78	82.4	63.2	5000	4.1	17.7
Medium – 10%	0.69	83	76.8	68.2	5760	4.4	19.5
High – 10%	0.79	69	63.4	59.3	6500	5.3	24.8

Analysis of lignins isolated from different feedstock particle sizes also allows us to infer differences in lignin extraction and intermolecular lignin reactions. We would expect that for larger particle sizes with lower volumetric surface area, IL diffusion into the particles and lignin diffusion out of the particles would be slower, reducing the rate of lignin extraction from the core of the particles. Moreover, the proton/IL concentration is expected to be lower at the particle core, giving rise to lower severity pretreatment conditions, producing lignins that are less depolymerized and less condensed.

Evidence for lesser extent of depolymerization was seen from increasing β -O-4', β - β' and β -5' linkage abundance in HSQC NMR analysis of lignins extracted from coarse *Miscanthus* chips. Consequently, the precipitated lignins also had higher molecular weight, as observed by GPC (Table II-16) where M_w values increased from 4900 to 6500 from fine to coarse particles. It is suspected that less condensed lignins were released from the core of lignin particles, which was confirmed from the higher G_6 and S and lower $G_{2,cond}$ and S_{cond} signal intensity in HSQC NMR. While less depolymerized/condensed lignins were released from the core of *Miscanthus* chips, smaller lignins would still be released from the surface, and this may be an explanation for increasing polydispersity seen with particle size. Overall, **lignins isolated from larger particles were less condensed with higher S/G ratio, but were also less depolymerized with higher molecular weight and polydispersity.**

Effect of stirring

Previous sections have illustrated the beneficial effect of agitation on ionoSolv pretreatment efficacy. However, the power requirements of mixing are non-negligible and demand optimization of slurry density and viscosity (dependent on solvent, solid loading and particle size), mixing velocity and agitator design¹. An **anchor agitator design** was selected for this study, after testing of other designs including an impeller, propeller and helical coil impeller showed these to be less effective for mixing IL-biomass slurries. The effect of stirring speed on pretreatment outcome was then assessed by conducting experiments in the 1.5-L reactor at a constant speed (150 rpm) and running the same experiments at the maximum speed that could be attained for a given slurry, which was limited by the motor power used to drive the anchor agitator. High (450 rpm) and moderate (400 rpm) speeds were attainable for fine particles and coarse chips at 10 wt% loading, while long fibers used for the medium particle size fraction could only be stirred at up to 250 rpm. It is likely that significant energy from the stirrer motor went into breaking biomass particles apart against the viscous forces; despite different 'maximal' stirring speeds, the amount of energy delivered per second to the IL-biomass slurry upon an increase in mixing speed was the same (i.e. the maximum stirrer power). This investigation thus provided an indication of the effect of increased energy input through stirring on pretreatment efficacy.

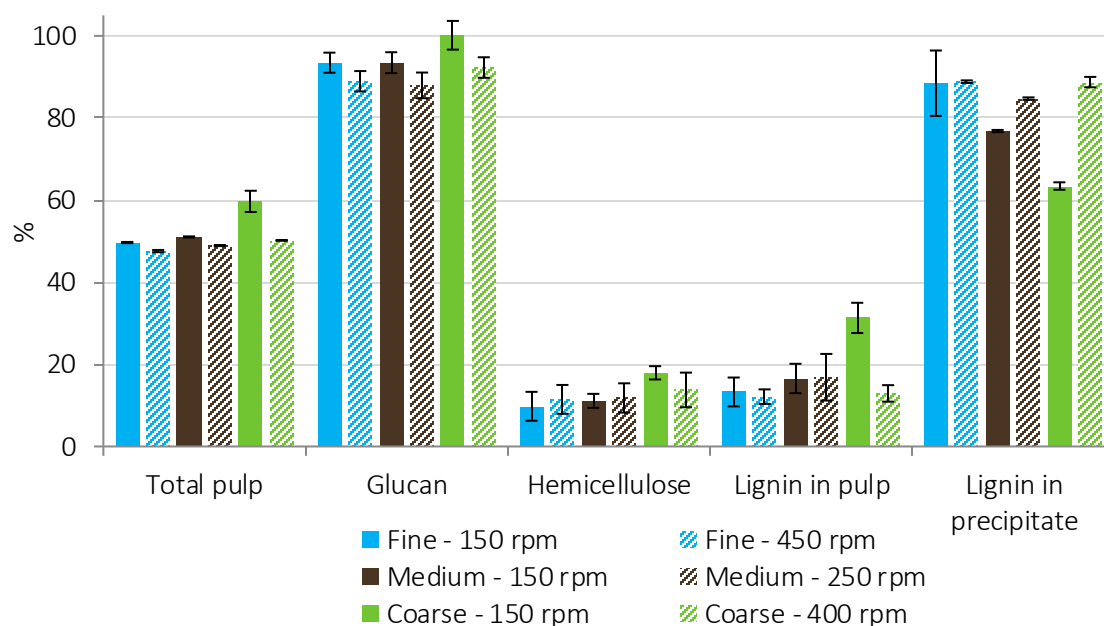


Figure II-45. Comparison of total solids (i.e. pulp), glucan, hemicellulose and lignin recovered in the pulp, and lignin precipitate yield, for fine, medium and coarse particle sizes as a function of stirring speed. Results shown as a proportion of component amount in untreated *Miscanthus*.

The product yields obtained (Figure II-45) show that an increase in stirring speed resulted in slightly lower pulp yield and higher lignin precipitate yield, suggesting improved lignin extraction. These effects became more prominent with increasing *Miscanthus* particle size. However, analysis of the recovered pulps revealed that pulp compositions were not significantly affected, though a slight decrease in glucan recovery and improvement in delignification were observed. Saccharification of the pulps produced surprising results, shown in Table II-17: increasing the stirring speed did *not* significantly affect saccharification yields. The exception was for the fine fraction, where glucose yield dropped drastically (from 79.1% to 66.1%) upon increasing stirring speed from 150 to 450 rpm. It is believed that more rapid lignin extraction may take place with greater mixing due to improved heat and mass transfer; however, as fine particles have higher volumetric surface area, this increased the available pulp surface for lignin re-precipitation. This effect may also have been worsened by particle size reduction during pretreatment, validated by the PSD curves shown in Figure II-46, which show smaller average particle size of the pulps with faster mixing. Consequently, the increase in lignin extraction with higher mixing speed was countered by more lignin sticking to the pulp surface, so that overall delignification of the pulp remained approximately constant with stirring speed (Table II-17). However, for the coarse particle size fraction, increasing the stirring speed from 150 to 400 rpm improved delignification (from 69% to 87%) though glucose yields were very similar (59%). At lower mixing speeds, lignin extraction is poorer but re-precipitation is also limited, while at higher mixing speeds it is strongly suspected that size reduction of the pulps due to the effect of faster mixing may have increased *Miscanthus* accessibility and hence also both lignin extraction and re-precipitation.

These results indicate that mixing speed should be optimized depending on the particle size of the

feedstock, as optimal outcomes are influenced by lignin extraction and lignin re-precipitation, both of which were favored by higher agitation rates, though faster mixing did not increase glucose yields. However, it was noted that the pretreatment conditions employed in this study were previously optimized based on results for the benchmark scale. Pretreatment outcomes could benefit from re-optimization for larger scale processing with mixing. The use of high stirring speeds at elevated temperatures for short residence times could help to maximize delignification and avoid prolonged contact of the pulp with the liquor to reduce lignin re-precipitation to achieve high glucose yields and minimize inhibitor formation.

Table II-17. Comparison of key pretreatment outcomes for pretreatment at 10 mL and 1 L-scale as a function of stirring speed between 150 rpm and maximal stirring speed that can be achieved (250 – 450 rpm).

	Delignification (wt%) ^a	Lignin mass balance (wt%) ^b	Saccharification yield (%)	IL liquor recovery (wt%)
Fine – 150 rpm	87±2	102±6	79.1±0.5	100.5±0.6
Fine – 450 rpm	88±2	101±2	66.1±4.6	99.2±0.6
Medium – 150 rpm	83±4	93±4	68.2±4.8	100.9±0.2
Medium – 250 rpm	83±6	93±6	67.8±3.0	102.0±0.2
Coarse – 150 rpm	69±4	95±5	59.3±10.5	101.8±0.6
Coarse – 400 rpm	87±2	102±3	59.0±7.4	100.7±0.6

The PSD determined by sieving for *Miscanthus* pulps before and after pretreatment with varying stirring speeds are shown in Figure II-46. Calculation of D_{50} values revealed that faster mixing gave rise to greater *in situ* size reduction, producing pulps with lower average particle size and hence higher volumetric surface area. Geometric mean length decreased dramatically during ionoSolv pretreatment, with D_{50} values decreasing by 40, 91 and 96% for fine, medium and coarse particles, respectively. Unsurprisingly, the decrease in D_{50} was greater than for unstirred experiments at the bench scale (Figure II-36), which were 40 and 68% respectively for fine and medium particles. These findings indicate that *in situ* size reduction of *Miscanthus* chips due to combined effects of agitation and ionoSolv deconstruction may reduce the need for mechanical grinding prior to pretreatment.

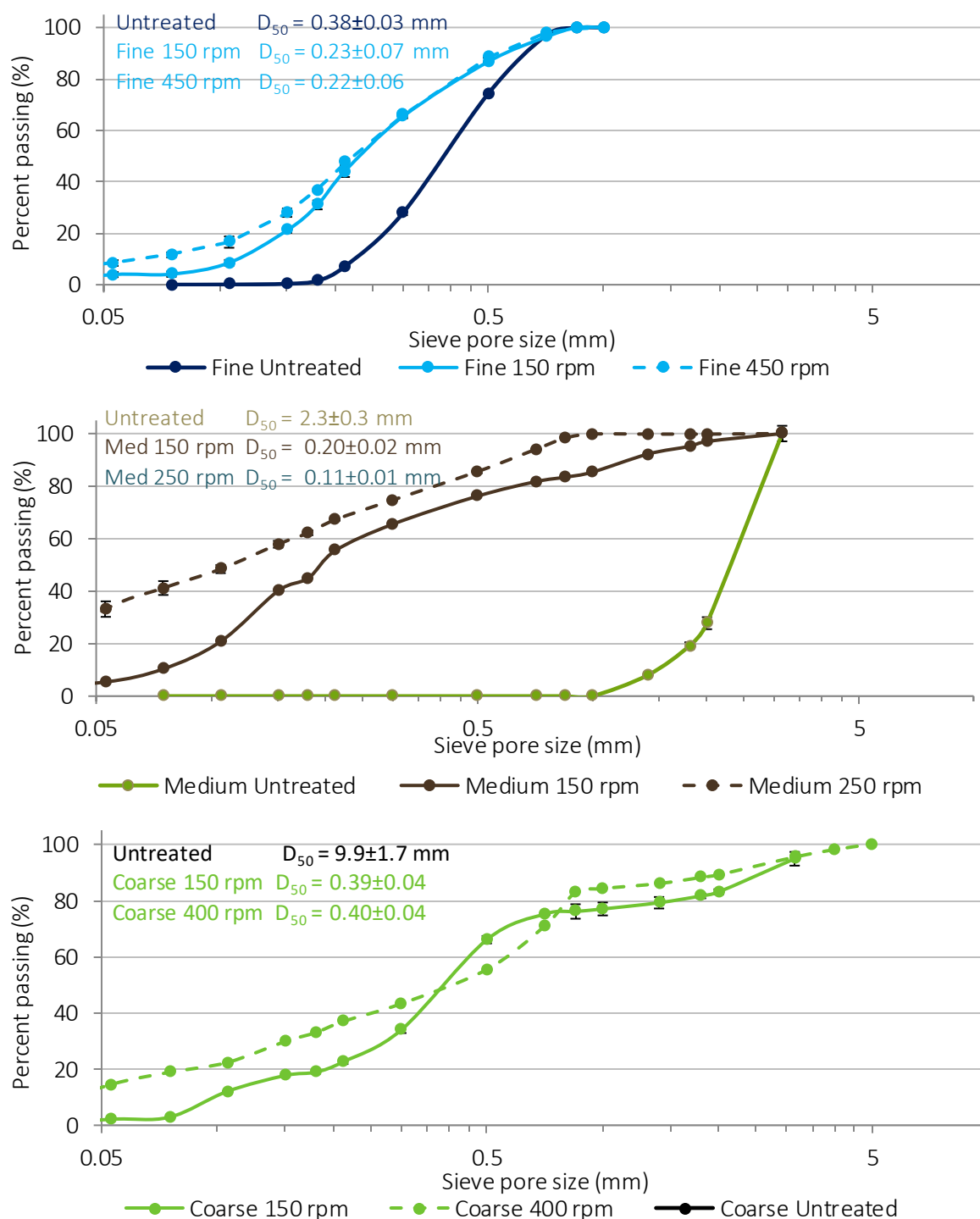


Figure II-46. Particle size distribution plotted as cumulative weight fraction of biomass particles passing through screens of successive sizes. Results are shown for fine (above), medium (middle) and coarse (below) size fractions of *Miscanthus* pretreated at different stirring speeds. Inset values indicate geometric mean particle size D_{50} .

The lignin precipitates recovered from 1 L-scale experiments at different mixing speeds were analyzed by HSQC NMR and GPC. Only subtle differences in lignin characteristics were observed. However, in all cases, faster mixing speeds produced lignins with a slightly lower abundance of β -O-4' ether linkages, suggesting it is more cleaved, possibly due to a higher probability of two species encountering each other and reacting with better mass transfer. The slight increase in signal intensity

for G₆ units seen from HSQC NMR, combined with lower amounts of G₂ and higher G_{2,cond} subunits, suggest that lignins extracted using faster mixing were also more condensed. The implication, based on analysis of the lignin yields and structure, is that faster mixing led to a transition from diffusion controlled to kinetically controlled reactions, leading to more condensation. GPC analysis of the lignins (Table II-18) showed that an increase in mixing speed produced lignin precipitate with *lower* molecular weight, which may be due to more condensation leading to greater likelihood of lignin macromolecules precipitating onto the (increased) pulp surface area with faster mixing. The differentiated results in lignin yield, delignification and lignin MW depending on particle size (i.e. volumetric surface area) illustrate the combined effects of lignin reactivity (producing more condensed lignins) and lignin re-precipitation (reducing the proportion of high MW lignins remaining in solution until the water addition step), both of which appear to become enhanced with stirring speeds. In summary, faster mixing seems to have increased lignin reactivity, accelerating condensation reactions and producing lignins with lower S/G ratio. However, *in situ* size reduction of pulps with faster mixing that increased surface area is strongly suspected to have increased re-precipitation of high MW lignins, with the resulting lignins remaining in solution during the ethanol wash having lower MW and polydispersity, but with adverse effects on delignification and hence glucose yields. **Given the high power requirements of stirring¹, it appears that minimal stirring (150 rpm) is preferable and could even be further reduced to minimize energy requirements.** Evidence in support of this, in the form of a preliminary energy assessment, is presented in the Appendix.

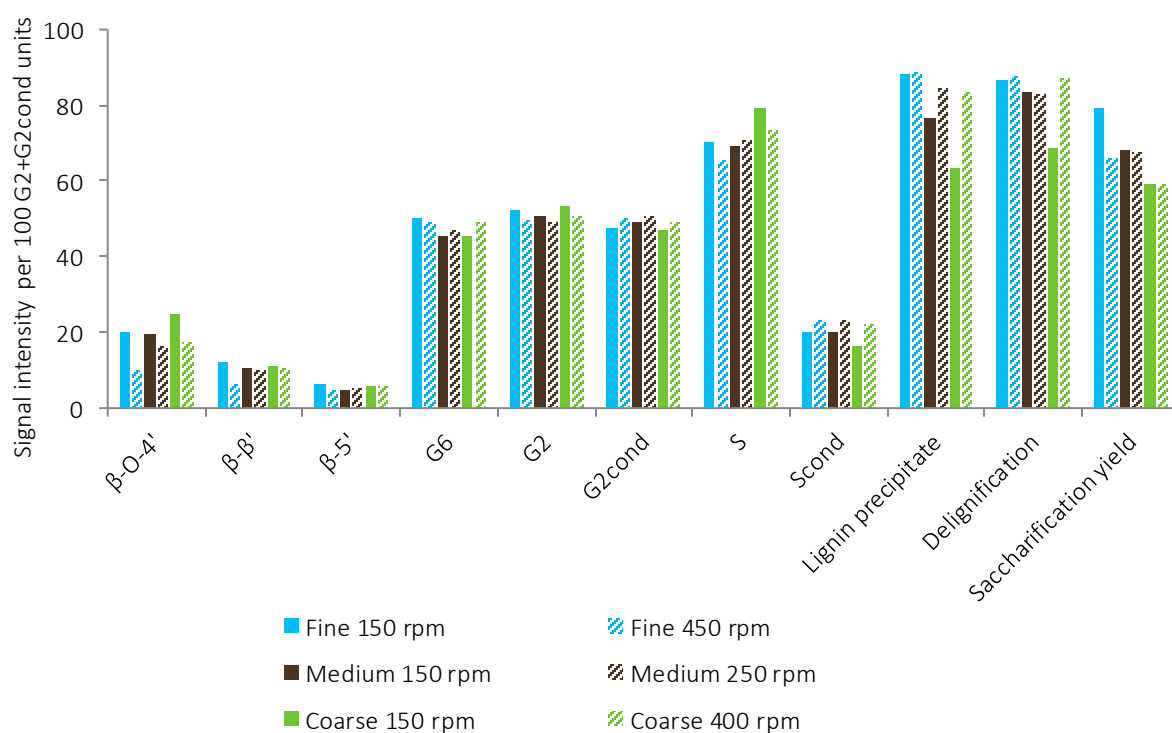


Figure II-47. HSQC NMR signal intensities of different lignin C-H units relative to 100 G₂+G_{2,cond} units, lignin yield and delignification for lignins isolated from fine, medium and coarse *Miscanthus* particles using different mixing speeds.

Table II-18. Effect of mixing speed on key indicators of pretreatment and lignin characteristics from *Miscanthus*.

	Stirring speed	Delignification (wt%)	Lignin yield (wt%)	Saccharification (%)	M _w (g/mol)	PDI (-)	S/G ratio (-)	β-O-4' abundance
Fine	Low	87	88.5	79.1	4900	4.1	0.70	20.3
	Fast	88	88.8	66.1	4620	3.7	0.66	10.2
Medium	Low	83	76.8	68.2	5760	4.4	0.69	19.5
	Fast	83	84.7	67.8	5720	5.4	0.71	16.5
Coarse	Low	69	63.4	59.3	6500	5.3	0.79	24.8
	Fast	87	83.7	59.0	5610	4.7	0.74	17.4

3.5 Conclusions and Future Work

Process intensification of the ionoSolv process was investigated at the 10 mL-scale in unstirred pretreatment tubes using the low-cost PIL [TEA][HSO₄]. The ionoSolv process was shown to successfully pretreat *Miscanthus* at solid loadings up to 50 wt%. Glucose yields decreased from 68% to 23% upon five-fold increase in loading from 10 to 50 wt%, which arose due to re-precipitation of the extracted lignin onto the pulp surface under higher lignin concentrations. Saccharification yields were improved to 66% by omitting the air-drying step and instead saccharifying wet pulps to reduce fiber hornification. Investigation of pretreatment of larger *Miscanthus* particle sizes from 0.18–10 mm was investigated with a view to reducing comminution energy. In unstirred conditions, the use of particle sizes of 1–3 mm was preferable over fine powders (0.18–0.85 mm) to reduce the surface area available for lignin re-precipitation, giving higher glucose yields while minimizing grinding energy needs. Comparison with dilute acid pretreatment applied to the same *Miscanthus* size fractions showed ionoSolv processing is far more tolerant to use of larger particle sizes.

100-fold scale-up (from 10 mL to 1 L) of ionoSolv pretreatment technology was demonstrated on *Miscanthus* after selection of optimal pulp washing protocols and agitator design. No drop in performance was seen as a function of scale-up; on the contrary, efficacy of ionoSolv pretreatment at the 1 L-scale was greater due to improved heat and mass transfer with slurry mixing. The introduction of stirring gave rise to higher delignification, reduced lignin condensation and improved lignin mass balance, notably for coarse particles (10–12 mm). Doubling of the solid loading at the 1 L-scale from 10 to 20 wt% showed reduced lignin re-precipitation onto the pulp surface than at the 10 mL-scale, giving higher glucose yields. Ionic liquid liquor recovery rates were found to be >99.2% in all cases, with excellent potential for recycling of the IL solvent. Substantial cost savings could be realized by tailoring the pre-processing size reduction stages to an individual feedstock.

The results suggest that stirred ionic liquid pretreatment offers great potential for industrialization and further process intensification by selection of optimum pretreatment conditions (temperature, residence time, stirring speed), particle size and biomass loading depending on the feedstock. Improved mixing could also reduce reaction times, thus increasing the space-time yield of the ionic liquid process. There is a need to further improve the pulp washing protocols to reduce the incidence of lignin precipitation in ethanol at high lignin concentrations and to reduce the water requirements of lignin washing. Further work to evaluate the effect of residual ionic liquid in the pulp on downstream processes including fermentation is also necessary to arrive at an integrated conversion process attaining high ethanol yields. Integrating these process parameters into improved process models of the ionic liquid process could enable identification of the remaining bottlenecks to achieve economic viability and estimate the life cycle greenhouse gas savings of scaled-up ionic liquid-based biorefining.

3.6 References

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Chapter 4: Lignin Fast Pyrolysis

4.1 Introduction

Lignin is nature's dominant source of aromatics and constitutes between 5 and 40 wt% of the dry weight of lignocellulosic biomass¹. The valorization of lignin holds opportunities to produce carbon fibers², polymers³, aromatic commodity chemicals (such as benzene or phenols)⁴ and fuels⁵. Pyrolysis, specifically fast pyrolysis, is a promising way to depolymerize lignin to obtain valuable chemicals^{6,7}. In this process, the various bonds within the lignin polymer are broken down to yield a mix of over 300 compounds⁸. Thermally-induced decomposition of lignin is thought to proceed *via* the initial formation of radicals^{9,10}, which can recombine easily and, in time, form undesired char. The liquid product of fast pyrolysis is bio-oil, which is thermally unstable and corrosive, particularly when derived from biomass¹¹.

Much research has been devoted to improvement of the quality of the products of pyrolysis, such as by upgrading the bio-oils by hydroprocessing or zeolite upgrading to produce components for transportation fuels^{8,12-17}. Another common approach is to attempt to increase liquid yields by using catalytic fast pyrolysis¹⁸⁻²³. Among the possible root causes of instability of bio-oils is the presence of water-soluble compounds such as organic acids and aldehydes, mostly originating from degradation of the oxygen-rich carbohydrates in biomass^{8,11}. Therefore, pyrolyzing polysaccharide-free lignins, which have lower oxygen content than biomass, is desirable.

The IonoSolv pretreatment process has previously been shown to enable control of lignin structure and, in particular, oxygen content²⁴, by careful selection of lignin isolation conditions. We therefore

investigated the link between IonoSolv pretreatment residence time and temperature, lignin structural and thermal characteristics, and the quality and yield of pyrolysis products. The results are presented in three sections: lignin characterization, pyrolysis product yields and characterization.

4.2 Background

4.2.1 Fast pyrolysis of biomass vs lignins

Though fast pyrolysis processes are well-established for conversion of whole biomass¹⁴, such as agro-industrial residues^{25–27}, energy crops²⁸ or wood^{11,29–33}, the study of lignin pyrolysis is more recent, spurring increasing research interest with the advent of abundant lignin streams from biorefineries^{4,11,34–42}. Biomass bio-oils are characterized by their acidity (pH ~2.5), high water content and high oxygen content, and they age after they are first produced¹¹. Phase separation is observed, alongside an increase in viscosity with time and temperature^{11,43}. Fast pyrolysis of whole biomass produces bio-oils with higher water content (15–30 wt%)¹⁵ than lignin bio-oils (3–15%)^{44,45} as dehydration reactions occur significantly during the decomposition of carbohydrates, which account for three-quarters of biomass dry weight. As a result, lignin bio-oil presents higher heating value (HHV) (about 30 MJ kg⁻¹)^{5,46} than biomass pyrolysis bio-oil (about 16–19 MJ kg⁻¹)¹⁵. Hemicellulose in biomass can contain as much as 10% acetyl substituents, resulting in the production of large amounts of acetic acid⁷. The presence of organic acids is thought to contribute to bio-oil degradation, as acids could work as catalysts to accelerate polymerization reactions between bio-oil compounds⁸. Though small quantities of acids are found in lignin bio-oils (0.3–0.9 wt%)^{6,46}, total acids are much more concentrated in biomass bio-oils (7–12 wt%)⁴⁷ as they derive mainly from decomposition of carbohydrates as well as from acetates and coumarates in lignin and hemicellulose, which are removed during pretreatment^{8,11}. High water and acid content are detrimental to bio-oil quality.

The presence of oxygen is believed to be a major reason for the difference in stability between biomass pyrolysis oils and hydrocarbon fuels. As lignin has a lower oxygen content (22–32%)⁶ than biomass (35–40 wt%)¹¹, lignin-derived pyrolysis oils contain fewer oxygenated compounds⁴⁶. An ability to tune the oxygen content of lignin feedstocks is therefore a potential parameter to reduce the water content and acidity and produce bio-oils with a higher energy density and improved thermal stability compared to biomass-derived pyrolysis oils. Based on these assertions, it can be theorized that lignin is potentially a more desirable feedstock for thermochemical conversion compared to biomass. Isolation of lignin from lignocellulose is therefore a crucial step in conversion of biomass into bio-oils for production of chemicals and fuels²⁴.

4.2.1 Lignin pyrolysis for aromatic chemicals production

Interest in lignin as a pyrolysis feedstock is motivated not only by the possibility of improving bio-oil properties, but also for production of aromatic chemicals, notably lower molecular weight compounds such as monophenols or BTX that are highly valuable^{9,48–50}. Amen-Chen *et al.*⁹ and Zakzeski *et al.*¹⁰ have suggested that free-radical depolymerization and fragmentation reactions during pyrolysis result in formation of monomeric phenol compounds. Kosa *et al.*⁴¹ report that lignin pyrolysis oils can contain 40–70 wt% of aromatic structures. Py-GC-MS studies carried out by Lin *et al.*⁵¹ indicated that phenols were the most abundant products from pyrolysis of cellulolytic enzyme lignin and alkali lignin isolated from two different agricultural residues at temperatures above 450 °C. Not only phenolic monomers but also phenolic dimers have been shown to make up a significant proportion of primary products during lignin pyrolysis^{52–54}; however, the separation and upgrading processes are complex and costly. While efficient conversion of lignin to value-added low molecular-weight aromatic compounds represents a pathway to highly profitable lignocellulosic biorefineries in the future, in the near-term, it is likely to be more cost-effective to focus on production of a blended fuel component, such as for the marine or automotive industries⁵⁵.

4.2.2 Effect of lignin structure on thermal decomposition

Lignin thermal decomposition is known to be influenced by a number of parameters, including pyrolysis temperature, reactor type, heating rate, residence time and feeding rate, and lignin characteristics^{6,7,35,52,56–58}; this study focuses exclusively on the latter, with all other pyrolysis conditions remaining the same in all experiments conducted.

Pyrolysis product distribution is highly dependent on the properties of the lignin polymer, notably according to the biomass source^{59–62}, part of the plant (e.g. stalk or leaf)⁶³, and processing conditions^{6,24}. The chemical structure of lignin is defined by the proportion of the three phenylpropane units (H, G and S); presence of various functional groups (e.g. hydroxyl and methoxyl) and elemental composition of lignin (notably O/C and H/C atomic ratio). Numerous studies have reported that the most significant structural parameter affecting lignin pyrolysis products was the abundance of the β -O-4' aryl ether linkage^{60,64,65}. Ether bond cleavage is widely associated with depolymerization of lignin, but the electrophilic carbocation formed by ether cleavage may also react with the adjacent aromatic ring to form condensation products^{5,66}. Other functional groups with significant influence on product yield are OH and OCH₃ groups, which have been correlated with the total volatile products formed⁶⁷. Another study found that OH and OCH₃ groups strongly affect bio-oil yield since radicals containing these side groups are easily polymerized to form char⁶⁸. The presence of functional groups is also determined by the relative proportions of phenylpropanoid sub-units, further influencing the chemical composition of bio-oil. Py-GC-MS experiments using S-rich lignins, which have less crosslinked

structures relative to G-rich lignins, have shown that these structures are more reactive and produce a lower char and higher liquid yield^{36,60,69}.

The elemental composition of lignin is also known to affect pyrolysis product distribution⁷. During industrial wood pulping, the introduction of sulfur into lignin samples, such as in Kraft lignin or lignosulphonates, can significantly lower bio-oil yield during pyrolysis⁶⁴. Technical lignins are a complex mixture of unconverted carbohydrates, lignin, minerals and process chemicals; they typically give low conversion rates and produce characteristic organic sulfur compounds upon pyrolysis and are therefore not desirable feedstocks^{41,54,70}. Sulfur-containing compounds are inclined to polymerize to form char, which also gives rise to lower aromatic hydrocarbon production^{46,71,72}. Bio-oil quality is frequently compared using the effective hydrogen index ratio (H/C_{eff}) introduced by Chen *et al.*⁷³, whereby increasing lignin H/C_{eff} increased bio-oil yield significantly during fast pyrolysis processes. H/C_{eff} also impacted the yield of aromatic products during non-catalytic fast pyrolysis, which were linked to higher hydrocarbon production⁷¹. Higher oxygen content in lignins is suspected to affect not only bio-oil stability, as discussed previously, but also product yields. This is because oxygen content could arise from higher content of reactive groups, such as hydroxyl and methoxyl groups, which are favorable for char formation and adverse to bio-oil production^{7,74}. Finally, the purity of lignin is a critical influence, especially due to presence of mineral compounds in ash which could present a catalytic effect on lignin conversion⁷⁵. Potassium, followed by sodium, was shown to have the highest catalytic activity, contributing to the reduction in liquid yield and increasing formation of gas, water and char^{27,76,77}.

There is no consensus on the impact of lignin molecular weight on pyrolysis characteristics^{78,79}. Lignin fractionation prior to pyrolysis has been used to gain insight into its impact^{78,79}. Guo *et al.*⁷⁸ found that lower MW had the effect of increasing the yield of aromatic products and CO₂ due to easier cracking and demethoxylation of the lignin molecule, while phenol yield was reduced. A similar study using alkali lignins fractionated by selective pH precipitation showed that lignin fractions with higher MW also produced more char and monomeric phenols, agreeing with Brandt *et al.*'s previous findings using ionoSolv lignins of varying MW^{24,79}. Overall, MW changes were difficult to divorce from changes in lignin chemical composition, though higher MW could be loosely linked to higher HHV, hydroxyl content and lower O/C ratio²⁴, which all impact product yields⁷.

As we have seen, the product spectrum of lignin pyrolysis is strongly dependent on the way lignin was isolated, for instance through biodegradation, industrial pulping or pretreatment processes⁶. Native or mildly isolated lignins tend to give rise to carbohydrate-derived peaks in the pyrographs, especially abundant in ball-milled or EMAL lignins, which have also been witnessed to generate larger amounts

of acids and aldehydes^{51,80}. A key study by Nowakowski *et al.*⁶ demonstrated that lignin feedstock processing has a significant effect on lignin thermal behavior and product yields. Key findings included that a highly impure lignin (~50wt% carbohydrates) behaved like biomass upon thermal degradation, producing a higher quantity of bio-oil and significantly lower char yield. In contrast, a highly purified herbaceous-type lignin produced a less oxygenated and more phenolic bio-oil with higher pH, higher carbon content and lower water content on average. More recently, Wang *et al.*⁸¹ pyrolyzed four lignins extracted from the same pine wood using alkali, acid, ethanol Organosolv and ball-milling, respectively, and found significant differences in their pyrolysis characteristics using TG-FTIR and Py-GC-MS. The results showed that MWL and alkali lignin, which were observed to be less thermally stable, produced more phenols at low temperature (400 °C) due to the breakage of abundant and weak ether linkages preserved in their structures.

A summary of the expected impact of lignin characteristics on pyrolysis product distribution may be found in Table II-19.

Table II-19. Influence of lignin characteristics on lignin fast pyrolysis product distribution.

Lignin characteristic	Influence on bio-oil yield	Influence on char yield	Reference
S/G unit ratio	↑, more substituted monoaromatics	↓	36,60,69
OH, OCH ₃ content	↓	↑	68,82
β-O-4' bond abundance	↑, more condensed products	↓	59,64,83
H/C _{eff} ratio	↑	↓	73
O/C ratio	↓	↑	7,75
Carbohydrate impurities	↑, acids and aldehydes, lower pH, lower carbon content and higher water content	↓	6,51,80
Ash content	↓	↑	27,76,77
Sulfur content	↓, less aromatics	↑	41,46,54,70–72
Molecular weight	↓(?)	↑(?)	78,79

4.2.5 Pyrolysis of biorefinery lignins

Biorefinery lignins may be isolated in high purity from lignocellulose using pretreatment solvents, such as acidified organic solvents (Organosolv processing)^{54,84,85} and ionic liquids (RTIL pretreatment)²⁴. Investigating the feasibility of using biorefinery lignins as a feedstock for fast pyrolysis remains a nascent field, and relatively few studies have linked the structural changes occurring in lignin as a result of pretreatment with pyrolysis products of the isolated lignins^{85,86}. De Wild *et al.*⁴⁰ produced

high-purity lignins from wheat straw using an Organosolv process and compared their behavior upon fast pyrolysis with that of hardwood Organosolv lignin and wheat straw soda lignin. Upon thermal decomposition, highly pure wheat straw Organosolv lignins gave typical product distributions of 45–55 wt% bio-oil, 35–45 wt% char and 15–20 wt% gas in a bubbling fluidized bed facility at 500 °C^{40,54}. Moreover, herbaceous-type Organosolv lignins produced a total phenolic (monomer + oligomer) yield of ~30 wt%, in contrast to hardwood Organosolv lignin which produced more char, less bio-oil and a lower yield of phenolics, suggesting that grass lignin was easier to crack⁴⁰. However, in these studies only one or two types of lignins were compared, making a study of the effect of pretreatment conditions on lignin pyrolysis bio-oil very challenging. Therefore, a systematic comparison of the pyrolysis behavior of lignin isolated under various conditions could help to elucidate the effect of structural changes on lignin pyrolysis products. The only known report of fast pyrolysis of ionoSolv lignins was carried out by Brandt *et al.*²⁴ using Py-GC-MS at 600 °C to compare untreated *Miscanthus × giganteus* (a herbaceous biomass) and *Miscanthus* lignins isolated at 120 °C. Comparing lignins isolated using increasing pretreatment times, GC-MS showed an increase in G and in H-type compounds, while S-type, PCA-type and FA-type compounds decreased²⁴. Higher yields of monomeric phenols such as phenol, guaiacol and syringol were observed, indicating that aromatic rings in the depolymerized/condensed lignin facilitated release of simple monomers. These findings further suggest that pyrolytic valorization of ionoSolv lignin is more promising than whole lignocellulose.

In order to investigate the link between pretreatment conditions, lignin properties and thermal behavior of lignin, ionic liquid pretreatment was employed to isolate lignins from biomass. Previous studies in our research group^{24,87,88} have shown that pretreatment with low-cost ionic liquids can be used to extract lignins that are low-sulfur, low-ash and free of carbohydrates and therefore especially attractive for pyrolysis. Moreover, selection of temperature, residence time, IL solvent acidity and other parameters can enable control of the lignin properties, notably the O/C ratio, a key indicator of fuel value, as demonstrated by Brandt *et al.*²⁴ Therefore, this study attempted to tune lignin properties to improve the quality and stability of bio-oil.

4.2.6 Sugarcane bagasse lignin

As seen previously in Chapter 2:, sugarcane bagasse (SCB) is an abundant agro-industrial residue that is currently underutilized with ca. 50 wt% of bagasse going to waste due to recent improvements in the boiler efficiency within sugar and ethanol mills⁸⁹. Sugarcane bagasse lignin is thus an excellent candidate material for lignin valorization studies^{89–91}. Compared with the thermal utilization of woody lignins, the investigation of non-woody lignin derived from agricultural residues is still rare⁹², although recent studies have highlighted its potential⁹³. Lignin isolated from sugarcane bagasse has been extensively studied^{89,90,94–99}. Sugarcane bagasse lignin is a grass lignin (S-G-H-type), resembling lignin

in herbaceous biomass, as found for other agricultural residues¹⁰⁰. As evidenced by several studies, bagasse lignin is mainly composed of ~80–85% β -O-4' ether bonds together with small amounts of β - β' , β -5' and 5-5' carbon-carbon linkages^{96,101}. Observations by Sun *et al.*⁹⁶ have also identified *p*-coumaric acid linked to SCB lignin by ester bonds and ferulic acid linked to lignin by both ether and ester bonds. Bagasse lignin is known for its high γ -COOH and γ -COOR group content with low OCH₃ and OH group content^{82,90}, which is conducive to higher bio-oil yields^{68,82}.

While the work of Brandt and Gschwend^{24,87,88} has shed considerable light on the structural changes occurring in Miscanthus lignins (also S-G-H-type) during IL pretreatment, only one previous study has reported the effects of IL treatment on bagasse lignins. Tan *et al.* (2009)¹⁰² used an imidazolium-based IL mixture to extract lignin from bagasse at temperatures between 170–190 °C. High-purity lignins with low MW (2220 g/mol) were obtained, though with non-zero ash and sulfur content¹⁰².

4.2.7 Fast pyrolysis reactor design

The fast pyrolysis of lignocellulosic materials is known to produce considerably larger liquid product yields and lower char yields than slow pyrolysis⁶. This study used an approach to flash pyrolysis initially described by Scott and Piskorz^{29–32}, which has the objective of maximizing liquid (bio-oil) product yields. By conducting atmospheric pressure flash pyrolysis process utilizing a fixed bed of solid as heat carrier, previous work found that organic liquid yields of up to 70 wt% on a moisture-free basis could be obtained from hardwoods, with up to 60 wt% yields obtained from agricultural wastes including bagasse²⁹. The optimum conditions (in terms of calorific value and H/C ratio of the liquid products) also gave the maximum liquid product yields³². Multiple studies found the optimal yield temperature for total liquid yield to occur at 500 °C^{29–31,103}. Therefore, a pyrolysis temperature of 500 °C was selected to enable the characterization of liquid products.

In order to achieve high liquid yield, high temperatures, using rapid heat transfer rates must be combined with short vapor residence and cooling times⁵¹. In this study, fast pyrolysis experiments were carried out in a fixed-bed downdraft pyrolysis reactor that allows semi-automated operation by batch feeding of solid fuels. The reactor design enables high heating rates ($>100\text{ }^{\circ}\text{C s}^{-1}$)^{115–117} comparable to those obtained in commercial, large-scale pyrolyzers, with excellent control of gas flowrates and temperatures allowing for high reproducibility¹⁰⁴. The fixed-bed design minimized the extent of secondary reactions, as the evolved gases and volatiles were immediately swept downstream and condensed in a cooled trap, reducing the vapor residence time and hence contact between primary pyrolysis products. Despite greater complexity and time consumption, it was decided to conduct fast pyrolysis experiments in this experimental rig rather than a fully automated pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) instrument. Although techniques such as Py-GC-MS require minimal sample preparation and provide rapid analysis of a feedstock, they do

not enable quantification of the liquid product yield, as only solid residue (i.e. char) yields can be determined⁶⁹. It is worth noting that while the fixed-bed pyrolysis reactor employed in this study enabled quantification of the solid, liquid and gaseous product yields, one limitation was that only very small quantities (<50 mg) of bio-oil were produced. This limited analysis to certain techniques and notably prevented determination of pH and water content of bio-oils.

4.2.8 Pyrolysis of IonoSolv sugarcane bagasse lignin

In this study, we examined the effect of IonoSolv pretreatment time and temperature on the structural characteristics of isolated lignins and their pyrolysis behaviors. For comparative analysis, enzymatic mild acidolysis lignin (EMAL) was used as a representative of native lignin, extracted from the same bagasse species. Chemical properties of all lignins were determined by elemental analysis and compositional analysis and content of selected metals was determined by acid digestion followed by inductively coupled plasma optical emission spectroscopy (ICP-OES). Molecular size distribution was determined by gel permeation chromatography (GPC) and structural features were characterized by ¹H-¹³C HSQC NMR, ¹³C NMR and ³¹P NMR. Furthermore, thermal decomposition behaviors were determined by TGA and DSC. On the basis of these results, the chemical characteristics of bagasse, EMAL and IonoSolv lignins were extensively compared.

Bagasse, EMAL and seven IonoSolv lignins were then pyrolyzed isothermally in the aforementioned fixed-bed downdraft pyrolysis reactor at 500 °C and 1.5 bara. Pyrolysis experimental conditions were kept identical for all experiments. The mass fraction of bio-oil, char and gas products and the elemental composition of the char and bio-oil were determined. Pyrolysis oil samples were collected and characterized with size exclusion chromatography (SEC), ultraviolet fluorescence (UV-F), ¹H NMR and elemental analysis to study their structural changes which were linked back to feedstock characteristics. In this way the pyrolysis product distributions and characterizations could be linked to the structural characterizations of lignin and biomass feedstocks.

4.2.9 Summary

- For chemicals production through fast pyrolysis, whole biomass should be fractionated first.
- High purity lignins with low oxygen content are more desirable *prima facie* for production of bio-oils with greater stability (i.e. lower acidity and water content) and higher energy content.
- Greater abundance of β-O-4' linkages was linked to higher volatiles production while greater extent of condensation, methoxyl and hydroxyl content linked to char-forming reactions.
- Expected product yields from fast pyrolysis of biorefinery lignins are 45–55% oil, 35–45% char and 15–20% gas.

4.3 Experimental

4.3.1 Materials

All reagents were obtained from VWR International or Sigma-Aldrich, unless stated otherwise, and used as received. The minimum purity of the starting materials was $\geq 99\%$. The synthesis of [TEA][HSO₄] was as described in section 1.3.2. The lignocellulosic feedstock used throughout this study was industrially depithed sugarcane bagasse (*Saccharum officinarum*) (henceforth ‘bagasse’) sourced from a pulp and paper mill in the KwaZulu-Natal province of South Africa. The bagasse was washed, air-dried ground and sieved (0.18–0.85 mm, -20/+80 US mesh scale). The biomass was then stored air dry in sealed plastic bags at ambient temperature. The summative composition of the depithed sugarcane bagasse used for pretreatment and fast pyrolysis is displayed in Table II-20. The Karl-Fisher titrator used in this work was a V20 volumetric Titrator (Mettler-Toledo) and the analytical balance was a Sartorius CPA 1003 S balance (± 0.001 g).

Table II-20. Summary of fuel analyses results for sugarcane bagasse.

	Air dry	Dry
Proximate analysis (wt%)^a		
Moisture	3.9	0
Ash	0.9	0.9
Volatiles	83.6	87.0
Fixed carbon	12.0	12.5
Ultimate analysis (wt%)^a		
Carbon	45.7	46.5
Hydrogen	5.6	5.7
Nitrogen	<0.10	<0.10
Sulfur	0.7	0.7
Oxygen ^b	48.0	47.1
Compositional analysis (wt% dry)		
Cellulose		43.5 $\pm$ 2.1
Hemicellulose		29.1 $\pm$ 6.9
Lignin		24.1 $\pm$ 3.7
Acid-soluble lignin		4.7 $\pm$ 3.7
Acid-insoluble lignin		19.4 $\pm$ 4.0
Ash		0.7 $\pm$ 0.0
Extractives		2.6 $\pm$ 0.0
Ash analysis (wt% dry basis, 600 °C)^a		
SiO ₂		50.8%
CaO		8.9%
Na ₂ O		7.3%
Fe ₂ O ₃		6.6%
MgO		4.5%
Al ₂ O ₃		3.6%
P ₂ O ₅		2.9%
K ₂ O		1.6%
SO ₃		1.3%

TiO ₂	0.3%
Mn ₃ O ₄	0.2%
NiO	0.1%
ZnO	0.07%
SrO	0.05%
BaO	<0.01%
CuO	<0.01%
Cr ₂ O ₃	<0.01%
HfO ₂	<0.01%
PbO	<0.01%
Vr ₂ O ₅	<0.01%
ZrO ₂	<0.01%
LOI	12%

^a Repeatability estimated to be ≤0.5% (absolute)

^b Oxygen content by difference.

4.3.2 Lignin isolation

ionoSolv lignin

ionoSolv lignins were isolated from industrially depithed sugarcane bagasse using a method based on the standard operating procedure of our laboratory¹⁰⁵ as described in detail in section 1.3.3. A biomass to solvent ratio of 1:5 g/g was used with 80 wt% [TEA][HSO₄] and 20 wt% water as the solvent. A pretreatment experiment matrix summarizing the treatment conditions used is shown in Table II-21.

Table II-21. Summary of treatment conditions used for lignin isolation.

Run no	Lignin type	Extraction method	Treatment conditions
1	EMAL	Enzymatic mild acidolysis	Native
2	120 °C, 2 h	ionoSolv pretreatment	120 °C, 2 h
3	120 °C, 4 h	ionoSolv pretreatment	120 °C, 4 h
4	120 °C, 8 h	ionoSolv pretreatment	120 °C, 8 h
5	120 °C, 16 h	ionoSolv pretreatment	120 °C, 16 h
6	120 °C, 48 h	ionoSolv pretreatment	120 °C, 48 h
7	150 °C, 2 h	ionoSolv pretreatment	150 °C, 2 h
8	170 °C, 2 h	ionoSolv pretreatment	170 °C, 2 h

Briefly, depithed sugarcane bagasse was treated in the IL-water mixture in unstirred glass pressure tubes at a temperature between 120 and 170 °C and for a time between 2 and 48 hours. The cellulose-rich material (pulp) was washed four times with ethanol and the IL/ethanol mixture was concentrated by evaporating the ethanol to leave a dried IL/lignin mixture. Lignin was precipitated by addition of deionized water as an antisolvent to the original IL mixture quantity (with a water to IL mixture ratio

of 4.5 g/g). The mixture was left to stand overnight to allow lignin to precipitate fully, then lignin was isolated by vacuum filtration as above and washed three more times with 4.5 g/g equivalents of water as before. The solid precipitate was collected, dried *in vacuo* at 45°C overnight, and Soxhlet extracted with deionized water (150 mL, 24 h) to remove any residual ionic liquid. The resulting purified IonoSolv lignins were dried *in vacuo* as above, ground and sieved (size fraction 0.106–0.150 mm; US mesh scale -140/+100) and stored in sealed glass jars at room temperature prior to characterization and pyrolysis. The cellulose-rich material (pulp) was kept and analyzed by saccharification. The IonoSolv lignins were analyzed as described in section 4.3.3.

Enzymatic mild acidolysis lignin (EMAL)

EMAL was isolated from depithed bagasse following the three-stage protocol for isolation of “native” lignin developed by Wu and Argyropoulos¹⁰⁶. The full method may be found in section 1.3.4.

4.3.3 Biomass and lignin characterization

Compositional analysis

The composition of the untreated biomass, pretreated biomass pulps and precipitated lignins obtained from pretreatment was determined according to the standard NREL protocol¹⁰⁷ as described in section 1.3.5. All samples were Soxhlet extracted for 24 hours (with ethanol for untreated biomass or pretreated pulps; with water in the case of lignins) and vacuum-dried for 48 h prior to compositional analysis. The ASL and AIL contents were combined and reported as the total lignin content.

Saccharification assay

Untreated depithed bagasse and the pretreated biomass pulps obtained during pretreatment were analyzed by enzymatic hydrolysis in triplicate using the standard NREL protocol¹⁰⁸ as an assay, using a cellulase enzyme blend (Cellic Ctec2) obtained from Novozymes with a hydrolysis time of 7 days.

Inductively coupled plasma optical emission spectroscopy (ICP-OES)

ICP-OES measurements were performed on a Perkin Elmer Optima 2000 DV spectrometer (Perkin Elmer Inc, USA). The values given in the results are averages of 3 measurements. Analyte solutions were prepared by mixing 100±10 mg of accurately weighed lignin with 10 wt% nitric acid. Plain 10% nitric acid was the reference. Standards were prepared using a multi-element standard solution in 10% nitric acid (Multi-element standard solution 1 for ICP, TraceCERT®, Sigma-Aldrich). The standard concentrations were (in mg/L) Na: 50, 10, 1; K: 100, 20, 2; Ca: 10, 2, 0.2; Fe: 10, 2, 0.2; Mg: 10, 2, 0.2; Al: 10, 2, 0.2. Two samples each were analyzed for biomass and for lignin produced for each pretreatment condition, while three independent samples were analyzed for 8 h lignin produced at 120°C, displaying excellent reproducibility.

Ash analysis by WD-XRF

Ash samples were obtained from depithed sugarcane bagasse by ashing at 575 °C to remove any organic material using a ramping program in a muffle furnace (Nabertherm + controller P 330). The heating program was as follows: heating from room temperature to 105 °C; hold at 105 °C for 12 min to remove moisture; heating to 250 °C at 10 °C/min; hold at 250 °C for 30 min; heating to 575 °C at 10 °C/min; hold at 575 °C for 3 hours; cool to room temperature. The ash obtained was a pink/orange powder and its composition was analyzed by quantitative WD-XRF. Sample preparation was by fused bead using an automated FluXana Vulcan system (Fluxana GmbH, Germany) using a standard 10:1 ratio of flux:material. This was carried out by ITRI Innovation Ltd (UK). The fused bead was analyzed using a PANalytical AxiosmAX minerals wavelength dispersive X-ray fluorescence spectrometer (PANalytical, Netherlands) equipped with a 60 kV generator and a 4 kW Rh SST-mAX X-ray tube. Analysis was carried out using Omnian software package for semi-quantitative XRF.

NMR spectroscopy of lignins

HSQC NMR: For HSQC experiments of ionoSolv lignins and EMAL, ca. 20 mg of lignin was dissolved in 0.25 mL of DMSO- d_6 and the solution transferred to a Shigemi NMR tube. Samples were analyzed semi-quantitatively following the method given in section 1.3.9 and referenced to the central DMSO- d_6 solvent peak (δC 39.520 ppm, δH 2.500 ppm). Assignment of the HSQC spectra was described elsewhere.²⁴ The full method, assignments and relevant HSQC spectra are found in the Appendix.

Quantitative ^{13}C NMR: Selected lignins (~70 mg) were dissolved in the appropriate deuterated solvents with slight heating and vortex stirring until completely dissolved. EMAL was dissolved in 0.30 mL of deuterated acetone- d_6 : D_2O (3:1 v/v) and ionoSolv lignins were dissolved in 0.30 mL of deuterated DMSO- d_6 . The solutions were transferred to a Shigemi NMR tube. NMR spectra were acquired on a Bruker Avance 500 MHz spectrometer at 50 °C in order to reduce the viscosity. An inverse-gated decoupling (Waltz-16) pulse sequence with a 30° pulse angle and 25 s pulse delay was used. The spectra were interpreted according to El Hage *et al.*¹⁰⁹ and Brandt *et al.*²⁴, counting the aromatic region at δC 160-102 ppm as 6.12 carbon atoms (representing all aromatic carbons plus a contribution of 0.12 from the unsaturated side chain). However, the amount of unsaturated side chains in more treated lignins is not known and could be larger or smaller than in early stage lignin. The ionic liquid signals (δC , DMSO- d_6 /ppm: 46.21 (N-CH₂), 9.15 (N-CH₂-CH₃)) were subtracted where necessary. Full spectra can be found in the Appendix.

^{31}P NMR: Quantitative ^{31}P NMR spectra of all lignin preparations were obtained using published procedures¹¹⁰. Briefly, a solution of 1.6:1 (v/v) of anhydrous pyridine and deuterated chloroform was prepared. This 'stock solution' was used to prepare: (1) a solution containing 20 mg/mL of the internal standard cyclohexanol dissolved; and (2) a solution containing 5.6 mg/mL of chromium (III)

acetylacetonate, the relaxation agent. The lignin was dried under vacuum (1 mbar) at 45 °C for 24 hours prior to analysis. 10mg dried lignin was accurately weighed into a clean HPLC glass vial. To each lignin sample, the following reagents were added: 100 μ L of anhydrous pyridine and deuterated chloroform stock solution, 50 μ L of the cyclohexanol solution, 50 μ L of the relaxation agent solution and finally 50 μ L of the phosphitylating reagent 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP). The sealed solution was vortex-mixed intensely for 5 minutes or until completely dissolved and transferred to a Shigemi tube. An inverse gated decoupling pulse sequence was used to obtain quantitative spectra on a Bruker Avance 500 MHz NMR machine using the following settings: 298 K (room temperature), relaxation delay 25 s, 30° pulses, 127 scans. The quantity of the various hydroxyl group types was determined by integration using MestReNova relative to the concentration of the internal standard cyclohexanol (δP , $CDCl_3$: 144.2 ppm). Integration regions that were used to assign the signals and the relative signal intensities used to calculate the concentration of hydroxyl groups are tabulated in the Appendix.

GPC of lignins

GPC measurements were performed as described in section 3.3.12. Standard deviations for the time course lignins were $M_n \pm 100$, $M_w \pm 300$ and $PDI \pm 0.4$ or less. Standard deviations for the temperature course lignins were $M_n \pm 100$, $M_w \pm 200$ and $PDI \pm 0.4$ or less.

Elemental analysis of lignins

CHNS analysis of lignins was performed in duplicates 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. Residual ionic liquid contamination was calculated using the nitrogen and sulfur content to calculate the cation and anion contributions, respectively; the sum of the two was used to obtain the residual ionic liquid content in the lignins.

Higher heating value

According to Demirbas (1997)¹¹¹, the higher heating value (HHV) of biomass fuels can be estimated from the C, H, O and N contents (daf) of the samples investigated. Thus, the HHV ($MJ\ kg^{-1}$) of lignocellulosic materials can be calculated from

(Equation 16:

$$HHV = (33.5[C] + 142.3[H] - 15.4[O] - 14.5[N]) \quad (\text{Equation 16})$$

where C = carbon content, H = hydrogen content, O = oxygen content, and N = nitrogen content. In this study, the elemental compositions of the raw materials were used as-received, rather than on a dry ash-free (daf) basis. HHV were calculated from elemental analysis values obtained in duplicates.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis of biomass and lignins was conducted on a TA Q500 (TA Instruments, USA) TGA analyzer fitted with a gas switching system, using a platinum pan, with N₂ as the purge gas (50 mL/min). 15±5 mg of biomass or dried lignin was loaded per run; each sample was analyzed in duplicate. For proximate analysis of sample composition, a drying step at 105 °C for 15 min was carried out before linearly ramping the temperature at 60 °C/min over a temperature window of 105 to 600 °C. The sample was held at 600 °C for 15 min before a burn-off step was initiated by switching the purge gas to 10% air and 90% nitrogen to remove all organic material and determine the ash content. The sample weight after 15 min (dry weight), 39 min (fixed carbon and ash weight) and 54 min (ash weight) were determined and fixed carbon content and ash content were calculated using

(Equation 17 and (Equation 18, respectively, where m_t is the weight fraction of the sample at a given time t expressed in minutes:

$$\text{Fixed carbon content} = \frac{m_{39 \text{ min}} - m_{54 \text{ min}}}{m_{15 \text{ min}}} \cdot 100\% \quad (\text{Equation 17})$$

$$\text{Ash content} = \frac{m_{54 \text{ min}}}{m_{15 \text{ min}}} \cdot 100\% \quad (\text{Equation 18})$$

Proximate analysis was performed using TA Universal Analysis software and in Microsoft Excel. For thermal analysis, the TGA weight loss curves and their first derivatives, i.e. the differential thermogravimetric (DTG) curves with respect to temperature for all lignins were plotted and used to identify the temperature at which the DTG curve showed the maximum rate of thermal composition (DTG_{max}). TGA experiments were conducted in duplicates.

Differential scanning calorimetry (DSC)

DSC measurements of lignin samples were performed on a TA instruments DSC Q2000 (TA Instruments, UK). The analyses were conducted using 6 ± 1 mg of lignin in hermetically sealed aluminium pans (Tzero pan and hermetic lid; 40 µL). Prior to running DSC scans, samples were freeze dried at 0.05 mbar for 72 h to avoid any solvent/water artefacts. The highly amorphous nature of lignin coupled with its complex hydrogen bonding interactions can induce significant endothermic enthalpy relaxation during the initial DSC scan of the original lignin^{112–114}. To overcome such a strong enthalpy relaxation and for the purposes of an accurate T_g determination, annealing all samples above their T_g using a heat-cool-heat experiment is often a standard part of the DSC protocol during T_g determination for lignins. In order to eliminate the thermal history of the sample, an initial heating, annealing and cooling cycle was carried out to clear the stored thermal history of the sample within the polymer's glassy state. The sample was subjected to an initial scan where it was heated from 20 °C to 160 °C with

a heating rate of 10 °C/min and maintained at 160 °C for 10 min. The sample was then cooled down to -20 °C with a cooling rate of 10 °C/min and maintained at this temperature for 10 min. The second heating run was used to determine the glass transition temperature (T_g) of the sample. The temperature was ramped from -20 °C to 200 °C at 20 °C/min (as the glass transition is more pronounced at faster heating rates), followed by an isothermal state at 200 °C for 10 min and cooling to room temperature at 30 °C/min. All DSC scans were conducted under a helium atmosphere with a flow rate of 50 mL/min. DSC analysis was performed using TA Universal Analysis software and in Microsoft Excel. DSC analysis was conducted in duplicates. The full DSC curves can be found in the Appendix.

4.3.4 Fast pyrolysis experiments

Fixed-bed reactor description

Pyrolysis experiments were conducted in a single-stage fixed-bed pyrolysis reactor that was designed and built by Dr. Matthew E. Boot-Handford and colleagues, as previously reported¹⁰⁴. A complete description of the reactor is given in the Appendix (see Fast pyrolysis reactor description and Figure S0-7). Briefly, the reactor body was constructed from a high temperature NB Incoloy 800HT alloy working tube (1" nominal bore, 350 mm length) with an internal quartz liner. A sintered quartz disk midway down the tube acts as a bed support and retains ash and residual char. Typically, sand (2.8 g, 500-700 µm) was loaded into the reactor to provide an inert bed (5 mm in height) and heat sink that the thermocouple could be placed in. A K-type thermocouple was immersed in a bed of sand during the pyrolysis to measure the heating rate (estimated as > 100 °C s⁻¹ based on literature reports of fixed-bed reactors with a similar design¹¹⁵⁻¹¹⁷). During the reaction, the evolved gases and volatiles are swept downstream into the main tar trap, which is cooled with refrigerant solution (30wt% ethylene glycol in distilled water; -2 °C) to cool the gases and condense the tars and water vapor contained in the reactor flue gas for analysis. The inner tube was packed with quartz wool (~6 g) to increase the surface area for condensation of bio-oils formed. A secondary line diverted the gas flow from the tar trap and to the reactor outlet; two ball valves enabled the gas flow to be diverted from the main tar trap after 90 s of reaction time had elapsed and closed off after the trap, so that the tars could be isolated from the hot gas flow and sealed off from the atmosphere prior to recovery. Two smaller quartz wool traps (not cooled, each containing ~1.5 g of quartz wool) were placed after the main tar trap outlet and at the diversion line outlet, respectively, to condense any remaining tars. Mass flow controllers (Bronkhorst High-Tech B.V.) were used to control and monitor the flowrate and composition of gas entering the reactor. The pressure was controlled and monitored by a pressure controller and pneumatic valve (Bronkhorst High-Tech B.V.) at the exit of the tar trap. The concentrations of CO, CO₂ and CH₄ were measured in real time at the exit of reactor throughout the

pyrolysis experiment using a multi-gas infra-red detector (CO, CO₂, CH₄) using an online multigas analyzer (MGA3000, ADC Analyzers, UK) that was connected after the pressure control valve. A constant purge of 600 ml min⁻¹ was maintained to the gas analyzer to ensure that the flow remained in the calibrated range for the analyzers. The gas exiting the analyzer train was vented into the fume cupboard. A control program written in Agilent VEE automated the entire process including fuel feeding, temperature control and switching, gas flow rate control and switching, pressure control and data monitoring and collection. All relevant information including measurements of the temperatures at different points in the reactor, flow rates, the system pressure and the signals from the gas analyzers were logged every second and saved to a designated text file.

Pyrolysis conditions

A standard set of conditions were developed and used when operating the fixed-bed reactor using a fast pyrolysis regime (see Table II-22). The reactor and traps were assembled and sand was pre-loaded into the quartz liner. The assembled reactor was pressure tested (~7 bara) and a cold gas calibration was performed using a certified calibration mixture (29.9 vol% CO₂, 9.7% CO, 10.1% CH₄, balance N₂) supplied by BOC. The reactor was then heated up to the experimental set point of 500±5 °C under N₂ and the temperature was allowed to stabilize for at least 30 min. Prior to feeding, the loaded sample cylinder was purged for 5 minutes with a 10 ml s⁻¹ flow of N₂ to displace any air that could interact with the sample and skew the results. The biomass sample (0.10 g) was then fired into the reactor from a pressurized 50 mL double-ended sample cylinder, pressurized to 0.5 bar above the reactor pressure (1.5 bara). During fuel feeding, 20 ml N s⁻¹ of flow was diverted to the feeder for sweeping the biomass particles from the sample feeder into the reactor. A superficial velocity of 0.25 m s⁻¹ was previously found to be optimal. After the sample was fed, the reactor was held at the set point temperature for 900 s to ensure complete pyrolysis was achieved. The pyrolysis tube was flushed with N₂ gas with a superficial velocity of 0.25 m s⁻¹ (60 ml N s⁻¹ at 500 °C) to sweep evolving volatiles from the sample bed downstream to the tar trap. After this time had elapsed, following the isothermal phase, the heating was either switched off and the reactor was allowed to cool, or a char combustion step was initiated. For the latter, the gas composition was switched to 10 vol% O₂ (balance N₂) causing the organic content of the char bed to combust. The char combustion step was allowed to continue for a further 10 min. This char combustion was used as part of the method for determining the char yield (discussed in 4.3.4: Product recovery). Following the char combustion step, the heating was switched off and the reactor allowed to cool. All pyrolysis experiments were conducted in duplicate measurements; depithed bagasse was analyzed in sextuplicate and 120 °C, 8h lignin was analyzed in triplicate to obtain a better estimate of the errors.

Table II-22. Summary of experimental operating parameters for pyrolysis experiments.

Operating conditions	Fixed-bed pyrolysis reactor
Feedstock	Depithed sugarcane bagasse; IonoSolv lignins
Feed weight	0.10 g
Feed particle size	106 – 150 μm
Temperature	500 $^{\circ}\text{C}$
Heating rate	Fast ($> 100\text{ }^{\circ}\text{C s}^{-1}$) ^a
Hold time	900 s
Pressure	1.0 bara
Carrier gas	Nitrogen
Carrier gas superficial velocity	60 mL N s^{-1} (0.25 m s^{-1})

^a Fast pyrolysis achieved by pressure feeding sample into reactor once the experimental set point temperature had been reached.

Product recovery

After each experiment, when the trap had cooled to $\sim 60\text{ }^{\circ}\text{C}$, the reactor and traps were dismantled and each of the reactor components (including all three quartz wool samples) was carefully washed with a 4:1 (v/v) solution of chloroform and methanol ($\sim 300\text{ mL}$) to collect any liquid products. The washings were filtered (Whatman no.1 filter paper) to collect any solid debris. The bulk of the solvent was removed under vacuum on a rotary evaporator (Rotavapor 3000, Buchi, Germany) operated at $60\text{ }^{\circ}\text{C}$. The concentrated bio-oil/solvent solution was then transferred to a pre-weighed aluminium beaker to be further dried for 2 hours in a circulating oven at $35\text{ }^{\circ}\text{C}$ under a N_2 purge to ensure complete solvent removal from the samples. The bio-oil was then weighed to determine the gravimetric yield as a percentage of the initial dry weight of the biomass/lignin sample. After the bio-oil yield was determined, the bio-oils were transferred to sealed and N_2 purged glass vials using $\sim 3\text{ mL}$ of 4:1 v/v chloroform:methanol solvent. The oils were stored in this solvent in the refrigerator ($-4\text{ }^{\circ}\text{C}$) if they were analyzed within a week; for oils which were analyzed later, they were dried under a N_2 purge and the dried oils which were stored in the freezer ($-20\text{ }^{\circ}\text{C}$).

To obtain a repeatable and precise char yield, the char was recovered from the bed as much as possible and analyzed for its carbon content to calculate the char yield. To overcome the issue of char sticking to the quartz liner walls and bed due to electrostatic interactions, a method developed by Boot-Handford¹⁰⁴ was employed to determine the char yield. This method involved running at least two experiments: in the first, a char combustion step was conducted after the isothermal pyrolysis phase, causing the char bed to combust (see 4.3.4: Pyrolysis conditions); in the second, the experiment was ended after the isothermal pyrolysis step such that the residual char could be recovered. The char yield could then be determined by comparing the carbon content of the gas produced during the

char combustion step in the first experiment with the carbon content of the char retrieved in the second step without the burn-off, as determined by elemental analysis. The total char yield for each set of experiments was then determined via

(Equation 20 and

(Equation 19,

(Equation 21:

$$C_{char,g} = Q_{out,293K} \times Ar_C \left(\frac{x_{CO_2}}{MR_{CO_2}} + \frac{x_{CO}}{MR_{CO}} + \frac{x_{CH_4}}{MR_{CH_4}} \right) \quad (\text{Equation 19})$$

$$W_{char,g} = \frac{C_{char}}{Ar_C \times x_{C,char}} \quad (\text{Equation 20})$$

$$W_{char,\%} = \frac{W_{char,g}}{W_{feedstock,g}} \% \quad (\text{Equation 21})$$

$C_{char,g}$ is the carbon content of the char from the combustion step, as determined from the concentrations of CO_2 , CO and CH_4 measured at the reactor outlet; $Q_{out,293K}$ is the total flowrate at the outlet of the reactor at 293 K and 1 bara (temperature and pressure of the gas analyzers); Ar_C is the atomic mass of carbon; x_i is the mole fraction of gaseous species i in the gas stream exiting the reactor; MR_i is the relative molecular mass of gaseous species i ; $W_{char,g}$ is the total calculated weight of char, including ash; $W_{char,\%}$ is the total yield of char relative to the dry feedstock fed into the reactor; $x_{C,char}$ is the mole fraction of carbon in the char where $Ar_C \times x_{C,char}$ is the fraction of carbon in the char as determined by elemental CHN analysis of the char.

To determine the gaseous product distribution, the concentrations of CO_2 , CO and CH_4 were measured in the outlet gas of the reactor after the tar trap via online gas detectors. The total gas yields were determined by integrating the signals from the IR detector with reference to the gas calibration conducted prior to each experiment.

4.3.5 Characterization of pyrolysis products

^1H NMR

Bio-oil samples were analyzed within max. 3 days after generation by fast pyrolysis. 30–40 mg of dried bio-oil was dissolved in 0.25 mL DMSO- d_6 and the solution transferred to a Shigemi tube. ^1H NMR spectra were acquired on a Bruker 600 MHz spectrometer using 1D pulse sequence zg30 and a 30° flip angle; the spectral width was 16 ppm, the acquisition time was 1.7 s and the number of scans was 16.

Size exclusion chromatography (SEC)

SEC was performed using a Mixed-D column with NMP as eluent and polystyrene standards, as described in section 2.3.7. The full chromatograms corresponding to a 270 nm detection wavelength

are shown in this study. These chromatograms have been area normalized for ease of interpretation. The ratio between the excluded ($t = 0\text{--}15$ min) and retained ($t = 15\text{--}30$) peak areas, i.e. $A_{E/R}$, was calculated by numerical integration in Microsoft Excel.

Ultraviolet fluorescence (UV-F)

The UV-F spectra of solutions of pyrolysis oil dissolved in NMP were recorded on a Perkin-Elmer LS55 Luminescence Spectrometer using a quartz cell of 10 mm path length. Synchronous spectra were recorded with 20 nm difference between excitation and emission over a wavelength of 254–600 nm. Spectra were peak normalized for ease of interpretation.

Elemental analysis

CHN analysis of bio-oils and chars was performed in duplicate by the Centre for Chemical Instrumentation and Analytical Services (CCIAS), Department of Chemistry, University of Sheffield, UK using a Perkin Elmer 2400. Oxygen content was obtained by difference. Accuracy is $\pm 0.30\%$ absolute. Higher heating values (HHV) were calculated from CHN results for both bio-oils and tars on an as-received basis, following the modified Dulong's formula given previously (section 4.3.3). HHV values were calculated based on duplicate CHN data.

4.4 Results and Discussion

4.4.1 Lignin isolation trends

Lignin mass balance

Lignin samples were isolated from sugarcane bagasse using the ionoSolv process at bench scale. Seven lignin precipitate samples were selected for characterization to assess the influence of major process parameters (temperature and treatment time) on lignin characteristics. Table II-23 gives an overview of lignin samples produced under varying process conditions ranging between 120–170 °C and 2–48 h. A number of indicators for pretreatment effectiveness are shown, reflecting the fate of the lignin precipitated versus in the pulp as well as the lignin mass balance (see Figure S0-8).

Clearly, ionoSolv process conditions have a strong influence on the lignin yield: residual lignin in the pulp progressively decreased, while isolated lignin yield concomitantly increased. Maximum lignin was isolated (67%) under treatment at 150 °C for 2 h. Under more severe conditions, the lignin mass balance exceeded 100%. As previously discussed in Chapter 2: and Chapter 3:, this is believed to be due to formation of pseudo-lignin¹¹⁸ that is indistinguishable from acid-insoluble lignin. Excessively positive lignin mass balances (129%, 166%) were witnessed under the most severe reaction conditions which saw the residual lignin in the pulp also exceed >100%. It is strongly suspected that this reflects not only pseudo-lignin formation but also extremely condensed lignins forming under severe conditions. The fact that the precipitated lignin yield *decreased* under these conditions is further evidence that highly cross-linked, high MW lignins formed that either precipitated during pretreatment or were too large to dissolve in the IL/ethanol liquor during the ethanol washing step and did not appear in the precipitate, fractionating instead with the pulp and reducing the lignin yield.

Table II-23. Lignin recovery and delignification of sugarcane bagasse following pretreatment with low-cost ionic liquid [TEA][HSO₄] under various conditions.

Sample	ionoSolv process condition ^a	Pulp yield (% dw)	Residual lignin in pulp (% dw) ^b	Isolated lignin yield (wt%) ^b	Lignin mass balance (wt%) ^c
1	120 °C, 2 h	59.6±0.1	32.1±0.4	35±2	67±2
2	120 °C, 4 h	53.8±2.7	26.3±0.2	59±5	86±4
3	120 °C, 8 h	51.5±0.4	29.3±0.2	58±2	88±2
4	120 °C, 16 h	57.0±0.9	60.9±0.0	56±9	117±8
5	120 °C, 48 h	65.4±0.2	108.3±1.3	20±1	129±2
6	150 °C, 2 h	50.7±0.3	46.0±0.1	67±6	114±5
7	170 °C, 2 h	46.7±0.9	123.7±1.7	40±3	166±4

^a Biomass-to-solvent ratio of 1:5 g/g. ^b Based on feedstock lignin content (AIL + ASL), as determined by compositional analysis of untreated SCB. ^c Sum of residual lignin in pulp and isolated lignin yield.

Enzymatic hydrolysis of bagasse pulps

A saccharification assay was conducted on the pretreated cellulose-rich pulps recovered to determine glucose release, an important indicator of effective pretreatment. Glucose yields are displayed together with other key pretreatment indicators in Figure II-48. The results indicate that a low process temperature and residence time (120 °C, 4 h) were optimal, releasing 74% of theoretical glucose from air-dried pulps. These reaction conditions coincided with high glucan recovery in the pulp (97%), maximal delignification (74%) and lignin yield (59%). Prolonged treatment times and higher reaction temperatures reduced glucose yields, likely due to formation of pseudo-lignin which is known to limit enzyme accessibility¹¹⁹. Surprisingly, the glucose yield increased again to 72% after 48 h of treatment; this result was highly unexpected given the irreversible formation of pseudolignin under severe treatment conditions^{119,120}. Though never previously observed during ianoSolv pretreatment, it is suspected that changes in cellulose crystallinity may have rendered glucose easier to hydrolyze enzymatically; this effect is still under investigation. However, a high glucose release does not guarantee high ethanol yields upon fermentation; this is worth investigating as severe pretreatment conditions employed are likely to produce higher concentrations of fermentation inhibitors, such as phenolics from lignin degradation and furfural and HMF from sugar degradation.

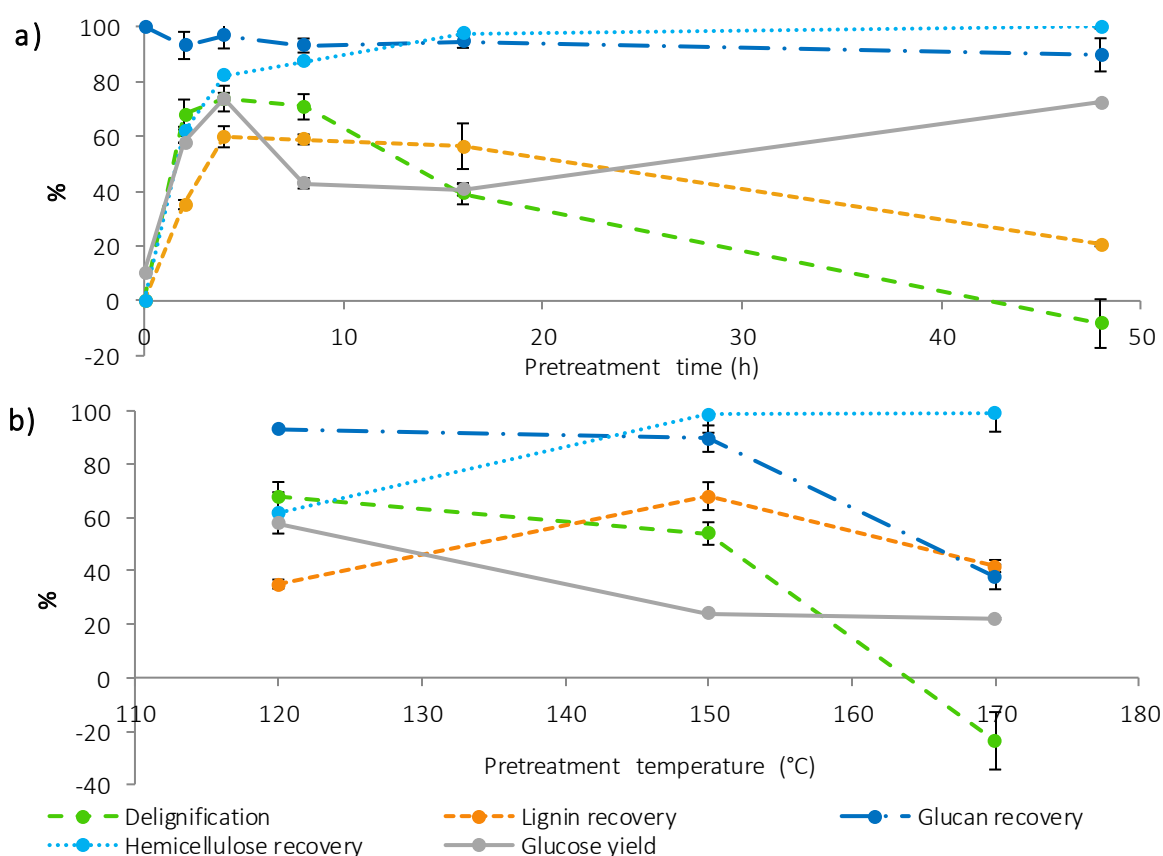


Figure II-48. Five indicators of ianoSolv pretreatment efficacy at varying pretreatment (a) times at 120 °C and (b) temperatures for 2 hours. Depithed sugarcane bagasse was pretreated at a 1:5 g/g biomass to solvent ratio in 80% [TEA][HSO₄] with 20 wt% water at bench scale without stirring.

Prolonged treatment times and high temperatures led to formation of pseudo-lignin and highly condensed lignins that were lost from the precipitate, fractionating instead with the pulp. Mild extraction conditions (120 °C, 4 h) gave optimal glucose yields and also produced acceptable lignin recovery, though the latter was maximized at higher temperatures for short times (150 °C, 2 h).

4.4.2 Lignin and biomass feedstock characterization

Seven lignin fractions were isolated by precipitation using water as anti-solvent following IonoSolv pretreatment under the aforementioned conditions. Precipitated lignin particles were sedimented by centrifugation, the liquor was decanted and the lignin was washed thrice more using distilled water. The lignins were then purified by Soxhlet extraction (H₂O, 24 h) to remove any residual ionic liquid²⁴. Enzymatic and mild acidolysis (EMAL) was isolated from bagasse as a reference for “native” lignin present in untreated biomass. This section describes the detailed structural, chemical and thermal characterization of these lignins prior to their subsequent fast pyrolysis.

Composition

The residual carbohydrate content of the bagasse and isolated lignin samples was compared using an analytical procedure for summative compositional analysis of biomass (Table II-24). As expected, bagasse showed a typical composition of whole lignocellulose with ~75% carbohydrates and 25% lignin. EMAL contained a relatively high carbohydrate content (7%), indicating that mild acidolysis did not cleave all of the lignin-carbohydrate linkages¹²¹. Though a residual sugar content <0.5% can be obtained, use of a milder acidolysis process is preferable to retain the native structure of lignin¹²². IonoSolv lignins have high purity (>98.6%) and low ash content (<0.6%), which is favorable for thermochemical conversion processes targeting high liquid yields¹²³. The amount of carbohydrate impurities varied between 0.0–1.1% seemingly independently of process conditions. This suggests that carbohydrates derive from imperfect washing protocols whereby solid pulp fragments may contaminate the precipitated lignin. It is worthy to note that the protocol used to determine lignin purity is designed for whole biomass and so low sugar concentrations in lignin samples are close to the detection limit of the HPLC instrument; in reality, carbohydrate levels are likely to be even lower.

Table II-24. Compositional analysis of bagasse, EMAL and lignins isolated from bagasse using the IonoSolv process. ALL: Acid-insoluble lignin. ASL: Acid-soluble lignin.

	Bagasse ^a	EMAL	120 °C, 2h	120 °C, 4h	120 °C, 8h	120 °C, 16h	120 °C, 48h	150 °C, 2h	170 °C, 2h
Sugars	74.5	7.0	1.1	0.0	0.0	0.0	1.1	1.1	0.3
Lignin	24.7	93.0	98.8	99.6	99.8	99.9	98.8	98.6	99.1
Ash ^b	0.7	0.0	0.1	0.4	0.2	0.1	0.0	0.3	0.6

^a Extractives-free basis.

^b Water- and ash-insoluble inorganic components.

Elemental analysis

Elemental analysis was used to estimate oxygen content, test for impurities (e.g. ionic liquid incorporation) and to assess fuel value²⁴. Table II-25 shows the elemental compositions as well as calculated IL content and higher heating values. Clearly, lignins have a much higher carbon content (60–65 wt%) compared with biomass (46%), with a concomitantly lower oxygen content (28–32 wt%) than biomass (47 wt%), indicating that ionoSolv lignins are highly deoxygenated.

The N and S content of ionoSolv lignins indicate residual IL contamination; nitrogen is representative of the triethylammonium cation which contains one nitrogen atom and sulfur is representative of the hydrogen sulfate anion, which contains one sulfur atom. The N and S contents were used to estimate IL incorporation, which amounted to between 4–12 wt%, in line with previous data from Brandt *et al.*²⁴. N content is believed to be evidence of covalent bonding between lignin and IL molecules, since all lignins were extensively washed and Soxhlet extracted with water for 24 h prior to drying and elemental analysis. EMAL, on the other hand, contained negligible N and S content, pointing to contamination in the ionoSolv samples rather than it deriving from biomass itself.

Table II-25. Carbon, hydrogen, nitrogen, sulfur and oxygen contents of the bagasse and lignins isolated under different conditions. Anion, cation and residual ionic liquid content of IonoSolv lignins were calculated from N and S values.

	Bagasse	EMAL	120 °C 2h	120 °C 4h	120 °C 8h	120 °C 16h	120 °C 48h	150 °C 2h	170 °C 2h
C (wt%)	46.4	61.9	61.2	61.2	60.5	61.5	64.5	63.6	65.4
H (wt%)	6.1	6.47	5.8	5.7	6.0	5.9	5.5	5.3	5.3
N (wt%)	0.21	0	0.5	0.6	1.0	1.0	0.6	0.3	0.4
S (wt%)	<0.10	0	0.7	0.9	1.7	1.8	0.8	0.5	0.6
O ^a (wt%)	47.3	31.7	31.8	31.6	30.8	29.9	28.6	30.3	28.3
H/C (mol/mol)	1.58	1.26	1.13	1.11	1.18	1.14	1.02	1.00	0.97
O/C (mol/mol)	0.76	0.38	0.39	0.39	0.38	0.36	0.33	0.36	0.33
IL content (wt%)	-	-	5.5	7.1	12.3	12.4	6.4	3.6	4.7
HHV ^b (MJ kg ⁻¹)	16.9	25.0	23.8	23.6	23.9	24.2	24.9	24.2	25.0

^a Calculated by difference ($O = 100 - C - H - N - S$ %) on an as-received basis (air-dry for bagasse; vacuum dried for lignins); ^b Higher heating value, calculated from the elemental composition according to Demirbas¹¹.

The most significant change in elemental composition with prolonged pretreatment time and higher temperature is that lignins became enriched in carbon from 61.2 to 65.4 wt%, which is compensated for by a loss in oxygen from 31.8 to 28.3 wt% and hydrogen from 5.8 to 5.3 wt%. These changes were visually observed by plotting a Van Krevelen plot of H/C vs O/C atomic ratio of lignins and the starting biomass (see Figure II-49).

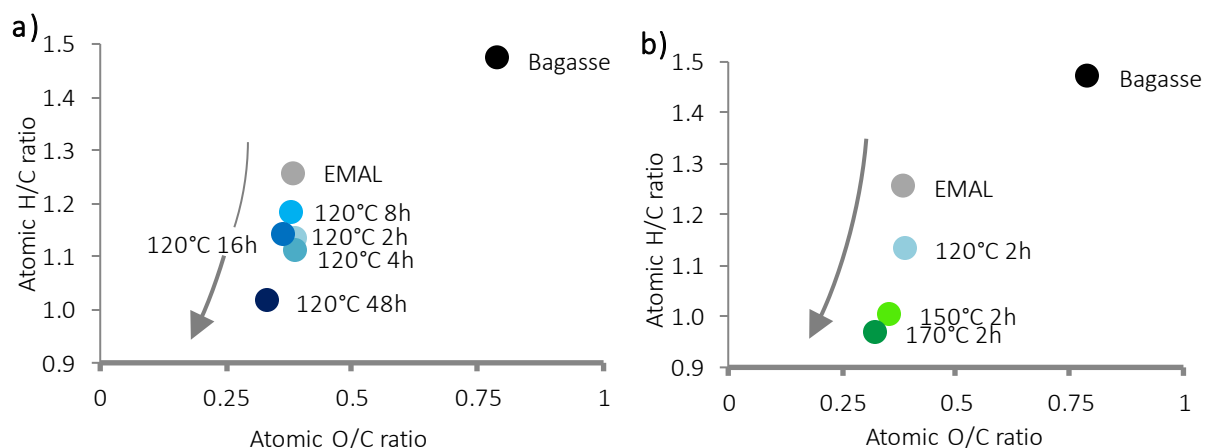


Figure II-49. Van Krevelen diagram of bagasse, EMAL and IonoSolv lignins produced under various pretreatment conditions, i.e. a) time and b) temperature.

H/C and O/C were highest for biomass followed by EMAL, in line with their carbohydrate contents. Loss of hydrogen and oxygen took place with increasing IonoSolv pretreatment severity, as previously observed by Brandt *et al.*²⁴ upon pretreating *Miscanthus* in acidic ionic liquid [Hbm][HSO₄]. Their analysis showed that lignin-carbohydrate-complex linkages were broken within 1 h at 120 °C, suggesting that loss of carbohydrates is unlikely to be the explanation for these trends. A more likely explanation is the loss of water molecules from the lignin macromolecule during condensation reactions which occur between lignin fragments and compounds cleaved from the native lignin during pretreatment in acidic solvent²⁴, whereby C–O bonds are replaced with C–C bonds.

Elemental compositions were used to calculate the theoretical higher heating value (HHV) for biomass and lignins, using a correlation developed by Demirbas and based on Dulong's formula¹¹¹. Bagasse HHV was calculated as 16.9 MJ kg⁻¹, agreeing closely with the average value of 16.0 MJ kg⁻¹ from the literature as measured for 60 sugarcane bagasse samples¹²⁵. The energy content of lignins increased with both pretreatment time and temperature, in line with carbon content. The calculated HHV values for IonoSolv lignins (23–25 MJ kg⁻¹) are within the expected range of 23.3–25.6 MJ kg⁻¹ reported in the literature¹²⁶. Based on their HHV values, as determined experimentally by Demirbas (2004)¹²⁷, lignins are better fuels for thermochemical conversion than lignocellulosic biomasses (14–21 MJ kg⁻¹). Interestingly, despite its high carbohydrate content, the HHV of EMAL (25.0 MJ kg⁻¹) is the same as that of the most severely treated lignin, possibly due to the higher hydrogen content accounting for the absence of nitrogen and sulfur (indicative of IL contamination) in native lignin.

These results underline a major advantage of the IonoSolv pretreatment process, namely the ability to control the oxygen content and fuel value of lignins by selecting isolation conditions.

Metal content

It is important to note that pyrolysis of biomass-derived materials is inherently a catalytic process given the catalytic effects of inorganic components¹²³. The influence of alkali, alkaline earth and other metals on the thermal decomposition behavior of biomass fuels is well known, notably the effect of 19 metals arising from inorganic species present in the biomass ash^{123,128–131}. The fairly high ash content of sugarcane bagasse can introduce a variety of inorganic components such as silica, Ca, K and Na salts that can end up in the lignin fraction. The concentrations of six naturally occurring metals (Fe, Mg, Ca, Al, Na and K) in all pyrolyzed materials were investigated by inductively coupled plasma optical emission spectroscopy (ICP-OES). Thermogravimetric analysis (TGA) was used as a second method to determine the inorganic content. The metal contents obtained from triplicate measurements of each lignin by ICP-OES were converted to their most abundant oxygenated form and compared with total inorganic material measured after ashing to 600 °C by TGA; the results are displayed in Figure II-50. We note that metal concentrations in EMAL were not determined; total ash content in the sample was 2.2 ± 0.2 wt%.

Whereas bagasse had 0.9% inorganic content, total inorganic matter ash in the lignin as determined by ashing was found to range between 0.3–0.8%. Brandt *et al.*²⁴ claimed that partitioning of inorganic matter from biomass within both the pulp and lignin is likely; these measurements confirm the presence of inorganic impurities in the lignins which could reasonably be expected to catalyze primary and secondary reactions during ionoSolv lignin pyrolysis. Nevertheless, the inorganic species concentration in all ionoSolv lignins analyzed was significantly lower than that of many unpurified technical lignins, such as Kraft lignin (27.1%) and lignosulfonate (9.3%)¹³². It is worth noting that total inorganic content represents metals in their oxygenated states, whereas ICP-OES measured six metallic species only, hence the total ash content is expected to exceed the metal content in all cases.

The levels of metal analytes within bagasse (results not shown) were very similar to the least treated lignin (120 °C, 2 h), with the exception of powerful catalyst Na^+ which was $\sim 6.5\times$ more concentrated in bagasse at (270 ± 5 ppm). Total inorganic content decreased sharply after 2 h of pretreatment at 120 °C and fell somewhat less sharply with increasing temperature. The contents of the six metals within the lignin samples analyzed followed the same trend; this decrease was not proportional to the increase in lignin recovery. The cause in the reduction in inorganic cation content is not clear at this point. The most prevalent metal cations were Ca^{2+} (known to have a strong catalytic effect) and Fe^{3+} , with the other analytes Al^{3+} , Mg^{2+} , K^+ and Na^+ present in concentrations < 100 ppm except in the least treated sample (120 °C, 2h).

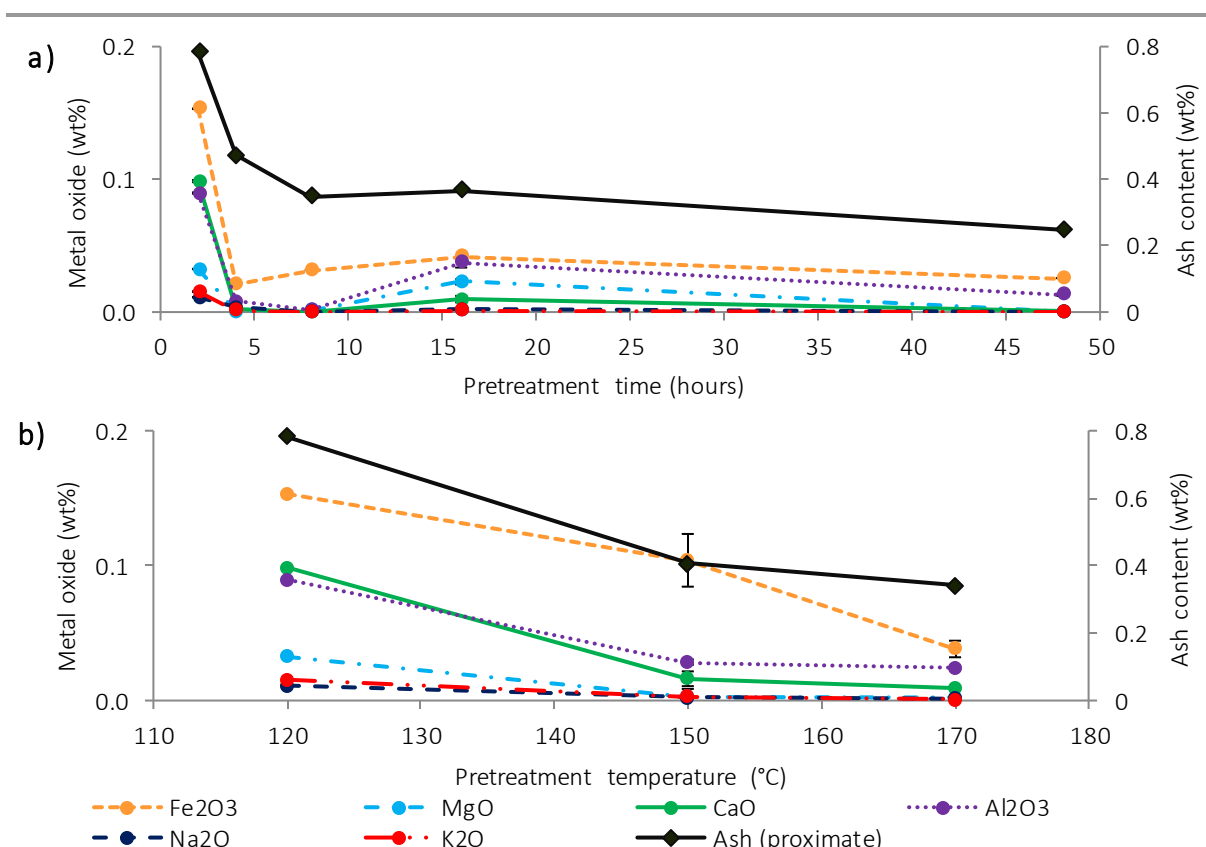


Figure II-50. Concentrations of six metal compounds determined by ICP-OES compared with the total inorganic material (ash) content determined by TGA after ashing to 600 °C. Data shown for ionoSolv lignins produced under various a) residence times at 120 °C, and b) treatment temperatures for 2 h.

Lignin structural analysis

HSQC NMR spectroscopy

As seen in Chapter 1:, 2D HSQC NMR spectroscopy can provide important insights into the structural transformations of lignin during pretreatment. Of particular interest in this study are the linkages between monolignols, which determine lignin reactivity, as well as the subunit composition (S-G-H ratio) and degree of condensation. These can be analyzed by comparing the peak intensities (i.e. volume integrals) of selected peaks over a time or temperature course¹³³. In this study, a semi-quantitative pulse sequence was used to provide sub-structural information. The abundance of different sub-units and linkages in sugarcane bagasse lignin was reported by using volume integration with reference to the signal from the 2-position of G2 units (i.e. the sum of the G_2 and $G_{2,cond}$ integrals), previously shown by Gschwend *et al.* to be stable over the conditions used^{88,118}. The various signals were assigned according to previous assignments in the literature¹³⁴⁻¹³⁶.

Figure II-51 depicts the key parts of the HSQC spectra for bagasse EMAL and lignins isolated with TEA HSO₄-water mixture at an early stage (4 h) and a late stage (48 h) of pretreatment at 120 °C. Both the aromatic ($\delta C/\delta H$ 100–135/6.0–8.5 ppm) and side chain ($\delta C/\delta H$ 50–90/2.5–5.8 ppm) regions are

highlighted. A notable feature of the side chain region is the presence of the three most common interunit linkages, β -O-4' ether (A), β - β' (resinol, B) and β -5' (phenylcoumaran, C), while the aromatic region shows the most common unsaturated subunit structures: syringyl ($S_{2,6}$, $S_{cond.}$), guaiacyl (G_2 , G_5 , G_6 , $G_{cond.}$), *p*-coumarate ($PCA_{2,6}$, PCA_β) and *p*-hydroxyphenyl ($H_{2,6}$).

The most drastic changes were observed in the side chain region, where many C-H signals disappeared completely during treatment. Loss of lignin side chains is consistent with falling oxygen content suspected from elemental analysis and falling H/C values. Signal intensity for the abundant β -O-4' aryl ether linkage (structure A) in EMAL underwent near-complete cleavage over time. This region also contained polysaccharides ($\delta C/\delta H$ 60–85/3.2–4.2) associated with residual carbohydrates; these correlations disappeared from the side chain region resulting in a carbohydrate-free lignin after 4 h of treatment. The aromatic region of the HSQC spectra contains information about the aromatic substructures in lignin. Bagasse lignin could be confirmed as an S-G-H lignin, with EMAL having a S- and H-rich monolignol composition of S:G:H 48:41:12 as expected for bagasse, though with higher H-content than expected for bagasse EMAL, possibly due to the method of isolation used¹³⁷. The subunit composition was seen to change over time, notably with the disappearance of G_2 and S_2 units and concomitant increase in $G_{2,cond}$ and $S_{2,cond}$ units. Similarly, signals associated with PCA subunits were observed to decrease while H units increased. These findings were very similar to previous observations by Brandt *et al.*^{24,87,88} who compared lignins isolated in acidic ionic liquid mixtures following pretreatment of *Miscanthus*.

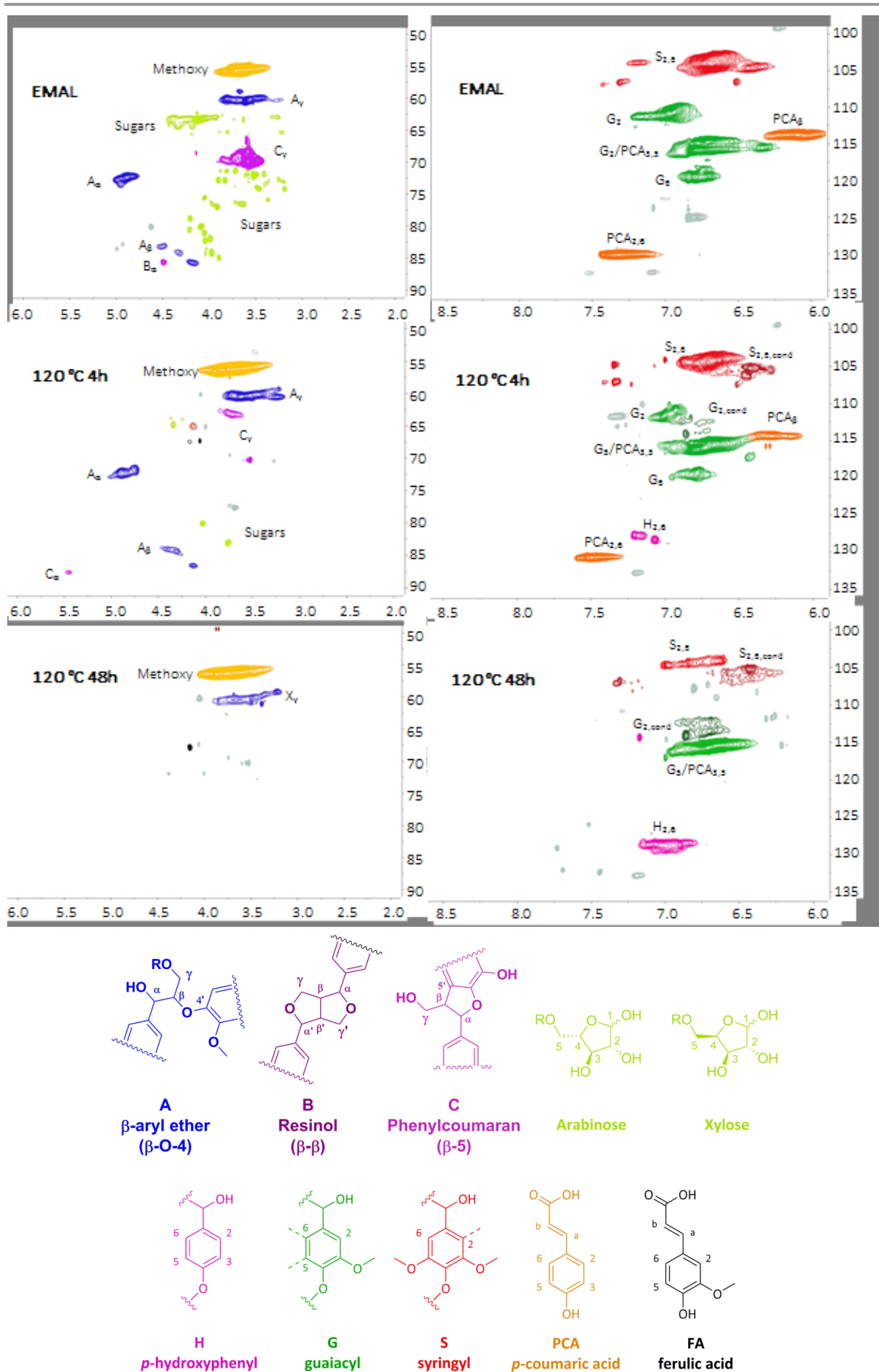


Figure II-51. HSQC NMR spectra of bagasse EMAL and bagasse lignins isolated after pretreatment for different times at 120 °C with [TEA][HSO₄] using a biomass-to-solvent ratio of 1:5 g/g and a final water content of 20wt%, showing the side chain region (left) and aromatic region (right). Representative substructures are shown above.

Cleavage of β -O-4' linkages is well known to be the most important depolymerization reaction in lignins, as scission of these bonds is catalyzed by the acidic pretreatment medium²⁴. Nearly 50% of linkages present in native lignin were cleaved within 4 h, while the disappearance of the β -O-4' linkage signal was nearly quantitative under the most severe treatment conditions employed in this study. A combination of breakage of β -O-4' ether bonds and carbohydrate-lignin linkages is known to facilitate solubilization of the newly generated lignin fragments into the pretreatment medium, from which they can then be isolated by precipitation^{24,87,88}. These changes are typically correlated with the hydrolytic degradation and/or depolymerization of lignin, which would lead to an expected decrease in molecular weight (investigated below). Decreased signal intensities assigned to β - β' and β -5' may seem to suggest cleavage with time and temperature; however, it is more likely these linkages were chemically altered, as they contain C-C bonds which are unlikely to have been broken.

Peak volume integration with respect to the total aromatic ($G_2 + G_{2,cond}$) units enabled more detailed examination of the trends in lignin structure with increasing pretreatment severity. Figure II-52 shows the changes in the major interunit linkages β -O-4', β -5' and β - β' while Figure II-53 examines changes in the relative abundance of major lignin subunits $S_{2,6}$, $H_{2,6}$, $PCA_{2,6}$, G_2 , G_6 , S_{cond} and $G_{2,cond}$ C-H bonds. In Figure II-52, we observe that native lignin (EMAL) is mostly made up of β -O-4' alkyl-aryl ether linkages, accounting for 77% of all interunit linkage types measured, followed by small amounts of β -5' phenylcoumarans (15%) and β - β' resinols (7%); this is consistent with Brazilian sugarcane bagasse MWL compositions reported in the literature (83%, 6% and 0%, respectively)¹⁰⁰. Upon an increase in pretreatment time and temperature, the most abundant β -O-4' bond was cleaved very rapidly while the less abundant β -5' and β - β' linkages reduced more gradually.

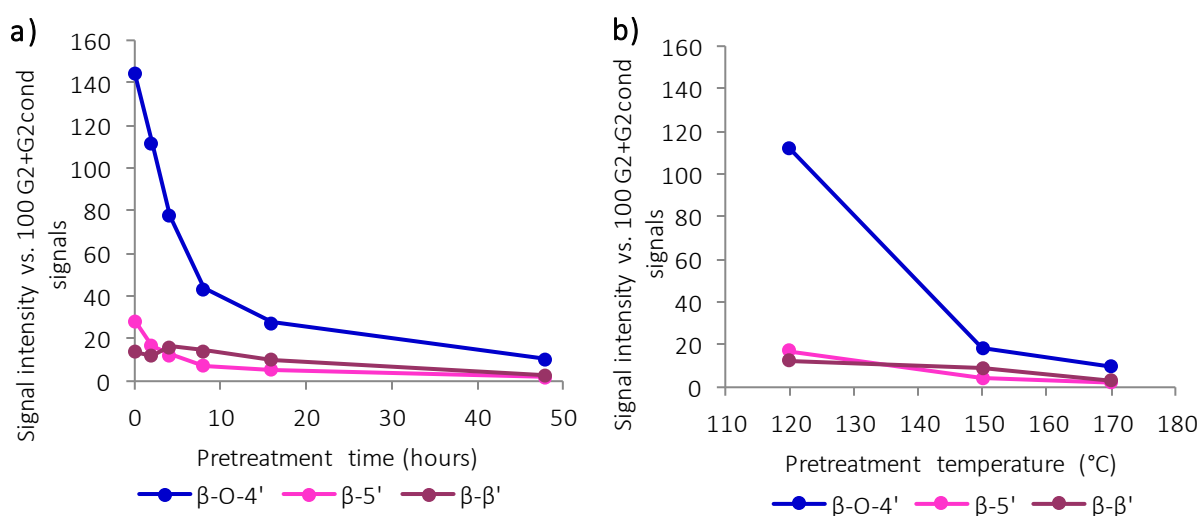


Figure II-52. Relative signal intensity of major lignin linkages in precipitated lignin relative to the combined $G_2/G_{2,cond}$ volume integral as evidenced HSQC NMR spectroscopy. Bagasse was pretreated in $[TEA][HSO_4]$ at a) 120 °C between 0 (EMAL) and 48 h; and at b) various temperatures for 2 h.

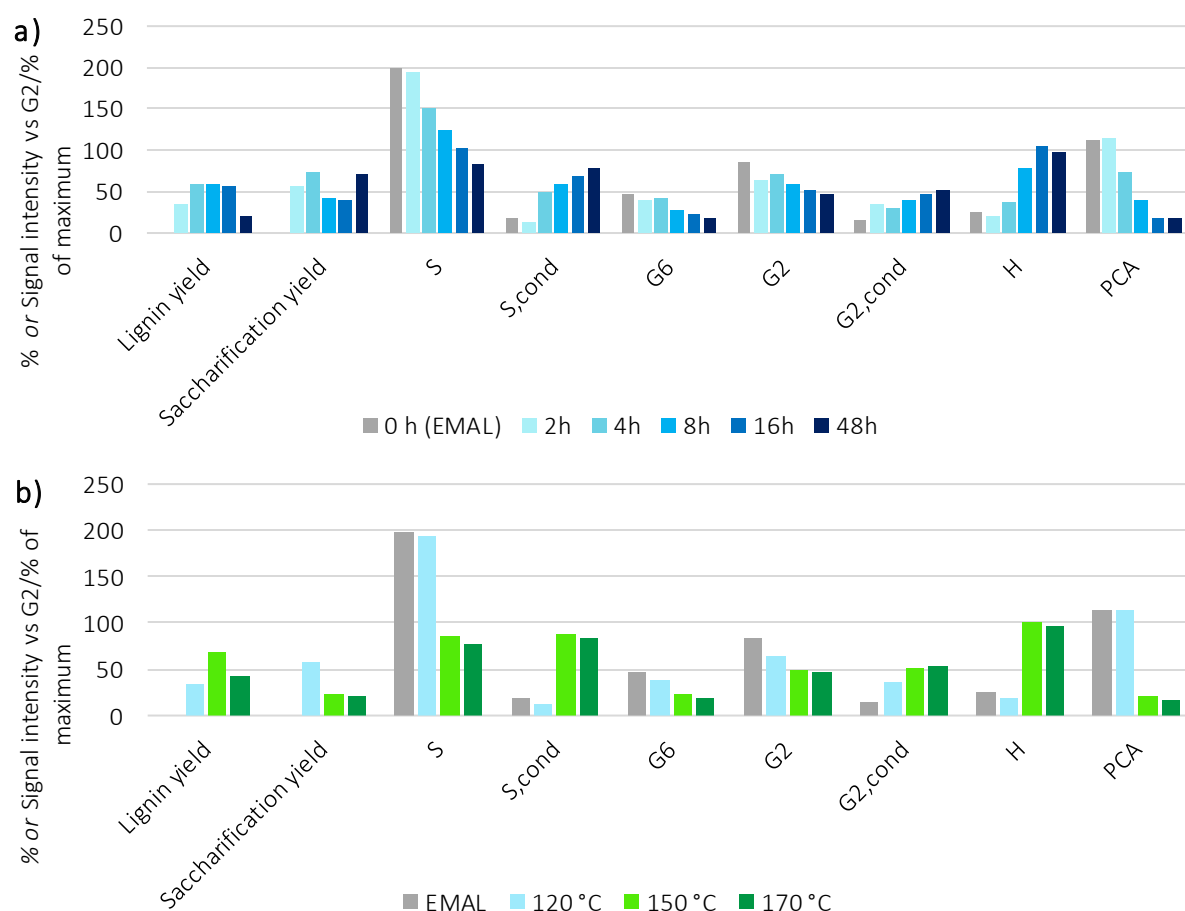


Figure II-53. Relative signal intensities of major lignin subunits in precipitated lignin relative to the combined G₂/G_{2,cond.} volume integral as evidenced HSQC NMR spectroscopy. Bagasse was pretreated in [TEA][HSO₄] at a) 120 °C for pretreatment times between 0 (EMAL) and 48 h; and at b) various temperatures for 2 h.

Figure II-53 captures the most prominent changes in the aromatic region of the HSQC spectra with prolonged treatment time and temperature. Similarly to findings reported for *Miscanthus* lignins by Hallett *et al.*^{24,87,88}, a decrease in the G₆ signal as well as in G₂ and an increase in the G_{2,cond} signal were observed. In addition, the S signal decreased while the S_{cond} signal increased. Previous work by Brandt *et al.*²⁴ concluded that the decrease in G₆ signal relative to G₂ is evidence of condensation (C–C bond formation) taking place at the G₆ ring positions in acidic ionic liquid solutions; the presumed mechanism is shown in Figure II-54. Moreover, lignin condensation was observed to progress irreversibly with prolonged treatment times and to become aggravated by elevated pretreatment temperatures, which may explain the decrease in saccharification yields under these conditions. Condensation can be considered to be a competing mechanism with β-aryl ether cleavage as it causes lignin fragments to recombine, whereas ether bond cleavage leads to fragmentation and/or depolymerization. The resulting effect on molecular weight of lignins is discussed below. Further types of polymerization were observed through changes in the H and PCA content (Figure II-53); apparent enrichment of H units was attributed to conversion of PCA into H-like oligomers via decarboxylation and polymerization reactions catalyzed by the acidic IL-water mixture²⁴.

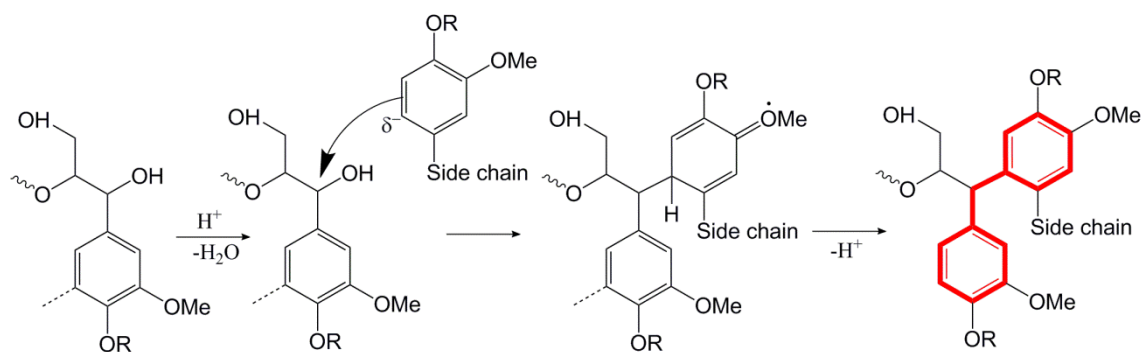


Figure II-54. Formation of condensed diphenylmethane structures at the G6 position during pretreatment in acidic solvents. Assumed mechanism indicated by Brandt *et al.*²⁴

Another structural alteration observed is a change in S/G ratio in lignin, calculated from the $S_{2,6} + S_{2,6\text{cond}}$ and $G_2 + G_{2,\text{cond}}$ signals in the HSQC NMR spectra (Table II-26). The S/G ratio of native bagasse lignin was found to be 1.08, comparing closely with the average value of 1.09 reported in the literature, indicating a slight excess of syringyl units⁹⁵. The data show that the S/G ratio remained relatively stable in the early hours of pretreatment relative to native lignin at ~ 1 , while in highly treated lignins S/G decreased to around 0.8. These findings agree with those of Brandt *et al.*²⁴ who postulated that apparent enrichment of guaiacyl units is due to preferred C–C bond formation (condensation) at the G₆ ring position, resulting in precipitation of G-rich lignin polymers.

To summarize, the major structural changes observed using HSQC NMR spectroscopy are the initial removal of carbohydrates, a dramatic reduction in β -O-4' ether bonds, gradual chemical changes in the β - β' and β -5' substructures, and condensation reactions taking place, leading to the formation of new C–C bonds and to a decrease in the S/G monolignol ratio.

Table II-26. Changes in the S/G ratio with pretreatment time and temperature, as determined from semi-quantitative HSQC NMR spectroscopy.

	EMAL	120 °C 2h	120 °C 4h	120 °C 8h	120 °C 16h	120 °C 48h	150 °C 2h	170 °C 2h
S/G ratio ^a	1.08	1.04	1.01	0.92	0.86	0.81	0.87	0.81

^a S/G ratio calculated as $0.5 \times (S_{2,6} + S_{2,\text{cond}}) / (G_2 + G_{2,\text{cond}})$; ^b Ratio of $G_{2,\text{cond}}$ and G_2 .

Quantitative ¹³C NMR Spectroscopy

To further investigate the fundamental lignin chemistry during the IonoSolv pretreatment process, quantitative ¹³C-NMR spectrum of EMAL, 4, 8 and 48 h lignins was obtained. Most of the observed signals have been previously assigned in herbaceous lignins^{24,90,138,139}. The values for the structural moieties are reported per aryl group, setting the integral at δ C 160–102 ppm as reference for 6.12 aromatic carbons (six aromatic carbons plus 0.12 from the aromatic side chain). The signal quantifications for key functional group clusters for lignins pretreated for 4, 8 and 48 h at 120 °C are displayed in Figure II-55.

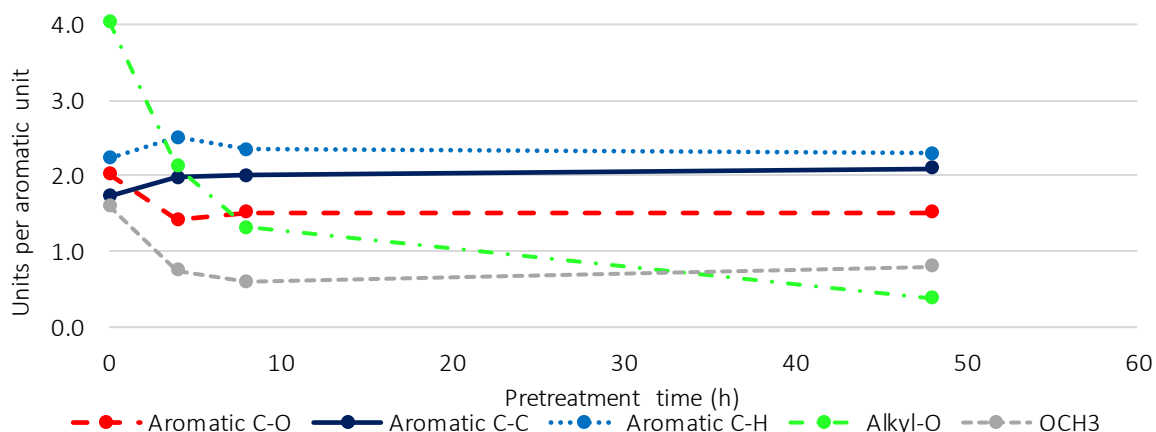


Figure II-55. Relative functional group abundance (per aryl ring) in quantitative ^{13}C spectra of EMAL and selected IonoSolv lignins.

The ^{13}C NMR spectra provided supplementary evidence for some of the changes observed by HSQC NMR and compositional analysis, notably the absence of residual polysaccharides in IonoSolv lignin spectra (δC 58–100 ppm)¹⁴⁰, cleavage β -O-4' linkages under acidic treatment conditions (δC 86.1, 72.4 and 60.1 ppm)¹⁴¹ and the S-G-H type nature of bagasse lignin¹³⁹. The results from integration of five key functional group clusters are shown in Figure II-55. This revealed a high aliphatic C–O bond content in EMAL, with around 4.0 alkyl-O groups per aryl ring. Strikingly, the alkyl-O content dropped drastically within the first hours of pretreatment to 10% of its original level within 48 h of treatment. This implied that the majority of aliphatic side chains were modified during IonoSolv pretreatment, due to dehydration of alkyl chains and shortening of the side chain²⁴.

Further evidence for the occurrence of condensation reactions with extended pretreatment was observed through increased abundance of C–C bonds and decrease in C–H bonds. An initial increase in C–H bonds in 4 h lignin (2.5 groups per aryl ring) compared to EMAL (2.2 groups per aryl ring) is attributable to cleavage of β - β' ethers, giving rise to the formation of two new aromatic C–H bonds (see Figure S0-9. Cleavage of ether bonds in lignin under acidic conditions. Reproduced from Gschwend, 2017⁸⁹. Figure S0-9). Thereafter, the C–H signal can be seen to decrease slowly to 2.3 groups per aryl ring after 48 h of pretreatment. This phenomenon illustrates the competition of the condensation reaction with β -O-ether hydrolysis. Ultimately, the condensation reaction seems to dominate. A gentle decrease in C–O bond abundance was also observed. This could be due to a reduction in the content of S units, which have three C–O bonds, compared to H and G units which have only one or two C–O bonds, respectively²⁴. Another notable change observed was the reduction by 50% in methoxy group content, which again could be linked to the reduction in S units which are OCH_3 -rich; this is promising for pyrolysis reactions to favor high liquid product yields^{68,82}. The S/G ratio was calculated from the ^{13}C NMR spectra using the $\text{S}_{2,6}$ and G_2 peak integrals (see Table S0-11), consistent with the previously observed trend of decreasing S/G ratio observed by Py-GCMS²⁴.

³¹P NMR Spectroscopy

Hydroxyl groups are one of the most important functionalities that determine the reactivity and physical properties of lignins upon fast pyrolysis^{142,143}. A quantitative ³¹P technique originally method developed by Argyropoulos *et al.*^{144–146} was applied to monitor the changes in hydroxyl groups present in the lignins; the results are shown in Figure II-56. The lignin was derivatized with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, which allows good quantification of aliphatic and aromatic hydroxyl groups, condensed hydroxyl units and carboxylic acids, in mmol g⁻¹.

Figure II-56 depicts the concentration changes in the lignin hydroxyl groups during pretreatment. As the syringyl signals overlap with signals of condensed G and S structures, the total phenolic hydroxyl groups (G, S/condensed phenolics, and H) are presented along with aliphatic hydroxyl, carboxylic acid and total hydroxyl groups measured. Similar to previous findings, ³¹P NMR analysis showed a net decrease in the concentration of aliphatic OH groups while the abundance of all other OH groups increased continuously over time^{24,118}. An increase in carboxylic acid groups has been associated with the hydrolysis of *p*-coumarate units, and partially from cleavage of ester bonds from lignin-carbohydrate linkages^{24,147}. Similar results were observed by Tan *et al.*¹⁴³ while monitoring the effect of Organosolv pretreatment on poplar lignin, and El Hage *et al.*¹³⁹ who compared OH concentrations in MWL and Organosolv *Miscanthus* lignins.

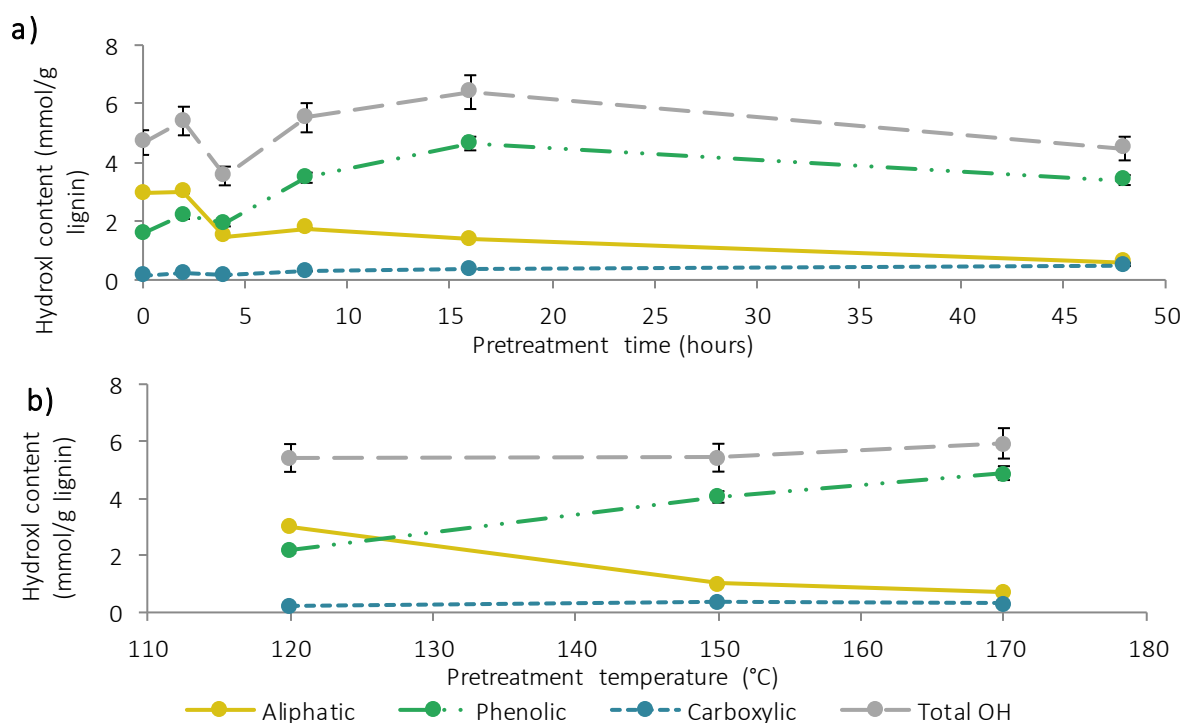


Figure II-56. Hydroxyl group concentrations in precipitated lignin as evidenced quantitative ³¹P NMR spectroscopy, showing the abundance of various OH functional group types with varying a) pretreatment time between 0 (EMAL) and 48 h at 120 °C; and b) pretreatment temperatures for 2 h of treatment.

The loss of aliphatic hydroxyl groups was mainly from the side-chain of lignin via acid-catalyzed elimination, since these lignins have low carbohydrate content¹⁴³. The initial strong decrease in aliphatic OH groups between 2 and 4 h is attributable to abundant β -O-4' bonds breaking relatively quickly, whereby an aliphatic OH group is replaced by an aromatic OH group (see proposed mechanisms in Figure S0-9). Gradual scission of β - β' and β -5' bonds at 120 °C, seen in HSQC NMR, produced new aliphatic and aromatic groups which could explain the slight increase in aliphatic OH groups between 4 and 8 h, beyond which aliphatic OH groups all but disappeared. The ratio of aliphatic to phenolic groups (Table S0-12) therefore decreased with pretreatment severity.

The phenolic OH content stayed relatively stable in the first 4 h of pretreatment, which suggests that the depolymerization may precede repolymerization reactions²⁴. However, as aliphatic OH groups gradually disappeared, the phenolic hydroxyl content continued to increase, particularly after 8 h when HSQC NMR indicates that most ether scissions had ceased. The enrichment of phenolic hydroxyl groups is believed to occur when fragments are added to the lignin polymers *via* condensation^{24,148}, in line with the increase in C-C bonds observed in ¹³C NMR. This likely helped to solubilize the newly formed lignin fragments. Somewhat surprisingly, the content of phenolic OH groups decreased after 48 h at 120 °C. This was attributed to the loss of highly condensed new lignins under severe pretreatment conditions, which became insoluble in the IL/ethanol solvent and therefore did not precipitate, as discussed previously. Accordingly, the signals for condensed and substituted phenolic hydroxyls (which account for nearly half of phenolic OH signals) decreased.

The total OH group content in EMAL was determined to be 4.7 mmol g⁻¹, within the upper range (2.5–5 mmol g⁻¹) reported in the literature for MWL extracted from grasses⁵. Total OH group content in IonoSolv lignins generally increased with pretreatment severity up to a maximum value of 6.4 mmol g⁻¹. The exception is after 4 h of pretreatment at 120 °C, where a decrease in aliphatic OH indicated the maximum extent of ether linkage hydrolysis before condensation reactions cause the phenolic OH to increase, followed by a steady increase in total OH group content. This behavior reflects the competition between β -aryl ether cleavage and condensation reactions^{24,90,147,149}.

Lignin molecular weight

Although NMR spectroscopy provides structural information about lignin, it contains only information about the average lignin macromolecule. GPC, on the other hand, enables insight into the relative size of macromolecules and the molecular size distribution. The molecular distributions of lignin samples were analyzed through DMSO-eluted gel permeation chromatography (GPC), and absolute molar masses (M_w and M_n and polydispersity, i.e. M_w/M_n) were determined by calibration with pullulan standards. Representative molecular mass distributions are displayed in Figure II-57 and relevant molecular markers are shown in Figure II-58.

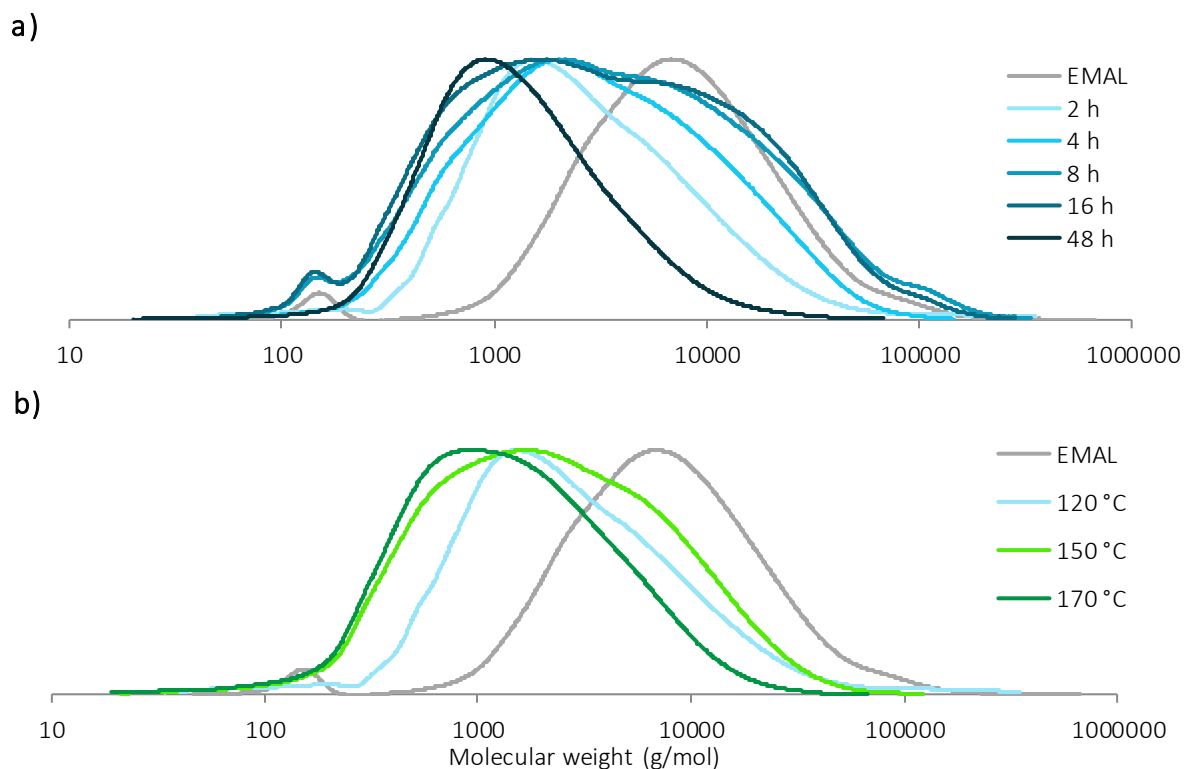


Figure II-57. Peak-normalized GPC profiles of bagasse EMAL and lignin isolated from 80% [TEA][HSO₄] with a biomass to solvent ratio of 1:5 g/g and a final water content of 20 wt%. Profiles are compared for lignins isolated under a) varying pretreatment times at 120 °C; and b) varying pretreatment temperatures for 2 hours.

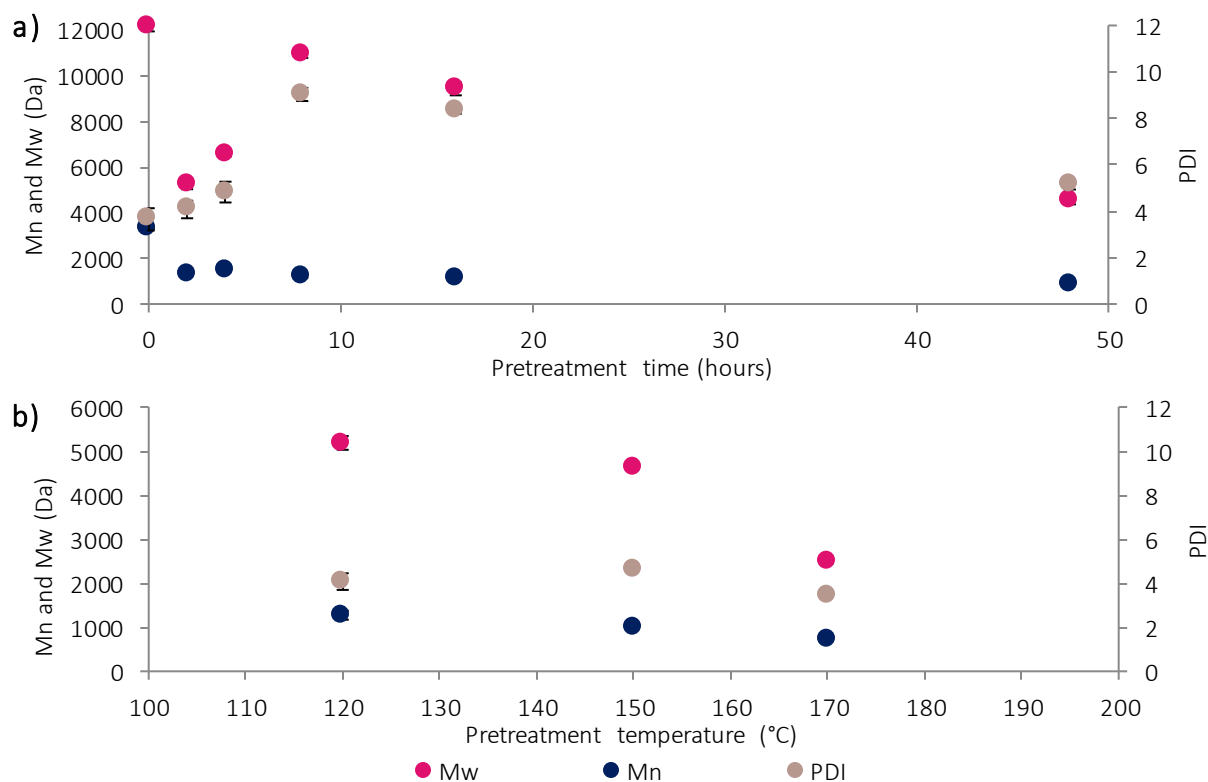


Figure II-58. Molecular markers of isolated bagasse EMAL and IonoSolv lignins. Bagasse was pretreated in [TEA][HSO₄] using varying a) pretreatment times between 0 h (EMAL) and 48 h at 120 °C; and b) pretreatment temperatures for 2 h. All experiments used a biomass-to-solvent ratio of 1/5 with a final water content of 20 wt%. M_w: weight-average molecular weight; M_n: number-average molecular weight; PDI: polydispersity index (M_w/M_n).

ionoSolv treatment conditions were seen to have a strong effect on molecular weight distribution.

Native bagasse lignin was found to have an M_w of 12,100 Da and M_n of 3,300 Da, agreeing closely fairly closely with reported values of 13,700 and 4,100 from the literature¹³⁵. M_w was seen to decrease from 12,100 to 5,200 Da while M_n reduced from 3,300 to 1300 Da in the first 2 h of pretreatment. This initial shortening of lignin chains can be linked to the reduction of ether linkage signals observed by HSQC NMR spectroscopy, indicating that lignin chains were progressively shortened by hydrolysis of β -aryl ether bonds in acidic ionic liquid solution.

The reduction in molecular weight during the first 2 h of pretreatment was followed by a steady increase in M_w to around 11,000 Da after 8 h of pretreatment, while the changes in M_n were more subtle, remaining at around 1100 Da. These diverging trends are reflected in a sharp increase in polydispersity index (PDI) from 4.0 to 9.0 between 2 and 8 h of pretreatment, indicating that there was a small fraction of polymers whose size increased to around 11,000 Da while the majority underwent gentle depolymerization. Since HSQC NMR spectroscopy revealed that condensation reactions are still underway beyond 8 h of pretreatment, further increases in M_w would be expected. However, M_w was seen to decrease from 11,000 Da to 9,500 Da at 16 h and further to 4,500 Da at 48 h. Since it is highly unlikely that condensation products became depolymerized by the scission of C–C bonds, this supports the hypothesis that high molecular weight lignin condensation products became insoluble in the ionic liquid solution or the ethanol-diluted IL after pretreatment and redeposited on the cellulose surface. Similar changes were observed upon an increase in pretreatment temperature (Figure II-58b). While a slight decrease in M_n and M_w occurred upon a temperature increase from 120 °C to 150 °C, the change was much more drastic at 170 °C. Under such severe conditions, condensation of lignin fragments to form new larger chains took place so rapidly that lignin macromolecules did not precipitate, leading to an apparent reduction in molecular weight. The “loss” of the most condensed lignins in the precipitate under prolonged pretreatment times or higher temperatures (see lignin mass balance presented in Figure S0-8) agrees with the findings from ³¹P NMR spectroscopy, leading to a decrease in substituted/condensed phenolic hydroxyl groups (Figure II-56 and Table S0-12).

Lignin thermal characteristics

Thermal properties of lignin were expected to be important for their application in fast pyrolysis. Thermogravimetric analysis (TGA) was used to assess the thermal stability, thermal oxidation stability, ash content and volatile- and char-forming capability of lignins. Similarly, differential scanning calorimetry (DSC) was used to study the thermal behavior of lignin, notably to determine the glass transition temperature T_g of lignin polymers.

Thermogravimetric analysis

TGA was used to study the pyrolytic thermal stability of lignins, as commonly determined under inert atmosphere^{6,40}. Proximate analysis was conducted employing an incremental heating process to quantify the composition of biomass-derived fuels in terms of gross components such as the moisture, volatile matter, ash and fixed carbon. Proximate analysis provides a quick sense of the stability and chemistry of lignins, indicating the volatile matter (VM) lost in heating biomass to a fixed temperature (600 °C), the ash content and the fixed carbon (FC) remainder obtained by difference. The TGA weight loss curves and their first derivatives (DTG) with respect to temperature for all lignins are shown in the Appendix (Figure S0-10). The peak in the DTG curve (DTG_{max}) corresponds to the temperature at which the lignins exhibit the maximum rate of degradation, and may be expressed as a single decomposition temperature and used to compare the relative thermal stability of lignins. These markers of thermal decomposition of lignins are tabulated in Table II-27.

As shown in the TGA curves (Figure S0-10), the thermal degradation of all lignins proceeded over a wide temperature range from approximately 225 °C to 600 °C, suggesting the presence of various oxygen-containing functional groups with different thermal stability. Only EMAL showed some limited weight loss between 175 and 225 °C, attributable to pyrolysis of hemicellulose residues¹⁵⁰. Between 225 and 350 °C, weight loss is associated with primary lignin pyrolysis, whereby fragmentation of interunit linkages results in the release of monomeric phenolic compounds into the vapor phase¹⁵¹. A shoulder in the DTG curve was observed in this range, with EMAL showing the lowest temperature shoulder (219 °C), reflected by its high volatile matter content of 80.6 wt%, topped only by bagasse (88.5 wt%). In such samples, easily degradable β -O-4' intramolecular ether linkages are more abundant compared with ionoSolv lignins. The release of volatile matter decreased sharply from 80.6–58.1% with increasing treatment severity, while the low-temperature shoulder all but disappeared, reflecting the fact that most ether linkages have already been cleaved as evidenced by HSQC NMR. Above 350 °C, the degradation process is possibly related to the slow decomposition of some aromatic rings in lignin⁵⁸. The main decomposition temperatures observed for bagasse (358 °C) and EMAL (380 °C) agreed with literature values for MWL (350–380 °C)^{61,152}. Peak decomposition temperatures generally increased with pretreatment severity until 120 °C, 8 h of treatment (398 °C), decreasing slightly for the most treated lignins such as 120 °C, 48h (392 °C). These results suggest that more treated lignins were more thermally stable, as evident from the higher DTG_{max} values. According to Guo *et al.*, thermal stability of lignins increases with incrementally higher molecular weight⁷⁸. With a greater degree of condensation of the lignin macromolecule, the thermal energy needed for bond cleavage increased^{140,152}. Similar findings were obtained by Tao *et al.*¹⁴³ who compared the maximal degradation temperatures for Organosolv lignin isolated from poplar, which increased from 369 °C to 384 °C with fractionation time.

At 600 °C, more than 21.9% of all lignin samples remained unvolatilized due to the formation of highly condensed aromatic structures. The amount of residue (fixed carbon on a dry ash-free basis) ranged between 21.9–42.7% and was seen to increase with pretreatment severity. This can be linked to higher C content and greater thermal stability of C–C bonds upon condensation. The ratio of volatile matter to fixed carbon (VM/FC) was calculated for the nine fuels analyzed. Bagasse was found to have a high VM/FC ratio of 6.9, in line with most biomass types (>4.0)¹⁵³, the VM/FC ratio for EMAL was 3.7, likely due to its residual carbohydrate content, while IonoSolv lignins had a VM/FC ratio that decreased from 3.1 to 1.3 for the most severely treated lignins.

Table II-27. Values of glass transition temperature (T_g), maximum of thermal decomposition temperature (DTG_{max}) and unvolatilized weight fraction at 600 °C (residue) for all lignin preparations.

Sample	T_g (°C)	Maximum decomposition temperature (°C)	Volatile matter (wt%) ^a	Fixed carbon (wt%) ^a	VM/FC ^a (wt/wt%)
Bagasse	ND	358	88.5	12.2	6.9
EMAL	136	380	80.6	21.9	3.7
120 °C 2 h	134	391	72.3	23.3	3.1
120 °C 4 h	144	396	66.6	33.5	2.1
120 °C 8 h	146	398	64.3	33.8	1.9
120 °C 16 h	147	395	61.9	36.2	1.7
120 °C 48 h	148	392	57.3	42.7	1.3
150 °C 2 h	155	390	56.6	41.7	1.4
170 °C 2 h	154	392	58.1	41.3	1.4

^a Residue reported on a dry ash-free basis.

^b Ratio of volatile matter to fixed carbon on a dry ash-free basis.

ND: Not determined; T_g : Glass transition temperature; DTG_{max}: temperature at which rate of weight change (i.e. gradient of differential thermogravimetric curve) with respect to temperature is steepest.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to determine the glass transition temperatures (T_g) of lignins, an essential parameter for lignin processing and valorization^{3,151,154,155}. T_g values are known to be strongly influenced by water and solvent content. For this reason, they were determined by DSC scans after extensive drying of the samples (45 °C for 48 h under vacuum, followed by freeze-drying) in order to release all of the moisture content prior to analysis. The T_g of each isolated lignin fraction is given in Table II-27. The glass transition temperature (T_g) showed a broad transition range (110–160 °C) for all the analyzed lignin samples (see Figure S0-11). The glass transition was not always clearly observed, suggesting lignin structures are fairly heterogeneous (reflected also in a PDI >3.5 for all samples, as shown by GPC). Higher ramping rates can be used during the DSC experiment to obtain

sharper transitions to observe T_g for such materials, though this was not feasible using the DSC instrument available. EMAL had among the lowest T_g values ($\sim 136^\circ\text{C}$), slightly higher than the T_g values of MWL preparations reported in the literature for grass lignins ($110\text{--}130^\circ\text{C}$)¹⁵⁶, possibly due to residual polysaccharides in EMAL⁸⁵. As expected, lignins isolated under higher temperatures had highest T_g values ($\sim 155^\circ\text{C}$), whereas the least treated lignin sample had the lowest ($\sim 134^\circ\text{C}$). T_g values for IonoSolv lignins were significantly higher than values reported for Organosolv lignin in the literature ($91\text{--}97^\circ\text{C}$) and compared more closely to Kraft lignin ($127\text{--}174^\circ\text{C}$), which contains highly condensed structures¹⁵⁶. This increase in T_g with increasing acidic treatment is in agreement with observations by Olsson and Salmén¹⁵⁷.

Lignin glass transition temperatures could be influenced by several molecular factors such as crosslinking, intermolecular forces and molecular weight¹⁴³. While T_g generally increases with increasing molecular weight, it is also known that the degree of condensation (i.e. the proportion of phenylpropanoid units involved in C–C bonds) has a strong impact on T_g values, as established by Baumberger *et al.* (2002)¹⁵⁶. A high T_g is associated with the formation of hydrogen bonding between the phenolic hydroxyl groups in the lignin main chain, as stronger interactions will reduce the thermal molecular motion of the lignin molecules^{142,143}; this observation agrees with the findings from ^{31}P NMR (Figure II-56). In order to reveal relationships between the measured characteristics of the precipitated lignins, T_g was plotted against several key variables observed by HSQC NMR, ^{31}P NMR and TGA (see Appendix, Figure S0-12). These plots indicated that the chemical characteristics of the lignin samples were strongly correlated to the thermal properties, such as T_g . Aliphatic OH and S/G ratio were negatively correlated to T_g whereas the degree of β -aryl ether bond cleavage and the extent of condensation were loosely positively correlated. The observed relationship between ether bond cleavage and increased T_g has been suggested to be indicative of increased crosslinking with time¹⁵⁸. No significant correlation was measured between T_g and molecular weight; Olsson and Salmén suggest that at such low temperatures, any difference in molecular weight between the lignin polymers would be too small to have an effect on T_g ¹⁵⁷. Neither was the content of phenolic OH groups or total OH groups correlated to T_g . Our results indicated that the effect of formation of hydrogen bonds was less significant than extent of condensation reactions in IonoSolv lignins, presumably given their dramatic effect on molecular weight, as seen by GPC. A strong positive correlation was found between T_g and the VM/FC and fixed carbon content of lignins determined by TGA, confirming the important role of C–C bond formation and condensation and the thermal properties of the precipitated lignins. In summary, **extended pretreatment produced lignins with greater thermal degradation resistance**. Condensation (C–C bond formation from severed β -O-4' linkages) decreased volatile matter yields in favor of char yields and produced lignins with higher T_g .

Summary of lignin changes during ionoSolv pretreatment

Based on the preceding results, a summary of key ionoSolv lignin structural and thermal characteristics is presented in Table II-28 and the major changes during ionoSolv pretreatment are summarized in Table II-29. Lignin structural characteristics were seen to change drastically in the early stages of pretreatment, with cleavage of β -O-4' ether linkages occurring rapidly, leading to a soaring phenolic OH content which helps to solubilize the newly generated lignin fragments. After 4 h of pretreatment, the lignin is most hydrolyzed and least condensed, reflected by a low proportion of condensed/non-condensed phenylpropanoid units as shown by HSQC NMR and low molecular weight seen in GPC. Beyond 4 h, the onset of condensation reactions dominates the trend in molecular weight, which increases sharply until 8 h of pretreatment. Evidence for condensation reactions via the formation of new C-C bonds was seen in the decreasing elemental oxygen content and increasing elemental carbon content and the increase in phenolic OH content in ^{31}P NMR. At higher temperatures and longer treatment times, the lignin contained a higher amount of condensed C-C linkages as shown by HSQC NMR and ^{13}C NMR. This trend was only reversed under extremely prolonged treatment times and very high temperatures, where lignins became so condensed that the highest MW fragments were no longer solubilized in the IL solvent, precipitated with the pulp and therefore the phenolic OH content and molecular weight of the most severely treated lignins was lower than expected.

Thermal analysis of the lignins showed that under harsher treatment conditions, lignin samples with enhanced thermal stability were obtained. Condensation reactions produced crosslinked lignins with higher glass transition temperatures that produced more char and less volatiles upon heating. T_g values could be linked to the degree of condensation as seen in HSQC NMR spectroscopy. Lignins with a low S/G ratio also exhibited a high T_g value, agreeing with observations in the literature^{151,159}; this was presumed to be because G moieties in lignin could lead to increased crosslinking.

Overall, ionoSolv treatment was seen to be able to produce lignins with a wide range of thermal and chemical properties though with a low ash and carbohydrate content. Changes in lignin characteristics with increasing pretreatment severity were summarized in Table II-29, building on the changes observed previously by Brandt *et al.*²⁴. **Milder treatments at low temperatures (120 °C up to 4 h) produced a fairly oxygenated lignin (32 wt% elemental O) with a relatively low MW and a low hydroxyl group content; it was found to be comparatively less thermally stable (T_g ~134 °C). Lignin polymers treated beyond this limit were observed to have higher carbon stability and to become more thermally recalcitrant with T_g values reaching ~155 °C, enriched in elemental carbon and depleted in oxygen (28 wt%) while the macromolecular structure became increasingly condensed, cross-linked and high molecular weight.**

Table II-28. Summary of lignin characteristics as determined from GPC, HSQC NMR spectroscopy, elemental analysis, TGA and DSC. Native bagasse EMAL was extracted and compared with lignins isolated using [TEA][HSO₄] with a biomass to solvent ratio of 1:5 g/g and a final water content of 20wt%.

	EMAL	120 °C 2h	120 °C 4h	120 °C 8h	120 °C 16h	120 °C 48h	150 °C 2h	170 °C 2h
Condensed/ Non-condensed ^a	0.18	0.55	0.41	0.67	0.91	1.08	1.07	1.14
Ether bond cleavage (%) ^b	0	24	43	65	77	92	84	92
M _n (Da)	3,300	1,300	1,400	1,200	1,140	900	1000	720
M _w (Da)	12,100	5,200	6,500	11,000	9,400	4,500	4,600	2,500
Phenolic OH	1.59	2.19	1.92	3.48	4.65	3.40	4.05	4.88
O by difference (wt%)	31.7	31.8	31.6	30.8	30.0	28.6	30.3	28.3
T _g (°C)	n.d.	134	144	146	147	148	155	154
S/G ratio ^b	1.08	1.04	1.01	0.92	0.86	0.81	0.87	0.81
Fixed carbon (wt%) ^c	21.9	23.3	33.5	33.8	36.2	42.7	41.7	41.3

^a Ratio of G_{2,cond} and G₂; ^b Proportion of ether bonds cleaved with reference to total ether bonds in EMAL; β -O-4', β - β' and β -5' α -signals were used.

Table II-29. Effect of IonoSolv pretreatment on isolated lignin characteristics with increasing pretreatment severity.

Impact	Experimental evidence	Technique
Carbohydrate-free	No carbohydrates detected, except at very short treatment times	Compositional analysis, HSQC NMR, ¹³ C NMR
Hydrolysis of ether and ester linkages	Disappearance of β -O-4' ether linkages Decreased molecular weight (M _n) Decrease in aliphatic hydroxyl content Increase in phenolic hydroxyl content	HSQC NMR, GPC, ³¹ P NMR
Changes in subunit composition over time	More H units, fewer PCA and S units More G, condensed G and condensed S	HSQC NMR, ¹³ C NMR
Condensation	Increased C–C aromatic bond content Increase in phenolic hydroxyl content Molecular weight increase Increased carbon content Decreased oxygen content	¹³ C NMR, HSQC NMR, ³¹ P NMR, GPC, EA, TGA, DSC
Thermal recalcitrance	Increased fixed carbon content Decreased volatile matter Increased T _g	TGA, DSC
Inorganic content	Decrease in alkali metal content Decrease in inorganic (ash) content	ICP-OES, TGA

GPC: gel permeation chromatography; EA: elemental analysis; TGA: thermogravimetric analysis; DSC: differential scanning calorimetry; ICP-OES: inductively coupled plasma optical emission spectroscopy.

4.4.3 Pyrolysis product distributions

Pyrolysis product distributions are very complex, and include bio-oils (typically made up of aromatic compounds, aldehydes, alcohols, esters, acids and water), char/ash and non-condensable gases. In this study, the mass fraction of bio-oil, char and the non-condensable gases CO₂, CO and CH₄ were determined. The product distributions for the pyrolysis of the different feedstocks are shown in Figure II-59, with the exception of EMAL for which gas and char yields could not be determined due to lack of sample. The product distributions demonstrated similar trends to those observed in proximate analysis, where the ratio of volatile matter to fixed carbon residue (VM/FC) emitted during TGA decreased sharply with both pretreatment time and temperature compared to native biomass and EMAL (Table II-27). Upon fast pyrolysis, more lightly treated lignins produced the largest quantity of volatiles (bio-oil and gases) while more treated lignins produced more char and less volatiles. The yield of volatiles has been linked to the amount of aromatic rings in lignin involved in β -O-4' bonds^{59,64,83}, whereas the remaining char mass is attributed to monomers connected by C-C bonds, or condensed units^{140,143}. The higher the degree of condensation and carbon stability, therefore, the greater the char yield obtained upon thermal degradation.

The highest bio-oil yield obtained was 54±5 wt% for sugarcane bagasse. George *et al.*¹⁶⁰ conducted fast pyrolysis of sugarcane bagasse at 500 °C and obtained 56.4 wt% bio-oil under similar operating conditions. Studies have shown that the yields of herbaceous biomass bio-oils are usually in the range of 36–56%^{11,161–163}, depending on the feedstock composition and pyrolysis conditions. Bio-oil yields from agricultural residues are typically lower than for woody feedstocks (60–95%), due to the catalytic effect of inorganic ash species on secondary bio-oil cracking and char-forming reactions¹⁶⁴.

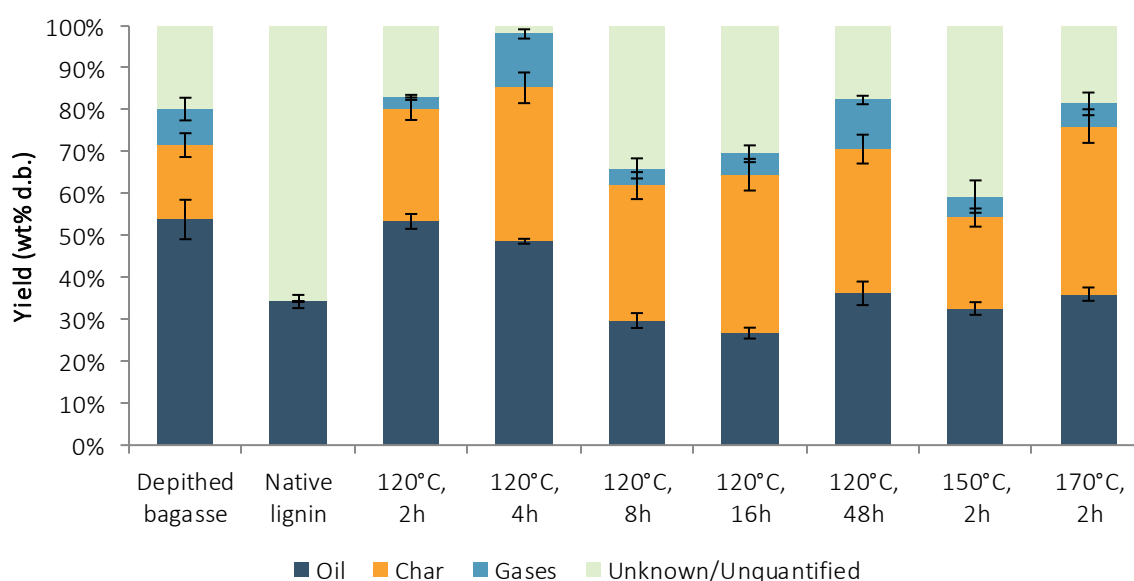


Figure II-59. Pyrolysis product distribution generated from fast pyrolysis of bagasse, EMAL and ionoSolv lignins. Gas yields include CO₂, CO and CH₄. Note: char and gas yields not quantified for EMAL due to lack of sample.

Bio-oil yields for lignins varied between 27–53%, with less treated lignins (e.g. 120 °C, 2h) producing the largest quantity of bio-oil (53%) and more treated lignins (e.g. 120 °C, 16 h) producing the least (27%). An exception was observed for the most severely treated lignins (120 °C, 48 h; 150 °C, 2h and 170 °C, 2 h) where the loss of high-MW lignins produced by condensation reactions can explain the higher than expected pyrolysis oil yields obtained (all 36%). These findings are consistent with the trends in OH group content in the feedstocks observed by ³¹P NMR, which are linked to lower bio-oil yields^{68,82}. The high bio-oil yields suggest that mildly treated lignins (e.g. 120 °C, 2–4 h) are promising candidate feedstocks for pyrolysis as they gave high bio-oil yields comparable to those previously reported for biorefinery lignins, in the range 45–55 wt%⁴⁰. EMAL gave an unexpectedly low bio-oil yield (34%) which was closer to yields obtained for condensed lignins than to biomass, perhaps due to its high ash content (2.2wt%)^{27,76,77}. Liquid yields from EMAL reported in the literature ranged from 40–60% and depended strongly on the purity^{165–167}. Given the low polysaccharide content, it is likely that devolatilization and conversion of EMAL polymers was limited by their high MW. Another possible explanation is feeding issues that occurred with EMAL, which frequently stuck to the feeding tube walls, reducing the observed product yields.

The amount of char produced during pyrolysis increased with condensation (i.e. proportion of phenylpropanoid units involved in C–C bonds), degree of cross-linking and elemental carbon content of the lignins as determined by HSQC NMR (Figure II-53). Char yields were in the range of 21–40 wt%, illustrating the widely varying degrees of condensation within the lignin samples. The fairly low char yields obtained for mildly treated lignins (27% and 37% for 120 °C, 2 h and 4 h lignin, respectively) indicate that IonoSolv lignins are promising feedstocks for pyrolysis. In contrast, char yields of 30–35 wt% were previously obtained for herbaceous Organosolv lignin by de Wild *et al.* in a fluidized bed pyrolysis reactor at 500 °C⁴⁰. Higher char yields from more treated lignins can be explained by the presence of very stable aromatic rings and C–C bonds in the macromolecule structure of lignin²³. These structural changes seem to outweigh the well-established correlation between alkali metal content (Figure II-50) and the incidence of char-forming reactions^{27,76,77}. Bagasse gave rather high char yields (18 wt%) given its low ash content (0.9 wt%), which was considerably higher than the 6.3 wt% reported by George *et al.*¹⁶⁰ as previously described. This result may be due to the fixed-bed reactor setup, as rather high char yields of 22 wt% were previously obtained by Boot-Handford for beech wood containing only 0.5 wt% ash using the same fixed-bed reactor¹⁰⁴.

The gas yields obtained were fairly low (3–13 wt%) and did not seem to correlate with the β-aryl ether content or degree of condensation of the lignins. CH₄ is known to result mainly from fragmentation of side chains and demethylation of OCH₃ groups⁸¹ (both were modified or decreased as seen in HSQC and ¹³C NMR spectroscopy) while CO₂ and CO are the main deoxygenation route from decomposition

of carboxyl, ester and carbonyl groups⁸¹; we would therefore expect gas yields to decrease with lignin treatment severity. It should be noted that due to the low gas concentrations and limited sensitivity of the gas detectors, the uncertainties in the gaseous product distribution were high and so the relationship between gas yields and feedstock characteristics cannot be reliably compared at this point. However, the total volatile yield expected from proximate analysis (58–89 wt%) was not exceeded within error, as the sum of bio-oil and gas yields quantified in the pyrolysis product distribution was limited to 62 wt%.

Between 2 and 40 wt% of the pyrolysis products could not be accounted for. Unidentified products are likely to include low molecular weight compounds such as light hydrocarbons, light bio-oil species, alcohols, water and hydrogen^{6,40,54}, that were either not effectively condensed in the tar trap during the experiment, or were lost during the rotary evaporation and oven-drying steps of product recovery procedure. Previous work with the same fixed-bed reactor¹⁰⁴ found that between 35–45 wt% of the pyrolysis products were unidentified. Up to half of the unidentified elements are likely to be water, which makes up ca. 5–15 wt% of lignin bio-oils and 10–25 wt% of biomass bio-oils. Water content measurement is an important element not only to close the mass balance but would also enable comparison of fuel value of bio-oils on a dry basis. However, this was not yet feasible using conventional techniques such as Karl-Fischer titration or ³¹P NMR spectroscopy at the time of this study, but is being investigated for future publications.

4.4.4 Pyrolysis product analysis

SEC analysis of bio-oils

Size exclusion chromatography (SEC) was employed to provide information about the molecular size distribution of the bio-oils produced from fast pyrolysis. Results from this method were compared with polymer-based calibrations, commonly used to convert elution times into estimates of molecular mass. SEC gives relative molecular mass distributions, since the absolute MW of compounds determined by SEC are dependent on the set-up¹⁶⁸. Figure II-60 shows bio-oil components are bimodally distributed with a low-intensity valley between two bands of signals, consistent with previous reports of biomass-derived samples using this SEC setup^{104,168}. The first (excluded) peak corresponded to the elution of molecules with high apparent molecular mass that are excluded by column porosity; these peaks are outside of the calibrated range of the column. The second (retained) peak in the SEC chromatograph corresponded to elution of lower molecular mass components which are retained and can be fully resolved. Comparison of the SEC profiles of the precursor materials and the resulting bio-oils, shown in Figure S0-13 in the Appendix, reveal that pyrolysis resulted in extensive thermal depolymerization of bagasse and lignins, as seen by a shift towards longer elution times and by a decrease in the proportion of larger molecular mass material excluded by the column relative to

retained material. Representative SEC profiles of the bio-oils obtained with the molecular mass corresponding to maximum peak intensities were calculated and are shown inset in Figure II-60 (see also Table S0-6).

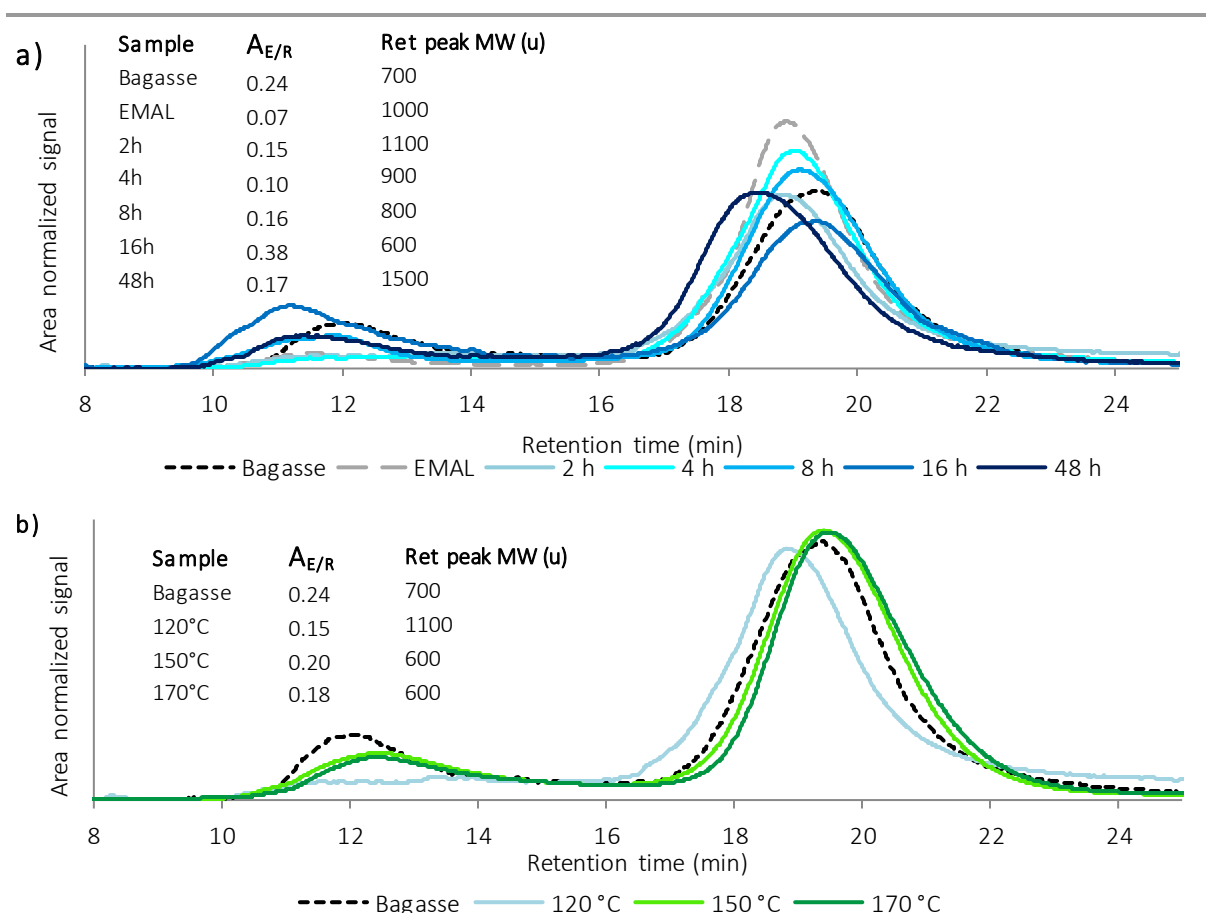


Figure II-60. Area normalized SEC profiles of bio-oils obtained from EMAL and ionoSolv lignins isolated from sugarcane bagasse. Depithed bagasse was pretreated at a biomass to solvent ratio of 1:5 g/g in 80% [TEA][HSO₄] with a final water content of 20 wt% at a) varying pretreatment times at 120 °C; and b) varying temperatures for 2 h treatment time. Inset: Ratio of excluded peak (9–15 min) to retained peak (15–25 min) and molecular weight associated with retained peak elution time and corresponding molecular mass values are shown inset.

Observing the relative areas of the excluded and retained peaks ($A_{E/R}$), we see that lignins pretreated under more severe conditions generally have a higher proportion of high MW molecules compared to low MW molecules. This change seems to reflect the increasing condensation, MW and thermal recalcitrance of lignin molecules themselves, producing bio-oils with a larger proportion of high MW material upon pyrolysis. Again an exception is noted for the most severe conditions applied (120 °C, 48h and 170 °C, 2h) where the excluded peak became less pronounced, attributed to the loss of the most high MW lignins to the pulp fraction under prolonged treatment (see Section 4.3.3). No correlation was established by comparing $A_{E/R}$ and the polydispersity of lignin precursors, however, suggesting that aggregation or interaction of bio-oil molecules with the column may also be occurring which would distort the proportion of retained molecules. The excluded peak in the bagasse bio-oil appeared at a longer elution time compared with all lignin bio-oils, suggesting that the heavier fraction of bagasse bio-oils have lower average molecular mass. This may be because bagasse contains ~75

wt% cellulose and hemicellulose which are less thermally recalcitrant than lignin, forming more small molecules during pyrolysis. For EMAL, a similar effect is observed, owing to its low degree of condensation and high ether linkage content; accordingly, the $A_{E/R}$ ratio is lowest (0.07) compared with IonoSolv lignins (0.10–0.38).

Retained peaks, corresponding to the lighter fraction of the bio-oils, were broader for lignins produced under more severe conditions, even where the peak maxima were similar. This suggests that mild pretreatment conditions, where lignin is hydrolyzed but not condensed, may be preferable to obtain a narrower molecular weight distribution in the resulting bio-oil. With increasing treatment of lignins, the retained peak positions were seen to shift to longer elution times by SEC, corresponding to lower average molecular weight; this mirrors the decrease in number-average molecular weight (M_n) of lignins seen by GPC. Lower average molecular weight in more depolymerized lignins accordingly produced lighter bio-oil components. These findings illustrate that the competition between scission of ether linkages and condensation reactions is also apparent in the oils: more treated lignins produce more polydisperse bio-oils with increasing $A_{E/R}$ while the lighter retained fraction of bio-oil components becomes progressively lower in MW. However, the bio-oil produced from severely treated lignin (120 °C, 48h) had the highest MW components, with the retained peak eluting earlier than for all other bio-oils. This could indicate a change in chemical composition of this bio-oil that would interact differently with the SEC column. Though 48h *lignin* was not among the highest in MW, HSQC NMR and TGA results revealed that it was the second most condensed and had the lowest VM/FC ratio of all lignins analyzed. Though comparable in chemical structure to 170 °C, 2h lignin, it had a higher MW (4500 vs 2500 u) and lower phenolic OH content, which could make this lignin significantly less reactive and this added thermal recalcitrance is likely to have produced bio-oils with a higher apparent molecular weight.

UV-F analysis of bio-oils

UV-fluorescence (UV-F) spectroscopy was used to provide information on the degree of conjugation and, to some extent, the size of the different species present in the tar samples. UV-F is only able to detect molecular species with masses below 3000 u, and so does not provide any information on the species appearing in the excluded region of SEC spectra¹⁰⁴. Figure II-61 presents height-normalized synchronous UV-F spectra of bagasse, EMAL and lignin bio-oils. The UV-F spectra were characterized by emission spectra spanning ~270–450 nm with three maxima appearing at ~310, 330 and 355 nm. The presence of these relatively well-defined peaks indicates that the lighter fraction of the bio-oils produced (as characterized by SEC) broadly consisted of three molecular groups that differed by the extent of conjugation and size of aromatic chromophores present. Lower wavelength features correspond to less conjugated components while higher wavelengths indicated greater conjugation.

Bagasse and EMAL had distinctly different spectral profiles to IonoSolv lignins, with increased fluorescence intensity in the first maximum at ~310 nm, suggesting there is a larger proportion of less conjugated bio-oil species in these tars. These UV-F spectra were similar to those reported by Boot-Handford¹⁰⁴, who compared bio-oils obtained from various agricultural residues. Given the major structural difference between these two samples and lignins is their carbohydrate content, it is highly likely that less conjugated (presumably aliphatic) bio-oil components arose from thermal decomposition of cellulose and hemicellulose present in biomass.

Lignin UV-F spectra were distinctly different, exhibiting two major peaks at ~330 and ~355 nm. Changes in spectral profiles were observed as lignin feedstocks became more condensed with treatment, as seen by NMR. A clear trend of increased fluorescence intensity at longer wavelengths was observed with increasing molecular size, as expected^{104,168}. The original substrate, lignin, contains mostly small single-ring aromatic nuclei, though condensed multiple-ring aromatic nuclei are more abundant in more treated lignins. In view of the relatively low temperature at which the bio-oils were prepared, it appears unlikely that destruction of these small aromatic nuclei would occur¹⁶⁹. Therefore, the changes in the UV-F spectra with increasing lignin treatment suggest that more condensed lignins produced bio-oils with more intensely substituted and cross-linked ring systems¹⁷⁰ and decreasing oxygenated substituent content¹⁶⁹. Spectral shifts to longer wavelengths are also expected with more heteroatom-containing aromatic substituent groups¹⁷¹, agreeing with the increasing phenolic OH groups seen in ³¹P NMR. UV-F results seem to be consistent with the SEC data, which suggest there is more variation in the conjugation extents of the bio-oil species that make up the lighter fraction of the bio-oils derived from more treated lignins. A notable outlier was the bio-oil deriving from lignin treated for 48 h at 120 °C, which showed increased intensity in the central (less conjugated) peak; this may arise from loss of the most condensed lignins.

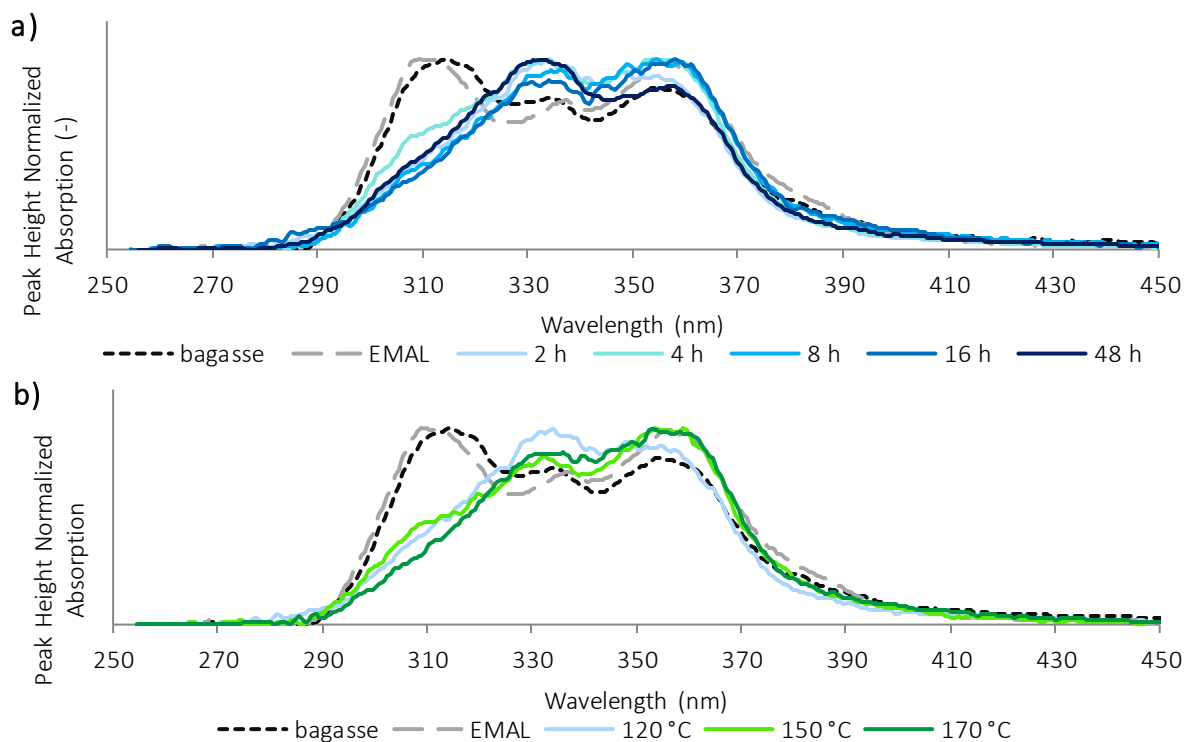


Figure II-61. Peak-normalized UV-F spectra of bio-oils obtained from EMAL and ionoSolv lignins isolated from bagasse. Depithed bagasse was pretreated at a biomass to solvent ratio of 1:5 g/g in 80% [TEA][HSO₄] with a final water content of 20 wt% at a) varying pretreatment times at 120 °C; and b) varying temperatures for 2h.

¹H NMR analysis of bio-oils

¹H NMR was used as a means of obtaining approximate ratios of the chemical environments of protons present in bio-oil. The integral values of selected regions of the spectra were calculated on a percentage basis after subtracting the region with significant water resonances which interfered with the spectra (δH 3.0–4.5), as the water content of the oils could not be quantified and was presumed to be significant. Figure II-62 shows changes in the hydrogen type distribution in the bio-oils in terms of the structural assignments outlined in the Appendix (Table S0-8).

From the spectra it is evident that there are major differences in the overall chemical makeup in the bio-oils produced from different feedstocks. Despite significant water peaks that distorted the analysis and the limited possibilities of assigning shifts to particular structural features, some trends could be discerned. For example, the alkane proton content of the bio-oils (δH 0.5–3.0 ppm) decreased while aromatic proton content increased with increasing lignin treatment. This is in line with the findings of aryl-ether cleavage and modified aliphatic side chains observed by HSQC NMR and ¹³C NMR spectroscopy. No clear trend was observed for in the region representing aliphatic protons that are adjacent to heteroatoms (O) or unsaturated groups (δH 1.5–3.0 ppm) with increasing lignin treatment. Bagasse, EMAL and heavily treated lignins had higher levels of these protons (~20–25%) reflecting their higher degree of conjugation, as witnessed by UV-F spectra. On the other hand, mildly treated

lignins (e.g. 120 °C, 4 h) had lower levels of these protons (~13%), which was linked to its low polysaccharide content but low degree of condensation.

Strikingly, bagasse bio-oil contained a *lower* content of alkane protons (~21%) compared with all lignin bio-oils (~35–50%). This can be explained by bagasse bio-oil having a significantly higher population in the region representing protons of carbohydrate-like molecules (δH 4.5–6.0). Further, bagasse bio-oil also had signals associated with intact carbohydrate content (~5 ppm)¹⁷². This was consistent with UV-F characterization, which showed higher levels of less conjugated (presumably aliphatic) bio-oil components arising from polysaccharide degradation in bagasse bio-oil compared with lignin bio-oils. Mullen and Boateng employed ¹H NMR to characterize bio-oils produced by fast pyrolysis of switchgrass and corn stover at 450–550 °C using a fluidized bed reactor¹⁷². These bio-oils showed high proportions (~30%) of carbohydrate-derived protons, similar to our findings for bagasse bio-oil (~25%). Some intensity (~2–7%) was also observed in this region for lignin bio-oils, most likely representing the presence of aromatic ether protons (i.e. lignin derived methoxyphenols). Aromatic ethers are likely to derive from side-chain reduction and their formation seems to have been favored by moderate lignin treatment (120 °C, 8h), possibly reducing thereafter due to increasing side chain modification. Another notable feature is that EMAL bio-oil contains a low proportion of these protons, reflecting the comparatively low polysaccharide content of the parent material.

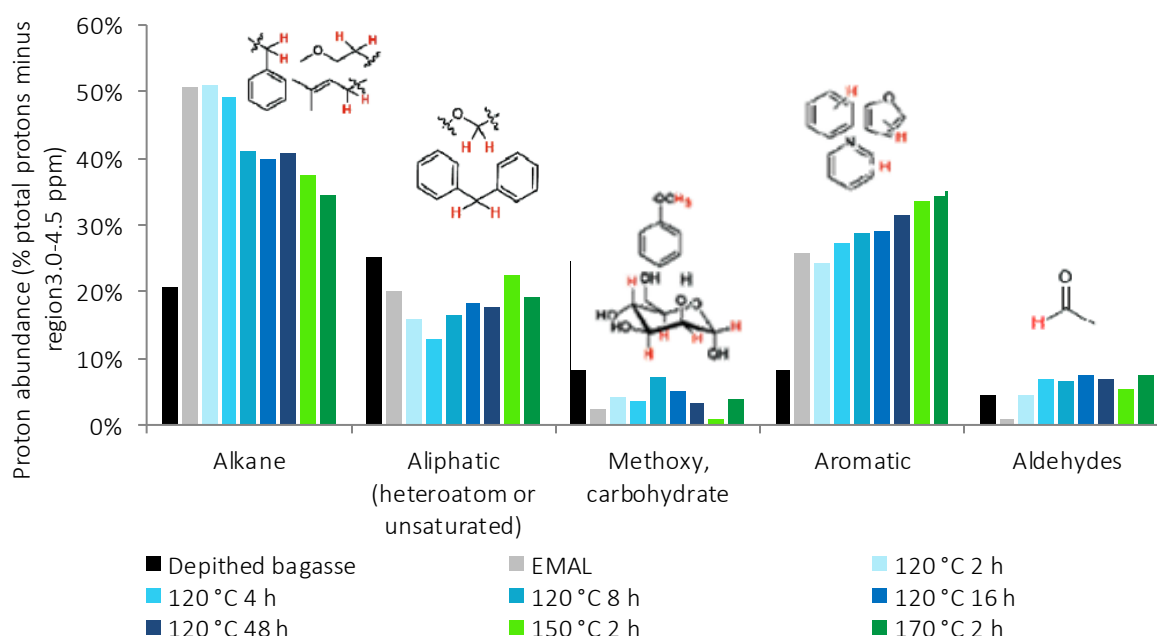


Figure II-62. ¹H NMR of liquid products obtained by fast pyrolysis of bagasse, EMAL and IonoSolv lignins. Spectra were normalized to the total number of protons after subtracting signals from the region δH 3.0–4.5 ppm.

The aromatic region (δH 6.0–8.5 ppm) of the spectra represents not only proton atoms in benzenoids, but also those in heteroaromatics containing O. These were highly abundant (~25–35%) in lignin bio-oils, as we would expect within lignin bio-oils which reportedly contain 40–70% aromatic structures

by weight⁴¹. As aromatization reactions are unlikely at the comparatively low temperature (500 °C) at which the bio-oils were prepared, the observed H-aromatization probably reflects the increasing concentration of benzenoids and aromatics in the bio-oils¹⁶⁹. During lignin pyrolysis, various reactions including hydrolysis and cleavage of the C–C and beta-aryl ether linkages occur, which would give rise to formation of phenolic monomers and dimers. Changes in proton abundance in this region can be attributed to differences in the lignin structure, with more condensed lignins giving a higher proportion of aromatic protons in the resulting bio-oil¹⁷². These findings were in alignment with UV-F analysis, which indicated an increase in conjugation and aromaticity of bio-oils.

The most downfield spectral region (δH 8.5–10 ppm) contains resonances that most likely arise from aldehydes or carboxylic acids. These resonances were most strongly observed for more treated lignins, although they were also detected in the spectrum for bagasse bio-oil; however, they were absent for EMAL bio-oil. Aldehydes can arise from multiple reaction pathways, including α -O-4', β -O-4' and β -1' cleavage⁴¹. Trace amounts of aldehyde have been detected in herbaceous biomass bio-oils, as noted by Mullen and Boateng^{172,173}; the authors also note that alkali metals in the bagasse ash have been known to change the mechanistic pathways of cellulose decomposition to produce hydroxyacetaldehyde rather than levoglucosan, which would appear in this region.

According to literature, during pyrolysis the remaining ether linkages will be the first to degrade, together with aliphatic hydroxyl groups⁴¹. The primary cleavage products are aliphatics, guaiacyl hydroxyls, styrenes, toluols and aldehydes while *p*-hydroxyphenols, catechols and cresols are products of further decomposition⁴¹. These products are likely to be represented in the regions between 0.5–3.0 and 4.5–8.5 ppm. These pathways are likely to occur in less treated lignins which contain a higher proportion of β -O-4' and α -O-4' linkages. In more treated lignins, β -1' and β - β' linkages predominate, cleavage of which forms methyl guaiacols, stilbenes, aldehydes and styrenes⁴¹, appearing in the region 4.5–10.0. Consequently, a decrease in the aliphatic/aromatic proton ratio from ~2:1 to ~1.1 between EMAL and severely treated lignin was observed in this study.

Overall, ¹H NMR results indicate that under the pyrolysis conditions employed, C–C linkages and ether linkages such as β -O-4' were easier to break, resulting in formation of phenolics, aromatic ethers and aldehydes; however, aromatic rings were not destroyed at pyrolysis temperatures of 500 °C. Accordingly, ¹H NMR results showed that pyrolysis of lignins with more condensed structures gave rise to a greater proportion of aromatic products compared with aliphatic products. However, given the complexity of lignin pyrolytic degradation pathways, NMR can only be used to support theoretical reaction routes; more powerful tools such as GC-MS are therefore necessary to provide more detailed analysis of the pyrolysis reaction products.

Elemental analysis of bio-oils

Table II-30 presents CHN analyses of the various bio-oils produced from bagasse and lignins. Given the trend in increasing carbon content and decreasing oxygen content in the parent lignins with pretreatment severity, one would reasonably expect a similar trend in the resulting lignin bio-oils. However, **no clear link between pretreatment severity and bio-oil elemental composition was observed**. It is possible that elemental compositions of the bio-oils were skewed by limitations in the bio-oil recovery procedure used, which involved washing of the condensed liquids using a chloroform-methanol solvent mixture, followed by drying *in vacuo* to obtain a dried bio-oil. Many of the volatile liquid products, such as highly oxygen-rich carboxylic acids, aldehydes, hydroxylaldehydes and some ketones with low boiling points⁶, almost certainly escaped during the drying step and their contribution to the elemental composition of the resulting bio-oils was therefore not accounted for. Further, water content could not be determined.

The elemental compositions of bio-oils with their respective parent biomass/lignins are displayed in (see Appendix, Table S0-7). In all cases, bio-oils possessed a higher carbon and hydrogen content and lower oxygen content than their parent materials. Pyrolysis reactions including dehydration, decarbonylation, demethoxylation, condensation as well as devolatilization (notably evolution of oxygen-rich acetic acid, H₂O, CO and CO₂) may have resulted in lower H/C ratios and higher O/C in the recovered bio-oil products compared with the parent biomass or lignin material⁵⁸.

A striking difference was seen between bagasse bio-oil which had markedly higher oxygen content (41.6 wt%) than all lignin bio-oils (24.0–31.1 wt%), as expected given thermal decomposition of polysaccharides in biomass produces a higher proportion of oxygenated compounds. Accordingly, the calculated HHV of bagasse bio-oil (20.5 MJ kg⁻¹) was significantly lower than for lignin bio-oils (26.7–30.2 MJ kg⁻¹). **This confirms the hypothesis that lignin produces bio-oils with higher fuel value than biomass, the contribution from water notwithstanding**. Lignin bio-oil HHV were higher than expected from literature (~19–26 MJ kg⁻¹)⁶ while bagasse bio-oil agreed closely with literature values (~20 MJ kg⁻¹)¹¹. Lignin bio-oil heating values also exceeded those of soda pulping lignin and acid hydrolysis lignin bio-oil⁶, illustrating the desirability of ionoSolv lignins as feedstocks due to their high purity. However, it was noted that energy contents of ionoSolv lignin bio-oils (27–30 MJ kg⁻¹) resembled their parent lignins (23–25 MJ kg⁻¹) more than those of crude oil (~40 MJ kg⁻¹)¹¹.

The fuel quality of bio-oils can also be evaluated by their O/C and H/C molar ratios, with low O/C and high H/C ratios indicating a higher quality liquid product¹¹. Nowakowski *et al.*⁶ compared bio-oils from technical and biorefinery lignins and reported H/C values between 1.3–1.8 while O/C values were reasonably low, between 0.4–0.7. Results from ionoSolv lignin pyrolysis were encouraging, with H/C values >1.8 and O/C values <0.4. These characteristics compare reasonably favorably with those for

petroleum-derived fuels, where we would expect H/C between 1.5–2.0, though with O/C close to zero, indicating the need for deoxygenation and/or catalytic upgrading of lignin bio-oils for use as transport fuels¹⁸. **Pyrolysis of more treated lignins led to a lower proportion of carbon retained in the liquid products and a greater extent of deoxygenation**, as carbon retention in the char increased since more condensed lignins showed greater carbon stability, as evidenced by TGA and DSC.

Table II-30. Summary of the elemental analysis and calculated HHV values for the bio-oils produced from flash pyrolysis of EMAL and IonoSolv lignins in a downdraft fixed-bed pyrolysis reactor.

Sample	Ultimate analysis (wt%)				Atomic ratio		HHV ^b (MJ kg ⁻¹)	wt% of Feedstock Carbon in Bio-oil
	C	H	N	O ^a	H/C	O/C		
Bagasse	51.2	6.9	0.3	41.6	2.15	0.61	20.5	59.3
EMAL	63.2	7.1	0	29.7	1.80	0.35	26.7	34.8
120 °C 2 h	67.0	8.0	0.9	24.0	1.92	0.27	30.2	58.5
120 °C 4 h	62.0	7.4	0	30.6	1.92	0.37	26.6	49.2
120 °C 8 h	62.1	7.0	1.4	29.5	1.80	0.36	26.2	30.5
120 °C 16 h	62.8	7.6	2.2	27.4	1.93	0.33	27.6	27.3
120 °C 48 h	64.9	7.1	0	28.0	1.75	0.32	27.5	36.4
150 °C 2 h	65.1	7.4	0.7	26.8	1.81	0.31	28.2	33.4
170 °C 2 h	61.1	7.6	0.3	31.1	1.98	0.38	26.4	33.6

^a The oxygen content was determined by difference. ^b Higher heating value, calculated from the elemental composition according to Demirbas¹¹².

Elemental analysis of chars

Table II-31 presents elemental analyses of the chars produced from bagasse and lignins. With increasing pretreatment time and temperature to produce the lignins, the carbon content increased and the oxygen content decreased, producing chars that showed an apparent trend of increasing HHV with treatment time. This is as expected given that more severely treated lignins had increasing fixed carbon content, C–C bond content and thermal stability, as determined by elemental analysis, NMR and TGA, leading to greater carbon retention within the solid products^{140,143}. It is also likely that less oxygenated lignin samples would form a cross-linked char during rapid heating at moderate temperatures, which is less susceptible to volatilize during pyrolysis. HHV of chars were generally lower than the HHV of the parent materials except for the most severely treated lignins. This could be explained by less ash incorporation into the chars in line with the trends observed by ICP-OES of the feedstocks, or to a greater tendency towards carbon retention during char-forming reactions which may be linked to carbon stability and C–C bond abundance in more treated lignins.

Table II-31. Summary of the elemental analysis and calculated HHV values for the chars produced from flash pyrolysis of EMAL and IonoSolv lignins in a downdraft fixed-bed pyrolysis reactor

Sample	Ultimate analysis (wt%)				Atomic ratio		HHV (MJ kg ⁻¹)
	C	H	N	O ^a	H/C	O/C	
Bagasse	52.2	3.1	0	44.7	0.96	0.64	15.0
EMAL*	-	-	-	-	-	-	-
120 °C 2 h	54.6	2.6	0.7	42.1	0.76	0.58	15.5
120 °C 4 h	68.7	3.2	0	28.1	0.75	0.31	23.2
120 °C 8 h	61.9	3.4	0	34.8	0.87	0.42	20.1
120 °C 16 h	67.0	3.3	0.5	29.1	0.79	0.33	22.7
120 °C 48 h	78.9	3.5	0	17.6	0.71	0.17	28.7
150 °C 2 h	77.3	3.4	0.3	18.9	0.71	0.18	27.9
170 °C 2 h	74.0	3.8	0	22.3	0.82	0.23	26.7

* CHNS analysis not possible for EMAL as insufficient could be separated from sand collected from the reactor bed.

Material and energy balance

Following simple material and heat balance calculations based on the input and output streams and using calculated HHV as reported in Table S0-7 (see Appendix), the energy recovery and efficiencies were computed (see Table II-32). We note that energy in the “lost” gaseous products and volatiles was not accounted for in this analysis. Boateng *et al.*¹⁶³ calculated that the energy requirement for bench-scale fluidized pyrolysis of switchgrass at 500 °C is ca. 20% of feedstock energy content, so this was used to estimate reactor heating requirements for pyrolysis. Total energy recovery, estimated as the ratio of the sum of the HHV of the raw products to that of the dry feedstock, ranged from 55–84%, though only 60–98% of the product mass was accounted for. For bagasse and for lignins subjected to mild pretreatment (120 °C, 2–4 h), a larger proportion of energy was recovered in the oil compared to the char; this was reversed for more treated lignins (120 °C, 8–48 h; 170 °C, 2 h) where char yields and char carbon retention were higher. Theoretically, there is sufficient energy in the char to sustain the endothermic pyrolysis reaction for all feedstocks except bagasse. **If we account for this coproduct as an energy source to replace or supplement the electrical power supplied, the energy efficiencies described above improve further to 63–96%. The energy and carbon recovery in the bio-oil also increase up to ~70% and ~60%, respectively.**

Table II-32. System energy balance calculated using estimated HHV values of input and output streams.

Sample	Energy in (MJ h ⁻¹)			Energy out (MJ h ⁻¹)				Energy recovery in oil (%)	Energy recovery in char (%)
	Reactor heating	Feedstock	Total	Bio-oil	Char	Gas	Total		
Bagasse	3.4	16.9	20.3	11.0	2.7	0	13.7	54.4	13.2
EMAL*	3.4	25.0	28.4	9.1	-	-	-	31.9	-
120 °C 2 h	3.4	23.7	27.1	16.1	4.2	0	20.3	59.5	15.4
120 °C 4 h	3.4	23.6	26.9	12.9	9.7	0	22.1	48.0	34.1
120 °C 8 h	3.4	23.7	27.1	7.8	8.4	0	16.2	28.7	31.1
120 °C 16 h	3.4	24.0	27.4	7.4	10.4	0	17.8	26.9	38.1
120 °C 48 h	3.4	24.9	28.2	10.0	9.5	0	21.4	35.3	40.6
150 °C 2 h	3.4	24.1	27.5	9.2	6.1	0	15.3	33.3	22.2
170 °C 2 h	3.4	24.9	28.3	9.5	10.6	0	20.1	33.6	37.4

4.5 Conclusions and Future Work

ionoSolv lignins were isolated from sugarcane bagasse and compared with bagasse EMAL and untreated bagasse as pyrolysis feedstocks. The degree of condensation, extent of aryl ether lignin cleavage and content of phenolic and aliphatic hydroxyl groups were found to be important parameters for characterizing how lignin polymer structure is affected by processing conditions. Lignin precipitates became more depolymerized and more condensed with increasing pretreatment time and temperature, producing polymers with increasing MW, total hydroxyl group content and thermal stability, except under severe conditions which led to loss of the highest MW lignins from the precipitate.

ionoSolv lignins were seen to be desirable feedstocks for fast pyrolysis with a view to bio-oil production, with high liquid product yields of 27–53 wt% obtained and up to ~70% of energy and ~60% of elemental carbon content in the feedstock recovered in the bio-oil. Mild pretreatment conditions (120 °C, 2–4 h) gave maximal enzymatic digestibility for bioethanol production combined with high yields of lignin precipitate. Further, these mildly treated lignins were less condensed and more hydrolyzed, favoring the yield of liquid pyrolysis products over char yields which remained low at 15–25 wt%. Condensed lignins produced under prolonged pretreatment times or at higher temperatures gave greater char yields with most of energy and carbon content found in the char product. Preliminary investigations of bio-oil composition revealed that mildly treated lignins produced less conjugated bio-oils with lower aromatic content, though further analysis of pH, water content and detailed composition are necessary to validate the trends observed.

4.6 References

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Chapter 5: Lignin Fractionation

5.1 Introduction

The heterogeneity of lignin, caused by variations in its composition, molecular weight, cross-linking and functional groups, makes its utilization quite challenging¹. Lignin has a high degree of variability in its structure which differs according to the biomass species and the extraction process used². Although the use of lignin for high-value applications has had some successes³, many potential applications, such as its use as a low-cost precursor for carbon fiber production, have not yet been achieved⁴. This is due, in part, to lignin's structural complexity, high polydispersity and complex and variable functional group distributions⁵.

Fractionation has been widely investigated as a means of obtaining lignin fractions with narrow molecular weight distribution, well-defined chemical composition and tunable chemical functionality⁶⁻⁸. To date, four main fractionation approaches have been reported in the literature: selective precipitation by acid addition⁹⁻¹¹, membrane-assisted ultrafiltration^{8,12-16}, extraction with solvents¹⁷⁻²⁰ and fractional precipitation by anti-solvent addition^{6,7,21,22}. These fractionation processes are mainly driven by molecular weight of lignin fractions, though it has been reported that the chemical structure as well as functional groups vary to some degree^{7,22}. Thus, fractionation can provide a means to obtain lignin fractions with fine-tuned weight distribution and chemical properties. In this study, fractional precipitation by anti-solvent precipitation was applied to ionoSolv lignins for the first time. Lignins were isolated from three biomass species and fractionated by fractional precipitation to produce lignins of decreasing molecular weight. The effect of fractional precipitation of ionoSolv lignins was then evaluated with a view to improving lignin characteristics for the production of high-value macromolecular products, such as carbon fibers.

5.2 Background

5.2.1 Rationale for lignin fractionation

Lignin has a polyphenolic structure and is well known for its role in lignocellulosic biomass in giving resistance to biological and chemical degradation²³. This is due to its hydrophobic nature and insolubility in aqueous systems²⁴. In industrial pulping processes, most large-scale processes remove and dissolve lignin by chemical degradation and modification under harsh alkaline conditions¹. The residual black liquor containing lignin is mostly burned to generate energy, though much energy is wasted on water evaporation²⁴. So-called “technical” lignins, such as Kraft lignin recovered from black liquor, have found limited high-value applications, with only ~2% of lignin used commercially at present¹. Heterogeneity, high sulfur and ash content and highly condensed polymeric structures are significant challenges for technical lignin utilization⁵.

With the advent of biorefinery processes, it has become possible to remove and recover high-purity lignin by chemical means before saccharification processes²⁵. Owing to their low ash and relatively low sulfur contents and the ability to tune lignin properties by selection of pretreatment severity²⁶⁻³¹, biorefinery lignins originating from ionoSolv and Organosolv processes are ideal potential precursors for high value applications^{30,32}. Despite these considerable advantages, randomized structure, high polydispersity and complex and variable functional group distributions remain a limitation for biorefinery lignin applications⁷. For instance, polydispersity indices in the range of 3–5 for ionoSolv^{26,27} and 4–7 for Organosolv lignins³¹ are commonly reported.

Lignin fractionation has been proposed as an efficient way to improve lignin miscibility in common solvents, enhance its compatibility with other polymers and to gain access to more controlled chemical and structure properties^{9,16,17}. The basic principle of fractionation techniques is the subdivision of the lignin sample into fractions due to the different solubility of the species³³. This can be achieved in different ways using both solvent/anti-solvent systems^{7,11}, mechanical means^{12-14,16} and temperature variation³⁴. Lignin solubility is primarily influenced by structural parameters of macromolecules, such as molecular weight, branching and chemical composition³³. The solubility of lignin is also affected by the proportion of functional groups, such as hydroxyl (phenolic and aliphatic), methoxyl, carbonyl and carboxyl groups (see Figure II-63)²⁴.

In its present configuration, the ionoSolv process involves extraction of lignin fragments into an IL-rich liquor. Lignin is then fully precipitated by addition of a large volume of water relative to the volume of IL (specifically, 3–5:1 water:IL on a wt:wt basis²⁶⁻²⁸). Subsequent concentration of the dilute IL-water mixture for IL recovery therefore entails high energy costs to evaporate a large volume of water.

Fractional precipitation using lower water volumes may therefore have the added benefit of reducing the water and energy requirements for lignin precipitation and IL recovery.

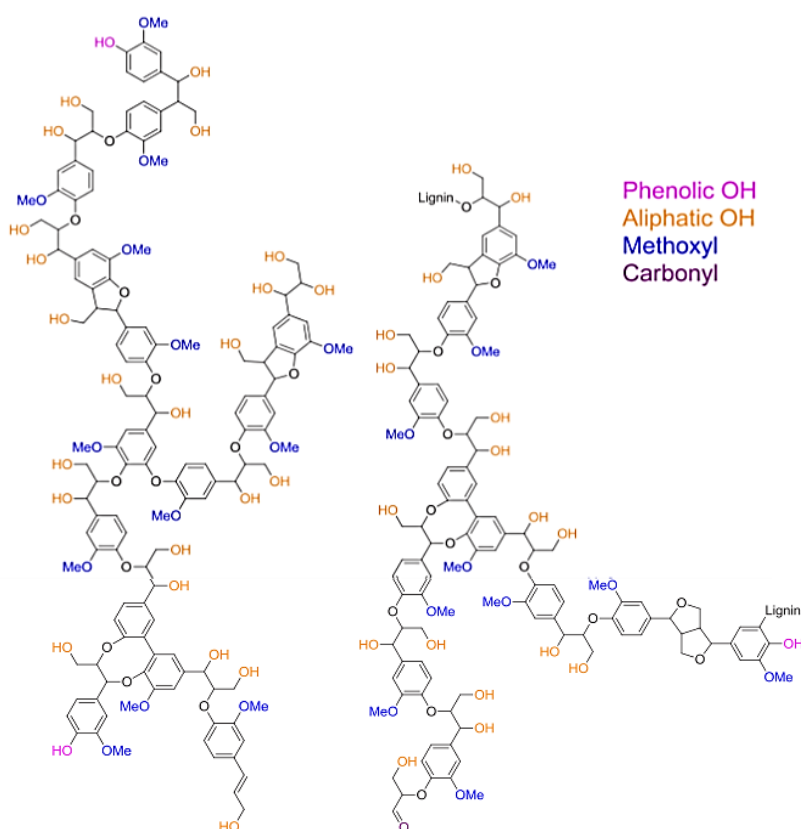


Figure II-63. Softwood lignin structure modified from Johannes and Gosselink²⁴.

To date, four main fractionation approaches have been reported in the literature: selective precipitation by acid addition^{9–11,14,35}, organic solvent extractions^{11,17–20}, membrane-assisted ultrafiltration^{8,12–16,36,37} and, more recently, fractional precipitation by anti-solvent addition^{6,7,21,22}. The respective advantages and drawbacks of each process are compared below with a view to producing lignin fractions more suitable for valorization.

5.2.2 Fractionation by acid precipitation

Sequential acid precipitation is applicable for fractionating Kraft lignins from wood pulping processes, the dominant source of industrial lignins^{9,11,16,17,19}. Kraft pulping involves the extraction of lignin from hardwood and softwood using harsh alkaline solvents (pH 14); the resulting waste stream, or ‘black liquor’, contains dissolved lignin fragments³⁸. Sequential acidification is a simple, easy and low-cost process that extracts lignin from ‘black liquor’ by gradually decreasing the pH by addition of a strong acid⁹. Kraft lignins are stable in alkaline solutions due to the negative charges of the deprotonated phenolic and carboxylic groups that repel one another by means of electrostatic interactions¹⁰. It is well-known that Kraft lignin forms colloidal structures in alkaline solution, and that protonation upon

addition of acid acts concomitantly to molecular mass to cause lignin precipitation^{9,10}. Thus, lignin with high molecular mass and low apparent pK_a tends to precipitate first due to lower solubility and weaker acid groups⁹. In its most common form, the pH (9–1 pH units) is reduced sequentially from a single sample to allow precipitation of fractions belonging to a specific pH⁹. Lourençon *et al.*⁹ precipitated industrial hardwood and softwood Kraft black liquors at pH 9, 7, 5, 3 and 1 and analyzed the different lignin fractions obtained⁹. Like other authors, they reported lignin fractionating according to molecular weight, with both phenolic hydroxyl and carboxyl content in the fractions increasing with decreasing molecular weight^{9,10}. Lignin yields decreased progressively with decreasing pH, and the resulting fractions also showed a reduction in acid content and increase in carbon content⁹. However, the ash-free fractions with high carbon content, the only promising products for valorization as carbon fibers, were obtained in very low yields (<5 wt%). **Low purity due to hemicellulose and ash contamination and lack of control over lignin molecular weight are the major disadvantages of acid precipitation**^{9,11}.

5.2.3 Fractionation by solvent extraction

The possibility to fractionate Kraft lignin based on its solubility in various organic solvents has been used to obtain low-polydispersity fractions of technical and biorefinery lignins since the 1950s^{11,20,39}. The ability of organic solvents to dissolve lignins was proposed to increase as the hydrogen-bonding capacities of the solvents increase⁴⁰. Solvents identified to significantly dissolve lignin include low molecular alcohols, dioxane, acetone, pyridine and DMSO^{41,42}. Early work by Mörck *et al.*^{19,43} established successive solvent extraction with solvents of increasing hydrogen-bonding capability or increasing solubility parameters to recover useable fractions from black liquors. Recently, Passoni *et al.*¹⁷ examined the link between extraction solvent properties and final properties of recovered Kraft lignin fractions. The amount of hydroxyl and carbonyl groups in the lignin fraction was correlated with the hydrogen bonding ability of the solvent. Poorer lignin solvents (with lower hydrogen bonding ability) limited the extraction to shorter macromolecular chains with lower T_g and lower char residue upon thermal degradation. Remarkable results were obtained, showing that fine-tuning of the lignin structural and chemical properties could be achieved by selective control of a suitable fractionation solvent¹⁷. This method was also demonstrated for Organosolv lignin by Li *et al.*²⁰. Lignins extracted with solvents of increasing hydrogen bonding capacity had higher molecular weight, condensation and a *decrease* in hydroxyl groups associated with severe condensation²⁰. However, the approach of sequential solvent extraction is limited by the limited set of available solvent types. **Scaling up of solvent extraction seems compromised by the use of high quantities of problematic and even hazardous organic solvents, leading to high process costs and safety risks**^{17,22}.

5.2.4 Fractionation by ultrafiltration

Membrane technologies such as nano- and ultra-filtration techniques for fractionating lignin have shown promise for Kraft and Organosolv lignins^{8,12–14,16}. The method has been demonstrated to produce less polydisperse lignin fractions, potentially offering better control of lignin reactivity in subsequent applications. Sevastyanova *et al.*¹⁶ used ultrafiltration to selectively extract lignin fractions with different thermomechanical properties from industrial Kraft black liquors. While lignin was efficiently fractionated by molecular weight, they noted an accumulation of sulfur-containing compounds in the (otherwise most valuable) low-molecular weight fractions. Thermal properties and functional groups also showed a good correlation with molecular weight – with decreasing molecular size, lignin fractions tended to contain fewer aliphatic hydroxyl groups and more phenolic hydroxyl groups, and T_g values decreased from 170 °C to 70 °C. These findings suggest it may be possible to tailor T_g of lignin using ultrafiltration methods. Toledano *et al.*¹⁴ compared the efficacy of ultrafiltration and compared it with acid precipitation of black liquor from *Miscanthus sinensis*. The removal of carbohydrate impurities seen by Sevastyanova *et al.* was confirmed^{14,16}, which would facilitate melt spinning for carbon fiber production. Ultrafiltration also enabled better control of lignin molecular weight and properties. However, a cost estimate provided by Jönsson *et al.*¹³ gave the cost of ultra- and nano-filtration as approximately \$0.39/kg, or over 5/6^{ths} of the target price of <\$0.45/kg for lignins necessary for commercial application such as carbon fiber production⁴⁴. **Filtration methods suffer from high energy requirements and capital cost of equipment¹⁴.**

5.2.5 Fractionation by anti-solvent addition

More recent work to fractionate lignin according to a specific narrow molecular weight fraction with high reproducibility has explored anti-solvent precipitation. Cui *et al.*²² demonstrated the use of a continuous solvent gradient involving two miscible organic solvents, one being a good solvent for softwood Kraft lignin (acetone) and the other a poor solvent (hexane). By gradually altering the polarity of the environment, a continuum of lignin fractions with narrow properties could be isolated²². As the volume fraction of hexane was increased, lignins possessed lower M_w of ~1,500 and PDI approaching 1.1 (see Figure II-64). This is a significant advance compared to solvent extraction efforts⁴⁵ where polydispersity of fractions generally exceeds 2. It was found that between 10 and 20 wt% of three different softwood Kraft lignins were composed of this highly monodisperse lignin fraction²².

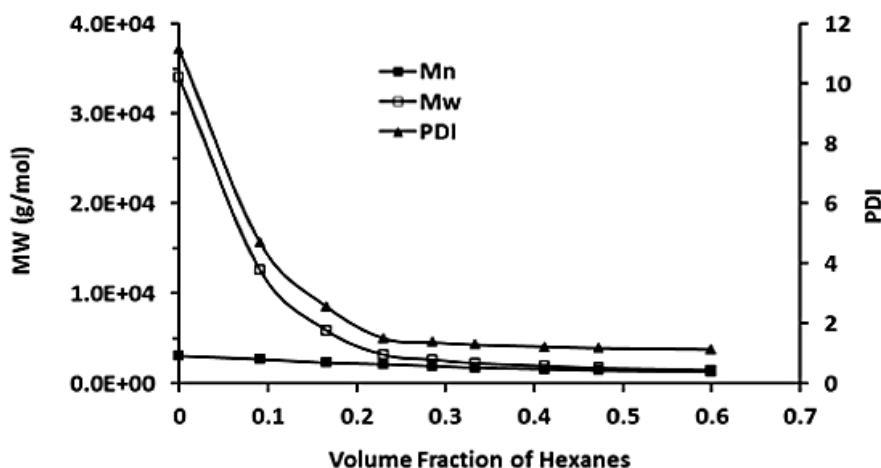


Figure II-64. M_n , M_w and PDI of fractions obtained by fractional precipitation of softwood Kraft lignin as a function of volume fraction of hexanes, as reported by Cui *et al.*²²

The fractional precipitation fractionation scheme proposed by Cui *et al.*²² for Kraft lignin was adapted by Sadeghifar *et al.*⁷ for non-technical lignins by substituting the hazardous chemical hexane with water. Switchgrass (herbaceous) and pine (softwood) Organosolv lignin fractions were dissolved in acetone and fractionated through fractional precipitation using mixtures of acetone and water. With increasing water proportion, lignin solubility decreased, producing lignins with lower molecular weight and polydispersity (from 4–5 to 2.7), decreasing aliphatic hydroxyl and increasing phenolic hydroxyl content and decreasing T_g .

Fractional precipitation, as demonstrated for Kraft²² and Organosolv⁷ lignins, namely, could also be extended to ionic solvents. ionic solvents have three major capabilities³²: the ability to break the linkages between carbohydrates and lignin and between lignin aromatic units, a high solubility for lignin fragments in the solvent, and an effective method to recover the lignin from the solution. Lignin is extracted and dissolved into a miscible solvent mixture consisting of ionic liquid (with high lignin solubility) and water (poor lignin solvent) from where it can be precipitated. Rather than fully precipitating the lignin by addition of excess water volumes, in this study water was added sequentially to the ionic liquid liquor. Enrichment of the anti-solvent leads to decrease of dissolution power of the solvent/anti-solvent mixture, resulting in fractional precipitation³³. Precipitation occurs in order of decreasing weight of lignin molecules with each addition of water, as seen in other fractional precipitation studies²². Liang *et al.*²¹ screened several low-boiling anti-solvents for lignin precipitation from IL-water mixtures. Water emerged as anti-solvent of choice due to its low cost, high hydrogen bonding capability and efficient precipitation ability²¹.

Fractional precipitation is based on partial solubility of lignin polymers in a solvent mixture³³. The ability of solvents to dissolve lignin can be characterized by the Hildebrandt solubility parameter (δ -value) and hydrogen bonding capacity⁴⁰. Generally, lignins preferentially dissolve in solvents with a δ -

value close to their own; the lignin-dissolving property also increases as the hydrogen-bonding capacity of the solvent increases at optimal δ -value. In a study on the solubility of Organosolv lignin⁴⁶, the δ -value of lignin was reported to be around 13–14 (cal cm⁻³)^{1/2}. The solubility parameters for water and ionic liquid are 22 and 11.3, respectively^{22,47}. Pure ionic liquid has a solubility parameter closer to lignin than water. Thus, with the addition of water into IL, the solubility parameter of the mixture will increase and become further from that of lignin, causing lignin to precipitate⁷.

5.2.6 Desired properties for lignin valorization

Opportunities arising from lignin utilization fit into three general categories: power and energy applications, macromolecules, and aromatics and fine chemicals, which were categorized by Holladay *et al.*² as near-, medium- and long-term applications, respectively. In this study, macromolecules such as carbon fibers and adhesives were prioritized as a possible application for ionic liquid lignins.

Near-term opportunities: Power, fuel and syngas

Thermochemical processes can be applied to many lignin streams in the near- to medium-term². Lignin combustion is widely practiced today¹ but does not add value beyond providing process heat. Lignin gasification can be used to produce a mixture of Fischer-Tropsch green fuels, low molecular weight alcohols and other green fuels⁴⁸. While black liquor gasification has been problematic within industry, it is anticipated that a wide range of lignins could be gasified, though the economics remain controversial². As for lignin pyrolysis technology to produce bio-oils, the exact specifications of desirable lignin fractions to maximize bio-oil quality/yield are unclear. Limited results have shown that low O/C molar ratio of lignin precursors is linked to higher fuel value of recovered bio-oils⁴⁹.

Medium-term opportunities: Carbon fibers

Owing to its abundance and high carbon content, lignin represents an ideal precursor for carbon fiber synthesis^{2,50}. Isolated lignin is first extruded into fibers, followed by thermal stabilization of the spun fibers in air to enable pyrolysis without melting or fusion⁵. The fibers then become carbonized through the elimination of volatiles to produce carbonized filaments, as depicted in Figure II-65⁵¹.

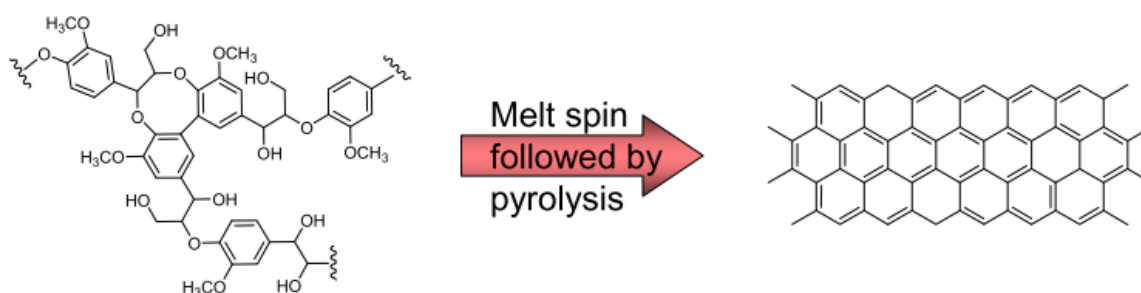


Figure II-65. Depiction of low-cost manufacture of carbon fibers from lignin, reproduced from Holladay *et al.*².

Ideal lignin monomers for this process should be of high purity, sulfur-free and fusible with a high carbon content ($\geq 60\%$) and low ash content (≤ 1000 ppm)⁵². Further 'specifications' proposed by Baker⁴⁴ include <5 wt% volatiles measured at 250 °C and <500 ppm non-melting particulate ($> 1\mu\text{m}$) content, at a target price of $<\$0.45/\text{kg}$ prior to melt-spinning. So far, lignin-based carbon fibers have largely derived from Kraft lignins, producing fibers with poor mechanical properties compared with polyacrylonitrile-based fibers⁵³. Elimination of high-molecular-weight fractions by solvent extraction or membrane fractionation has improved melt-processing of softwood and hardwood Kraft lignins^{54–56}. Minimization of reactive C–O bonds, notably β -O-aryl ether linkages, higher abundance of C–C linkages that are chemically less reactive, and unoccupied C₅ sites in high G content lignins (grasses and softwood) are considered favorable lignin properties for high carbon yields, good mechanical properties and low carbon fiber production time⁵³; on the other hand, highly condensed and cross-linked structures were linked to can cause difficulty in melt-spinning^{5,53}. Glass transition properties are also critical to assure low cost and minimal processing prior to fiber spinning; desirable ranges for T_g are between ~ 90 and 160 °C^{4,44}. Significant process has been made in recent years in use of purified Organosolv lignins⁴⁴ using a solvent extraction procedure, shown to produce lignin carbon fibers with mechanical properties at ~ 50 - 60% of the target for commercialization.

Medium term opportunities: Plastics and composites

Another high-volume application of lignin macromolecules is the production of plant-derived plastics, polymeric foams and membranes^{2,57}. Currently, research focuses on production of polymer modifiers in the form of simple, low-cost fillers^{2,57}. Notable successful examples have included the use of lignosulfonates as expanders for lead acid batteries³ and blends with synthetic epoxy resin blends⁵⁸ and phenol-formaldehyde resins⁵⁹. Resins and adhesives, notably formaldehyde-free resins⁵⁹, offer a particularly large-volume opportunity for lignins^{57,58,60}. Technical requirements for lignins in this area center on effective means for viscosity and molecular weight control (notably for polyurethane synthesis)⁶¹, functional group enhancement to improve oxidative and thermal stability and control of lignin color^{1,2,57}. Future research should move towards production of high-value additives to improve polymer performance or properties². Favorable properties for composite applications are minimal impurities, low molecular weight and polydispersity and controllable molecular weight and reactive and polyelectrolytic functionality^{58,625}.

Long-term opportunities: Aromatics and fine chemicals

A longer-term goal is to produce value-added low molecular weight chemicals from lignin^{5,63}. With its polyphenolic structure, in theory a wide variety of bulk and fine chemicals, particularly aromatic compounds, are potentially obtainable⁶³. Lignin depolymerization is challenging given the broad range of C–O and C–C interunit linkages and subunit variation in lignin and the tendency for smaller lignin

fragments to undergo condensation to produce more recalcitrant species⁵. Depending on the envisioned application, production of fuels and specialty chemicals from lignin requires low molecular-weight precursors with controlled degree of linearity, polydispersity and chemical functionalities to facilitate their transformation to value-added products⁵. Advanced separations processes will need to be developed to overcome the challenging product distribution arising from lignin subunit variations.

5.2.7 Summary

- Existing fractionation techniques (i.e. acid precipitation, ultrafiltration and solvent extraction) are controlled by lignin molecular weight, with some influence of chemical functionality.
- Sequential acid precipitation is a simple technique with the lowest energy and materials consumption; ultrafiltration enabled removal of hemicellulose impurities and organic solvent extraction enabled fine-tuning of lignin molecular weight and chemical properties.
- Fractional precipitation by (non-toxic) anti-solvent addition shows great promise for flexible manipulation of the yield and molecular composition of lignin fractions with narrow properties.
- Highly soluble lignin fractions generally possessed low molar mass (1,000–1,500 g mol⁻¹), more phenolic hydroxyl groups (4–6 mmol g⁻¹), lower degree of condensation and lower T_g .

The effect of fractional precipitation of ionic lignins needs to be evaluated with a view to improving lignin characteristics for the production of high-value macromolecular products.

5.3 Materials and methods

All chemicals used were reagent grade from Sigma-Aldrich and used as received, unless otherwise noted. *Miscanthus x giganteus* was obtained from Silwood Park campus (Imperial College London, UK) in April 2016. Willow (*Salix redheriana* ‘Endurance’ variety, Plot 22 Trial C8689) was obtained from Rothamsted Research Ltd (Harpenden, UK) in January 2014. Virgin pine (*Pinus sylvestris*) was obtained from Metla (Finish Forest Research Institute). All feedstocks were air-dried at room temperature for 72 h. They were then ground (Retsch AS200 cutting mill, 1 cm square mesh) and sieved (0.18–0.85 mm, US mesh scale -20/+80) and stored air-dry in sealed plastic bags at ambient temperature.

5.3.1 Ionic liquid synthesis

All pretreatment experiments in this study used a solution of 80 wt% *N,N*-dimethylbutylammonium hydrogen sulfate ([DMBA][HSO₄]) in DI water as pretreatment solvent. [DMBA][HSO₄], also known as or DMBA HSO₄, is a protic ionic liquid synthesized by mixing acid and base together. The ionic liquid was synthesized with an acid:base molar ratio (a:b) of 1:1 and 1.03:1. *N,N*-dimethylbutylamine (360.5 g, 3.56 mol) was placed in a 2 L round-bottom flask in an ice bath. Under stirring, a solution of 66.33 wt% H₂SO₄ (526.71 g for a:b = 1:1; 542.52 g for a:b = 1.03:1) was added dropwise to the stirred round-bottom flask over 1 hour. The reaction mixture was stirred for 3 hours at 0 °C. The ionic liquid was

recovered as a colorless, viscous liquid. Four batches of ionic liquid were produced for each a:b ratio and the batches were combined to form two separate reaction mixtures with respective a:b ratio of 1:1 and 1.03:1 (“excess acid”). The water content was adjusted in order to obtain ionic liquid solutions with a water content of 20 wt%. The water content was verified by Karl-Fischer titration (V20 volumetric titrator, Mettler Toledo) in triplicate according to (Equation 22):

$$\%water = \frac{m_{IL} \cdot wc_{IL} + m_{BM} \cdot mc_{BM} + m_{water}}{m_{IL} + m_{water}} \quad (\text{Equation 22})$$

where m_{IL} is the mass of the IL solution, wc_{IL} is the water content of the IL solution determined by Karl-Fischer titration, m_{BM} is the biomass weight, mc_{BM} is the moisture content of the biomass and m_{water} is the weight of the water added in order to reach 20wt%. The moisture content was taken into account given the high biomass loading used (20 wt%) since the amount of moisture in the biomass becomes more significant at higher loadings.

^1H NMR: δH (400 MHz, DMSO- d_6)/ppm: 9.24 (s, 1H, N-H), 3.02 (dt, $J = 12.9, 5.0$ Hz, 2H, N- CH_2), 2.76 (d, $J = 4.3$ Hz, 6H, N-(CH_3) $_2$), 1.64 – 1.51 (m, 2H, N- CH_2 - CH_2), 1.29 (h, $J = 7.4$ Hz, 2H, N- CH_2 - CH_2 - CH_2), 0.89 (t, $J = 7.4$ Hz, 3H, N- CH_2 - CH_2 - CH_2 - CH_3).

5.3.2 Acid base ratio determination

To determine the acid:base molar ratio, the synthesized ionic liquid was characterized by pH measurement (Jenway 3510 equipped with VWR Ag/AgCl symphony electrode) and the acid:base ratio was determined via titration against pre-titrated 0.1 M NaOH of known concentration (G20S compact titrator, Mettler Toledo). For pH measurements, 125 mg of [DMBA][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 agitated to ensure good mixing. The acid:base ratio, defined as the ratio of protonated dimethylbutylammonium cation [DMBA] $^+$ and the hydrogen sulfate anion [HSO_4] $^-$, was calculated from the titration measurement using (Equation 23). All measurements were performed in triplicate.

$$a:b \approx \frac{m_{dry-IL} + mol_{NaOH} \cdot MW_{base}}{2 \cdot mass_{dry-IL} - mol_{NaOH} \cdot MW_{base}} \quad (\text{Equation 23})$$

where $a:b$ is the acid:base molar ratio, m_{dry-IL} is the mass of IL added on a dry basis (g), mol_{NaOH} is the molar amount of NaOH consumed until the first equivalence point was reached (mol), MW_{base} is the molar mass of base (101.19 g mol $^{-1}$) and MW_{acid} is the molar mass of sulfuric acid (98.08 g mol $^{-1}$). The

experimentally determined acid:base values were as follows: 1.01 ± 0.007 (1:1) and 1.03 ± 0.008 (1.03:1). The measured pH values for the 1 wt% solutions of each ionic liquid in water, following the method presented in section 3.3.5, were as follows: 1.56 ± 0.02 (1:1) and 1.46 ± 0.01 (1.03:1).

5.3.3 Ionic liquid pretreatment and liquor recovery

The three feedstocks were pretreated according to the scaled-up pretreatment procedure described in Chapter 3; carried out in 1.5-L jacketed borosilicate glass pressure vessel with stirring (Büchi AG, Switzerland). The reactor vessel was equipped with a proportional relief valve set to 4.5 barg and a bursting disk (6 barg, with lowest rating of 5 barg at 150 °C). Reactions were carried out at 150 °C using a process volume of 1 L and stirring was achieved using a 50 mm anchor agitator with flat blades (100 rpm). A biomass to solvent ratio of 1:5 g/g was used (equivalent to 10 wt% loading) with 1000 g of 80 wt% [DMBA][HSO₄] and 200 g of biomass on a dry basis in all experiments. Reactions were carried out in monoplicate; the three feedstocks were pretreated at 150 °C for different reaction times: *Miscanthus*, willow and pine were pretreated for 120, 90 and 90 minutes, respectively. These pretreatment times were selected based on previous time course studies (using [TEA][HSO₄] for *Miscanthus*, willow and pine pretreatment) as the residence time giving the highest lignin precipitate yield for each feedstock^{26,28}. The pretreatment conditions applied are summarized in Table II-33.

Table II-33. Four experimental conditions used for generating lignin-rich IL liquor prior to lignin precipitation. Experiments were carried out with a volume of 1 L, stirring at 100 rpm and biomass loading of 20 wt%.

	Pretreatment experiment (monoplicate)			
	<i>Miscanthus</i>	<i>Miscanthus</i>	Willow	Pine
Feedstock / Ionic liquid solvent	[DMBA]:[HSO ₄] = 1:1	[DMBA]:[HSO ₄] = 1.03:1	[DMBA]:[HSO ₄] = 1:1	[DMBA]:[HSO ₄] = 1:1
Pretreatment condition	150 °C, 120 min	150 °C, 120 min	150 °C, 90 min	150 °C, 90 min

Briefly, the biomass was loaded into the reactor and the oil jacket was pre-heated to 95 °C. The ionic liquid preheated to 95 °C was added and the IL-biomass slurry was heated to 150 °C under stirring. The reaction was considered to begin when the slurry temperature reached 140 °C. After the reaction time had elapsed, the vessel was cooled down to 60 °C and the vessel contents unloaded by washing with 1 L of absolute ethanol. The IL-biomass-ethanol slurry was then transferred to four 500 mL centrifuge tubes (Corning, USA). The solution was allowed to equilibrate for 1 h and centrifuged (3000 rpm, 50 min) and the liquid phase decanted and collected in a 2 L round-bottom flask. The pulp washing was repeated 14 more times by addition of 1 L of ethanol split among the four centrifuge tubes for each wash. The IL-free pulp was then air-dried for 72 h in a fume cupboard to allow remaining ethanol to evaporate and the pulp yield was determined.

Ethanol was removed from the IL-ethanol liquor recovered from pulp washing by rotary evaporation (40 °C). The liquor (~1 L) was fully dried by rotary evaporation for at least 3 h until no further ethanol was removed. The recovered IL liquor (containing dissolved lignin) was collected and stored in a 1 L sealed glass bottle for a maximum period of 4 weeks before lignin precipitation was carried out.

5.3.4 Lignin fractionation by precipitation

Fractionation experiments using *Miscanthus*, willow and pine were carried out by other group members: initial trial experiments were carried out by Daniel Ogbonna (UROP summer student 2015), while lignin fractionation and characterization described in this study was carried out by Vivi Fitriyanti (MRes Green Chemistry student 2016-17).

After complete evaporation of ethanol, the water content of the liquor was measured by Karl-Fischer titration in triplicate (found to be ~5–10 wt%). Lignin was fractionally precipitated by sequential addition of anti-solvent (DI water) in order to precipitate lignin from solution. The liquor was then split into six different fractions: three fractions of ~50–75 g liquor each were used for “full precipitation” of lignin, while three batches of ~250 g liquor each were used for “fractional precipitation” of lignin. The amount of water added was calculated by weight “equivalents” of water which was compared to the amount of dry ionic liquid present in the liquor. (Equation 24 and (Equation 24 were used to calculate the equivalents of water in a given liquor sample:

$$Equivalents = \frac{m_{water,f}}{m_{dry\ IL}} = \frac{m_{liquor,f} \times WC_{liquor,f}}{m_{liquor,f} \times (1 - WC_{liquor,f})} \quad (\text{Equation 24})$$

$$\text{where} \quad m_{water,f} + m_{dry\ IL} = m_{liquor,f} \quad (\text{Equation 25})$$

where $m_{water,f}$ is the final amount of water in the liquor (g), $m_{dry\ IL}$ is the weight of dry ionic liquid in the liquor (g), $m_{liquor,f}$ is the final mass of liquor sample (g) and $WC_{liquor,f}$ is the final liquor water content as determined by Karl-Fischer titration (wt/wt).

For “**full precipitation**”: an excess quantity of water (5 equivalents) was added to the liquor in order to fully precipitate the lignin present in the ionic liquid liquor. For “**fractional precipitation**”: a smaller quantity of water (0.25, 0.5, 1, 1.5, 2, 2.5 or 5 equivalents) was added to the liquor sequentially in order to partially precipitate lignin present in the ionic liquid liquor.

The amount of water $m_{water\ added}$ to be added to a given ionic liquid liquor with initial water content $wc_{liquor,i}$ as determined by Karl-Fischer titration, in order to attain a specific number of equivalents Eq with final water content $wc_{liquor,f}$ was calculated using (Equation 26 and Equation 27):

$$m_{water\ added} = Eq \times [m_{liquor,i} \times (1 - wc_{liquor,i})] - m_{liquor,i} \times wc_{liquor,i} \quad (\text{Equation 26})$$

$$\text{where } wc_{liquor,f} = \frac{m_{water\ added} + m_{liquor,i} \times wc_{liquor,i}}{m_{water\ added} + m_{liquor,i}} \quad (\text{Equation 27})$$

where we start with an initial liquor with total weight $m_{liquor,i}$ (g) which has an initial water content as determined by Karl-Fischer titration in triplicates of $wc_{liquor,i}$ (wt/wt). In order to obtain a final solution with Eq equivalents of water present, we need to add an amount of water $m_{water\ added}$ (g) to precipitate the next lignin fraction. $m_{liquor,f}$ is the final mass of liquor (g) after adding the water and $wc_{liquor,f}$ is the final liquor water content as determined by Karl-Fischer titration (wt/wt) after addition of water.

The method of lignin full precipitation was carried out by addition of 5 equivalents of water to fully precipitate the lignin which was subsequently recovered in a single precipitation step. Starting with an initial batch of ~50–75 g of liquor weighed into a 500 mL centrifuge tube, the amount of water to be added to obtain $Eq = 5$ was calculated using (Equation 26– Equation 27 above). The desired amount of water was added in a 500 mL centrifuge tube (Corning, USA), the solution allowed to equilibrate overnight and then the precipitated lignin was separated by centrifugation (3500 rpm, 120 min) to sediment the lignin. The supernatant was carefully decanted and discarded. The lignin solid was then washed four more times, each by mixing the solid sediment with 500 mL of DI water, equilibration for 1 h followed by centrifugation as above, to remove residual IL from the lignin. After the washing was complete, the lignin solid was freeze-dried (Labconco Freezone 6, 0.016 mbar, -54 °C) for 48 h. The dried lignin was then transferred to a piece of foil and weighed and the lignin weight recorded. Full lignin precipitation was carried out in triplicate (i.e. using three batches of ~50–75 g of the original liquor recovered from each pretreatment experiment) for each of the four experimental conditions used.

The method of fractional lignin fractionation was carried out by precipitating lignin by addition of 0.25, 0.5, 1, 1.5, 2, 2.5 and 5 equivalents of water sequentially to obtain up to 7 precipitates per initial liquor

sample. Starting with an initial batch of ~250 g of liquor weighted into a 500 mL centrifuge tube, the amount of water to be added to obtain $E_q = 0.25$ was calculated using (Equation 26–

(Equation 27 above. The desired amount of water was added and the solution allowed to equilibrate for 1 h; subsequently, the precipitated lignin was separated by centrifugation (3500 rpm, 120 min) to sediment the lignin. The supernatant was carefully decanted into a clean pre-weighed 500 mL centrifuge tube and the weight of liquor was recorded. The sample was split into several clean centrifuge tubes when the volume of a single sample exceeded the capacity of one centrifuge tube (500 mL); the lignin products were later recombined to obtain the total yield. The solid precipitate was washed four times with DI water as for full precipitation to recover the “0.25 Eq” precipitate. If no solid precipitate was observed, the supernatant was transferred as before and it was proceeded to the next precipitation step. Next, the amount of water to be added in order to have a total water content of $E_q = 0.5$ was calculated and added to the supernatant (i.e. diluted IL liquor). The lignin precipitate was recovered as before and the supernatant again transferred to a clean 500 mL centrifuge tube. The procedure was repeated for $E_q = 0.5, 1, 1.5, 2, 2.5$ and 5 equivalents. The procedure for fractional precipitation is shown diagrammatically in Figure II-66. Fractional precipitation was carried out in triplicate (i.e. using three batches of ~250 g of the original liquor recovered from each pretreatment experiment) for each of the four experimental conditions used.

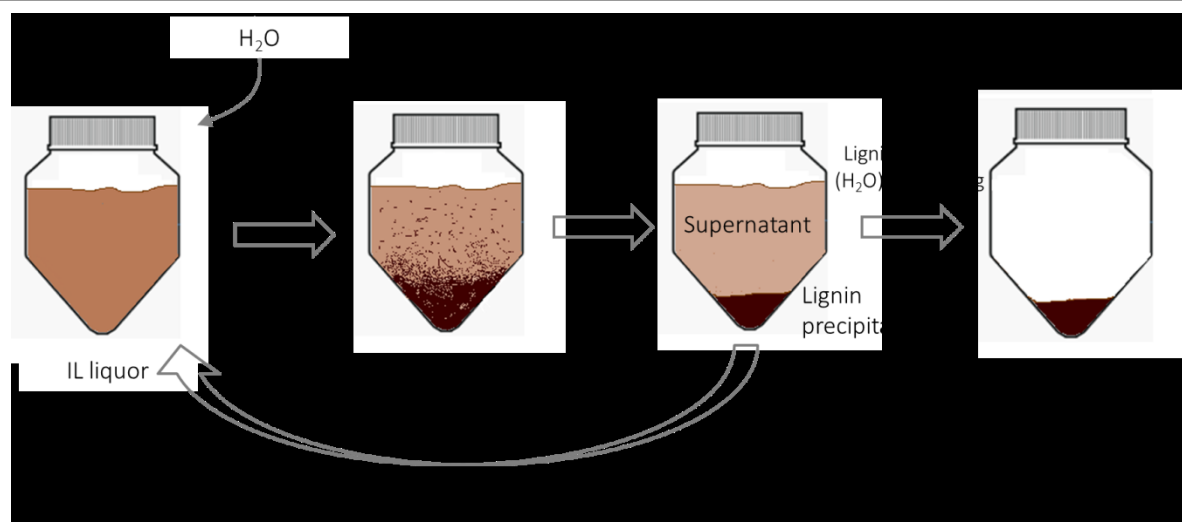


Figure II-66. Schematic representation of the lignin fractionation process by which lignin precipitates were recovered by sequential anti-solvent addition.

The lignin mass balance was calculated by weighing each of the dried lignin samples obtained from precipitation. The lignin weight for each sample recovered after cumulative addition of x water equivalents (full, $Eq = 0.25, 0.5, 1, 1.5, 2, 2.5$ and 5), $m_{lignin,x}$, was then normalized to the amount of dried IL in the initial liquor, $m_{liquor,i,x}$ in **each precipitation step**, as shown in

(Equation 28 and

(Equation 29:

$$Yield (x Eq) = \frac{m_{lignin,x}}{m_{dry IL,x}} \times 100 \quad (\text{Equation 28})$$

$$\text{where } m_{dry IL,x} = m_{liquor,i,x} \times (1 - wc_{liquor,i,x}) \quad (\text{Equation 29})$$

where $m_{lignin,x}$ is the mass of lignin (g) recovered after addition of x equivalents of water in total to a given liquor with initial liquor mass $m_{liquor,i,x}$ (g) and water content $wc_{liquor,i,x}$ (wt/wt).

The total amount of lignin obtained by full precipitation was calculated for each of the triplicates and the mean value reported in units of g lignin per 100 g of dry IL liquor. The total amount of lignin precipitated by fractional precipitation (which should, theoretically, be equal to the amount obtained by full precipitation given $Eq = 5$ were added to each liquor in total) was calculated as the average sum of all of the lignin yields obtained from (Equation 28 for a given fractional precipitation experiment.

5.3.5 Compositional analysis

Prior to undertaking compositional analysis, the extractives were removed from untreated biomass using NREL-TP-510-42619⁶⁴. Moisture content of untreated material was determined following NREL/TP-510-42618⁶⁵. The moisture contents of untreated *Miscanthus*, willow and pine were 6.8%, 5.2% and 6.7%, respectively. Untreated biomass feedstocks were Soxhlet extracted for 24 hours (with ethanol for untreated biomass or pretreated pulps) and air-dried for 48 h prior to compositional analysis. Compositional analysis of untreated extractives-free biomass was carried out following the NREL protocol 'Determination of Structural Carbohydrates and Lignin in Biomass', NREL/TP-510-42618⁶⁵ in triplicate, as described fully in section 1.3.5.

5.3.6 Saccharification assay

Saccharification assays were carried out according to an adapted procedure by the NREL in triplicates with blanks (also triplicates) as described fully in section 1.3.6. Untreated and pretreated *Miscanthus* samples were saccharified with 20 μ L of Novozymes Ctec2 enzyme mixture per sample while untreated and pretreated willow and pine samples employed 50 μ L of Novozymes Ctec2 enzyme per

sample. The hydrolysis time was 7 days. Glucose yields were calculated based on the glucan and xylan content of untreated biomass.

5.3.7 Gel permeation chromatography

GPC measurements were performed following the method given previously in section 3.3.12.

5.3.8 HSQC NMR

HSQC experiments of precipitated lignins were carried out following the method given in section 1.3.9. The spectra were analyzed by employing MestReNova 11.0 and referenced to the DMSO solvent peak at $\delta H/\delta C$ 2.50/39.5 ppm. Integration of lignin signals was conducted according to previous assignments in the literature^{26–28} by following the same contours for lignins of the same feedstock. The signal intensities were normalized to the total abundance of $G_2 + G_{2,cond}$ units. The total ($G_2 + G_{2,cond}$) and ($S_2 + S_{2,cond}$) were used to calculate the S/G ratio, after halving the contribution from S units to avoid double-counting for the symmetrical syringyl unit.

5.3.9 Quantitative ^{31}P NMR

Quantitative ^{31}P NMR spectra of all lignin preparations were obtained using published procedures⁶⁶ according to the method given in section 4.3.3. Vortex mixing of the prepared samples was carried out for at least 20 min or until the sample was fully dissolved. ^{31}P NMR experiments were recorded in triplicate for selected lignin fractions (the first two precipitates from fractional precipitation as well as the $E_q = 5$ and full precipitate) for each experimental condition.

5.3.10 Elemental analysis and HHV estimation

CHNS analysis of all lignin precipitates was performed in duplicate using an elemental analyzer (vario MICRO element analyzer, Elementar Analysensysteme GmbH). Each run used ~2 mg of freeze-dried lignin. Oxygen content was obtained by difference. Accuracy is $\pm 0.30\%$ absolute. Residual ionic liquid contamination was calculated using the nitrogen and sulfur content to calculate the cation and anion contributions, respectively; the sum of the two was used to obtain the residual ionic liquid content in the lignins. The higher heating values were calculated according to a correlation developed by Demirbas⁶⁷ as in Chapter 4: (section 4.3.3). In this study, the elemental compositions of the raw materials were used as-received, rather than on a dry ash-free (daf) basis. HHV were calculated from elemental analysis values obtained in duplicates.

5.3.11 Thermogravimetric analysis

Proximate analysis of lignins was conducted on a TA Q500 (TA Instruments, USA) TGA analyzer as described in section 4.3.3. Briefly, 3 ± 2 mg of freeze-dried lignin was loaded per run; each sample was analyzed in duplicate. Proximate analysis was performed using TA Universal Analysis software and in Microsoft Excel as described previously. For thermal analysis, the TGA weight loss curves and their

first derivatives, i.e. the differential thermogravimetric curves (DTG) with respect to temperature for all lignins were plotted and used to identify the temperature at which the DTG curve showed the maximum rate of thermal composition (DTG_{max}).

5.4 Results and Discussion

In this study, lignin fractionation by anti-solvent fractional precipitation was investigated for ionoSolv lignins from three biomass types. *Miscanthus*, willow and pine were used as representative species of grassy biomass, hardwood and softwood, respectively. Each feedstock was pretreated using the low-cost PIL [DMBA][HSO₄] at 150 °C between 90 and 120 min, under conditions shown by previous time course studies using PILs [TEA][HSO₄] and [Hbim][HSO₄] to give maximal lignin yield²⁶⁻²⁸. The mass balance was determined for each of the lignin fractions obtained by fractional precipitation and compared with full precipitation by addition of 5 equivalents instantaneously. Trends in the molecular weight, chemical functionality and thermal properties of the lignin fractions were analyzed. Results are presented for three feedstocks pretreated with [DMBA][HSO₄] (a:b = 1:1) and for *Miscanthus* treated using the same IL containing excess acid (a:b = 1.03:1). Lignin fractionation and characterization were carried out by Vivi Fitriyanti (MRes student).

5.4.1 Mass balance

Lignin was extracted from *Miscanthus*, willow and pine into [DMBA][HSO₄] (a:b = 1:1) and then fractionally precipitated by addition of water as antisolvent ($Eq = 0.25, 0.5, 1, 1.5, 2, 2.5$ and 5). The mass balance of lignin fractions as a proportion of the total mass of lignin from fractional precipitation is shown for each feedstock in Figure II-67. No precipitation was observed after addition of $Eq = 0.25$. Different amounts of lignin were precipitated from each feedstock upon addition of the same quantities of water, reflecting differences in lignin solubility. Addition of $Eq = 0.5$ was sufficient to precipitate 45 wt% and 28 wt% of *Miscanthus* and pine lignin (relative to the amount recovered *via* addition of $Eq = 5$), respectively, whereas no lignin precipitation was observed from willow until $Eq = 1$. For *Miscanthus* and pine, >90 wt% of lignin was precipitated after addition of $Eq = 1$. This suggests *Miscanthus* and pine lignins are more hydrophobic with higher molecular weight than willow lignins (72%), due to treatment conditions and intrinsic lignin structure.

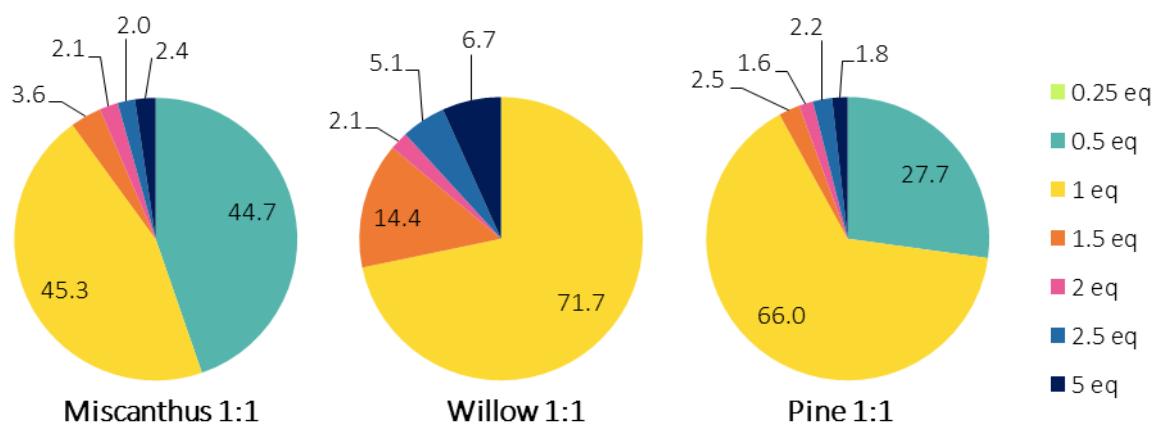


Figure II-67. Mass balance of lignins isolated by fractional precipitation for *Miscanthus*, willow and pine feedstock. Lignin was extracted into [DMBA][HSO₄]-water solution with 1:1 acid: base ratio.

Lignin yields were compared with those from full precipitation ($Eq = 5$) in Table II-33. The total lignin obtained from fractional precipitation up to $Eq = 5$ was comparable within standard error to the total weight of lignin recovered from full precipitation of lignin, as expected. Based on the lignin yield from full precipitation, the total amount of lignin increased as 6.0, 7.6 and 8.4 g lignin per 100 g of dry IL in the liquor in the order of willow, *Miscanthus* and pine, respectively. This is unexpected based on the lignin content of the materials (26, 26 and 32 wt% respectively) and suggests that willow lignin was less pretreated (with less lignin extracted) than *Miscanthus*. For willow lignin, the total lignin mass from fractional precipitation (7.5 ± 1.2 g/100g) appeared to exceed that from full precipitation (6.0 ± 0.7 g/100g); this was suspected to be due to IL contamination which would artificially increase the precipitate yield from willow. Correction of the mass balance by subtracting the residual IL is further discussed in Section 5.4.4. Normalizing to the lignin content in each material, it was calculated that these yields corresponded to recovery of 137%, 99% and 108% of lignin in native *Miscanthus*, willow and pine. Under the same pretreatment conditions at the 10 mL-scale without stirring in similar PILs, the lignin recoveries were 71%, 54% and 50% respectively. In all cases the lignin recovery at the 1 L-scale was roughly double of that reported for 10 mL-scale experiments without stirring; this was attributed to improved mass transfer with mixing. The lignin recovery for *Miscanthus* at 1 L-scale exceeded the amount in native biomass; this may be due to formation of insoluble lignin-hemicellulose reaction products that artificially increased the lignin yield, an effect which is likely to have been worsened by the prolonged treatment time used for low-recalcitrance *Miscanthus* (120 min) compared to the other feedstocks (90 min).

Table II-34. Mass balance comparing lignin precipitated during fractional fractionation and by full precipitation. Lignin was isolated by pretreatment of *Miscanthus*, willow and pine using [DMBA][HSO₄] with a molar acid:base ratio of 1:1.

Weight of lignin isolated (g/100g dry ionic liquid in liquor)			
Fractional Precipitation	<i>Miscanthus</i>	Willow	Pine

	[DMBA]:[HSO ₄] = 1:1	[DMBA]:[HSO ₄] = 1:1	[DMBA]:[HSO ₄] = 1:1
Equivalents of Water Added			
0.25 equivalents	-	-	-
0.5 equivalents	3.39 ± 0.15	-	2.22±0.25
1 equivalent	3.43 ± 0.22	5.38± 0.88	5.29 ± 0.76
1.5 equivalents	0.27 ± 0.04	1.08 ± 0.18	0.20 ± 0.01
2 equivalents	0.16 ± 0.06	0.16± 0.02	0.13 ± 0.05
2.5 equivalents	0.15 ± 0.03	0.38 ± 0.03	0.18 ± 0.03
5 equivalents	0.18 ± 0.05	0.50 ± 0.08	0.14 ± 0.02
Total from Seq. Precipitation	7.59 ± 0.55	7.49 ± 1.18	8.16 ± 1.13
Full Precipitation	7.61 ± 0.32	5.99± 0.72	8.36± 0.76

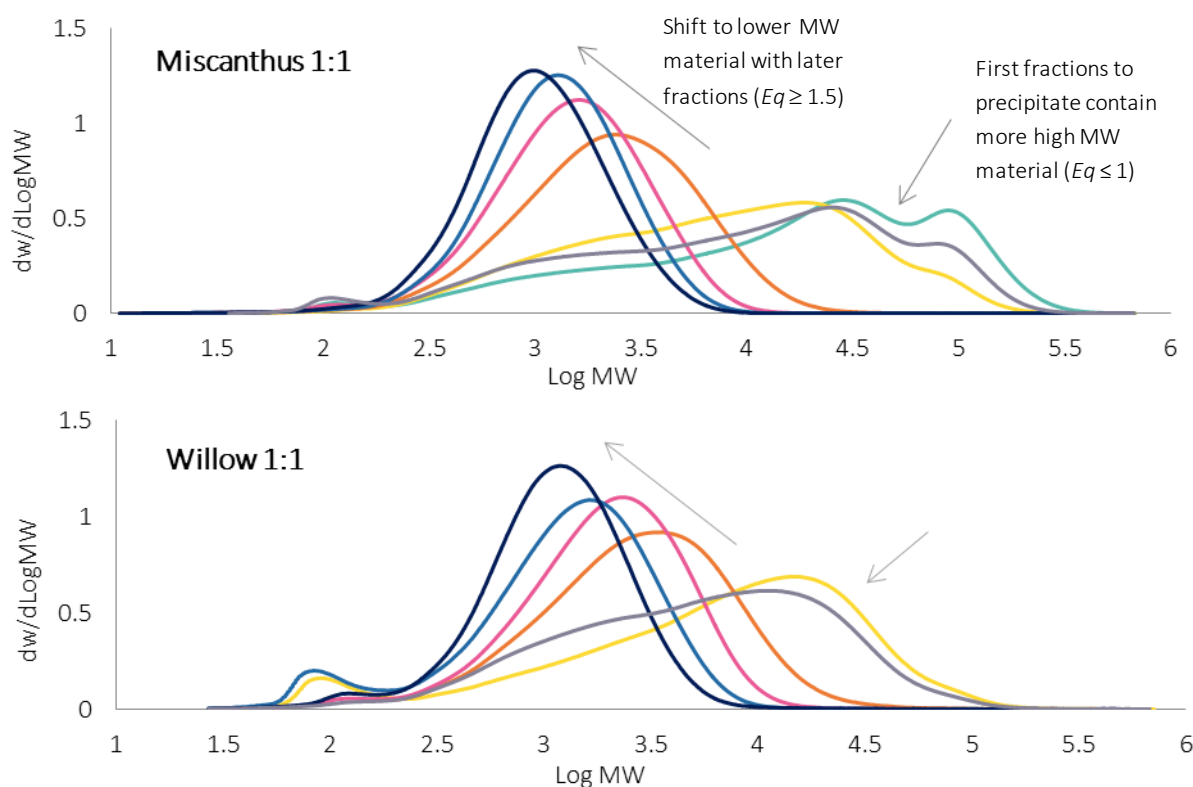
5.4.2 Molecular weight distribution

Different solubilities of lignin fractions in IL/water mixtures can first and foremost be attributed to differences in molecular weight of macromolecules³³. GPC was used to obtain insight into the molecular weight distributions of fractional and full precipitates. The eluted molecular weight profiles for *Miscanthus*, willow and pine lignin fractions are shown in Figure II-68. Key molecular markers for lignins, including the weight-average molecular weight (M_w), number-average molecular weight (M_n) and polydispersity index ($PDI = M_w/M_n$) are displayed in Table II-35–Table II-37.

GPC findings confirmed that lignin fractionation by fractional precipitation using anti-solvent addition was predominantly influenced by lignin molecular weight. The GPC profiles exhibited a marked shift towards lower molecular mass material with increasing anti-solvent addition, i.e. as the dissolution power of the solvent/anti-solvent mixture decreased. This trend agrees with previous reports from organic solvent and fractional precipitation studies^{7,17,22,33}. Initial precipitates ($Eq \leq 1$) had highest molecular weight, with a large proportion of material having mass $>30,000 \text{ g mol}^{-1}$. These macromolecules would be most hydrophobic and would precipitate first. It appears that smaller lignin fragments precipitated together with the larger molecules, as the elution profiles showed a significant population with molecular mass of $1,000\text{--}10,000 \text{ g mol}^{-1}$, resulting in high polydispersity. In contrast, later fractions ($Eq \geq 1.5$) showed narrow molecular profiles, with peak molecular mass occurring around a molecular mass of $1,000\text{--}3,000 \text{ g mol}^{-1}$. These smaller lignin molecules remained solubilized in the IL/water mixture before precipitating with further anti-solvent addition. The full precipitate elution profiles were very polydisperse (unsurprising as they contain all lignin fragments from large to small) and most closely resembled the GPC profile of the initial lignin precipitates (notably $Eq = 1$). This is expected given that the fraction $Eq = 1$ made up the largest proportion of the fractions by weight (Figure II-67). For *Miscanthus*, the full precipitate elution profile was intermediate between the profiles for $Eq = 0.5$ and 1, which is consistent with the finding that these fractions made up over 90

wt% of lignin by mass in a nearly equal mass ratio. Considering the elution profile for willow and pine, the full precipitate was closest to the $E_q = 1$ fraction which made up 72 wt% and 66 wt% of willow and pine lignin, respectively.

Key molecular markers for *Miscanthus* (Table II-35) revealed that the initial precipitate ($E_q = 0.5$) had high M_w of $\sim 40,000 \text{ g mol}^{-1}$ with polydispersity 18.1, with the moderate M_n (2190 g mol^{-1}) indicating small molecular mass material precipitating together with macromolecules. These small molecules may have modified alkoxy side chains, for instance after dehydration reactions, rendering them less soluble³². M_w and M_n decreased sharply to values below 3400 g mol^{-1} for $E_q \geq 1.5$, with the last fraction to precipitate ($E_q = 5$) having very low M_n of $\sim 700 \text{ g mol}^{-1}$, i.e. lignin oligomers with only 3–4 aromatic units assuming an average molar mass of $\sim 200 \text{ g mol}^{-1}$ per lignin aromatic unit⁶⁸. The polydispersity of lignin fractions also fell to 1.8 for the last fractions precipitated from IL:water mixtures. This finding shows very narrow molecular weight fractions could be obtained, comparable to results from organic solvent fractionation¹⁷ and approaching values of 1.1 and 1.2 reported for fractional precipitation using acetone/hexane and acetone/water mixtures^{7,22}. However, yields for the most monodisperse fractions were 4–14 wt%, whereas yields of 10–20 wt% were obtained by previous authors^{7,22}.



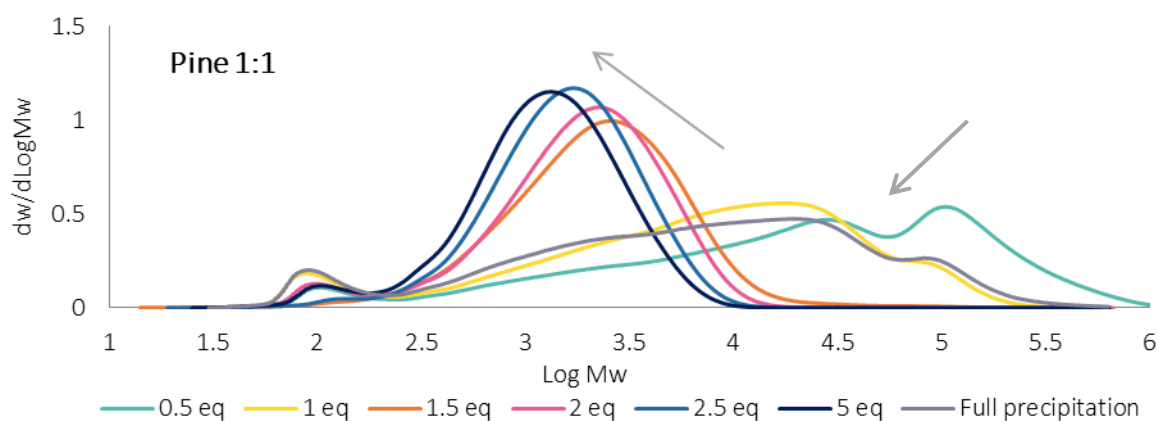


Figure II-68. GPC Chromatograms of full precipitation and fractionally fractionated lignin as a function of water amount added. Lignins were isolated from *Miscanthus* (top), willow (middle) and pine (below).

Table II-35. Yields and molecular markers for fractional and full precipitates isolated from *Miscanthus* (IL acid:base ratio 1:1).

Lignin Fractions	Yields (wt%)	M _n (g/mol)	M _w (g/mol)	PDI
0.5 equivalents	44.7±1.9	2190	39700	18.1
1 equivalent	45.3±2.9	2080	18200	8.7
1.5 equivalents	3.6±0.6	1190	3400	2.8
2 equivalents	2.1±0.8	900	2000	2.2
2.5 equivalents	2.0±0.4	860	1600	1.9
5 equivalents	2.4±0.7	720	1300	1.8
Full Precipitation	100	1590	26300	16.6

^a Mass recovery shown as a proportion of the total lignin obtained from fractional precipitation and for full precipitation.

Table II-36. Yields and molecular markers for fractional and full precipitates isolated from willow (IL acid:base ratio 1:1).

Lignin Fractions	Yields (wt%)	M _n (g/mol)	M _w (g/mol)	PDI
1 equivalent	71.7±11.7	1080	14400	13.4
1.5 equivalents	14.4±2.4	1630	4400	2.7
2 equivalents	2.1±0.3	1100	2600	2.4
2.5 equivalents	5.1±0.4	590	1900	3.2
5 equivalents	6.7±1.0	750	1700	2.3
Full Precipitation	100	1630	11800	7.2

^a Mass recovery shown as a proportion of the total lignin obtained from fractional precipitation and for full precipitation.

Table II-37. Yields and molecular markers for fractional and full precipitates isolated from pine (IL acid:base ratio 1:1).

Lignin Fractions	Yields (wt%)	M _n (g/mol)	M _w (g/mol)	PDI
0.5 equivalents	27.2±3.1	1750	75200	42.9

1 equivalent	64.8.8±9.3	990	20900	21.1
1.5 equivalents	2.4±0.1	1160	3900	3.3
2 equivalents	1.6±0.6	870	2700	3.2
2.5 equivalents	2.2±0.3	1000	2200	2.3
5 equivalents	1.8±0.3	730	1700	2.4
Full Precipitation	100	910	25000	27.5

^a Mass recovery shown as a proportion of the total lignin obtained from fractional precipitation and for full precipitation.

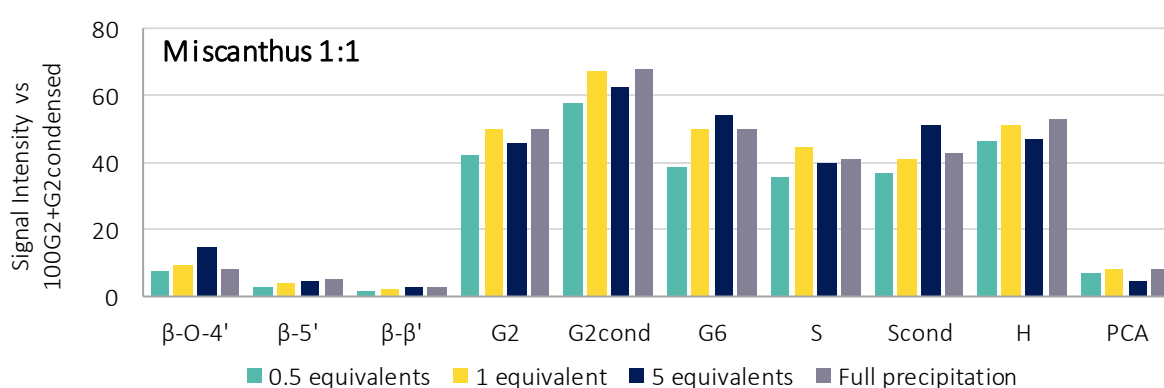
Molecular markers for willow (Table II-36) and pine (Table II-37) served to illustrate the differences between these biomass species, both pretreated at 150 °C for 90 min. The first lignin fraction to precipitate ($Eq = 1$) for willow had M_w of 14,000 g mol⁻¹, less than half of that observed for *Miscanthus*, likely due to the shorter pretreatment time used and a lower propensity for willow lignins to undergo condensation reactions³². However, the M_w values for $Eq = 1$ onwards across different feedstocks were very similar once the condensed species were removed from solution. Molecular weight was seen to decrease in a similar fashion with fractional precipitation, with the last fraction to precipitate ($Eq = 5$) having M_w of ~750 g mol⁻¹. Polydispersities were lower than for *Miscanthus*, ranging from 13.4 for full precipitation to only 2.3 for later fractions. For pine, the initial fraction had higher molecular weight than hardwood or grass lignins, as expected from literature⁶⁹. $Eq = 0.5$ had ultra-high M_w of 75,000 and polydispersity of 43, which reduced sharply to 21,000 g mol⁻¹ for $Eq = 1$; however, later fractions had comparable molecular weight of ~1000 g mol⁻¹ and low polydispersity of 2–3 as seen for other feedstocks. Molecular weight for lignin fractions increased in the order willow < *Miscanthus* < pine. This is in agreement with findings by Lapierre *et al.*⁷⁰ who found that native lignins from softwood and grasses are more highly branched by interunit linkages, which could result in a high molecular weight contributing to hydrophobicity. This may also explain the higher lignin yields obtained for $Eq = 0.5$ for *Miscanthus* and pine whereas no precipitation was observed for willow until $Eq = 1$. The fact that a larger proportion of lignin precipitated for $Eq = 0.5$ for *Miscanthus* than for pine, despite pine lignin having higher molecular weight, indicates that branching (i.e. molecular shape) may play a role in addition to lignin molecular mass. While GPC techniques are calibrated against standards of varying hydrodynamic volume (or molecular weight), the degree of branching and cross-linking is also expected to play a role⁷¹. The findings suggest that *Miscanthus* lignins may be less branched than softwood lignins: the greater steric bulkiness and lower volumetric surface area of linear polymers would restrict intermolecular interactions with water molecules, making them less soluble in the IL/water mixture. Lapierre *et al.*⁷⁰ provided experimental support for the fact that pine lignins are more branched than grass lignins, based on thioacidolysis of native lignins. Further analysis of lignin branching by Mark-Houwink plots²⁴ needs to be carried out to better understand the solubilities and fractionation properties of lignin fractions.

In summary, GPC profiles revealed the key role of lignin molecular mass in lignin fractionation. Full precipitates had high polydispersity (16.6, 7.2 and 27.5 for *Miscanthus*, willow and pine respectively) and high average molecular weight ($M_w = 12,000\text{--}25,000$) comparing closely to that for $E_q = 1$. As increasing anti-solvent was added, lignin oligomers with low molecular weight $\sim 700\text{--}1000\text{ g mol}^{-1}$ and narrow molecular weight distribution of ~ 2 were obtained. Lignins with these desirable properties accounted for $\sim 4\text{--}14\%$ of lignin mass, suggesting fractionation could be a worthwhile technique to separate valuable lignins from polydisperse macromolecules.

5.4.3 Chemical functionality

2D HSQC NMR was employed to gain insights into chemical functionality. In particular, solubility of lignin is affected by hydroxyl, methoxyl and carboxyl groups^{24,32}. The methoxylation degree has been correlated with the $\beta\text{-O-4'}$ abundance, contributing to flexibility of the lignin polymer chain and aiding solubility²⁴. Thus, ether linkage content and degree of C–C condensation were observed by 2D HSQC NMR while hydroxyl group abundance was measured by ^{31}P NMR spectroscopy.

Figure II-69 shows the signal intensities from HSQC NMR analysis of the first two lignin fractions ($E_q = 0.5, 1$ or 1.5), the last lignin fraction ($E_q = 5$) from fractional precipitation and full precipitate isolated from *Miscanthus*, willow and pine. The results show that as more equivalents of water were added sequentially, the lignin fractions had a greater abundance of $\beta\text{-O-4'}$ bonds and other ether linkages. Moreover, the content of G_2 and G_6 structures was slightly higher, indicating that these lignins were less condensed. These structural properties of higher $\beta\text{-O-4'}$ linkage (and therefore methoxyl substitution²⁴) may contribute to lignin solubilization by rendering the molecules more hydrophilic.



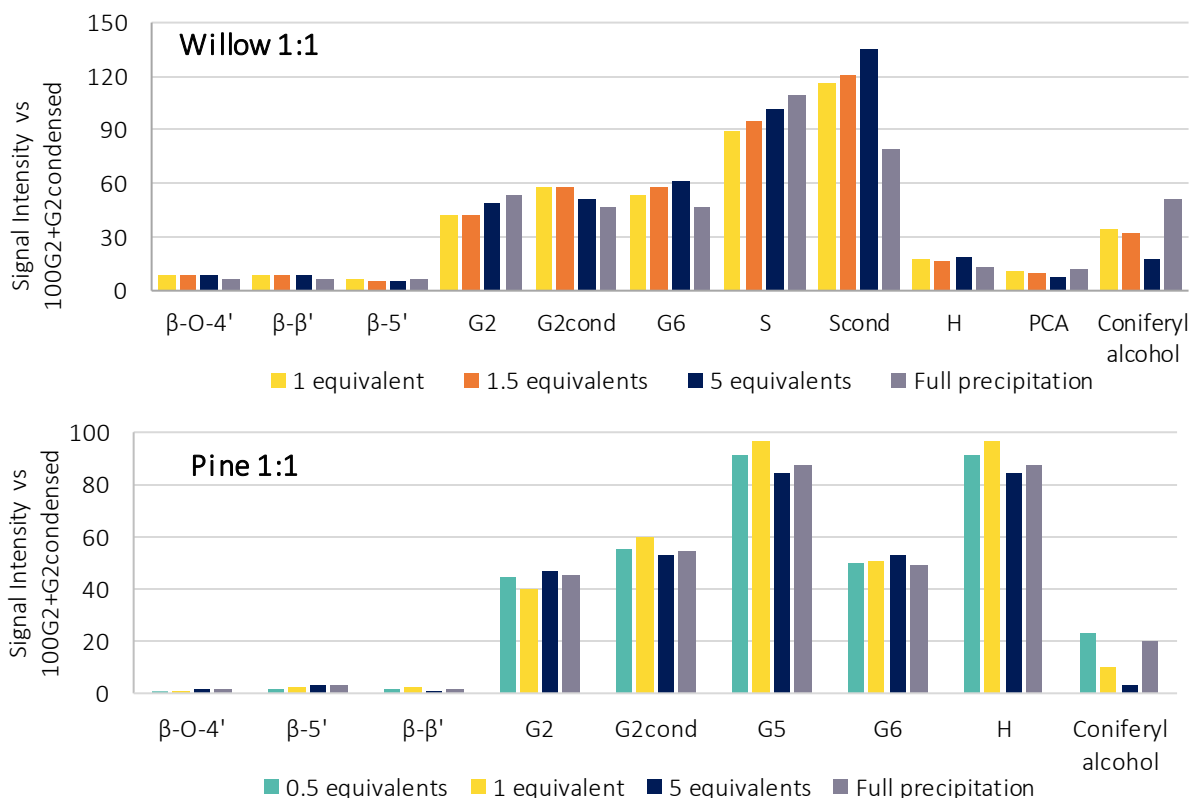


Figure II-69. HSQC NMR signal intensity of interunit linkages and subunits in lignin isolated from *Miscanthus* (above), willow (middle) and pine (below) as a function of sequential water addition and for full precipitation.

HSQC NMR results were in line with GPC findings, where lignin from increasing anti-solvent addition, i.e. lower dissolution power, had lower molecular weight associated with lower extent of condensation, agreeing with previous reports for fractionated Organosolv lignins obtained by sequential solvent extraction²⁰. Signal intensities for full precipitate most closely resembled those of $Eq = 0.5$ and 1 for *Miscanthus* and pine. However, for willow lignin, the full precipitate appeared *less* condensed than fractional precipitates; these findings cannot be explained at present.

S/G ratios and the degree of condensation were compared for the same lignin fractions in Table II-38. Based on literature reports, the S/G ratios of native *Miscanthus*, willow and pine EMAL are ca. 0.85, 1.67 and 0, respectively^{32,72}. After pretreatment, their S/G ratios were in the range ~0.4, 1.1 and 0, respectively; this reduction in the S/G ratio following pretreatment was observed by Brandt *et al.*³² for *Miscanthus* and was seen in Chapter 2: for sugarcane bagasse; however, this is contrary to the trend observed by Weigand *et al.*²⁸ who saw an *increase* in S/G ratio of willow lignins with increasing treatment time. The differences in S/G ratios and degrees of condensation between lignin fractions from the same feedstock were not substantial. However, there appeared to be a general trend of increasing S/G ratio and decrease in condensation for lignins obtained with fractional precipitation. Compared to the full precipitate lignin, the later fraction ($Eq = 5$) showed the starkest differences, notably with more G₅, G₆ and S_{cond} and lower PCA and coniferyl alcohol units while also having slightly

higher β -aryl ether content. **The lignin chemical structure, notably the degree of condensation and S/G ratio, thus likely influenced lignin molecular weight and, in turn, its solubility in water.** HSQC NMR also provided information on other lignin subunits that could aid in understanding the potential applications of these fractions, explored further in section 5.4.6. As more equivalents of water were added sequentially, lignin fractions showed decreasing abundance of coniferyl alcohol (Figure II-69).

Table II-38. S/G ratio and degree of condensation as a function of water addition for lignin fractional precipitates and full precipitate isolated from *Miscanthus*, willow and pine using acid:base ratios of 1:1 and excess acid.

		0.25 eq	0.5 eq	1 eq	1.5eq	5 eq	Full precip.
Miscanthus 1:1	S/G ratio ^a	-	0.36	0.42	-	0.45	0.42
	Cond/non-cond ^b	-	1.4	1.3	-	1.3	1.4
Miscanthus Excess Acid	S/G ratio ^a	0.26	0.23	-	-	0.28	0.25
	Cond/non-cond ^b	2.7	2.9	-	-	1.9	2.5
Willow 1:1	S/G ratio ^a	-	-	1.02	1.08	1.19	0.95
	Cond/non-cond ^b	-	-	1.4	1.4	1.0	0.9
Pine 1:1	S/G ratio ^a	-	0	0	-	0	0
	Cond/non-cond ^b	-	1.2	1.5	-	1.1	1.2

^a $S/G = 0.5(S_2 + S_{2cond})/(G_2 + G_{2cond})$; ^b $Cond/non-cond = G_{2cond}/(G_2 + G_{2cond})$

Hydroxyl groups are another key functionality that influence lignin solubility³² and are an important parameter for lignin valorization pathways⁶⁶. ³¹P NMR is well-known as a technique to accurately resolve the abundance of hydroxyl groups in lignins⁷³. Quantitative integrals from ³¹P NMR analysis are displayed in Figure II-70. A trend of increasing syringyl, guaiacyl, condensed guaiacyl and *p*-hydroxyphenyl (i.e. phenolic) OH, aliphatic OH and carboxylic OH groups was seen with molecular weight. Total hydroxyl group content increased from 2.8–3.0 mmol g⁻¹ lignin for initial precipitates (*Eq* = 0.5) to 4.3–6.5 g mol⁻¹ for the last precipitate (*Eq* = 5). An exception was seen for willow lignin, where initial precipitate (*Eq* = 1) had an unexpectedly high hydroxyl content across all groups; this phenomenon could be linked to the high S_{cond} content and is under further investigation. The trends observed in ³¹P NMR were consistent with those of previous sequential solvent extraction, ultrafiltration and anti-solvent precipitation studies, whereby phenolic hydroxyl and total hydroxyl groups showed a good correlation with decreasing molecular size^{7,16–18,20,22}, with total OH group contents of 4–7 mmol g⁻¹ for the most monodisperse fractions^{7,22}. **On account of their high hydroxyl group content, together with low molecular size, later precipitates were less hydrophobic allowing them to be separated from polydisperse macromolecules by fractional precipitation.**

Willow lignins had higher hydroxyl group content than *Miscanthus* and pine lignins. Hosseinaei *et al.*⁵³ reported that hardwood lignin contained more hydroxyls than herbaceous lignin. All lignins had a

higher proportion of phenolic to aliphatic OH groups, which is generally observed for lignins isolated following dilute acid, Organosolv and ianoSolv pretreatment^{32,66,74}. For pine lignin, due to the absence of S unit signals (which overlap with condensed G and S unit signals in hardwood and herbaceous lignins⁶⁶), the abundance of condensed G groups could be observed⁶⁶. G_{cond}/G was found to decrease as 0.37, 0.34 and 0.25 as $E_q = 0.5 < E_q = 1 < E_q = 5$, with full precipitate having ratio of 0.31. This agreed with the trend observed in HSQC NMR for $G_{2,\text{cond}}:G_2$ units.

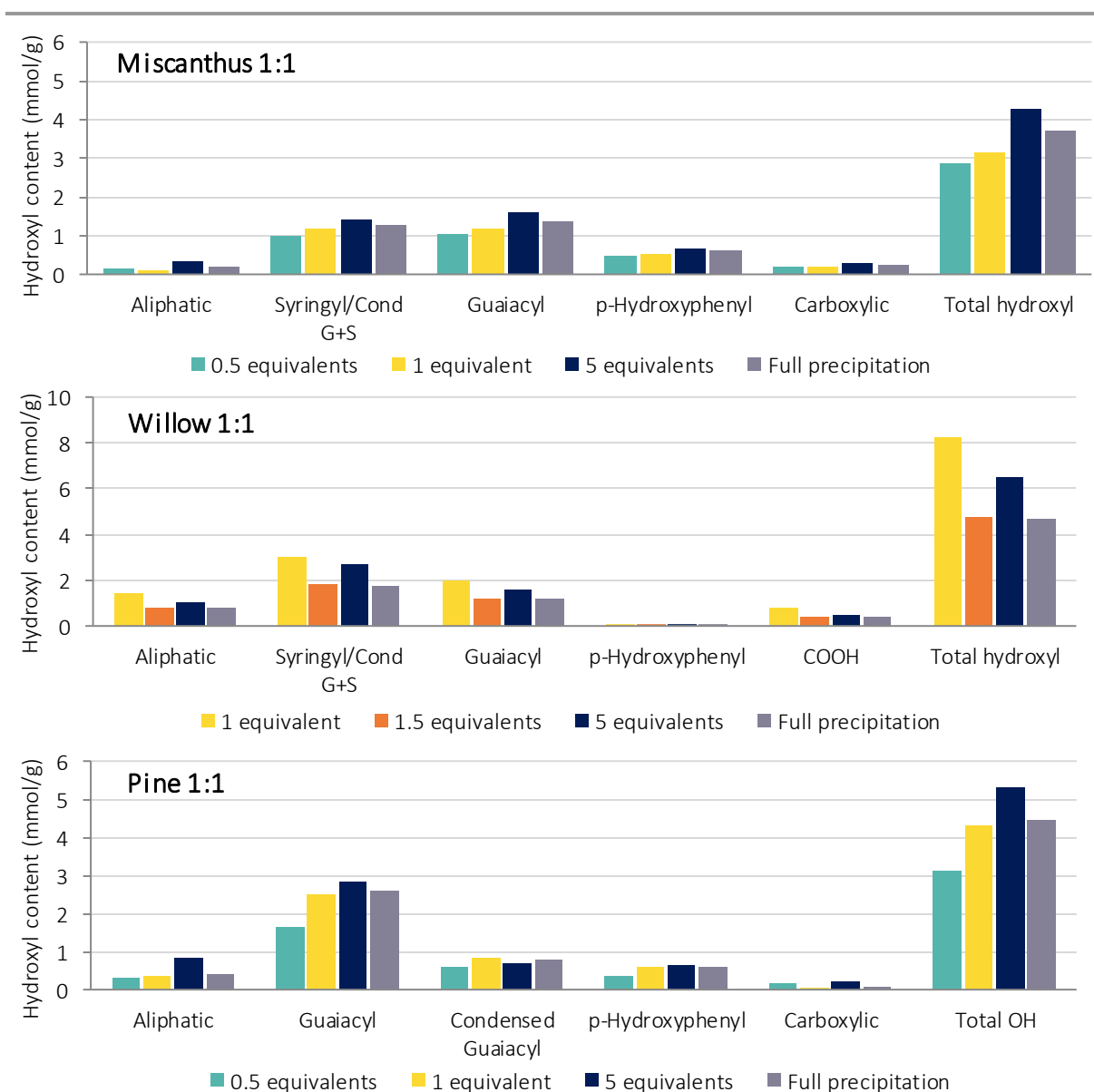


Figure II-70. Quantitative ³¹P NMR integrals of phosphitylated lignin from *Miscanthus* (above), willow (middle) and pine (below) lignin pretreated in 1:1 [DMBA][HSO₄] and fractionally precipitated with water.

5.4.4 Elemental composition

The elemental composition of lignin is helpful to assess lignin purity and IL contamination³². All lignin precipitates were washed four times with DI water to remove residual IL; however, this method was previously shown to be imperfect³². Significant quantities of nitrogen and sulfur (presumably derived from the alkylammonium cation and hydrogen sulfate anion, respectively) were detected, equivalent to up to 30 wt% IL contamination in some samples. Analysis of lignin fractions from sets of triplicates showed that contamination appeared to be random, though it tended to be worse for the largest macromolecules (which would be more likely to trap IL molecules in the lignin matrix). This could explain discrepancies in the full and fractional precipitation lignin mass balance, the implications of which are under further investigation. However, given the small size of IL molecules ($M_R = 199$) compared to lignin molecules ($M_R \sim 1,000 - 200,000$) and non-overlap of IL and lignin resonances, IL contamination is **not** believed to affect the trends observed in GPC, HSQC and ³¹P NMR analysis.

In order to focus on the lignin portion of the elemental analysis, we calculated the carbon, hydrogen and oxygen content of the “IL-free” portion of the lignin samples. The corrected elemental carbon content, molar H/C and O/C ratios and estimated HHV (calculated from Dulong’s formula⁶⁷) are displayed in Table II-39 for each lignin fraction. The carbon content of the IL-free lignin decreased substantially as fractional precipitation progressed by addition of increasing anti-solvent. A concomitant trend of increasing H/C ratio and increasing O/C ratio was observed when comparing initial ($E_q \leq 1.5$) and final ($E_q = 5$) precipitates. This finding agrees with data from HSQC NMR and ³¹P NMR that revealed that later fractions were less condensed, so the replacement of C–O bonds with C–C bonds is less extensive. **Initial precipitates tended to have higher fuel value, as we would expect due to their enrichment in elemental carbon, supporting their use for energy recovery.** Pine lignins had slightly higher HHV than willow and *Miscanthus* lignins due to greater condensation.

Table II-39. Elemental carbon, molar H/C and O/C ratios and estimated higher heating value (HHV) of lignin fractions isolated from *Miscanthus*, willow and pine using [DMBA][HSO₄] with molar acid:base ratios of 1:1 and excess acid on an IL-free basis.

		C (wt%)	H/C (mol/mol)	O/C (mol/mol)	HHV (MJ kg ⁻¹) ^a
Miscanthus 1:1	0.5 equivalent	63.0	0.7	0.4	21.6
	1 equivalent	65.0	0.6	0.4	21.8
	5 equivalent	61.9	0.9	0.4	22.2
	Full precipitation	63.3	1.1	0.4	24.3
Miscanthus Excess Acid	0.25 equivalent	75.9	0.9	0.2	31.1
	0.5 equivalent	67.2	1.0	0.3	26.5
	5 equivalent	63.0	1.1	0.4	24.9
	Full precipitation	60.5	1.0	0.4	21.8

Willow 1:1	1 equivalent	65.5	0.7	0.3	22.9
	1.5 equivalent	61.5	1.1	0.4	23.8
	5 equivalent	53.0	1.4	0.8	18.3
	Full precipitation	59.0	1.2	0.4	22.9
Pine 1:1	0.5 equivalent	68.6	0.6	0.3	23.8
	1 equivalent	62.1	0.5	0.4	19.1
	5 equivalent	63.8	1.0	0.4	24.2
	Full precipitation	66.3	0.5	0.3	21.8

N.B. All values reported on an ionic liquid-free basis (i.e. subtracting the contribution from N and S components attributed with IL contamination). ^a Estimated using Dulong's formula⁶⁷.

5.4.5 Thermal properties

Thermal degradation behavior of lignins is key for its utilization as a fuel, carbon fiber precursor, feedstock for pyrolysis or gasification and other valorization pathways^{4,48,49}. Many high-value lignin applications require low ash content and high char residue, notably production of carbon fibers⁵³. For isolated lignins, the overall degradation is a very complex process involving the simultaneous formation/degradation of interunit linkages, complicating the interpretation of TGA profiles^{16,75}. In the present study, we used differential thermal analysis to determine the maximum decomposition temperature (DTG_{max}) to compare the relative thermal stability of lignin fractions⁷⁵. We then applied proximate analysis to compare the ratio of volatiles to char residue following thermal degradation.

A trend of decreasing thermal stability was seen from the DTG_{max} values with decreasing molecular size, shown in Figure II-71; a similar result was reported by several authors of previous fractionation studies^{7,11,17,22}. The position of DTG_{max} could also be influenced by the greater yield of volatile products of low molecular mass which were generated by cleavage of functional groups⁷⁶, more abundant in smaller lignin fragments agreeing with HSQC NMR results. Full precipitates degraded at a temperature comparable to initial precipitates ($E_q \leq 1$), owing to their similar molecular weight and composition. The ratio of volatiles compared to char residue obtained after heating to 600 °C (VM/FC) is shown in Table II-40. Fractional precipitates obtained with increasing anti-solvent addition released increasing volatile matter and less char residue upon thermal degradation. Thermal behavior is mostly determined by molecular weight, so these changes are likely driven by the presence of highly cross-linked and condensed lignin macromolecules in initial precipitates; however, chemical composition is also known to play a role¹⁶. HSQC NMR showed that initial fractions were more condensed and ³¹P NMR indicated lower hydroxyl group content, both associated with a higher amounts of char residues⁷⁵. Residual char from fractionated lignins ranged between 38–45 wt% (see Appendix, Table S0-9). If lignin is used as a carbon precursor to produce carbon fiber, *Miscanthus* fraction $E_q = 0.5$ with high char yield (45.3 wt%) could be more valuable than other fractions. The glass transition

temperature (T_g) is a key parameter that remains to be investigated, especially with regard to assessing lignin fraction suitability for production of carbon fibers⁴.

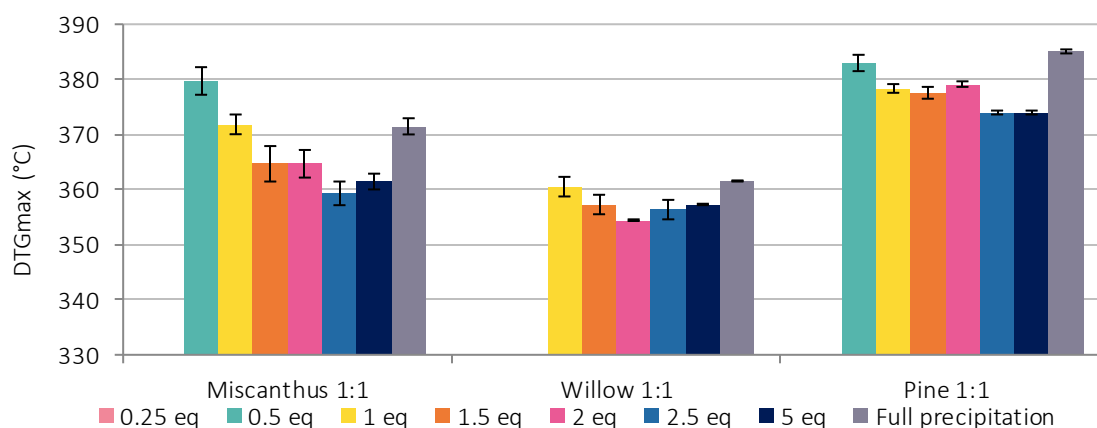


Figure II-71. Temperature of maximum thermal degradation (DTG_{max}) obtained from thermogravimetric analysis of lignin fractions isolated from *Miscanthus*, willow and pine using [DMBA][HSO₄] with molar acid:base ratios of 1:1.

Table II-40. Volatile matter to fixed carbon ratio (VM/FC) on a dry ash-free basis for lignin fractions isolated from *Miscanthus*, willow and pine using [DMBA][HSO₄] with molar acid:base ratios of 1:1.

	Miscanthus 1:1	Miscanthus Excess Acid	Willow 1:1	Pine 1:1
0.25 eq	-	0.87±0.01	-	-
0.5 eq	1.21±0.07	0.93±0.06	-	1.37±0.07
1 eq	1.27±0.07	1.29±0.06	1.35±0.13	1.35±0.01
1.5 eq	1.24±0.07	1.24±0.06	1.38±0.09	1.30±0.06
2 eq	1.22±0.01	1.35±0.06	1.42±0.07	1.47±0.03
2.5 eq	1.37±0.07	1.38±0.06	1.47±0.09	1.28±0.06
5 eq	1.39±0.06	1.39±0.06	1.60±0.19	1.48±0.21
Full precipitation	1.24±0.01	1.08±0.06	1.15±0.04	1.34±0.01

Several previous studies of fractionated lignin have cited the link between thermal properties and lignin molecular weight^{11,14,16,17}. Hence, the number-average molecular weight M_n was plotted against the VM/FC ratio (Figure II-72). A loose inverse correlation could be seen between the molecular size and ratio of volatile matter to fixed carbon. Such behavior has already been observed by others working with Kraft lignin due to higher carbon recalcitrance of large lignins^{9,19}.

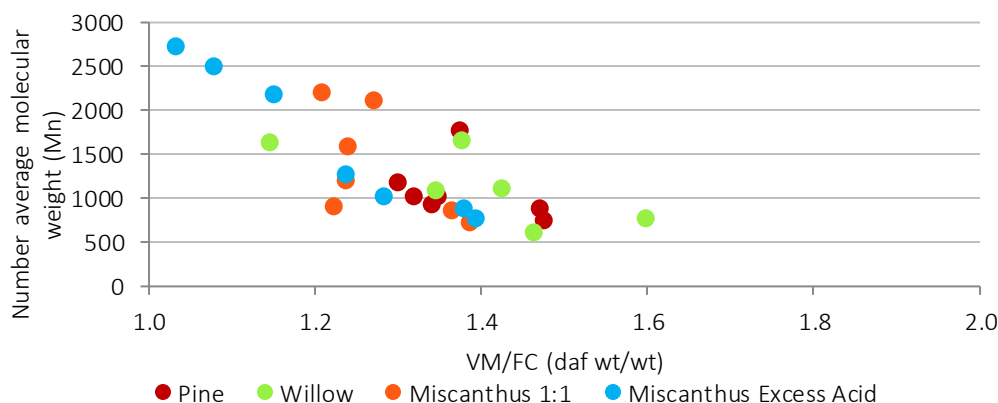


Figure II-72. Volatile matter to fixed carbon ratio obtained from TGA plotted against M_n as observed by GPC.

5.4.6 Effect of excess solvent acid on lignin fractionation performance

Protic ionic liquids can be synthesized with almost any acid:base ratio, rather than using a 1:1 stoichiometric mixture. Increased solvent acidity was shown to accelerate *Miscanthus* pretreatment in a previous study using [Hbim][HSO₄]⁷⁷. This would also reduce IL cost (as sulfuric acid is less costly than alkylamines) and reduce reactor cost due to higher throughput²⁷. A recent study examined the recovered lignin after *Miscanthus* pretreatment with 9% excess acid in TEA HSO₄²⁶. Harsher conditions accelerated ether cleavage and lignin condensation as seen by HSQC NMR and GPC data. Lignin recovery was also reduced at prolonged treatment times²⁶.

Lignin was isolated from *Miscanthus* using [DMBA][HSO₄] with 3% excess acid (a:b = 1.03:1), applying the same experimental conditions and precipitation method used previously. Unlike the lignin isolated from *Miscanthus* using a:b = 1:1, a precipitate was recovered with the addition of $E_q = 0.25$ (Figure II-73). Moreover, fractional precipitation with $E_q = 0.5$ precipitated nearly 88.1 wt% of lignin, whereas only 44.7 wt% were isolated using the same volume of anti-solvent using IL without excess acid. This result indicates that the lignin isolated with excess acid in the IL was strongly hydrophobic, was extremely condensed and had high molecular weight, so addition of small quantities of water were sufficient to cause it to crash out of the IL-water solution. The mass balance of lignin isolated from fractional precipitation was seen to be equal within error to the lignin weight recovered from full precipitation, as shown in Table II-41. Excess acid resulted in an increase in lignin yield, as ~10.2 g/100 g dry IL (~195% of native lignin) was obtained from excess acid compared to ~7.6 g/100 g dry IL (~137%) for stoichiometric 1:1 ratio.

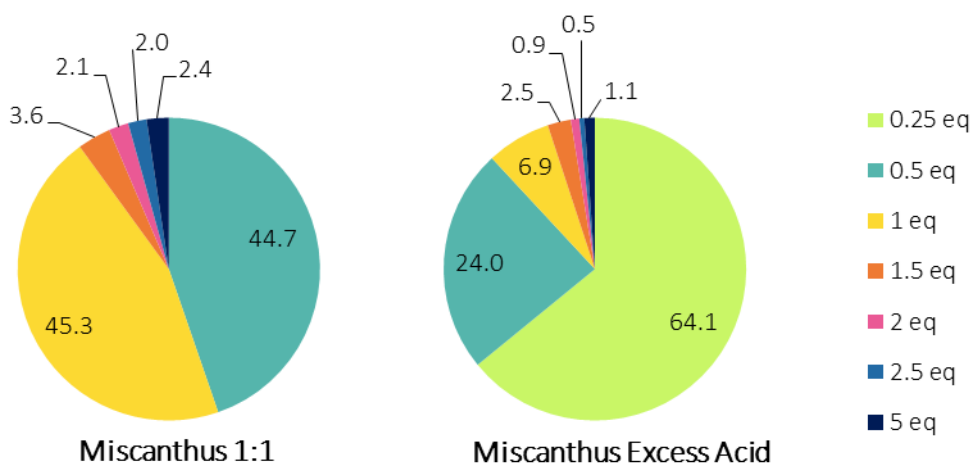


Figure II-73. Mass balance showing relative contribution of lignins isolated by fractional precipitation with increasing amounts of water. Lignin was extracted from *Miscanthus* using [DMBA][HSO₄]-water solution with 1:1 molar acid:base ratio and excess acid. 1 eq. = 1 g lignin precipitated/100 g of dry IL liquor in solution.

Table II-41. Mass balance comparing lignin precipitated during fractional precipitation and by full precipitation. Lignin was isolated by pretreatment of *Miscanthus* at 150 °C for 2 h using [DMBA][HSO₄] with a molar acid:base ratio of 1:1 and 1.03:1.

Fractional Precipitation	Weight of lignin isolated (g/100g dry ionic liquid in liquor)	
	<i>Miscanthus</i> [DMBA]:[HSO ₄] = 1:1	<i>Miscanthus</i> [DMBA]:[HSO ₄] > 1:1
Equivalents of Water Added		
0.25 equivalents	-	8.4 ± 0.4
0.5 equivalents	3.39 ± 0.15	3.1 ± 0.1
1 equivalent	3.43 ± 0.22	0.90 ± 0.01
1.5 equivalents	0.27 ± 0.04	0.33 ± 0.02
2 equivalents	0.16 ± 0.06	0.12 ± 0.03
2.5 equivalents	0.15 ± 0.03	0.07 ± 0.03
5 equivalents	0.18 ± 0.05	0.15 ± 0.1
Total from Fractional Precipitation	7.59 ± 0.55	13.1 ± 0.6
Full Precipitation	7.61 ± 0.32	10.2 ± 1.3

This reflects the strong effect of acid on increasing the propensity of condensation reactions which would have the effect of causing smaller lignin fragments to form high molecular weight lignins that are easily precipitated by addition of water, increasing the lignin yield; further evidence for this effect was obtained by subsequent analysis. This is a highly interesting finding, indicating the **ionoSolv process can be configured to optimize energy intensity** (using acidic ILs reduces reaction times and produces easily precipitated lignins with low water input) **or product value** (milder ILs for less condensed lignins using higher water input) by selection of IL acidity.

The GPC data, shown in Figure II-74 and Table II-42, confirmed that initial precipitates ($Eq = 0.25$ and 0.5) from *Miscanthus* pretreated using excess acid contained material with M_w exceeding 150,000 g

mol⁻¹. In contrast, the analogue lignin fractions obtained using a:b = 1:1 had M_w of 18,000 and 36,000, respectively. The E_q = 1 fraction obtained using excess acid showed unique properties with a molecular weight profile intermediate between the most condensed fractions and the more monodisperse later precipitates, which was not observed with a:b = 1:1. This suggests acid may alter molecular weight and hydrophobicity somewhat independently. Decreasing molecular size of polymers was observed after the addition of E_q ≥ 1.5; these later fractions resembled those from a:b = 1:1 and had narrow molecular weight distribution, low molecular weight (M_n < 1,000) and low polydispersity (2–3).

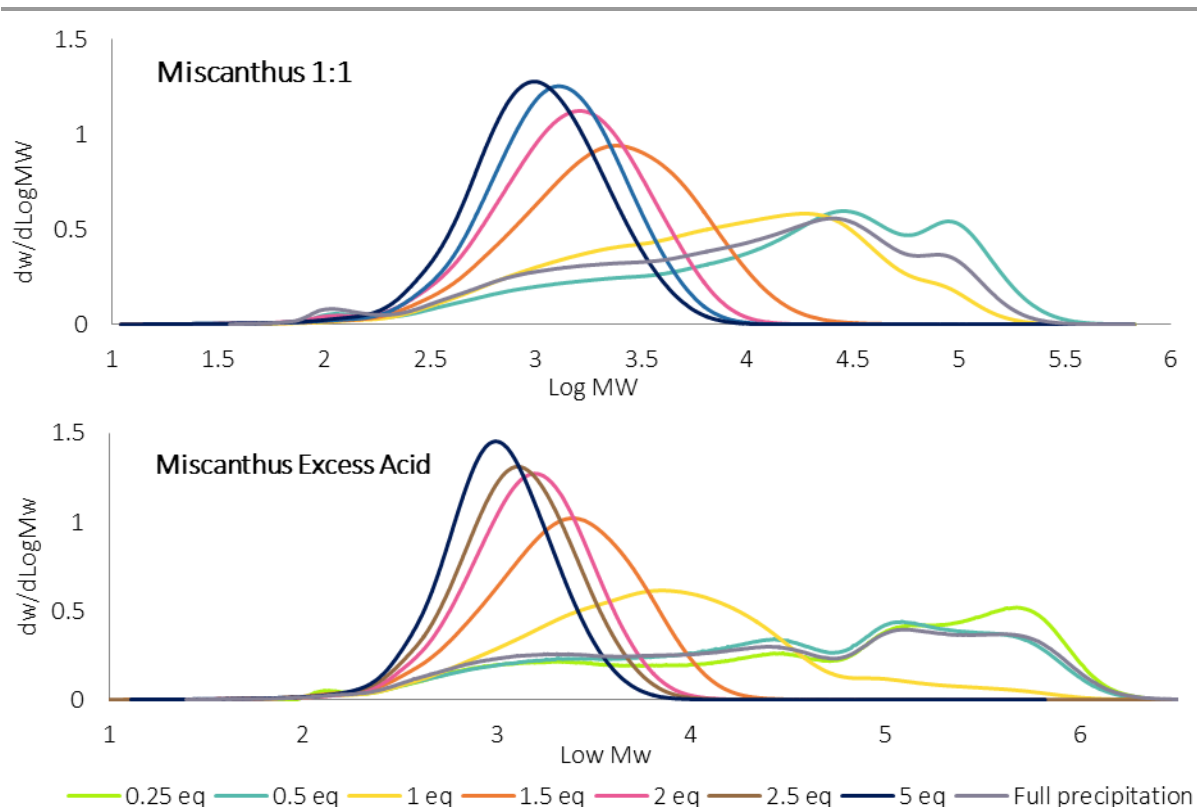


Figure II-74. GPC Chromatograms of full precipitation and fractional precipitates of *Miscanthus lignin* as a function of acid:base ratio, using molar 1:1 ratio (above) and excess acid (below).

Table II-42. Yields and molecular markers for fractional and full precipitates isolated from *Miscanthus* (IL acid:base ratio 1.03:1).

Lignin Fractions	Yields (wt%)	M _n (g/mol)	M _w (g/mol)	PDI
0.25 equivalents	64.1±2.7	2940	207000	70.5
0.5 equivalents	24.0±0.3	2710	151800	56.1
1 equivalent	6.9±0.1	2170	32100	14.8
1.5 equivalents	2.5±0.2	1250	3300	2.7
2 equivalents	0.9±0.2	1010	1900	1.9
2.5 equivalents	0.5±0.2	890	2700	3.1
5 equivalents	1.1±0.6	760	1400	1.8
Full Precipitation	100	2470	163800	66.2

^a Mass recovery shown as a proportion of the total lignin obtained from fractional precipitation and for full precipitation.

The chemical structure of lignin fractions was further probed by HSQC NMR and ³¹P NMR as before. Comparison of the key interunit linkage and subunit signal intensities Figure II-75 showed that initial lignin fractions (*Eq* = 0.25 and 5) had merely one-third of the β-*O*-4' ether linkage content compared to the last fraction (*Eq* = 5); in addition, the average ether bond content was reduced as bond cleavage is known to be acid-catalyzed. The presence of excess acid also accelerated condensation reactions, seen in the increase in G₆ and G₅ and decrease in G_{2cond} signals in later fractions compared to initial precipitates. **The effect of acid addition was to exacerbate the trends observed, producing lignin fractions with more distinct characteristics, though lignins were on average more condensed. Acid may also enable molecular weight and hydrophobicity to be tailored somewhat independently.**

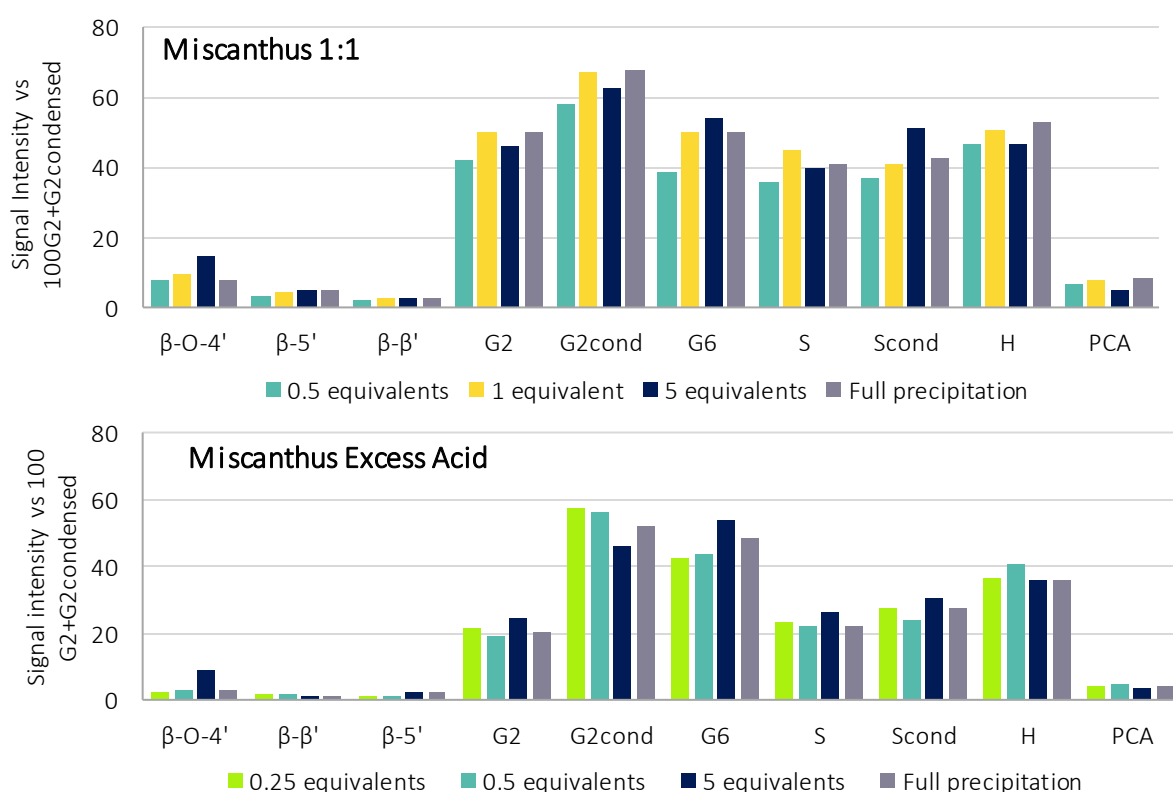


Figure II-75. HSQC NMR signal intensity of interunit linkages and subunits in lignin isolated from *Miscanthus* as a function of fractional water addition for IL molar acid:base ratio of 1:1 (above) and 1.03:1 (below).

³¹P NMR results (Figure II-75) showed that the increase in hydroxyl group content with fractional precipitation was also intensified with addition of excess acid, with the last fraction (*Eq* = 5) having over 65% more total OH group content compared to *Eq* = 0.25. However, the *average* hydroxyl group content in all other precipitates and in full precipitate was *lower* with the addition of acid. This may be linked to the dramatic increase in molecular weight with greater condensation, as lignin fragments recombined to produce fewer free end groups in the remaining lignin fragments. Lower hydroxyl

group content in lignins isolated using acidic IL may also have reduced their solubility, facilitating their precipitation in anti-solvent and giving higher lignin recoveries.

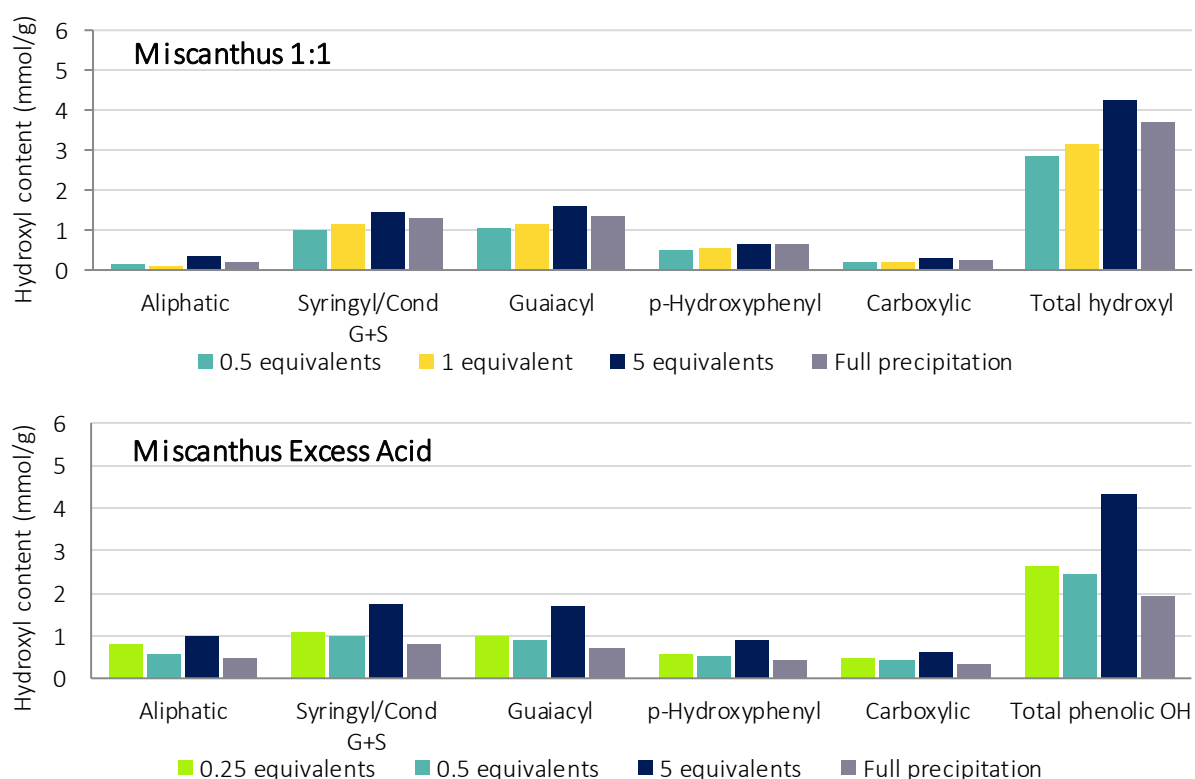


Figure II-76. Quantitative ^{31}P NMR integrals of phosphitylated lignin from *Miscanthus* pretreated in $[\text{DMBA}][\text{HSO}_4]$ with acid:base molar ratio of 1:1 (above) and excess acid (below) and fractionally precipitated with water.

Finally, the effect of acid addition on thermal properties of lignins was evaluated. Owing to the acceleration of lignin extraction and condensation with addition of excess acid^{26,77}, individual lignin fractions were most distinct and so differences in thermal properties could be more clearly observed. The thermograms and differential thermograms for lignin fractions from a:b = 1.03 applied to *Miscanthus* are shown in Figure II-77. The first mass loss (after moisture evaporation at $\sim 105^\circ\text{C}$), observed at $\sim 200^\circ\text{C}$, was similar for all lignin fractions, though middle precipitates ($E_q = 1$ and 1.5) began to degrade at slightly lower temperature, indicating the presence of lower molecular mass components. The next thermal degradation event was observed around $300\text{--}400^\circ\text{C}$, where the initial precipitates ($E_q \leq 0.5$) displayed a slower degradation pattern. Lignin degradation occurred in a broad temperature range, ranging from around 300°C when aliphatic side chains start to break off, to the cleavage of more stable C–C bonds at $370\text{--}400^\circ\text{C}$ ⁷⁵. The residual mass, attributed to non-volatile char and ash residues remaining at 600°C , increased with higher molecular mass of lignin fragments (as $E_q = 5 \rightarrow 0.25$). Such behavior was reflected in decreasing VM/FC values (Table II-40) and has already been observed by multiple authors working with Kraft lignin fractionation^{14,169,11}. Based on the DTG curves, the temperature at which maximal thermal degradation occurred shifted from 363°C for $E_q =$

5 to 379 °C for $E_q = 0.25$, the latter matching the DTG inflection point for full precipitate at 382 °C (see Figure II-78).

This trend of increasing thermal stability for earlier precipitates could be linked not only to their molecular size, observed by GPC, but also to the lower abundance of thermally labile aryl ether linkages and phenolic hydroxyl groups seen by ^{31}P NMR¹⁴. The appearance of multiple inflection points in the DTG traces as $E_q = 5 \rightarrow 0.25$ also reflected the decreasing homogeneity and high polydispersity of lignin fractions, as seen by GPC.

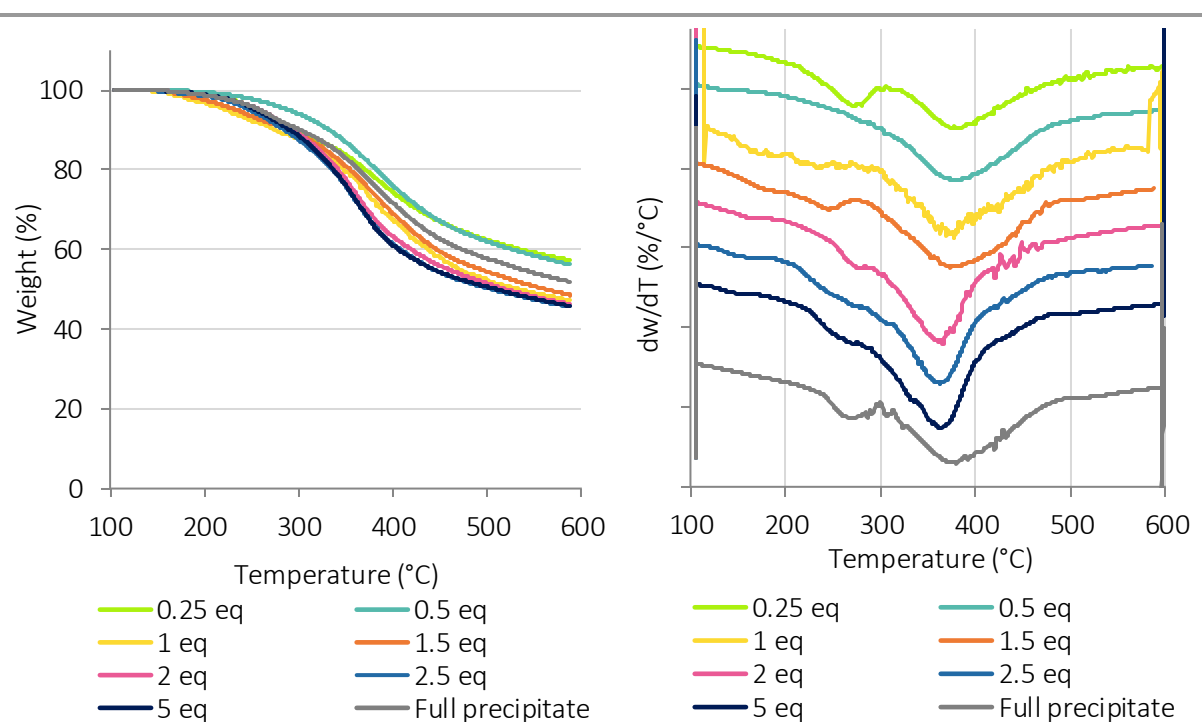


Figure II-77. Thermograms (TG) and derivative thermograms (DTG) of fractions obtained by fractional precipitation of *Miscanthus* using excess acid in the ionic liquid solvent (molar a:b of 1.03:1).

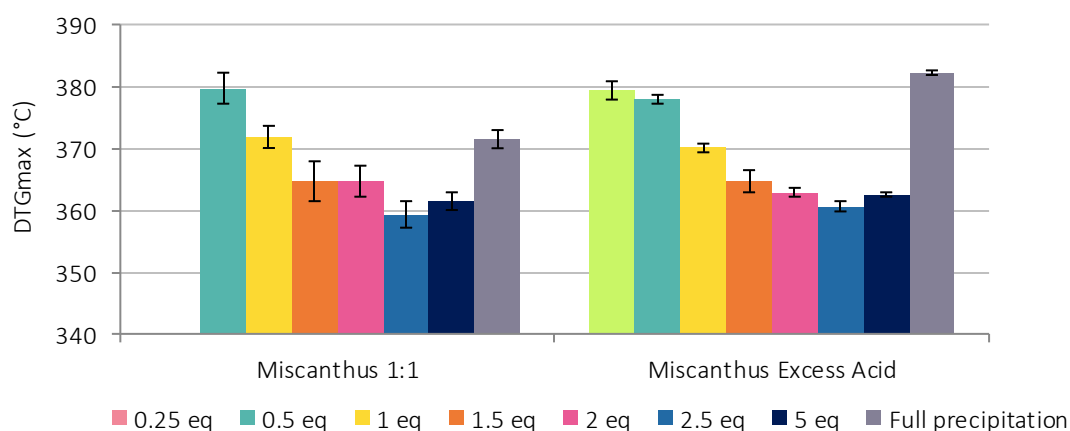


Figure II-78. Temperature of maximum thermal degradation (DTG_{max}) obtained from thermogravimetric analysis of lignin fractions isolated from *Miscanthus* using $[\text{DMBA}][\text{HSO}_4]$ with molar acid:base ratios of 1:1 and 1.03:1.

5.4.7 Outlook of fractionation towards valorization

Lignin fractionation has been studied with the aim of producing relatively homogeneous lignin samples for valorization applications and to minimize energy requirements during IL concentration and recovery (where water added for precipitation is evaporated). The trends obtained as increasing amounts of anti-solvent were added to a given ionic liquid liquor are summarized in Table II-43. **Fractional precipitation of lignins thus appeared to be controlled mainly by lignin molecular weight.**

Table II-43. Summary of trends observed for lignin precipitate fractions with increasing anti-solvent addition.

Lignin characteristic	Trend	Experimental evidence
Proportion of lignin mass	↓ ↓	Mass balance
Molecular weight	↓ ↓ ↓	GPC
Polydispersity	↓ ↓	GPC
β-O-4' bond abundance	↑	HSQC NMR, ³¹ P NMR, GPC
Degree of condensation (C–C aromatic bond content)	↓	HSQC NMR, ³¹ P NMR, GPC, EA, TGA, DSC
S/G ratio	↑	HSQC NMR
Total OH group content	↑	³¹ P NMR
Elemental carbon content	↓	EA
Higher heating value	↓	EA
Thermal recalcitrance	↓	TGA, DSC
Glass transition temperature (T_g)	↓	DSC
Char residue	↓	TGA
Inorganic content (i.e. ash)	—	TGA

GPC: gel permeation chromatography; EA: elemental analysis; TGA: thermogravimetric analysis; DSC: differential scanning calorimetry

The characteristics of fractionated lignins can be integrated with potential applications and feasible commercial direction for lignin valorization, though these will depend on the apparent trade-off between low energy intensity and high product value.

Given the requirement for process heat in the IonoSolv process, some lignin will always be needed for CHP. The initial precipitates ($E_q = 0.25, 0.5, 1$)_{seq} comprising up to 90% of lignin weight, are the obvious candidate for use as a fuel given their high level of condensation that precludes valorization coupled with high carbon content and fuel value. This is especially the case if acidic ILs are used, producing extremely condensed lignins with low water addition that are like to be suitable only for combustion.

By reducing process severity, less condensed lignins could be obtained with characteristics suitable for valorization. These polydisperse lignins benefit from high carbon content (63–76 wt%) and relatively low O/C molar ratios. Depending on their T_g values, which could be further tailored by selection of pretreatment conditions, they could be suitable for lignin pyrolysis to produce bio-oils with high fuel value. Based on the volatiles yield obtained upon thermal degradation, 50–58% of lignin weight could be recovered in the form of bio-oil or useful gases. Coniferyl alcohol has a high condensation reactivity⁷⁸ which would lead to the tendency to form char during lignin pyrolysis; this component was highest for initial fractions of hardwood and softwood lignins, suggesting *Miscanthus* lignins may be most suitable for lignin pyrolysis. Alternatively, these lignins could be further fractionated, for instance by sequential organic solvent extraction, to fine-tune the T_g value for production of carbon fibers or activated carbons. Low volatile content and thermal stability of these fractions would help avoid formation of pores and defects in carbon fibers⁵³. Higher *p*-hydroxyphenyl content (derived from H and PCA) units can negatively impact melt-spinning performance, suggesting pine lignins may be most suitable for carbon fiber production⁵³.

Middle fractions ($Eq = 1.5$ and $2)_{seq}$ were characterized by relatively low molecular weight and low polydispersity with low free phenolic hydroxyl group content. These fractions were insufficiently characterized but could be used as precursors for melt spinning to produce carbon fiber. A moderate degree of condensation and thermal stability (and, presumably, T_g) could be advantageous in addition to the high purity and low ash content of these fractions. The later fractions ($Eq = 2.5, 5)_{seq}$ were most monodisperse with polydispersity close to 2 and had low molecular weight ~ 700 – $1,000$. A high free phenolic hydroxyl group content suggests good reactivity toward polymer synthesis such as by reaction with formaldehyde to produce phenol-formaldehyde resins, or for elaboration of polyesters or polyurethanes⁷⁹. Duval and Lawoko⁷⁹ suggest that guaiacyl-type softwood lignins, for instance from pine, are particularly suitable for such applications owing to the presence of a free orthoposition (C_5) on the phenolic ring.

Ultimately, the desired applications from lignin fractions will be constrained by other process requirements, such as maximization of space-time yields (favored by high IL acidity, short reaction times and high temperatures) and minimization of process energy inputs for low-carbon fuel production (achieved by high IL acidity and low water addition). Ultimately, techno-economic analyses and life cycle assessments are required to evaluate these trade-offs.

5.5 Conclusions and Future Work

ionoSolv lignin was fractionally precipitated into portions of different molecular weights by sequential addition of water as anti-solvent to IL/water mixtures. The resulting fractions were more homogeneous than lignin obtained by full precipitation using an excess amount of anti-solvent. Lignins isolated from *Miscanthus* (herbaceous), willow (hardwood) and pine (softwood) were shown to consist of ~4–14 wt% highly monodisperse lignins with polydispersity index of ~2 and low molecular weight around $\sim 1000 \text{ g mol}^{-1}$ which could be isolated by fractional precipitation. Fractionation appeared to be controlled by molecular weight of lignin polymers. However, some structural differences were also found in these fractions, notably in that precipitates isolated with increasing amounts of anti-solvent (i.e. decreasing dissolution power of the solvent mixture) possessed (1) low molecular weight and narrow molecular weight distribution, (2) high phenolic hydroxyl and total hydroxyl content, (3) low degree of condensation, (4) low carbon content and (5) reduced thermal stability, compared to the initial macromolecules that precipitated. The effect of excess acid in the ionic liquid solvent was seen to exacerbate the differences between lignin fractions and to enable molecular weight and hydrophobicity to be tailored somewhat independently. Increasing severity of pretreatment increased lignin yield and produced more condensed (i.e. high molecular weight) lignins. Accordingly, by varying process severity and anti-solvent addition, there is great potential to tailor the lignin product streams depending on the desired outcomes of pretreatment. Lignin fractional precipitation also offers a unique separation platform for lignin, simplifying the biorefining process by fractionating lignin within the lignin precipitation itself, rather than conducting a separate fractionation step.

The valorization of fractionated lignin should depend on the unique characteristics of each lignin fraction. While the most condensed precipitates are likely to be burned for energy recovery, high molecular weight fractions may also be suitable for pyrolysis or elaboration of activated carbons, while moderate molecular weight polymers could be excellent candidates for carbon fiber production. Low molecular weight, narrow-polydisperse fractions with high reactivity and low thermal stability are highly desirable for synthesis of value-added products, such as polymers. It is worth noting that these later precipitates were obtained by addition of large volumes of water to the IL/water mixture; removal of this water during IL recycling is highly energy intensive and can be justified only with lignin valorization. Selection of IL acidity may also enable process optimization according to energy intensity or product value. If the target is low-carbon biofuel, then excess acidity in the IL is preferable to increase space-time yields and reduce water required for precipitation, lowering energy costs (and capital costs due to reduction in reactor volumes); however, the majority lignin produced is of poor

quality and has value only as a fuel. If maximum value is desired, milder ILs should be used, leading to a more costly process but higher product value.

Further work on this topic should concentrate on further analysis of lignin thermal properties, notably to compare trends in lignin glass transition temperature T_g , an essential property for many valorization routes such as carbon fiber production through melt spinning. T_g is known to be closely related to lignin molecular weight¹¹; this could not be validated at the time of this study. Methoxy content analysis could be used to determine the molecular formulae of the fractions while compositional analysis could be used to determine the lignin purity (ash and hemicellulose content). Further avenues for research could also examine the effect of different anti-solvents other than water, for instance ethanol, on fractional precipitation. It was noted that many of the lignin fractions produced in this study were highly treated (i.e. depolymerized and condensed); future studies employing fractionated lignin may benefit from using less severe pretreatment conditions, achieved by using high pretreatment temperatures for short residence times and milder IL acidity. Addition of 1 g of water per g of dry ionic liquid in the liquor was able to precipitate up to 90 wt% of lignin fragments compared to the lignin mass isolated by excess water addition (5 g/g). Calculation of energy savings relative to cost benefits remain to be evaluated. Finally, the effect of non-complete lignin precipitation to reduce water and energy requirements on solvent recycling at steady state needs to be examined.

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Part III. Conclusions

Key Experimental Findings

This PhD project covered different approaches to improving the cost-effectiveness of lignocellulose conversion to bioethanol using ionic liquid-based biorefining. Low-cost protic ionic liquids were used to pretreat abundant agricultural residues, energy crops and woody biomass with the aim of producing a highly digestible cellulose pulp and lignin stream suitable for further valorization.

In Chapter 1, it was shown that TEA HSO₄-water mixtures could successfully pretreat agroresidues with high ash (up to 13 wt%) and lignin content (up to 28 wt%). Under the optimized pretreatment conditions of 170 °C and 30–45 min, subsequent enzymatic hydrolysis of the pulps gave glucose yields of ~90% for rice straw, wheat straw and sugarcane bagasse and ~75% for rice husk. Glucose release from the pulps was enhanced by saccharifying wet pulps without an air-drying step to reduce hornification. The pulp glucan content was nearly quantitatively hydrolyzed, enabling facile separation of inorganic matter and simplifying processing. Rice husk and sugarcane bagasse were found to be more recalcitrant to deconstruction, in line with their higher lignin contents, dense cell wall structures and lignin compositions. The lignins recovered were fairly condensed, suggesting a slight reduction in residence times could further increase space-time yields while also reducing reactor volumes and capital costs.

These results were expanded in Chapter 2, which examined pretreatment of the geographically widespread and underutilized agro-industrial residue sugarcane bagasse. Five bagasse fractions, consisting of whole, industrially depithed, laboratory depithed and pith bagasse, were compared but did not show substantial differences in chemical composition or glucose release after pretreatment. Evidence was presented that depithing is unnecessary prior to sugarcane bagasse conversion, affording significant cost and energy savings; instead, particle size distribution and aspect ratio appeared to be major factors in bagasse conversion owing to the propensity for dissolved lignin to precipitate onto the pulp surface under prolonged treatment. The glucose yields obtained for bagasse using TEA HSO₄-water mixtures were ~75% as high as for the aprotic IL [Emim][OAc]; this result is highly promising given that TEA HSO₄ is 40 times less expensive. As in Chapter 1, the biomass ash was seen to partition with the pulp and could in theory be recovered from the post-hydrolysis solids.

Chapter 3 saw major steps taken in the intensification of the ionoSolv process by increasing the solids loading, particle size and mixing speeds. Energy crop *Miscanthus* was successfully pretreated using a biomass to solvent ratio of 1:2 g/g, equivalent to a solids loading of 50 wt%. Lignin precipitation onto

the pulp surface modified the properties of the recovered lignin precipitate, highlighting the need for improved pulp washing methods following pretreatment. The optimum size fraction for *Miscanthus* pretreatment without stirring appeared to be 1–3 mm, with great potential for reducing comminution energy requirements prior to or post-pretreatment. Pretreatment performance of ILs with dilute acid solvents was compared, with ILs offering major advantages, notably the ability to effectively pretreat larger particle sizes up to 10 mm.

Subsequently, 100-fold scale-up of the ionoSolv process (from 10 mL to 1 L) was performed with the introduction of mixing using an anchor impeller. The pulp washing protocol was adapted for this scale to consist of multiple vacuum filtration steps, offering ~25% ethanol savings compared to the bench scale protocol. The efficacy of pretreatment, evaluated by pulp digestibility, delignification and lignin recovery, was greater upon scale-up due to better heat and mass transfer with slurry agitation. Mixing had a larger effect for larger particle sizes, notably in accelerating lignin extraction, reducing the level of condensation and reducing lignin re-precipitation, compared to unstirred reactions. Experiments with varying mixing speeds indicated that much of stirring energy went into breaking up the biomass particles. Process efficacy would benefit from optimizing the mixing speed (and pretreatment time and temperature) based on the particle size of the feedstock. IL recoveries of over 99.3% were attained at the 1 L-scale, which is favorable to IL recycling for continuous processing. A mass balance suggested that losses were mainly due to hemicellulose conversion to volatile degradation products.

Having demonstrated ample potential for further scale-up of the ionoSolv pretreatment process, attention was then turned to lignin valorization. Chapter 4 examined the link between pretreatment conditions, lignin properties and their fast pyrolysis. Pretreatment time and temperature used to isolate lignin were varied to control its oxygen content, molecular weight and functionality. Milder treatments produced more oxygenated lignin with low molecular weight and hydroxyl group content; these lignins were also less thermally stable ($T_g \sim 135^\circ\text{C}$). Lignins isolated from more severe pretreatment conditions had higher carbon stability ($T_g \sim 155^\circ\text{C}$) and were increasingly condensed, cross-linked and of high molecular weight. Fast pyrolysis of these lignins gave bio-oil yields between 27–53%. Less treated lignins produced the largest quantity of bio-oil while more condensed lignins had higher propensity for char formation. Pyrolysis of mildly treated lignins also led to a higher proportion of carbon retained in the liquid product and gave the highest energy and carbon recovery in the bio-oil of ~70% and ~60%, respectively, assuming char is used for meeting the energy requirements of pyrolysis. Preliminary investigations of bio-oil composition revealed that more treated lignins produced more conjugated bio-oils with higher aromatic content. Though all lignin bio-oils were estimated to have higher fuel value than biomass bio-oil, no clear link between pretreatment

severity and bio-oil oxygenation or heating value was observed. Due to limited data on bio-oil water content, acidity and chemical composition, the effect of lignin characteristics on bio-oil components and thermal stability remains to be assessed.

Lignin fractional precipitation by addition of water as anti-solvent was then investigated in Chapter 5. Compared to other lignin fractionation techniques such as neutralization, filtration and solvent fractionation, fractional precipitation offers the advantages of high control over lignin structure without the use of hazardous organic solvents while minimizing process and equipment costs. Fractional precipitation was governed by lignin molecular weight, influenced by ether cleavage and C–C condensation reactions that occurred during pretreatment in the acidic IL. The lignin chemical structure, notably the hydroxyl group content, may also have influenced lignin solubility, allowing polydisperse macromolecules to be separated from smaller lignin fragments. With increasing anti-solvent addition, precipitates possessed drastically lower molecular weight and lower polydispersity, higher ether bond abundance and lower condensation, lower carbon content and reduced thermal stability, compared to the macromolecules that precipitated with small amounts of anti-solvent addition. The effect of higher IL acidity was seen to exacerbate these trends and though lignins were on average more condensed, they required lower anti-solvent volumes to be precipitated.

This final Chapter illustrated another major advantage of the ionoSolv process, namely the ability to select process conditions (such as IL acidity, residence time, temperature, anti-solvent volume) to tailor the product distribution, depending on whether low-carbon biofuels or high-value products are the main objective. Using fractional precipitation, lignin fractions with desirable properties of low molecular weight of 700–1000 g mol⁻¹ and narrow polydispersity of ~2 could be isolated in yields of 4–14 wt%. This section was concluded with an outlook on the possible applications of different lignin fractions. It was proposed that the most condensed fractions were best suited as a fuel for energy recovery. Synthesis of polymers or other products from low-molecular-weight lignin fractions could be justified if high product selling prices can be obtained.

Perspective for Commercialization

The work undertaken in this project has improved the economics of the ionoSolv process through scale-up and introduction of stirring. Low-cost ionic liquids were shown to produce highly digestible pulps and quality lignin from a range of abundant lignocellulosic feedstocks. Optimum process parameters of residence time and temperature were found for lignin- and ash-heavy agricultural residues including rice husk and rice straw. The process was shown to be robust, maintaining its performance with high biomass to solvent ratios and large particle sizes, offering potential for significant energy and materials savings. The ionoSolv process was successfully scaled up 100-fold,

which was shown to further increase space-time yields due to improved heat and mass transfer with slurry mixing. Additionally, lignin valorization through fast pyrolysis was investigated. High liquid product yields and excellent energy recovery in the bio-oil were obtained for mildly treated lignins, establishing a link between process severity and product distribution. Another highlight is fractional precipitation to obtain narrow monodisperse lignin fractions using minimal water for lignin precipitation, reducing energy requirements for evaporation. Lignin fractionation also offers a unique separation platform for lignin within the lignin precipitation operation itself, rather than adding additional capital and operational costs by conducting a separate fractionation.

In summary, the work presented here is a key step towards commercialization of the IonoSolv process for the production of various bio-derived products from various woody and grassy feedstocks. There remains, however, a lot of further work to improve overall performance and make renewable liquid transportation fuels, chemicals and materials sustainable and affordable.

Remaining Gaps and Proposed Future Work

Though pretreatment process intensification was investigated by means of increasing solids loading and particle size, further work is needed to determine the energy intensity of the process at industrial scale. Development of predictive models that integrate heat, mass and momentum transport are essential to improve reactor and agitator design and minimize energy requirements of heating and mixing. Improved mixing could also reduce reaction times to further increase the space-time yield and capital cost of process equipment. There is a need to tailor pretreatment conditions to a chosen process scale, feedstock, particle size fraction and reactor configuration, bearing in mind targets for energy/carbon intensity and product value need to be met. Process models should then be validated this against experimental data to further optimize process parameters (temperature, residence time, stirring speed and biomass loading) on specific individual feedstocks or feedstock blends. Techno-economic and life cycle analysis are required to address the question of how much lignin to precipitate, which will have a bearing on the energy consumption of the process and subsequent recycling operations.

As the section on hornification and pulp washing protocols has highlighted, there is a need to improve pulp washing and materials handling to reduce the incidence of lignin precipitation in ethanol at high lignin concentrations and to reduce the solvent requirements of washing. The 10 vacuum filtration steps used in this work are unfeasible industrially; instead, combinations of crude (e.g. filtration) and high-efficiency (e.g. centrifugation) separation steps could be appropriate for trials at pilot scale. Use of ethanol or DMSO is undesirable for pulp washing owing to low volatility and high solvent volumes, respectively. The use of (aqueous) hot ionic liquid may be an alternative that remains to be

investigated. Solid-liquid separations in commercial operations are very capital intensive and can represent a large proportion of capital and operating costs. There is therefore a need to improve separations through selection of centrifugation, sedimentation, filtration or multi-step processing.

This work has neglected the final step in bioethanol production, namely fermentation processes, and the effect of different carbohydrate and lignin degradation products or impurities found in the pulps on ethanol yields is unknown. Further work is needed to evaluate the effect of residual ionic liquid in the pulp on downstream processes and to arrive at an integrated conversion process attaining high bioethanol yields. Beyond the scope of this work, improvements in process equipment, enzymes and microbial systems are critical determinants of the successful implementation of biorefineries. Commercialization of the ionoSolv process will also require identification of go-to-market product segments from the pulp and lignin.

Returning to the challenge of lignin valorization, it is desirable to further investigate ionoSolv lignin thermal properties, notably the glass transition temperature, to enable conversion of high-volume products such as polymeric materials and carbon fibers. Lignin fractionation and recovery could then be matched to desired lignin applications. Preliminary investigations of lignin bio-oils revealed a lower degree of conjugation in bio-oils generated from pyrolysis of less treated lignins; however, further analysis of bio-oil pH, water content and composition are necessary to validate the trends observed. Future studies of lignin fractionation would benefit from comparing different process severities, with a view to either maximizing the yield of narrow-disperse lignins or to minimizing the anti-solvent and energy requirements. Calculation of the energy savings from reduced water addition relative to the cost benefits of fractionation remain to be investigated. Finally, the effect of incomplete lignin precipitation to reduce water and energy requirements on solvent recycling at steady state needs to be examined.

On a more general note, there is ample potential for biorefining to offer low-carbon alternatives to the materials, fuels, chemicals and energy we use every day. While lignocellulosic biofuels have immense potential on paper, their continued development should target sectors in urgent need of modernization, such as the production of marine, airplane and heavy automotive fuels, to reduce the carbon footprint of long-distance transportation. Further R&D in technologies such as ionoSolv biorefining must also go hand in hand with raising awareness among the public about the true benefits (and caveats) of biofuels, life cycle analyses to establish best practices for sustainable production in different geographies, development of markets for new bio-based products and clear policies that consider long-term sustainability when developing bio-based value chains.

Appendix

Chapter 1

Sample error calculation

In this thesis, all experimental errors were reported as one standard deviation, calculated using the STDEV.P formula in Microsoft Excel, which assumes that the dataset is the entire population. For example, let us take the datapoint for the saccharification yield at 170 °C and 60 minutes, as reported in Figure II-3 (Chapter 1). The saccharification yield values obtained from triplicate measurements were: 41.1%, 52.6%, 45.9%. The standard deviation, SD, was calculated using Equation S1:

$$SD = \sqrt{\frac{\sum |x - \mu|^2}{N}} \quad (\text{Equation S1})$$

where x is a data point, μ is the mean and N is the sample size ($N = 3$).

Therefore for the values given above, μ is 46.5 and $SD = 4.7$; therefore the data was plotted as having a value of 46.5 with a standard error of 4.7, i.e. $46.5 \pm 4.7\%$ glucose yield.

Delignification vs. saccharification



Figure S0-1. Saccharification yields vs. delignification of pulps before and after pretreatment of rice husk, rice straw, wheat straw and depithed sugarcane bagasse under various conditions.

Glucose release

Table S0-1. Glucose release after 7 day enzymatic saccharification experiments for a range of pretreated samples and untreated biomass. Data is expressed as four different percentages of glucose release.

Glucose release				
	As % of untreated BM	As % of glucan in untreated BM	As % of pulp	As % of glucan in pretreated pulp
Rice husk UT	1.08±0.02	2.4±0.0	-	-
Bagasse UT	4.8±0.1	11.4±2.8	-	-
Wheat straw UT	6.7±0.1	15.4±0.2	-	-
Rice straw UT	11.4±3.4	26.0±6.3	-	-
Rice husk 170 °C 45 min	31.0±1.4	73.0±4.2	57.5±2.4	98.2±0.2
Bagasse 170 °C 45 min	38.9±2.9	89.4±5.5	100.1±5.6	109.8±5.6
Wheat straw 170 °C 30 min	37.6±1.0	86.5±1.9	90.4±3.7	98±0.2
Rice straw 170 °C 30 min	38.8±0.1	88.2±0.2	88.0±5.3	102.0±0.2

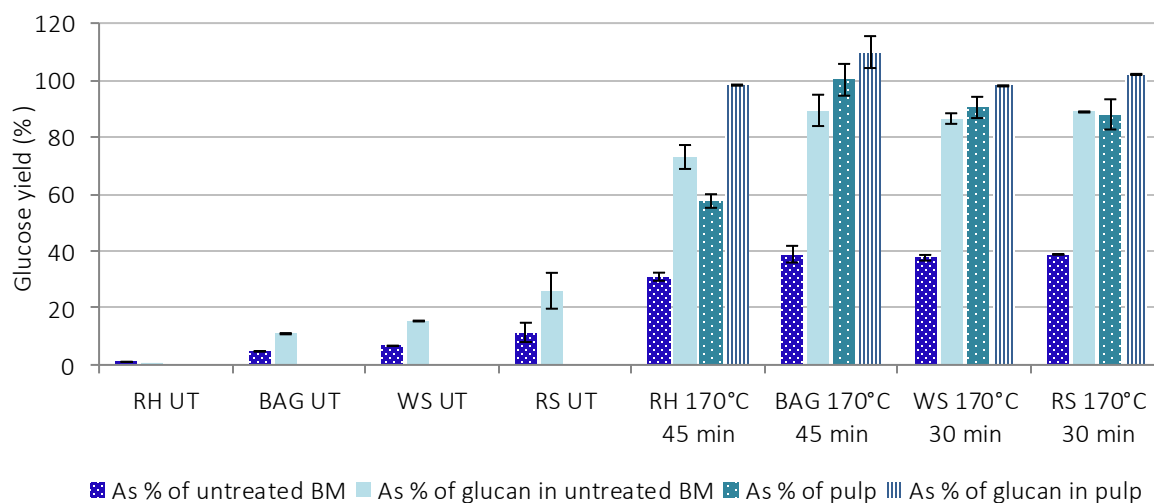


Figure S0-2. Glucose release after 7 day enzymatic saccharification experiments for a range of pretreated samples and untreated biomass. Data is expressed as four different percentages of glucose release.

Optimized saccharification yields

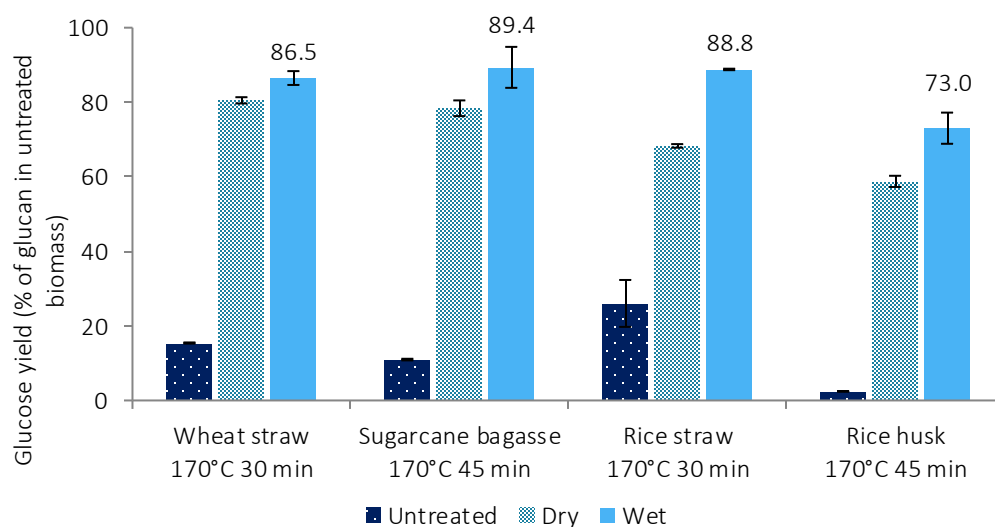


Figure S0-3. Saccharification yields for untreated, pretreated air-dried pulp and pretreated pulp without air-drying for various agricultural residues under optimal conditions at 170 °C.

Chapter 3

Scale-up reactor heating curve

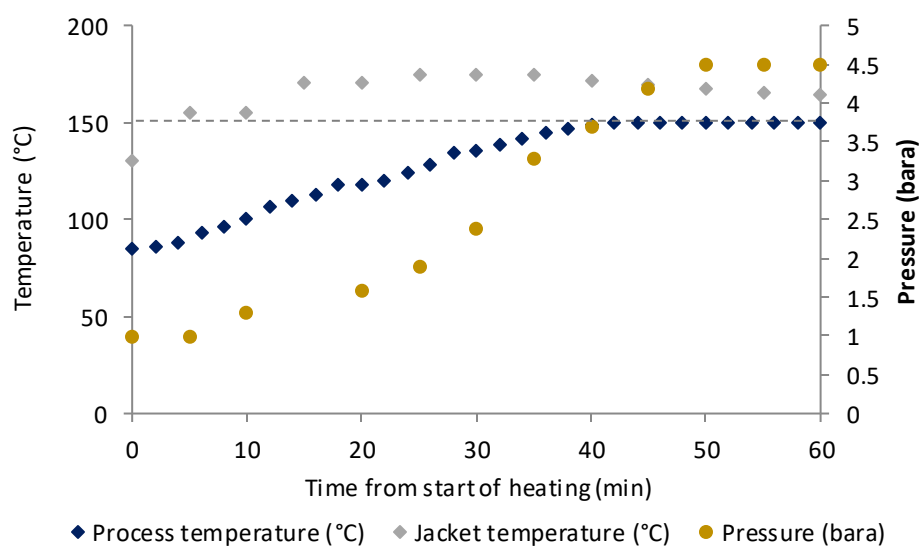


Figure S0-4. Sample heating curve and process pressure for [TEA][HSO₄]-biomass slurry (20 wt% loading) from 85 to at 150 °C using an oil jacket in a 1 L-glass reactor.

Thermal stability of [TEA][HSO₄]

Objective: To compare the short-term thermal stability of [TEA][HSO₄] used in this study with aprotic ionic liquid [Emim][OAc]. To verify the long-term isothermal stability of [TEA][HSO₄] by ¹H NMR spectroscopy before and after heating for 100 hours at 150 °C.

Methods used: The thermal stability of (dry) ionic liquids were compared using thermogravimetric analysis by temperature ramping up to 700 °C. The T_{onset} , which is the intersection of the tangent at the steepest point of the thermogravimetric curve with the baseline weight, has been reported. A second isothermal experiment was conducted by placing ~1g of fresh IL into a sealed glass vial and holding at 150 °C for 100 h. ¹H NMR spectra were recorded for both dilute (20 wt% water) and neat fresh ionic liquid and after ageing at 150 °C for 100 hours continuously to observe the possible formation of by-products. This technique would flag levels of impurities greater than 1% relative to the IL. The full spectrum with all peaks is shown in Figure S0-5. Peaks that dominate the spectrum can be assigned to the cation, NMR solvent (DMSO-d₆) and the protons on water/hydrogen sulfate. Significant signals for other proton-containing compounds were not discovered.

Findings: TGA analysis (Figure S0-5) showed that [Emim][OAc] is less thermally stable than [TEA][HSO₄] as observed by its lower T_{onset} value of around 230 °C compared to 275 °C for [TEA][HSO₄]. This finding is in agreement with values of ~220–250 °C reported for [Emim][OAc] in the literature⁸². However, T_{onset} values are often considerably higher than the temperature at which the IL is stable for a long time period⁸² and therefore the maximum temperature where the IL can be used in a process without significant thermal weight loss.

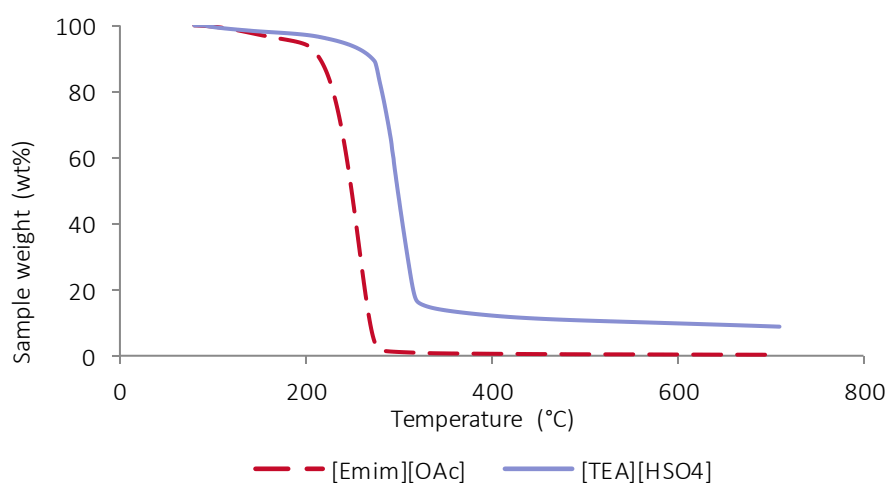


Figure S0-5. Thermogravimetric curves for [Emim][OAc] and [TEA][HSO₄] upon temperature ramping to 700 °C in nitrogen atmosphere indicating the T_{onset} values.

The long-term stability of [TEA][HSO₄] upon constant heating in air was compared by ¹H NMR spectroscopy before and after heating at 150 °C for 100 hours. Figure S0-6 shows spectra that are visibly identical with no change in peak position or integrals after 100 h, indicating no chemical changes producing products above 1% concentration have taken place. The results indicate that decomposition reactions are undetectable or produce minimal levels of decomposition products at these temperatures, indicating these ILs are thermally stable at up to 150 °C.

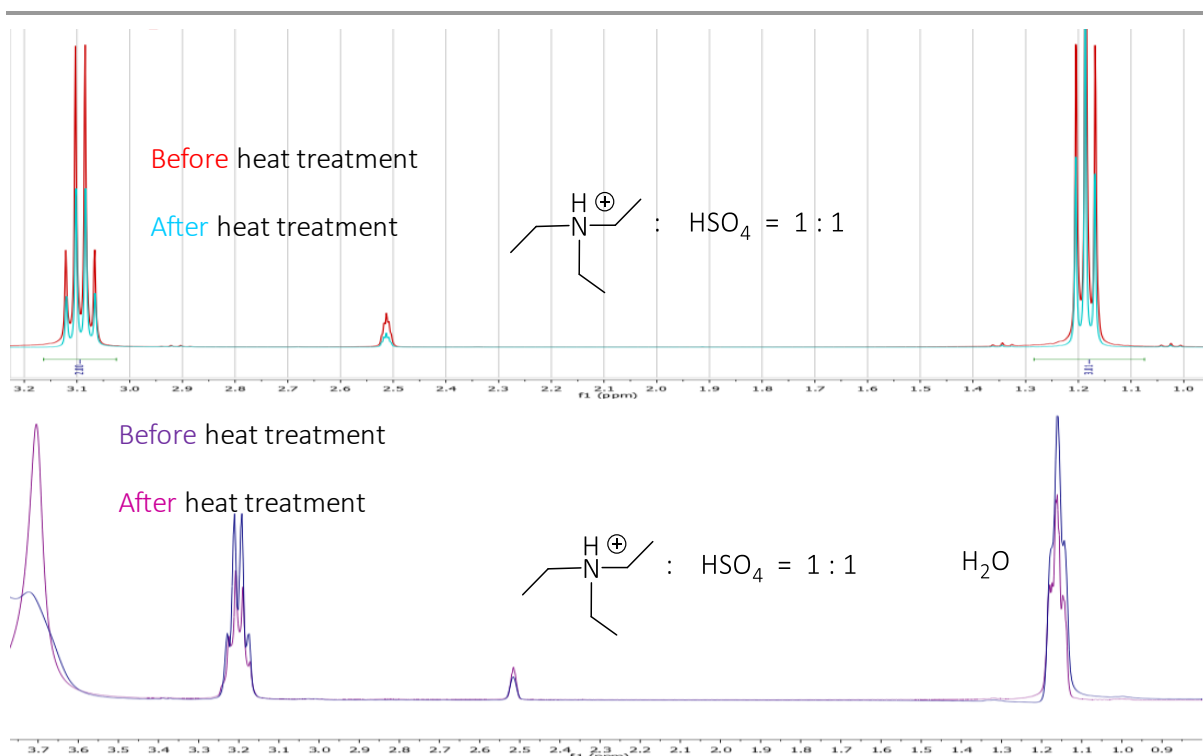


Figure S0-6. ¹H NMR spectrum (400 MHz; DMSO-d₆) of [TEA][HSO₄] with a water content of 20 wt% and acid:base ratio of 1:1 before (red) and after (blue) treatment at 150 °C for 100 hours. Shifts were reported in ppm with respect to TMS. Spectra shown are for (a) neat ionic liquid and (b) ionic liquid containing 20 wt% water.

Ionic liquid pH measurements

Table S0-2. Change of IL solution acidity with biomass loading. Miscanthus was pretreated at a biomass loading between 10 and 50 wt% in 80% [TEA][HSO₄] with 20 wt% at 120 °C for 6 hours.

Sample	pH of 1 wt% solution ^a	[H ⁺] concentration in IL (mmol/g) ^b	H ⁺ consumed with respect to fresh IL (%)
Fresh TEA HSO ₄	1.65±0.01	0.224±0.001	-
10 wt% loading	1.70±0.01	0.201±0.001	11
20 wt% loading	1.73±0.02	0.188±0.002	19
30 wt% loading	1.73±0.02	0.185±0.002	21
50 wt% loading	1.75±0.01	0.178±0.001	26

^a Diluted 1:100 wt:wt in water on a dry basis. ^b calculated as H⁺ concentration in undiluted IL on a dry basis.

Energy requirement calculations

Key assumptions

The work of Miao *et al.*⁷⁴ found a power relationship between the specific comminution energy requirement (kJ kg^{-1} dry matter), E_{sr} , and the geometric mean length (mm), x , of *Miscanthus* particles. The correlation obtained (validated against experimental data for $0.37 < D_{50} < 1.29$ mm) was extrapolated for fine, medium and coarse particles with respective calculated D_{50} values of 0.38, 2.3 and 9.9 mm, respectively (see Figure II-46).

According to Miao *et al.*⁷⁴:

$$E_{sr} = 26.79x^{-3.521} \quad (\text{Equation S2})$$

Inputting the D_{50} values above gave energy requirements for size reduction of air-dried *Miscanthus* of 808, 1.4 and 0.01 kJ kg^{-1} dry matter, respectively for fine, medium and coarse particles.

The power input of mixing, E_{mix} , was estimated by extrapolation of established correlations given in the work of Blanch and Clark⁸³, which provided a correlation between the Reynolds number, Re , and the power number N_p , for various agitated slurries. The relevant dimensionless parameters are defined below:

$$Re = \frac{ND^2\rho}{\mu} \quad (\text{Equation S3})$$

$$N_p = \frac{P}{\rho N^3 D^5} \quad (\text{Equation S4})$$

$$E_{mix} = P \times t \quad (\text{Equation S5})$$

$$E_{tot} = E_{sr} + E_{mix} \quad (\text{Equation S6})$$

where N is the stirring speed (s^{-1}), D is the agitator diameter (m), ρ is the slurry viscosity (kg m^{-3}), μ is the dynamic viscosity of the slurry ($\text{kg s}^{-1} \text{m}^{-1}$), P is the power requirement of mixing (W) and t is the residence time for pretreatment (s).

The relevant data points required for calculation of the power input for mixing at each pretreatment condition are given in Table S0-3 below. The calculated values for Re and N_p are found in Table S0-4.

Table S0-3. Summary of data values used for mixing energy calculations.

Parameter	Pretreatment condition ^a	Value ^b	Unit
ρ	10 wt% loading	1100	kg m ⁻³
	20 wt% loading	1025	kg m ⁻³
μ	10 wt% loading	100	kg s ⁻¹ m ⁻¹
	20 wt% loading	1000	kg s ⁻¹ m ⁻¹
N	150 rpm	2.5	s ⁻¹
	250 rpm	4.17	s ⁻¹
	400 rpm	6.67	s ⁻¹
	450 rpm	7.5	s ⁻¹
D	All scale-up experiments	0.05	m
t	All scale-up experiments	21600	S

^a Based on dynamic viscosity measurements for APIL [Emim][OAc] and switchgrass powder at loadings between 5 and 50 wt% measured by Cruz *et al.*¹³ ^b Based on density of [TEA][HSO₄] as 1197 kg m⁻³ and *Miscanthus* as 150 kg m⁻³.

Table S0-4. Calculated Reynolds number and power number dimensionless numbers used to estimate the energy input of mixing.

Particle size, D_{50} value and loading	Stirring speed (rpm)	Re (-)	N_P (-)	Energy input of mixing E_{mix}
Fine, 0.38 mm, 10 wt% loading	150	0.07	700	813
	450	0.21	350	10979
Medium, 2.3 mm, 10 wt% loading	150	0.07	700	813
	250	0.11	700	3765
Coarse, 9.9 mm, 10 wt% loading	150	0.07	700	813
	400	0.18	350	3856
Fine, 0.38 mm, 10 wt% loading	150	0.006	8000	4322

Other assumptions: Amount of biomass pretreated per run for experiments using 10 and 20 wt% loading was 0.1 and 0.2 kg, respectively. The glucan content of untreated *Miscanthus* (based on compositional analysis) is 46.4wt%.

Energy assessment

While the results in Chapter 3 individually assessed the effect of solid loading, particle size, mixing speed and scale-up on ionoSolv effectiveness, process optimization requires simultaneous assessment of process parameters to arrive at acceptable energy inputs for a desired glucose (and eventually, ethanol) yield. To gain a preliminary understanding of the optimal process configuration, an energy assessment was conducted. The PSD and D_{50} values for untreated biomass were used to estimate the energy input of size reduction to produce pulps of a specific geometric mean length. The estimated energy input for stirring was estimated based on established formulae⁸³. Thus, the total energy input required to produce a given glucose yield was calculated, taking into account the energy requirement for size reduction and mixing, neglecting the energy for heating which was assumed to be constant for reactions at 120 °C 6h.

The results of the calculations, displayed in Table S0-5, reveal that the energy input for mixing is significant compared to the energy input for size reduction, and dominates for larger particles. Taking into account the glucose yields obtained, maximum glucose release with minimal energy input was achieved for medium *Miscanthus* particles (1–3 mm) with low stirring speed (150 rpm), which gave a respectable glucose yield for air-dried biomass of $68.2 \pm 4.8\%$. This glucose yield could be further improved by avoiding hornification (i.e. saccharifying wet pulps without an air-drying step) which would reduce energy consumption of drying the pulp. Substantial cost savings could therefore be realized by tailoring the pre-processing size reduction stages to an individual feedstock. However, as a herbaceous biomass feedstock, *Miscanthus* is comparatively less energy-intensive to size-reduce compared to softwood feedstocks^{23,43}. The energy savings for post-ionoSolv pretreatment size reduction of woody feedstocks are potentially much higher, and investigation of these effects is necessary to evaluate the full potential of ionoSolv pretreatment for offering a low-energy, high-yield pathway to biofuels production from lignocellulosic biomass feedstocks.

It is important to note that these calculations are intended as a comparison of *relative* energy inputs, but the energy requirements cannot be directly translated to industrially relevant scales. Energy inputs varied between 26 and 384 MJ kg⁻¹ glucose released, all of which exceed the energy content of glucose itself (15.6 MJ kg⁻¹). The true power consumption of mixing is known to be strongly dependent on the system geometry, type of agitator, the rheological characteristics of the IL-biomass slurry, and the kinematic conditions in the pretreatment vessel⁸⁴. In reality, the power consumption is expected to be significantly lower than indicated in this study, given use of more efficient biomass milling equipment and improved vessel and agitator design at industrial scales.

Table S0-5. Estimation of energy input for ionoSolv pretreatment process as a function of process conditions.

Particle size, solid loading and D_{50} value	Energy input for grinding (kJ kg ⁻¹ biomass) ^a	Stirring speed (rpm)	Energy input of mixing (kJ kg ⁻¹ biomass) ^b	Total energy input (kJ kg ⁻¹ biomass) ^c	Glucose released in run (kg)	Total energy input (MJ kg ⁻¹ glucose) ^d
Fine – 10wt% D_{50} = 0.38 mm	808	150	810	1620	0.037	44
		450	10980	11790	0.031	380
Medium – 10wt% D_{50} = 2.3 mm	1.4	150	810	820	0.032	26
		250	3770	3770	0.031	120
Coarse – 10wt% D_{50} = 9.9 mm	0.01	150	810	810	0.028	30
		400	3860	3860	0.027	140
Fine – 20wt% D_{50} = 0.38 mm	808	150	4320	5130	0.073	70

^a Estimated from extrapolated correlations based on the work of Miao et al.⁷⁴ for hammer milling of air-dried Miscanthus, where the energy of grinding E varies with geometric length as $E = 26.79x^{3.521}$.

^b Estimated from standard correlations in the literature; detailed calculations found in the Appendix.

^c Sum of the energy of size reduction and energy requirement of mixing, excluding energy for heating.

^d Total energy required excluding heating divided by glucose released (kg released in each 1 L run).

Chapter 4

Fast pyrolysis reactor description

The fast pyrolysis reactor used was previously designed, built and commissioned by Dr Matthew E. Boot-Handford; the original description may be found in his 2016 thesis¹⁰⁵. It is a fixed-bed downdraft pyrolysis reactor that was operated with a single-stage configuration (see Figure S0-7). The reactor body was constructed from a high temperature NB Incoloy 800HT alloy working tube (1" nominal bore, 350 mm length) with an internal quartz liner. The connections between the reactor body, gas supply system and tar trap utilized Swagelok pressure fittings. A solid fuel feeder (50 mL double-ended sample cylinder) was placed above the main reactor body, such that the solid fuel could be pre-loaded and fed in a batch-wise manner. The ability to feed solid fuels in this way enabled samples to be fed into the reactor after it had been heated to the experimental setpoint, allowing for high heating rates (>100 °C/s) to be achieved that are more comparable to the heating rates of commercial, large-scale pyrolysers.

Gases were introduced through a T-piece at the top of the reactor. Nitrogen gas was introduced at the top of the reactor such that the fuel could be pyrolysed in the first stage, generating a fuel or exhaust gas which could be analyzed directly. Inert quartz tubes were designed to line the inside of the reactor and prevent undesirable reactants between the gaseous reactants and products with the Incoloy 800 HT reactor walls. A high temperature polymer gasket was placed at the top of the quartz tube to

prevent gas bypass. A bespoke design spring at the base of the quartz tube provided the force required for sealing and compensated any change in reactor length due to thermal expansion during the experiment. Inert quartz tubes incorporating sintered quartz disks midway down the tube acted as a bed support and retained ash and residual char, while evolved gases and volatiles were swept downstream into a tar trap. Typically, sand (2.8 g, 500-700 μm) was loaded into the reactor to provide an inert bed (5 mm in height) and heat sink that the thermocouple could be placed in. A K-type thermocouple was immersed in a bed of sand during the pyrolysis to measure the heating rate.

During the reaction, the evolved gases and volatiles were swept downstream into the main tar trap. The tar trap has an annular heat-exchanger design consisting of a 1000 mm stainless steel (1/2" OD) inner tube with a 1" OD outer tube connected around the outside of the inner tube via two 1" T-piece Swagelok connections. The gas exited the reactor through the internal 1/2" tube, which was cooled with refrigerant solution (30 wt% ethylene glycol in distilled water; -2 °C) to cool the gases and condense the tars and water vapour contained in the reactor flue gas for analysis, as illustrated in the schematic. The inner tube exit was loosely packed with previously dried quartz wool (~6 g) to increase the surface area for condensation of bio-oils formed. A secondary line diverted the gas flow from the tar trap and towards a second reactor outlet; a ball valve enabled the gas flow to be diverted from the main tar trap after 90 s of reaction time had elapsed, so that the tars could be isolated from the hot gas flow and sealed off from the atmosphere prior to recovery. Two smaller quartz wool traps (not cooled, each containing ~1.5 g of quartz wool) were placed after the main tar trap outlet and at the diversion line outlet, respectively, to condense any remaining tars.

Mass flow controllers (Bronkhorst High-Tech B.V.) were used to control and monitor the flowrate and composition of gas entering the reactor. The pressure was controlled and monitored by a pressure controller and pneumatic valve (Bronkhorst High-Tech B.V.) at the exit of the tar trap. The concentrations of CO, CO₂, CH₄ and H₂ were measured in real time at the exit of reactor throughout the pyrolysis experiment using a multi-gas infra-red detector (CO, CO₂, CH₄) using an online multigas analyser (MGA3000, ADC Analysers, UK) that was connected after the pressure control valve. A constant purge of 600 ml min⁻¹ was maintained to the gas analyser to ensure that the flow remained in the calibrated range for the analysers. The gas exiting the analyser train was vented into the fume cupboard. A control program written in Agilent VEE automated the entire process including fuel feeding, temperature control and switching, gas flow rate control and switching, pressure control and data monitoring and collection. All relevant information including measurements of the temperatures at different points in the reactor, flow rates, the system pressure and the signals from the gas analysers were logged every second and saved to a designated text file.

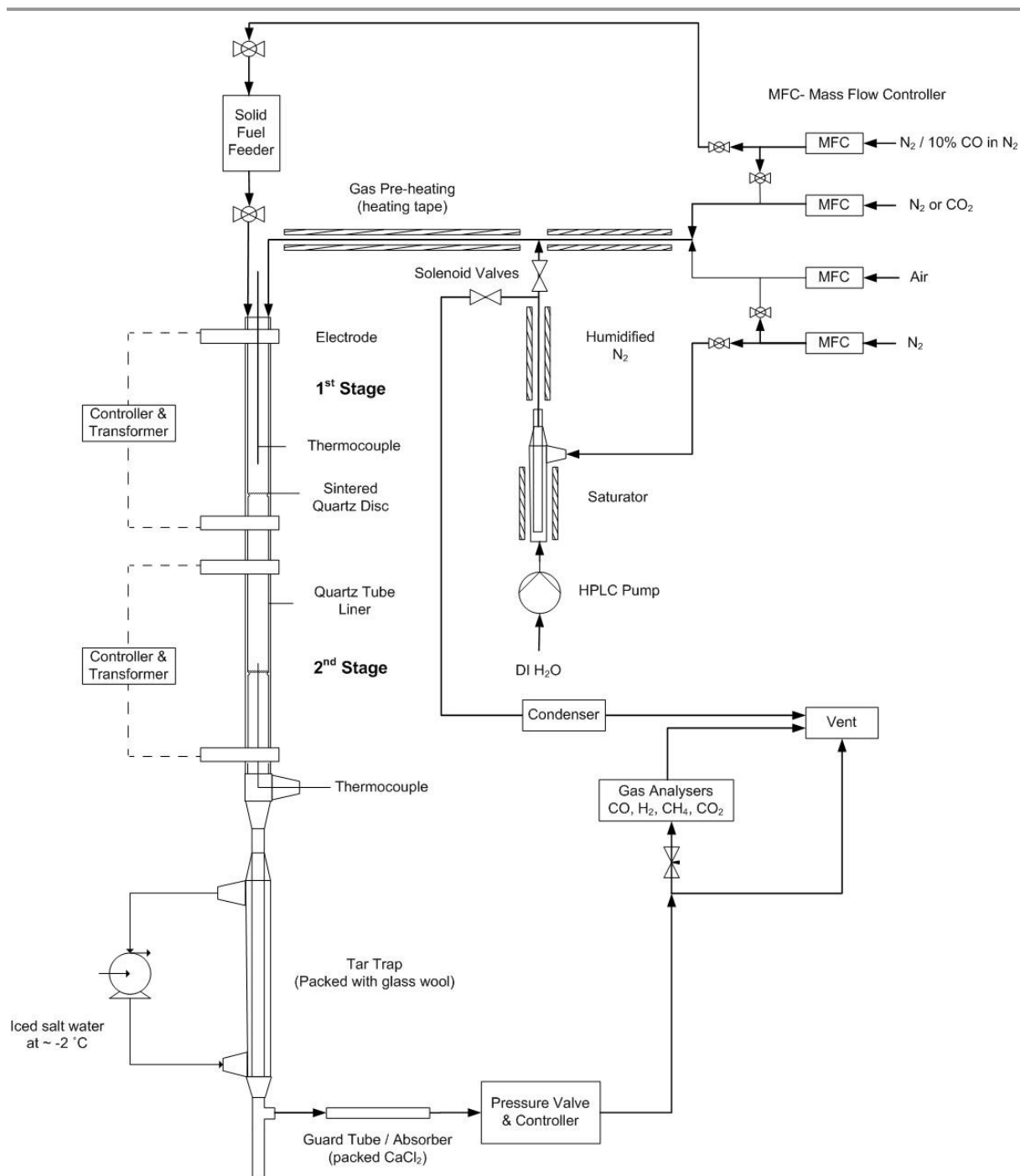


Figure S0-7. Schematic of experimental set-up for fast pyrolysis experiments using a single-stage fixed-bed reactor; shown here in two-stage configuration though the second stage was not used during experiments.

Lignin mass balance

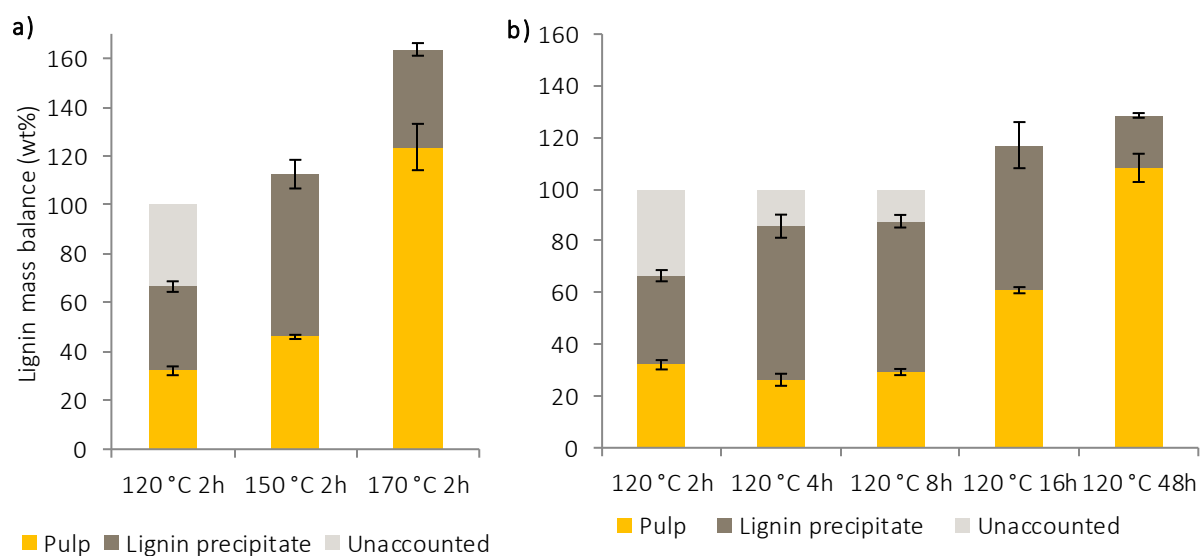


Figure S0-8. Mass balance for lignin (relative to original lignin content in native sugarcane bagasse). Depithed bagasse was pretreated at a biomass to solvent ratio of 1:5 g/g in 80% [TEA][HSO₄] with a final water content of 20 wt% at a) varying pretreatment times at 120 °C; and b) varying temperatures for 2 h treatment time.

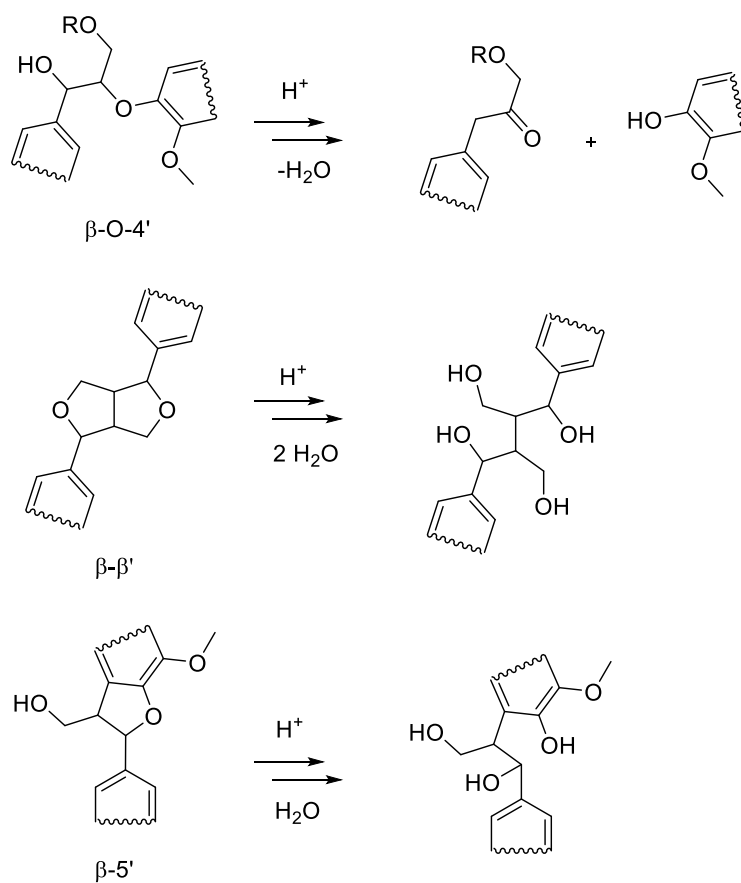


Figure S0-9. Cleavage of ether bonds in lignin under acidic conditions. Reproduced from Gschwend, 2017⁸⁹.

Thermogravimetric analysis

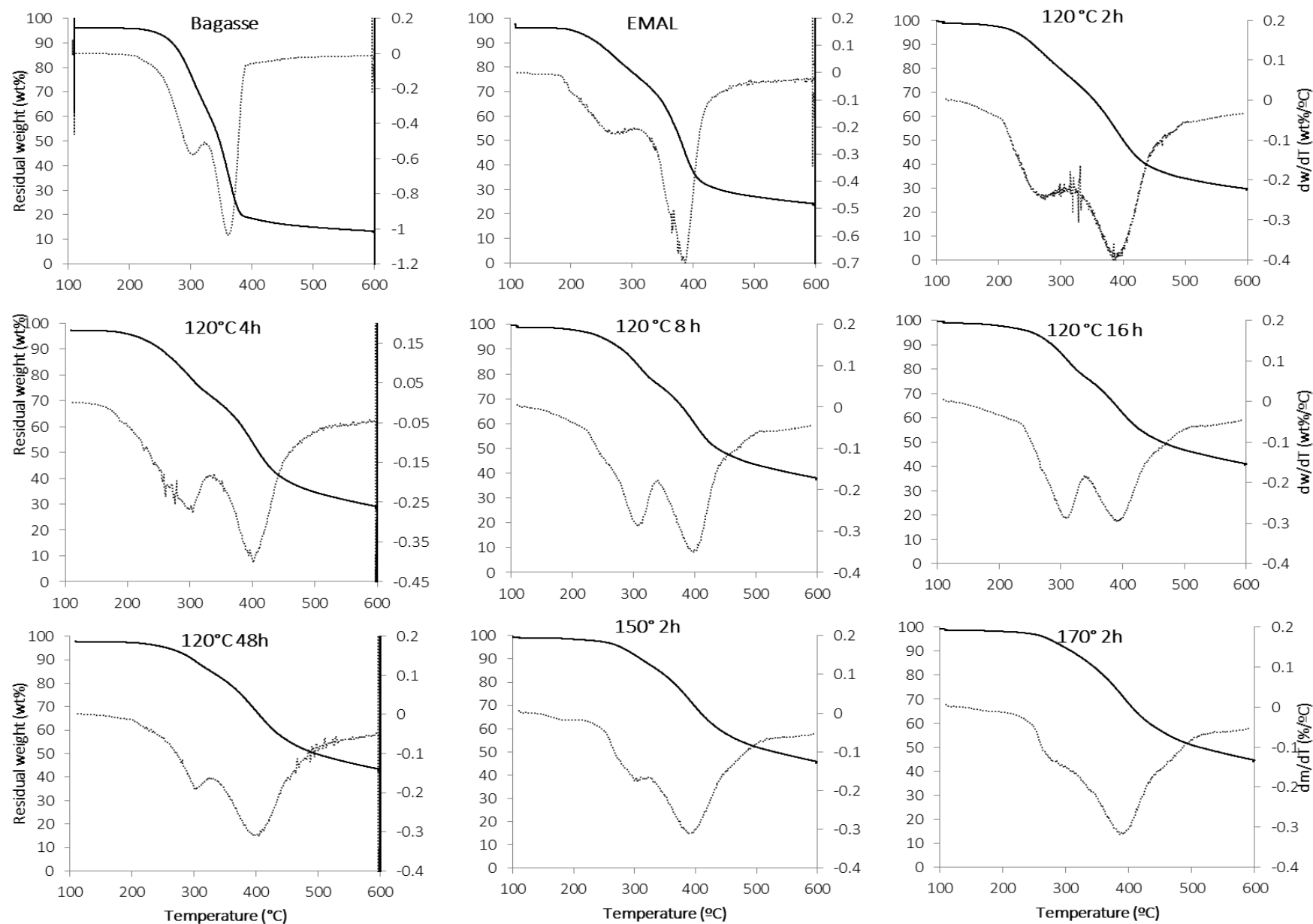


Figure S0-10. Thermogravimetric (TGA) and differential thermogravimetric (DTG) curves of bagasse, EMAL and IonoSolv lignins.

Differential scanning calorimetry

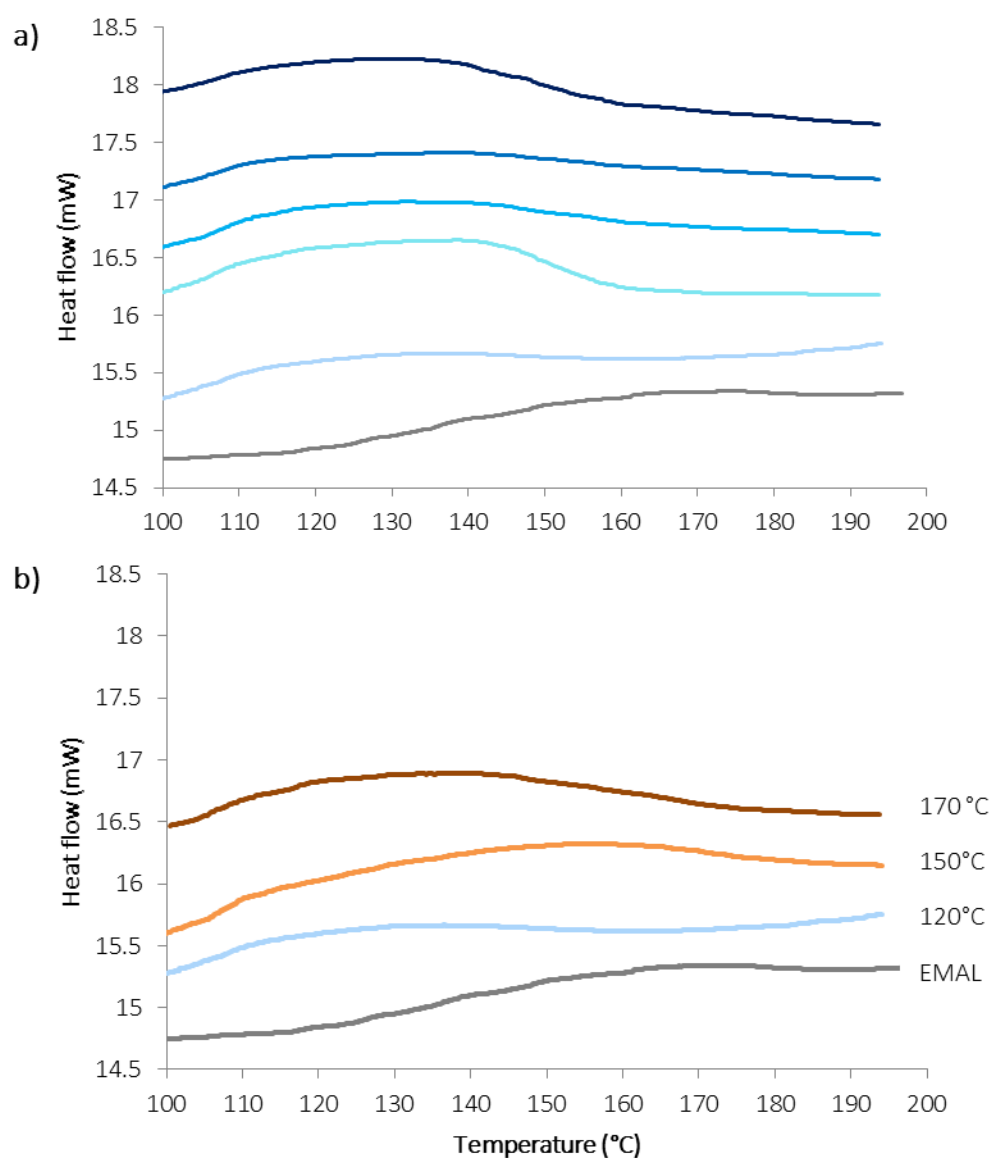


Figure S0-11. DSC curves for EMAL and lonoSolv lignins. Depithed bagasse was pretreated at a biomass to solvent ratio of 1:5 g/g in 80% [TEA][HSO₄] with a final water content of 20 wt% at a) varying pretreatment times at 120 °C; and b) varying temperatures for 2 h treatment time.

Glass transition vs lignin properties

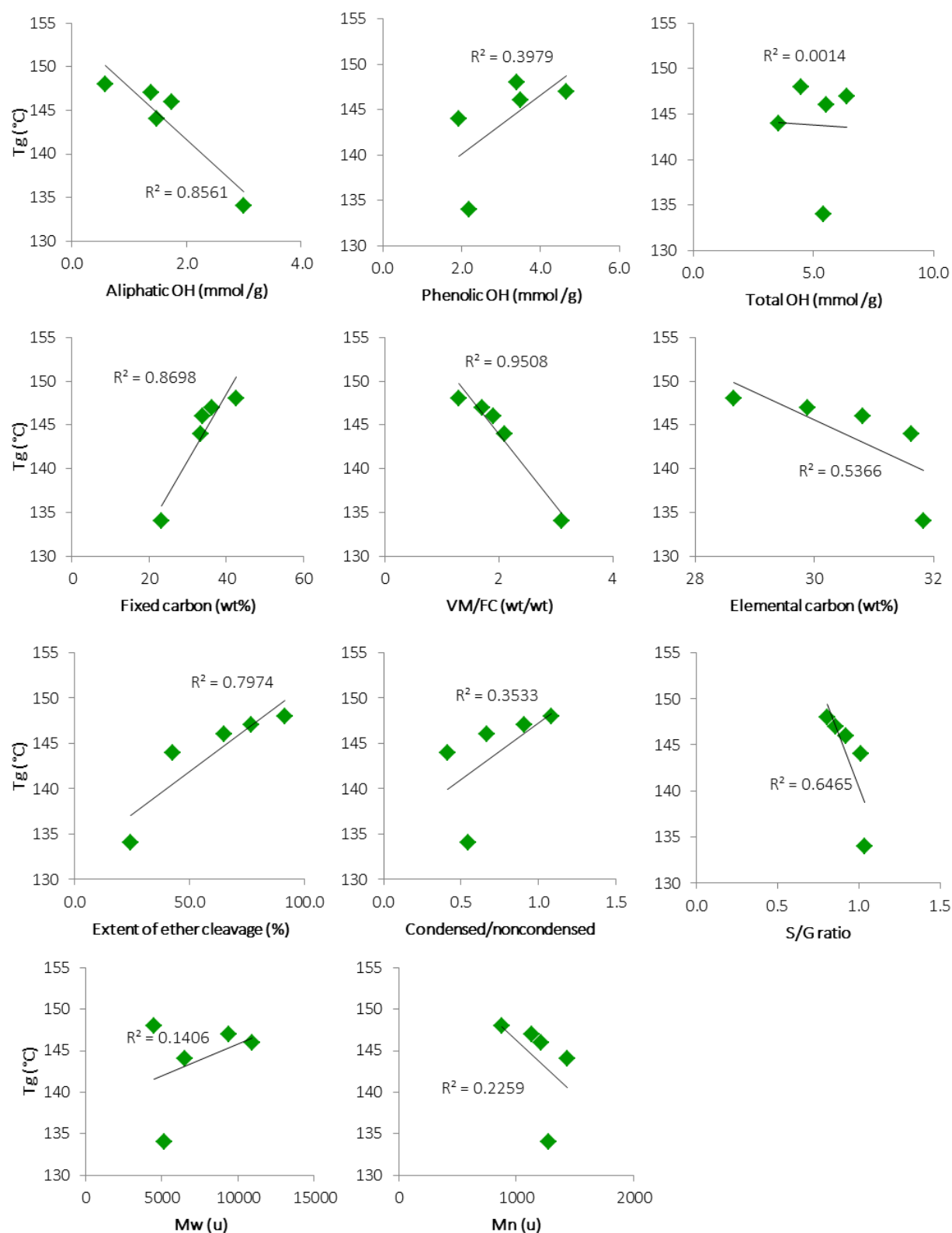


Figure S0-12. The correlation between lignin chemical properties observed through, quantitative ^{31}P NMR spectroscopy (aliphatic, phenolic and total hydroxyl group content), thermogravimetric analysis (fixed carbon, volatile matter to fixed carbon ratio), elemental analysis (elemental carbon content), HSQC NMR spectroscopy (extent of ether bond cleavage, condensed/non-condensed ratio and S/G ratio), GPC (M_w and M_n) and T_g .

Bio-oil Size Exclusion Chromatography

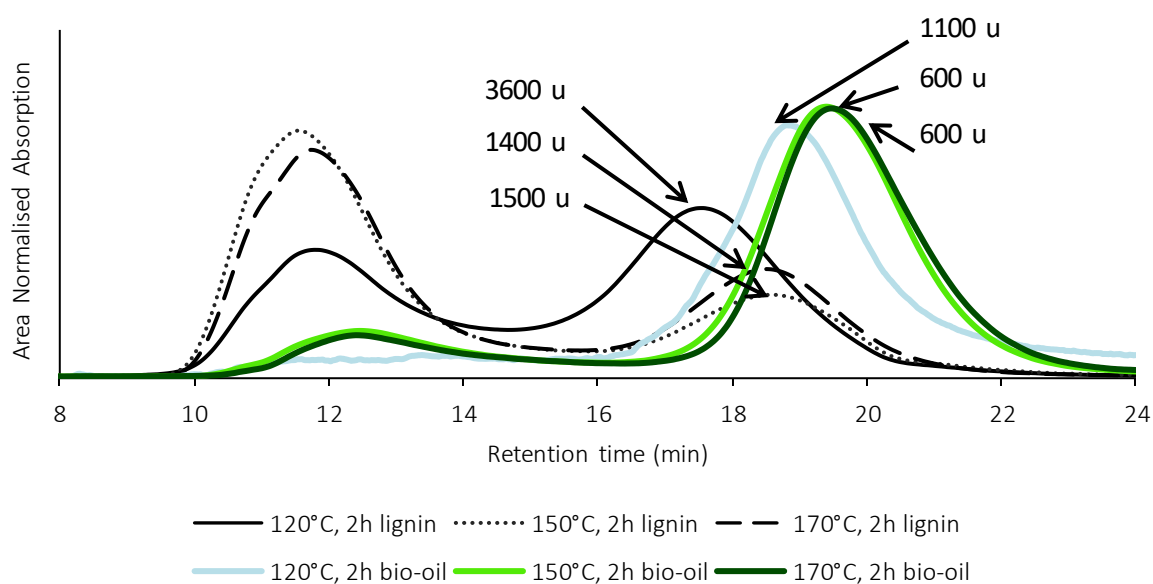


Figure S0-13. Comparison of the area-normalized SEC profiles of lonoSolv lignins with the respective bio-oils. Depithed bagasse was pretreated at a biomass to solvent ratio of 1:5 g/g in 80% [TEA][HSO₄] with a final water content of 20 wt% at varying temperatures for 2 h treatment time.

Table S0-6. Key parameters obtained from size exclusion chromatography of bagasse, EMAL and lonoSolv lignins bio-oils. Depithed bagasse was pretreated at a biomass to solvent ratio of 1:5 g/g in 80% [TEA][HSO₄] with a final water content of 20 wt% at varying temperatures for 2 h treatment time.

Sample	Excluded peak t (min)	Retained peak t (min)	Retained peak MW (Da)	Excluded:Retained peak ratio (-)
Bagasse	12.06	19.33	669	0.24
EMAL	11.57	18.93	970	0.06
120 °C 2 h	12.15	18.85	1048	0.10
120 °C 4 h	13.27	19.07	857	0.08
120 °C 8 h	11.83	19.12	818	0.16
120 °C 16 h	11.22	19.38	638	0.37
120 °C 48 h	11.37	18.45	1520	0.17
150 °C 2 h	12.47	19.40	628	0.20
170 °C 2 h	12.42	19.48	582	0.17

Elemental analysis of feedstocks, bio-oils and char

Table S0-7. Elemental analyses, atomic ratios and HHV values of biomass and lignins and the resulting bio-oils and chars produced by fast pyrolysis at 500 °C.

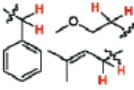
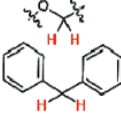
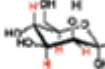
Sample	Ultimate analysis (wt% daf)					Atomic ratio		HHV ^b
All materials	C	H	N	S	O ^a	H/C	O/C	(MJ kg ⁻¹)
Bagasse	46.4	6.1	<0.10	0.2	47.5	2.10	0.77	16.9
Bagasse oil	51.2	6.9	0.3	nd	41.6	2.15	0.61	20.5
Bagasse char	52.2	3.1	0	nd	44.7	0.96	0.64	15.0
EMAL	61.9	6.5	0	0	31.7	1.67	0.38	25.0
EMAL oil	63.2	7.1	0.0	nd	29.7	1.80	0.35	26.7
EMAL char	-	-	-	-	-	-	-	-
120 °C, 2h lignin	61.2	5.8	0.5	0.7	32.6	1.51	0.40	23.7
120 °C, 2h oil	67.0	8.0	0.9	nd	24.0	1.92	0.27	30.2
120 °C, 2h char	54.6	2.6	0.7	nd	42.1	0.76	0.58	15.5
120 °C, 4h lignin	61.2	5.7	0.6	0.9	32.5	1.48	0.40	23.6
120 °C, 4h oil	62.0	7.4	0	nd	30.6	1.92	0.37	26.6
120 °C, 4h char	68.7	3.2	0	nd	28.1	0.75	0.31	23.2
120 °C, 8h lignin	60.5	6.0	1.0	1.7	32.6	1.57	0.40	23.7
120 °C, 8h oil	62.1	7.0	1.4	nd	29.5	1.80	0.36	26.2
120 °C, 8h char	61.9	3.4	0	nd	34.8	0.87	0.42	20.1
120 °C, 16h lignin	61.5	5.9	1.0	1.8	31.7	1.52	0.39	24.0
120 °C, 16h oil	62.8	7.6	2.2	nd	27.4	1.93	0.33	27.6
120 °C, 16h char	67.0	3.3	0.5	nd	29.1	0.79	0.33	22.7
120 °C, 48h lignin	64.5	5.5	0.6	0.8	29.5	1.35	0.34	24.9
120 °C, 48h oil	64.9	7.1	0	nd	28.0	1.75	0.32	27.5
120 °C, 48h char	78.9	3.5	0	nd	17.6	0.71	0.17	28.7
150 °C, 2h lignin	63.6	5.3	0.3	0.5	30.8	1.34	0.36	24.1
150 °C, 2h oil	65.1	7.4	0.7	nd	26.8	1.81	0.31	28.1
150 °C, 2h char	77.3	3.4	0.3	nd	18.9	0.71	0.18	27.9
170 °C, 2h lignin	65.4	5.3	0.4	0.6	29.0	1.29	0.33	24.9
170 °C, 2h oil	61.1	7.6	0.3	nd	31.1	1.98	0.38	26.4
170 °C, 2h char	74.0	3.8	0	nd	22.3	0.82	0.23	26.7

^a Calculated by difference (O = 100 – C – H – N – S wt%).

^b Higher heating value, calculated from the elemental composition according to Demirbas¹¹².

¹H NMR spectroscopy of bio-oils

Table S0-8. Spectral assignment and hydrogen type distribution from bio-oils from ¹H NMR spectra with different feedstocks and lignin pretreatment conditions. Data presented as a % of total protons present (δH 0.5-10.0) after subtracting methoxyl region (δH 3.0-4.5).

Chemical shifts (ppm)	Proton Assignment	Bagasse	EMAL	120 °C 2h	120 °C 4h	120 °C 8h	120 °C 16h	120 °C 48h	150 °C 2h	170 °C 2h
0.5-1.5	 Alkanes	21%	51%	51%	49%	41%	40%	41%	38%	35%
1.5-3.0	 Alcohols, methylene-dibenzene^a	25%	20%	16%	13%	16%	18%	18%	23%	19%
3.0-4.5	 Methoxy, carbohydrate	—	—	—	—	—	—	—	—	—
4.5-6.0	 Aromatic protons	24%	2%	4%	4%	7%	5%	3%	1%	4%
6.0-8.5	 Aldehydes	25%	26%	24%	27%	29%	29%	31%	34%	35%
8.5-10.0	 Aldehydes	4%	1%	4%	7%	7%	7%	7%	5%	8%

^a This region included significant peaks from water which distorted the analysis, so was excluded from the analysis.

Chapter 5

Proximate analysis of lignin fractions from fractional precipitation

Table S0-9. Volatile matter and fixed carbon (i.e. char residue) on a dry ash-free basis for lignin fractions isolated from *Miscanthus*, willow and pine using [DMBA][HSO₄] with molar acid:base ratios of 1:1 and excess acid with 1.03:1.

	Miscanthus 1:1		Miscanthus Excess Acid		Willow 1:1		Pine 1:1	
	VM	FC	VM	FC	VM	FC	VM	FC
0.25 equivalents	-	-	46.5	53.5	-	-	-	-
0.5 equivalents	54.7	45.3	50.8	49.2	-	-	57.9	42.1
1 equivalent	56.0	44.0	53.2	46.3	57.4	42.6	57.4	42.6
1.5 equivalents	55.3	44.7	55.3	44.7	57.9	42.1	57.0	43.8
2 equivalents	55.0	45.0	56.2	43.8	58.8	41.2	59.5	40.5
2.5 equivalents	57.7	42.3	59.0	42.0	59.4	40.6	56.9	43.1
5 equivalents	58.1	41.9	58.3	41.8	61.5	38.5	59.6	40.4
Full precipitation	55.4	44.6	51.9	48.1	53.4	46.6	57.3	42.7

Raw Spectra

2D HSQC NMR spectroscopy of lignins

Method description

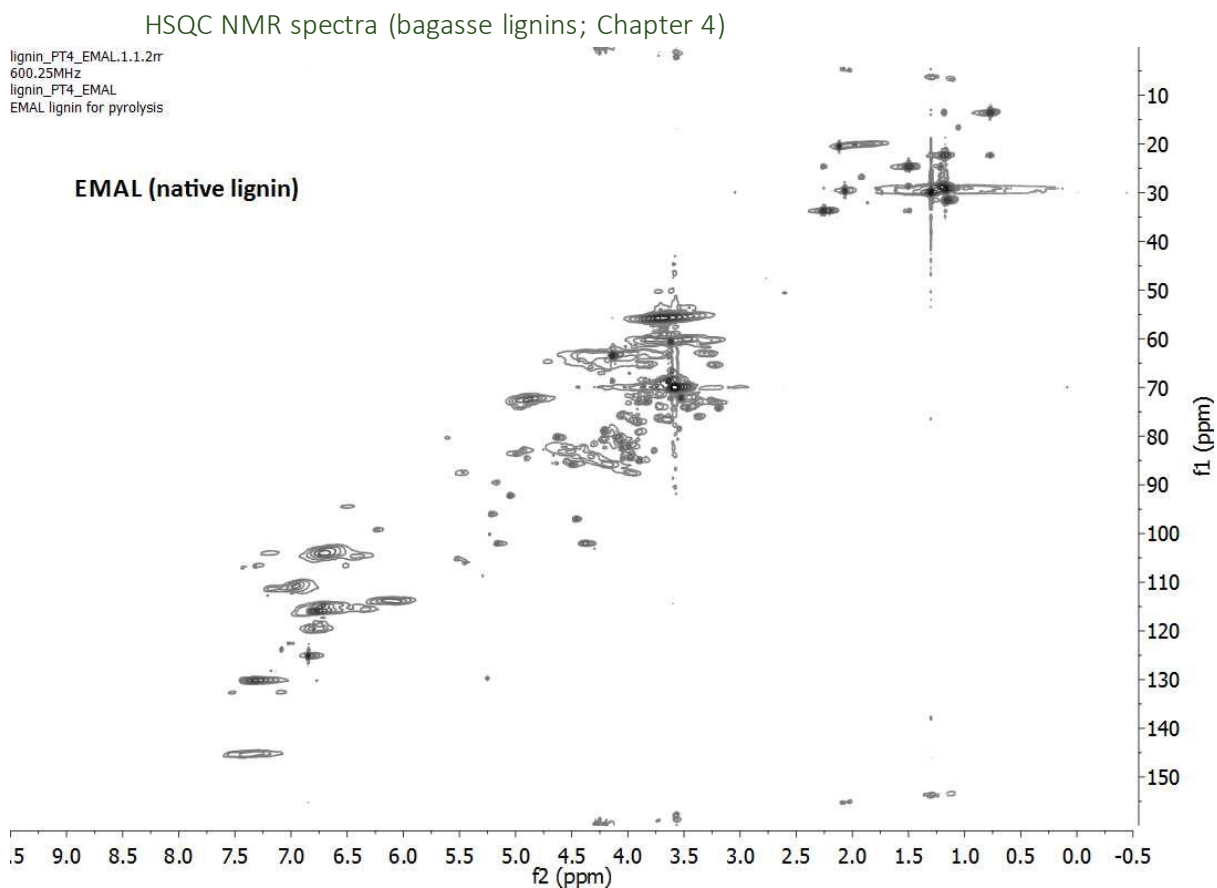
Obtaining fully quantitative data is hindered by the rather long relaxation time of the ¹³C nucleus, making these experiments extremely time-consuming. Instead, semi-quantitative data were obtained using shortened experiments by comparing the peak volume of a certain correlation with respect to another correlation that is considered constant over a range of experiments. Literature reports state that the volume of the G₂ signal on the guaiacyl (G) sub-unit can be used as such a reference in the case of softwood lignin, which consists predominantly of G units and condensation in the 2-position is highly unlikely¹³⁶. Grass lignin, such as from sugarcane bagasse, contains all three units, G, S and H, as well as some PCA and FA⁹⁷, resulting in some signal overlap which makes it difficult to have a reliable representation of the total number of phenolic units. However, previous results reported by Gschwend *et al.* have shown that, despite the presence of other phenolic moieties, the G₂ + G_{2,cond} integral is very stable over a 23 h time period for *Miscanthus* lignin^{89,121}. In the experiments presented here, the abundance of different sub-units and linkages in sugarcane bagasse lignin are reported by using volume integration with reference to the G₂ + G_{2,cond} integral, obtained using a pulse sequence that gives only semi-quantitative information. This was deemed suitable to gain some insight into the

change in lignin subunits. Sub-structural information was obtained by assigning the various signals and estimating the $S_{2,6}/G_2+G_{2\text{cond}}$ ratio according to previous assignments^{137–139}. Full spectra are presented below.

Signal assignment

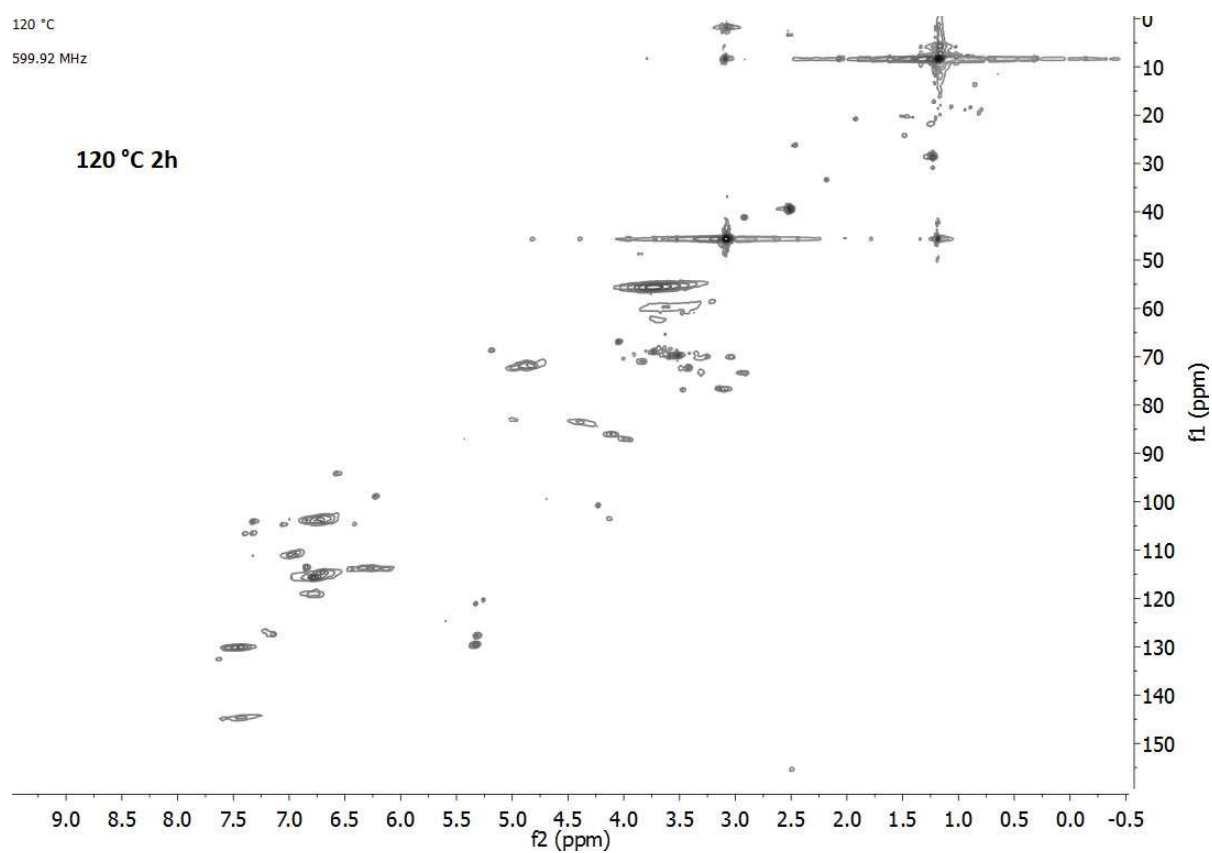
Table S0-10. Signal assignment showing center of integrated area for HSQC NMR spectra of lignins.

Label	Assignment	$\delta\text{H/ppm}$	$\delta\text{C/ppm}$
A_α	$C_\alpha\text{-H}_\alpha$ in $\beta\text{-O-4'}$ linkage (A)	4.91	72.33
B_α	$C_\alpha\text{-H}_\alpha$ in resinol $\beta\text{-5'}$ linkage (B)	4.95	83.21
C_α	$C_\alpha\text{-H}_\alpha$ in phenylcoumaran $\beta\text{-}\beta'$ linkage (C)	5.49	87.81
$G_5/H_{3,5}/PCA_{3,5}$	$C_5\text{-G}_5$ in guaiacyl units (G), $C_3\text{-H}_3$ and $C_5\text{-H}_5$ in <i>p</i> -hydroxyphenyl (H), $C_3\text{-H}_3$ and $C_5\text{-H}_5$ in <i>p</i> -coumarate (PCA)	6.66	115.25
G_6	$C_6\text{-G}_6$ in guaiacyl units (G)	6.76	119.41
G_2	$C_2\text{-G}_2$ in guaiacyl units (G)	7.00	110.99
$G_{2,\text{cond}}$	$C_2\text{-G}_2$ in condensed guaiacyl units (G)	6.76	112.64
$S_{2,6}$	$C_2\text{-H}_2$ and $C_6\text{-H}_6$ in syringyl units (S)	6.80	103.4
$S_{2,6,\text{cond}}$	$C_2\text{-H}_2$ and $C_6\text{-H}_6$ in condensed syringyl units (S)	6.33	106.82
$H_{2,6}$	$C_2\text{-H}_2$ and $C_6\text{-H}_6$ in <i>p</i> -hydroxyphenyl units (H)	6.91	128.54
$PCA_{2,6}$	$C_2\text{-H}_2$ and $C_6\text{-H}_6$ in <i>p</i> -coumarate (PCA)	7.49	130.69



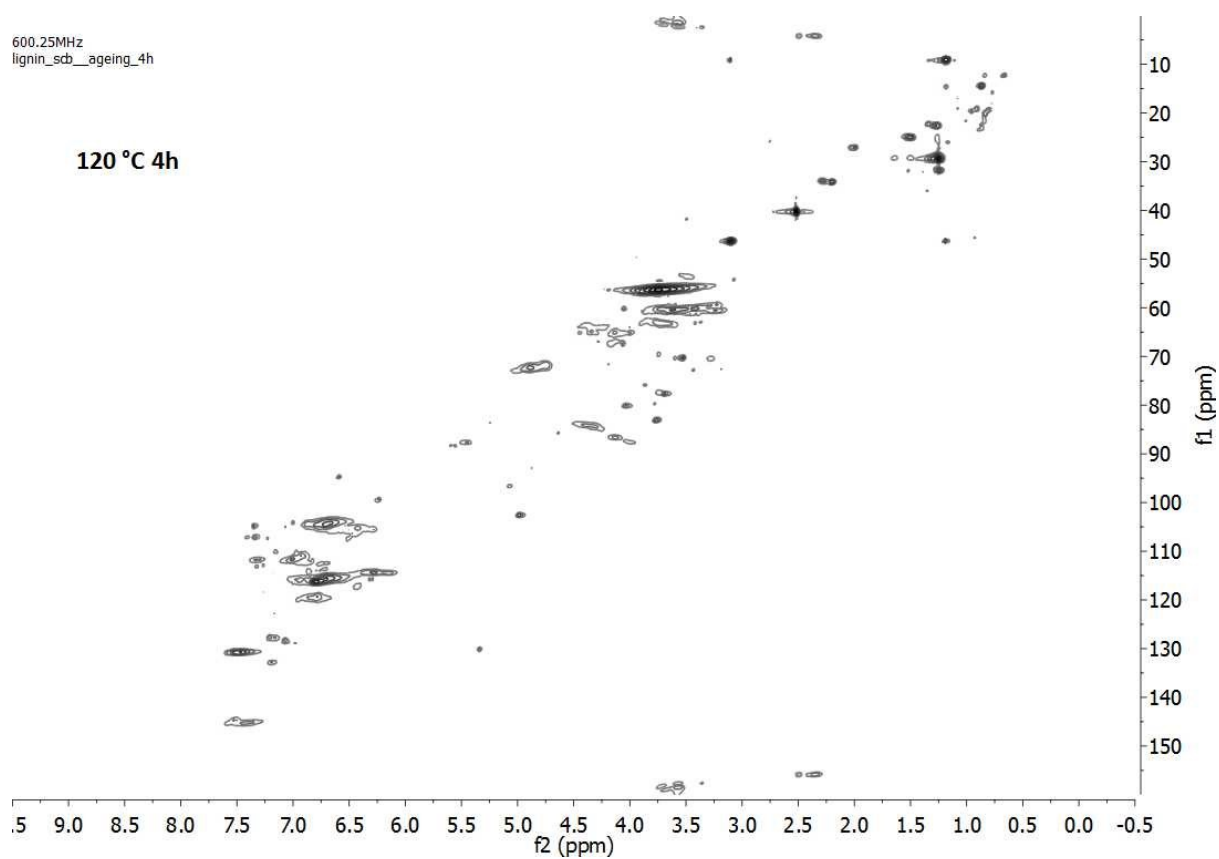
120 °C
599.92 MHz

120 °C 2h



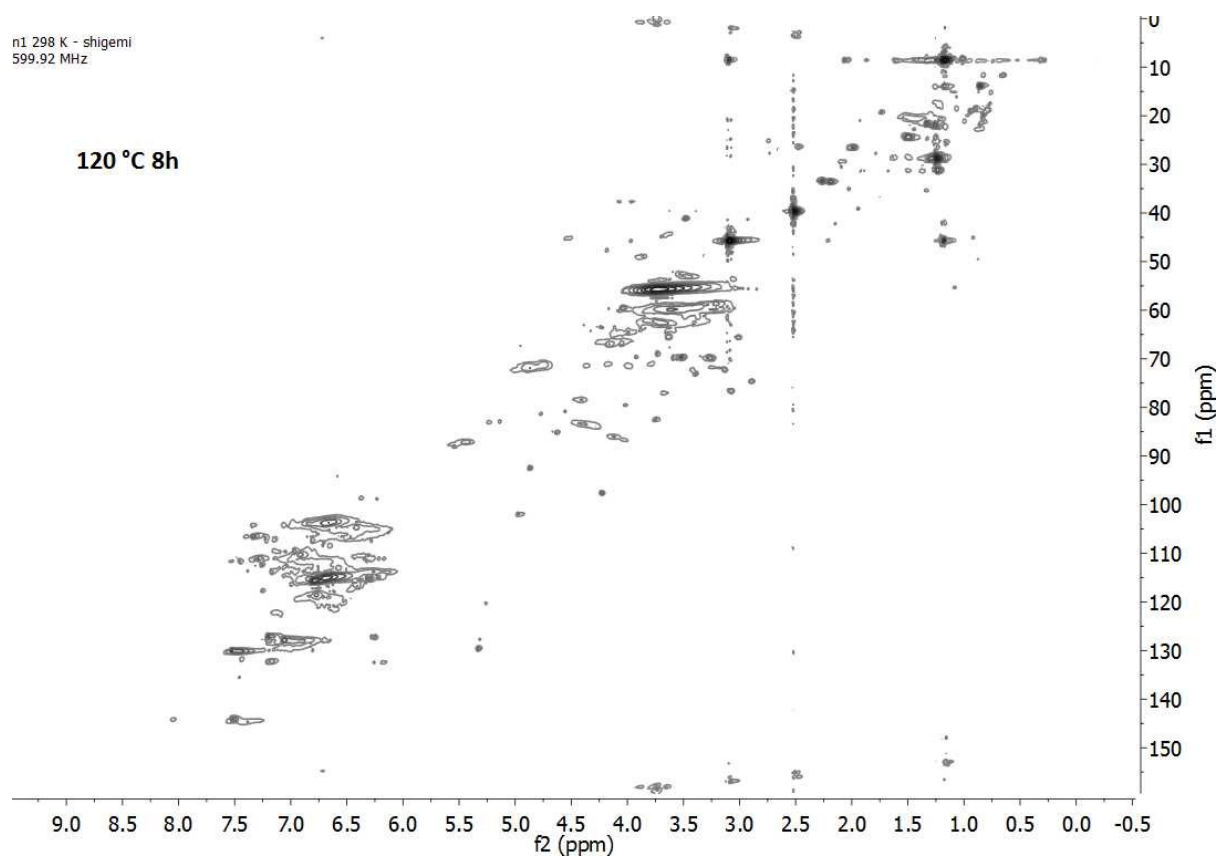
600.25MHz
lignin_scb_ageing_4h

120 °C 4h



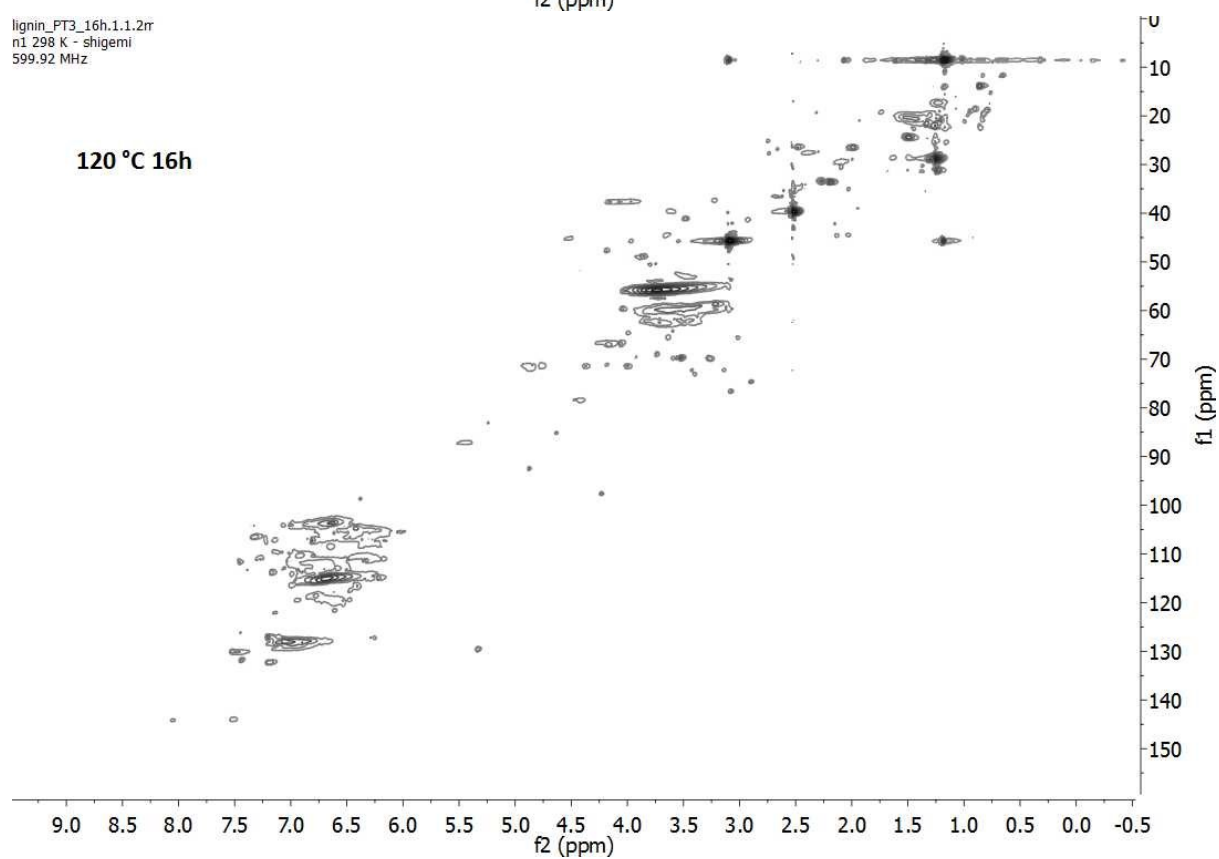
n1 298 K - shigemii
599.92 MHz

120 °C 8h



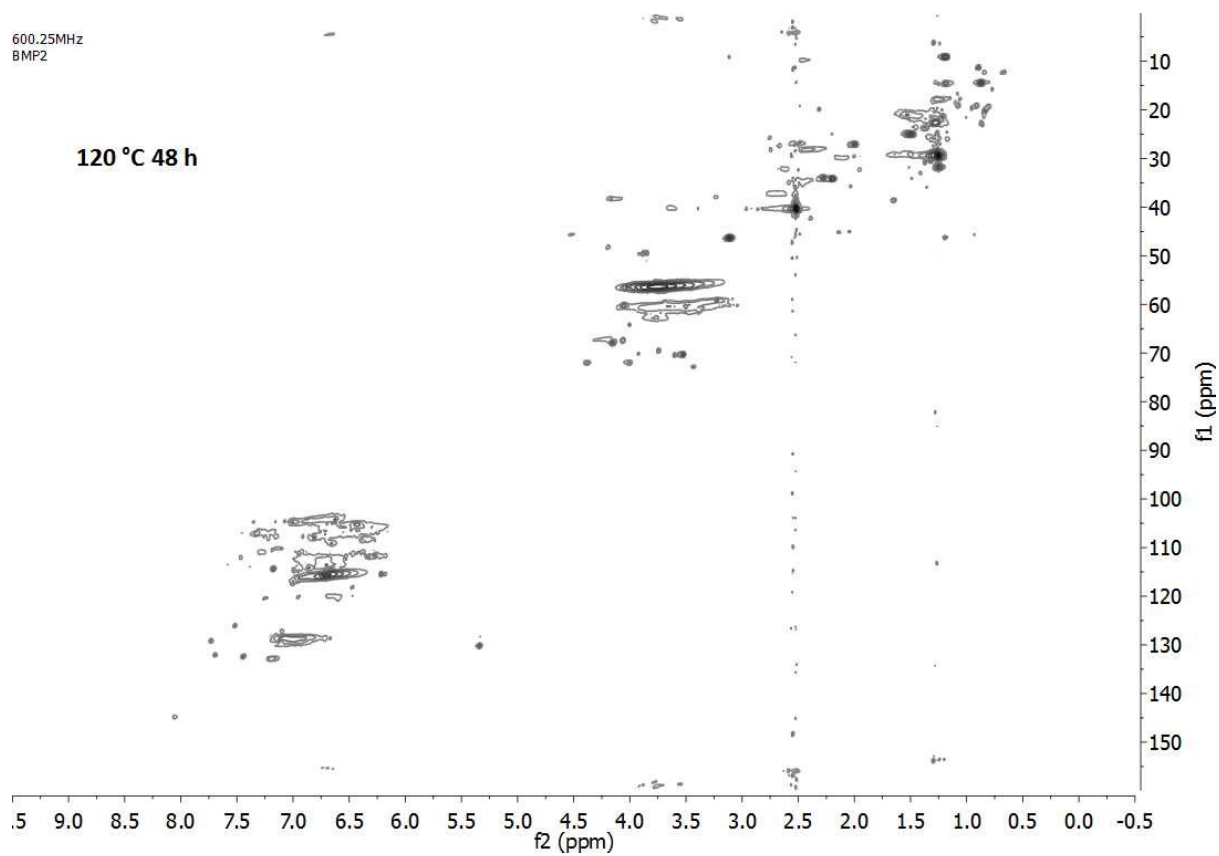
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n1 298 K - shigemii
599.92 MHz

120 °C 16h



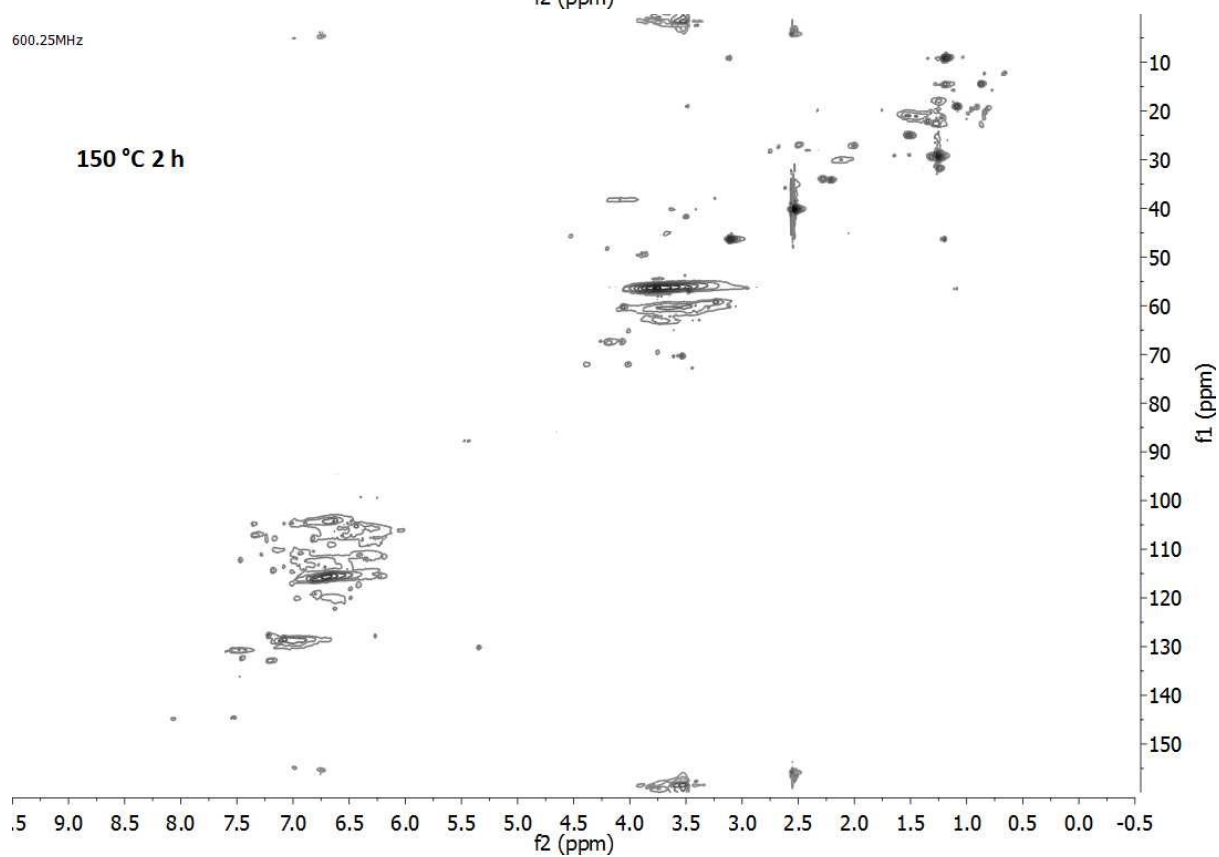
600.25MHz
BMP2

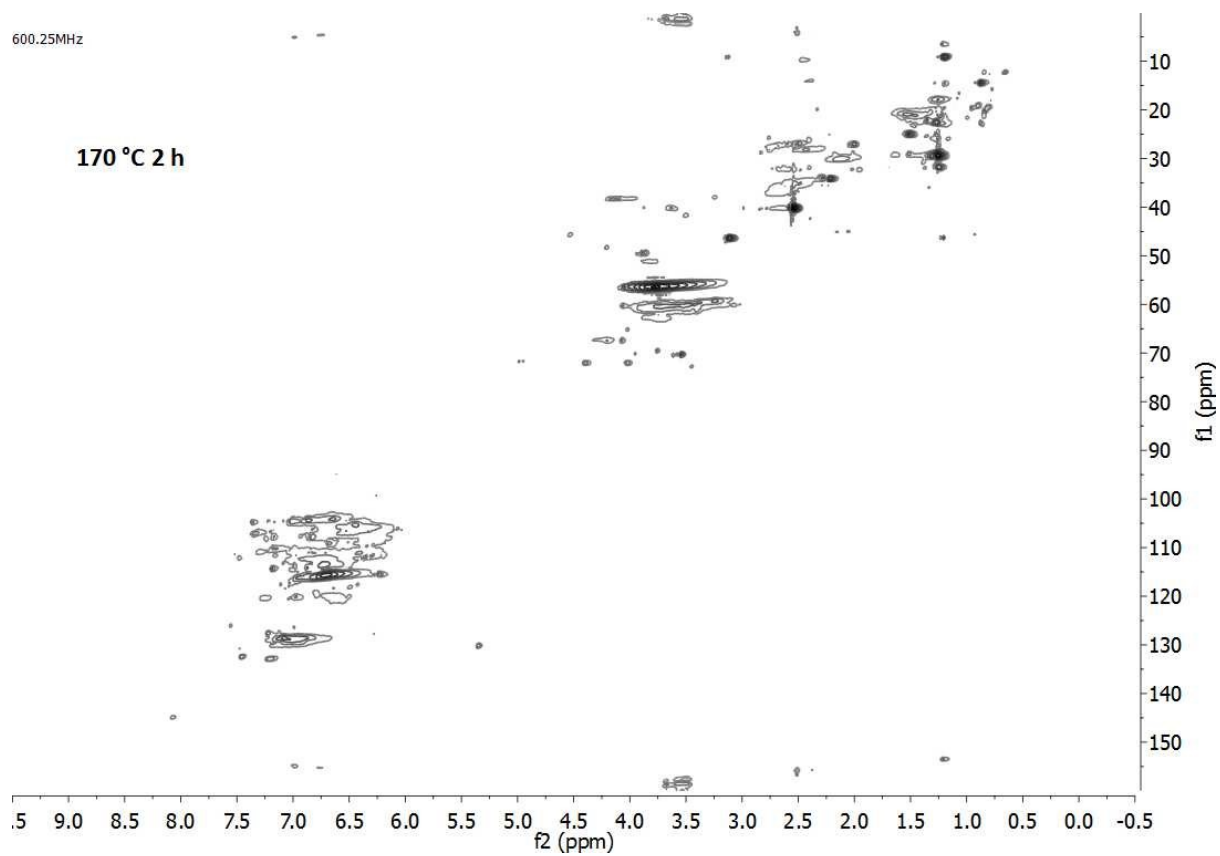
120 °C 48 h



600.25MHz

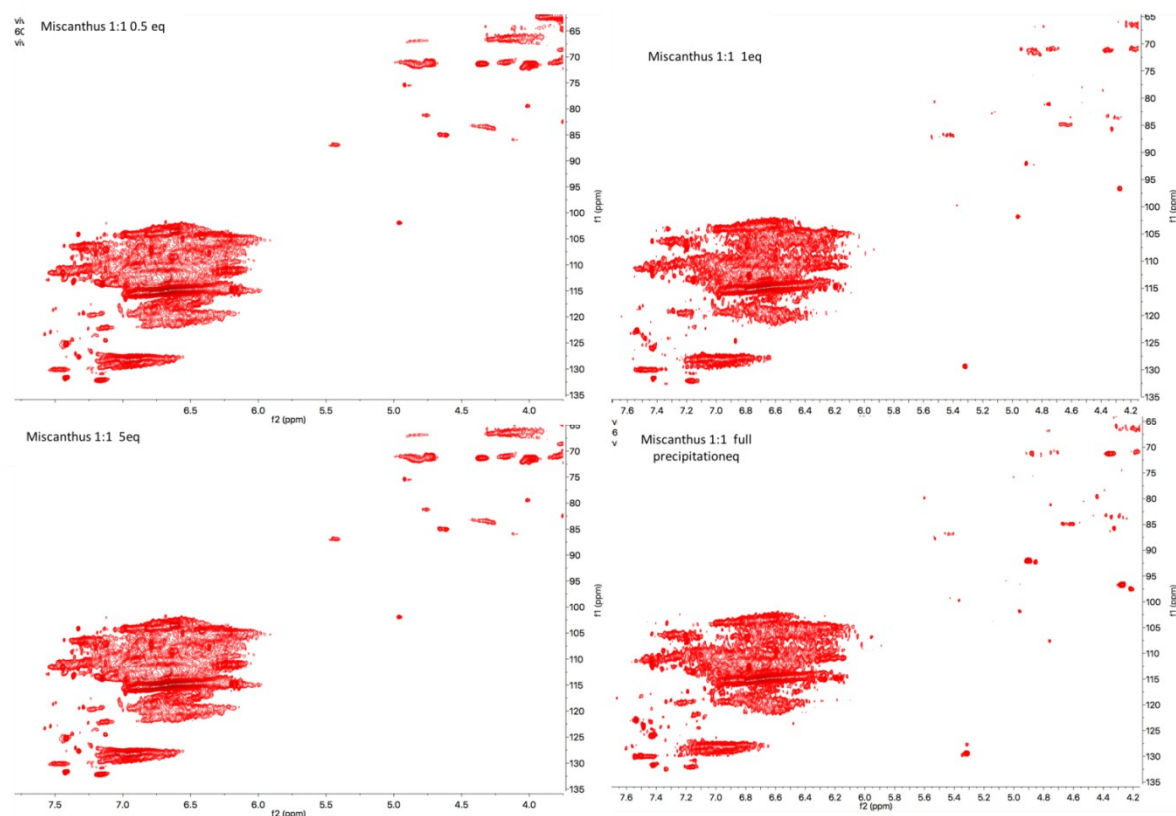
150 °C 2 h



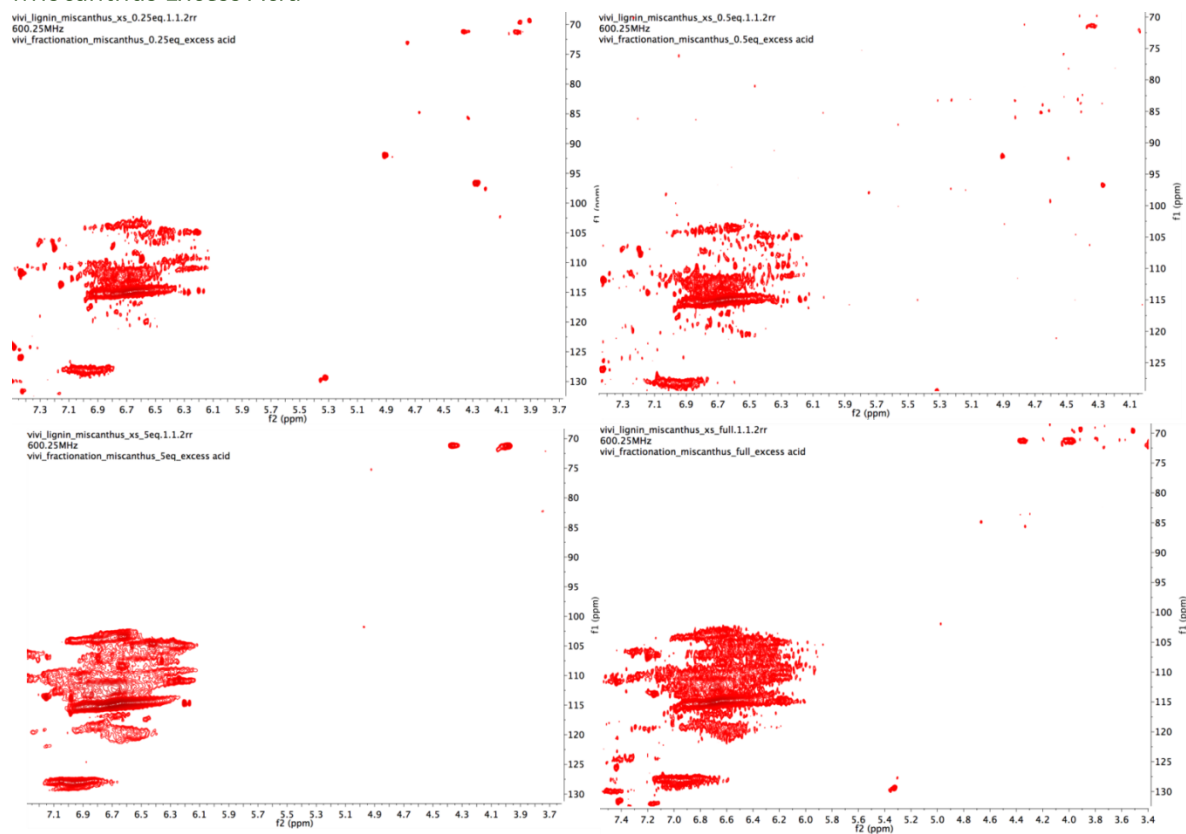


HSQC NMR spectra (Miscanthus, willow and pine lignins; Chapter 5)

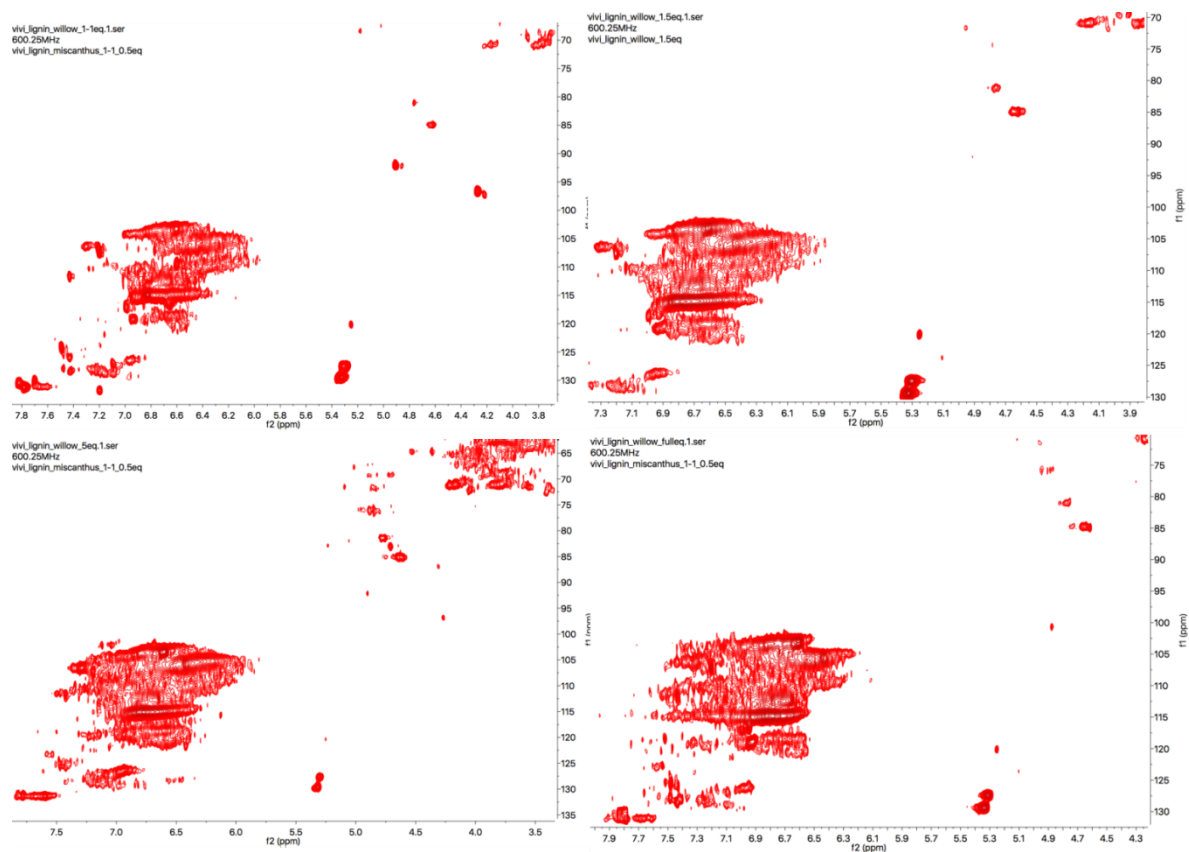
Miscanthus 1:1



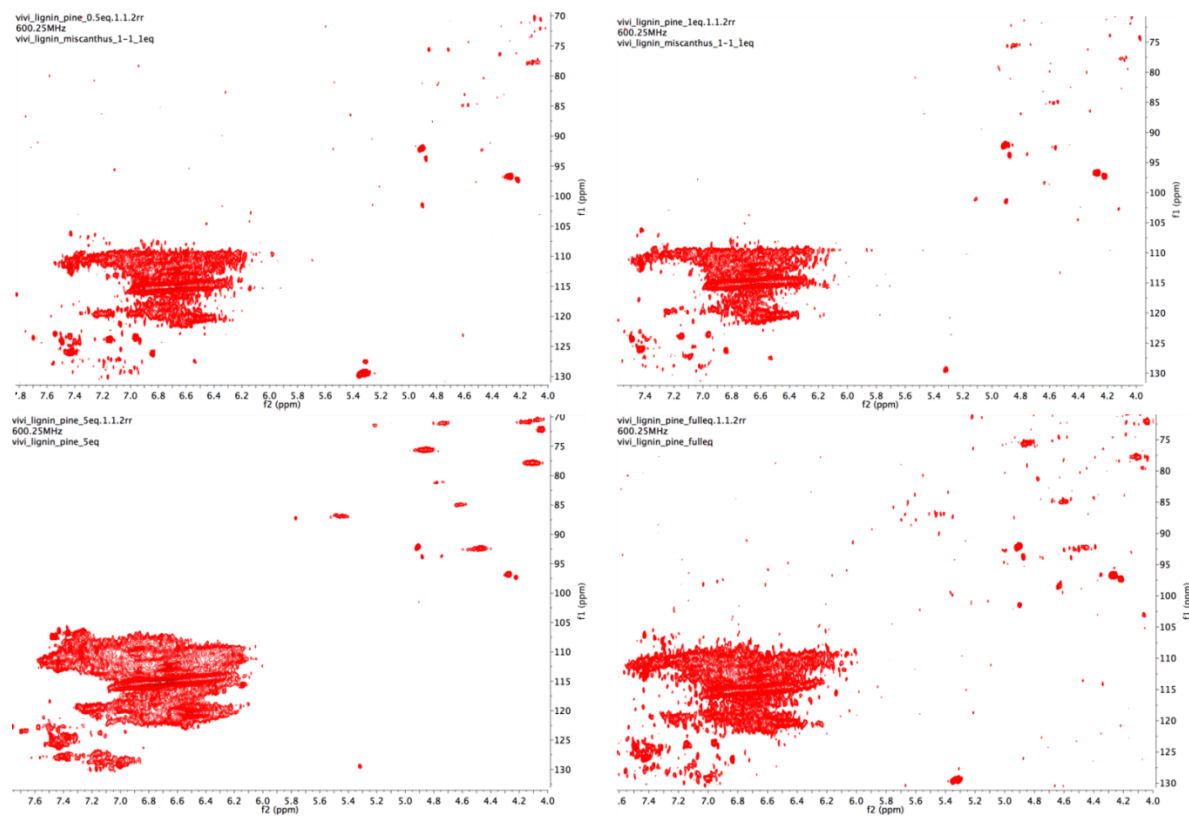
Miscanthus Excess Acid



Willow 1:1



Pine 1:1



Quantitative ^{13}C NMR spectroscopy of lignins

Table S0-11. Signal assignment and relative functional group abundance (per aryl ring) in quantitative ^{13}C spectra and semi-quantitative HSQC spectra of EMAL and selected IonoSolv lignins.

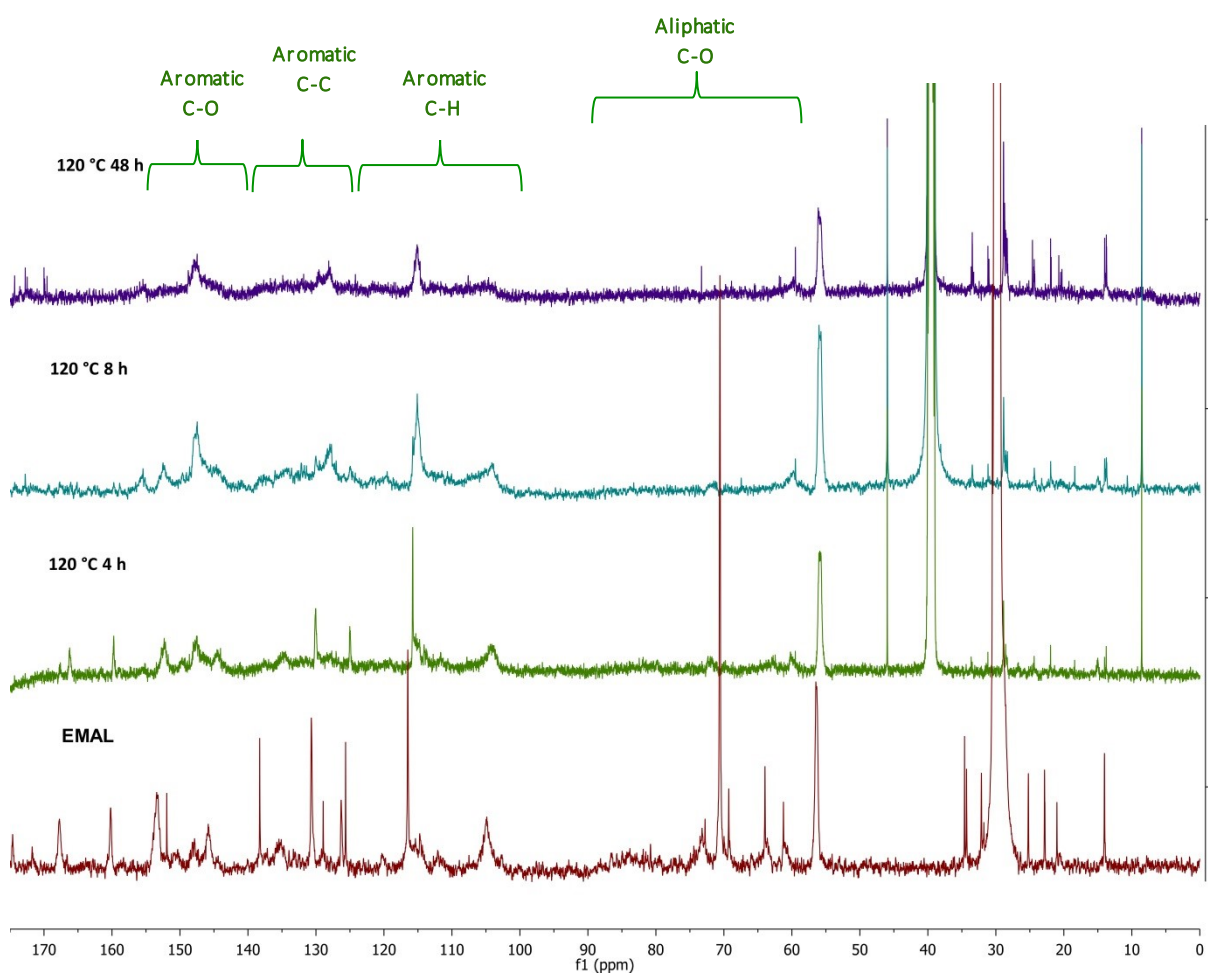
Chemical shift (ppm)	Assignment	Native lignin (EMAL) ^a	120 °C 2h	120 °C 8 h	120 °C 48 h
155 – 142	Aromatic C-O	2.02	1.42	1.51	1.51
142 – 124	Aromatic C-C	1.73	1.98	2.01	2.09
124 – 102	Aromatic C-H	2.23	2.51	2.35	2.30
89 – 58	Aliphatic C-O	4.04	2.12	1.32	0.38
56	OCH ₃	1.60	0.75	0.60	0.80
-	S/G (^{13}C) ^b	0.89	0.79	0.60	0.54
-	S/G (HSQC) ^c	1.08	1.01	0.92	0.81

^a Enzymatic mild acidolysis lignin (EMAL) recovered using a 2-step protocol.

^b Calculated from ^{13}C NMR spectra as $S_{2,6}/G_2$.

^c Calculated from HSQC NMR spectra as $0.5(S_2+S_{2\text{cond}})/(G_2+G_{2\text{cond}})$.

^{13}C NMR spectra (bagasse lignins, Chapter 4)



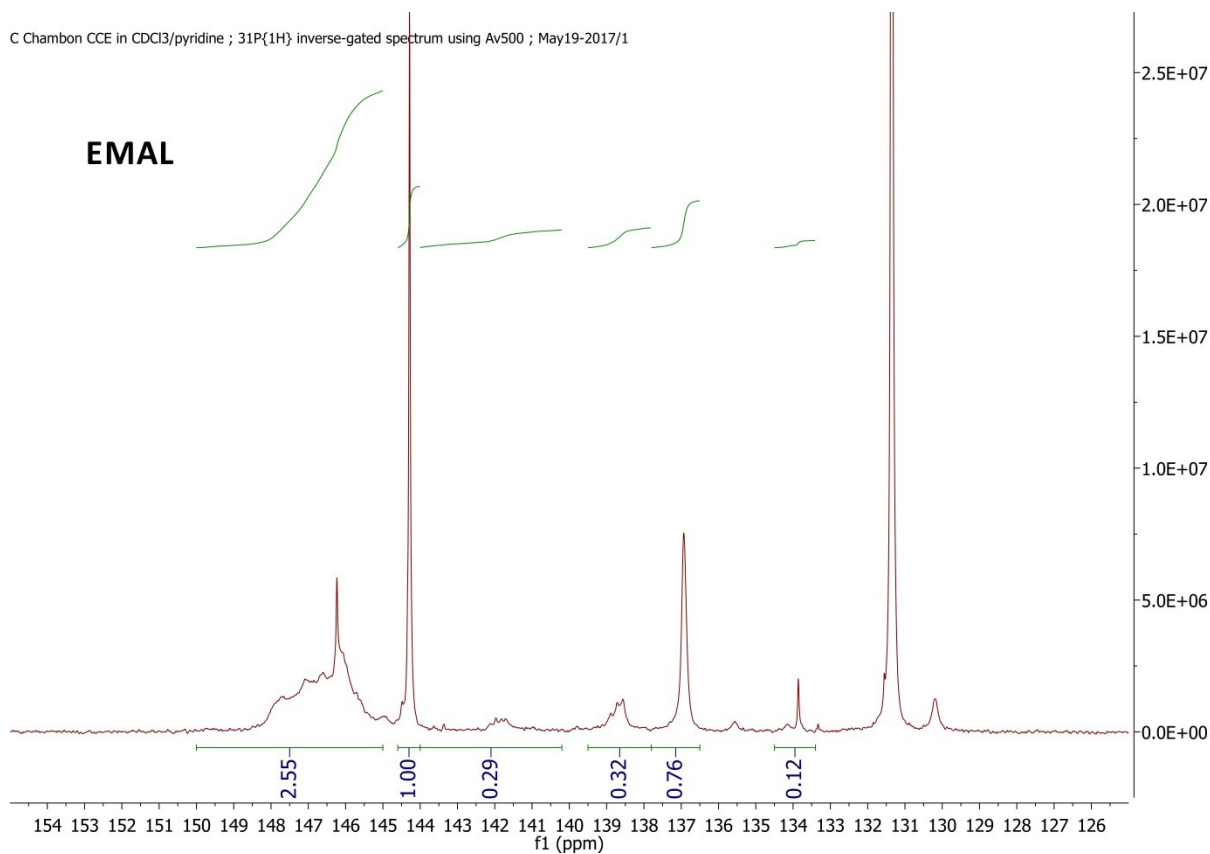
Quantitative ^{31}P NMR spectroscopy of lignins

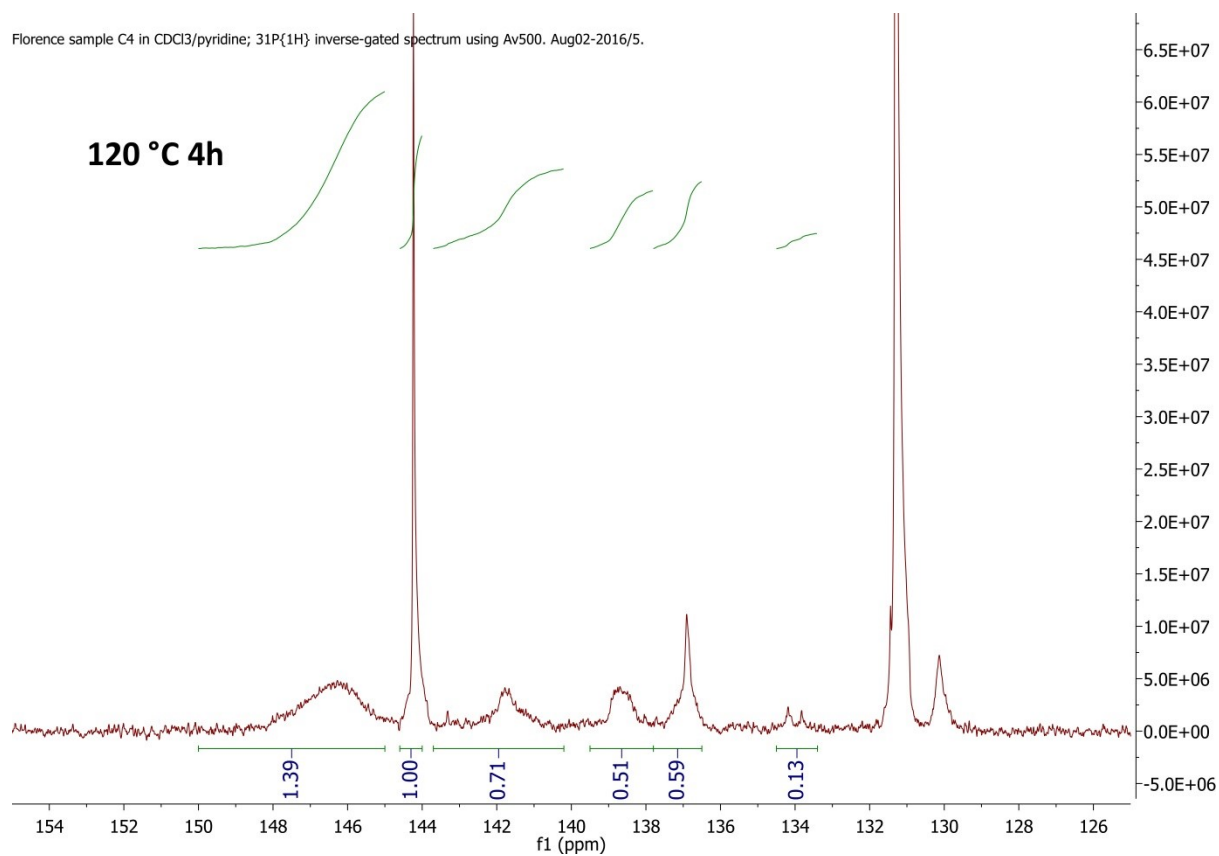
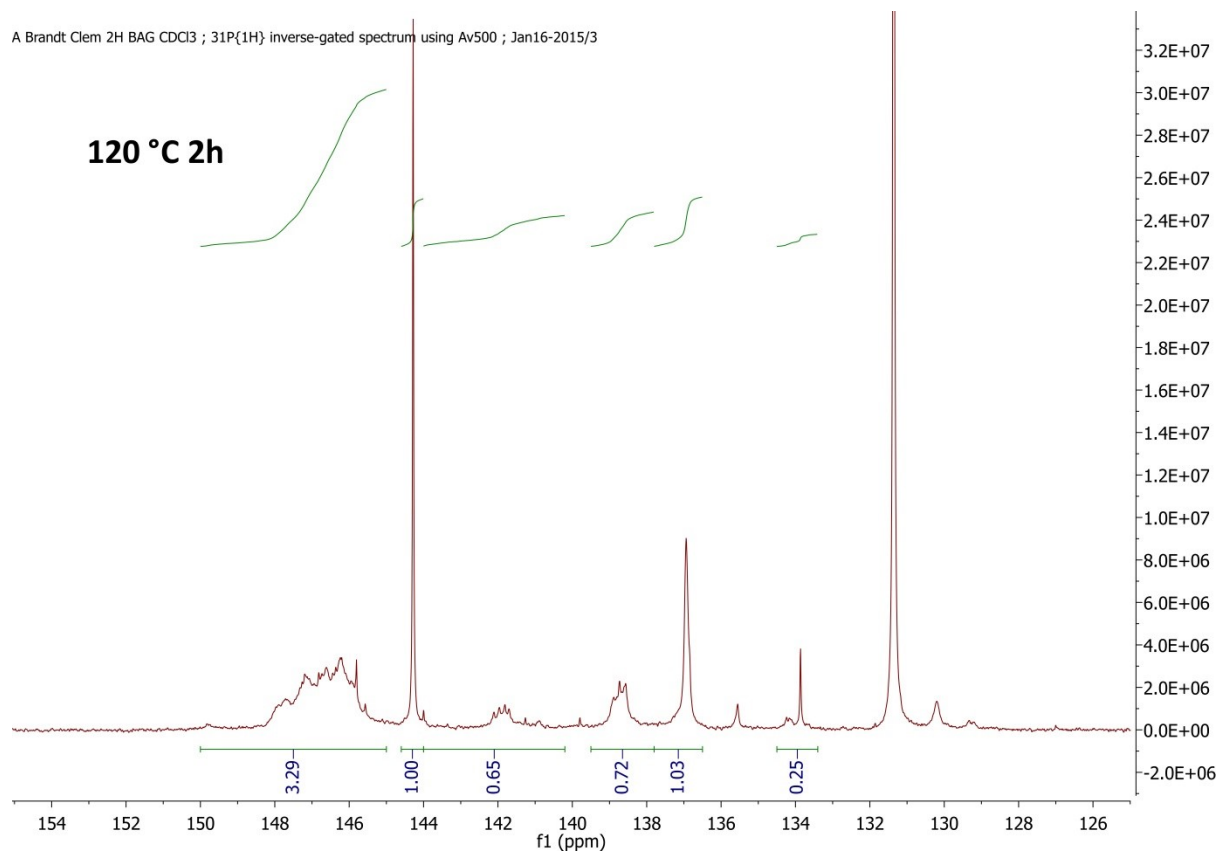
Table S0-12. Signal assignment and relative functional group abundance (mmol OH per g lignin) in quantitative ^{31}P NMR spectra of EMAL and lignins isolated from sugarcane bagasse.

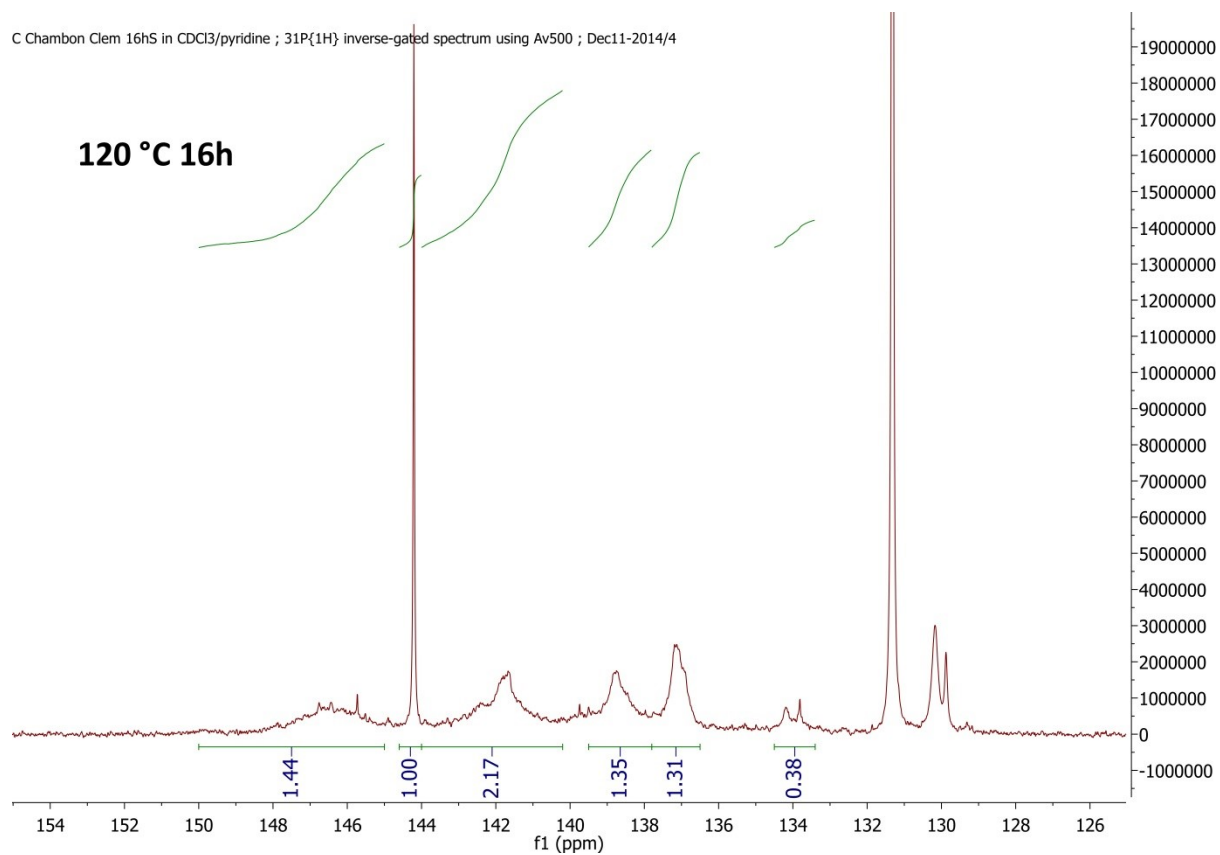
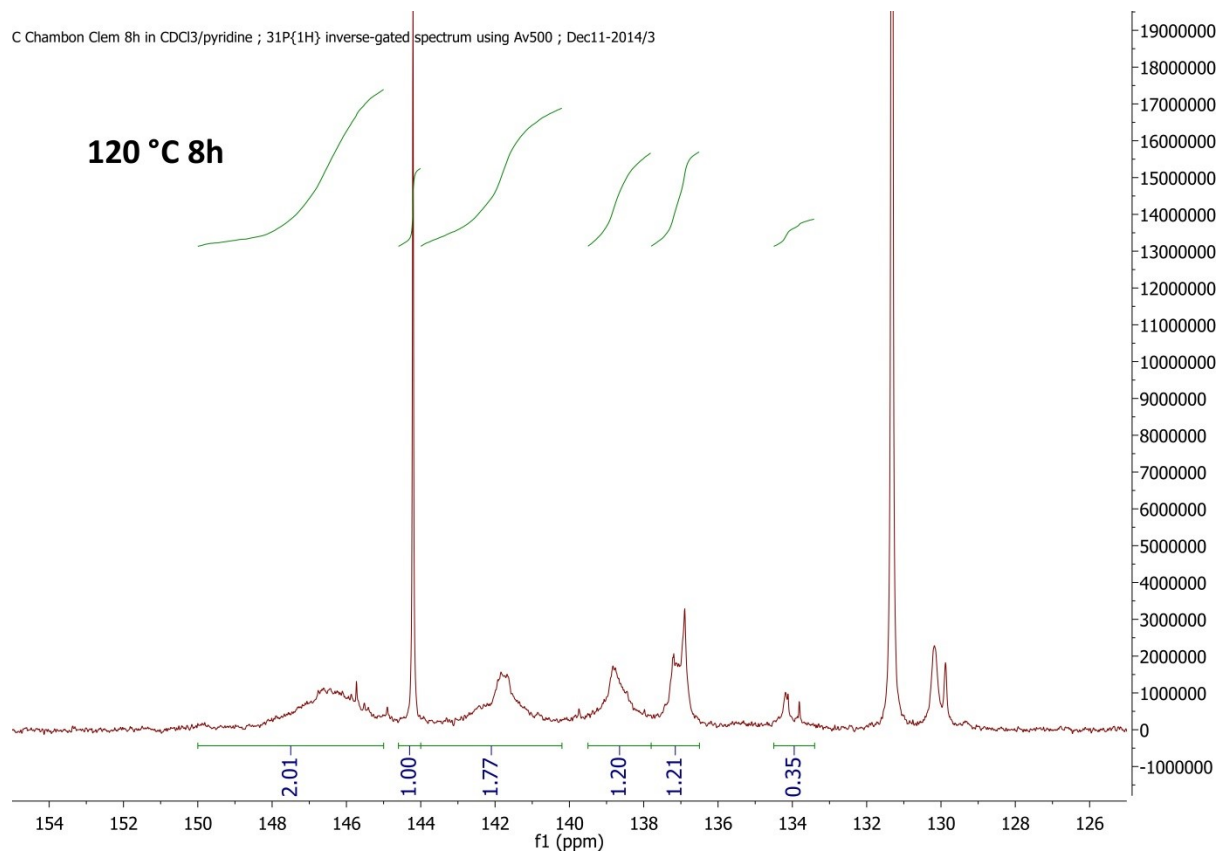
	Aliphatic	C ₅ substituted/ syringyl	Guai- acyl	<i>p</i> - hydroxy- phenyl	Carbox- ylic	Total OH	Total Phenolic	Aliphatic / Phenolic ^a
Chemical shift (ppm)	150.0– 145.0	144.0– 140.2	139.5– 138.0	137.5– 136.5	134.5– 133.4	–	–	–
EMAL	3.0	0.3	0.4	0.9	0.1	4.7	1.6	1.9
120 °C 2h	3.0	0.6	0.7	0.9	0.2	5.4	2.2	1.4
120 °C 4h	1.5	0.7	0.6	0.6	0.1	3.5	1.9	0.8
120 °C 8h	1.7	1.4	1.0	1.0	0.3	5.5	3.5	0.5
120 °C 16h	1.4	2.1	1.3	1.3	0.4	6.4	4.6	0.3
120 °C 48h	0.6	1.5	1.0	0.9	0.5	4.5	3.4	0.2
150 °C 2h	1.0	1.8	1.1	1.1	0.4	5.4	4.0	0.2
170 °C 2h	0.7	2.3	1.4	1.2	0.3	5.9	4.9	0.1

^a Ratio of aliphatic OH groups to sum of phenolic OH groups.

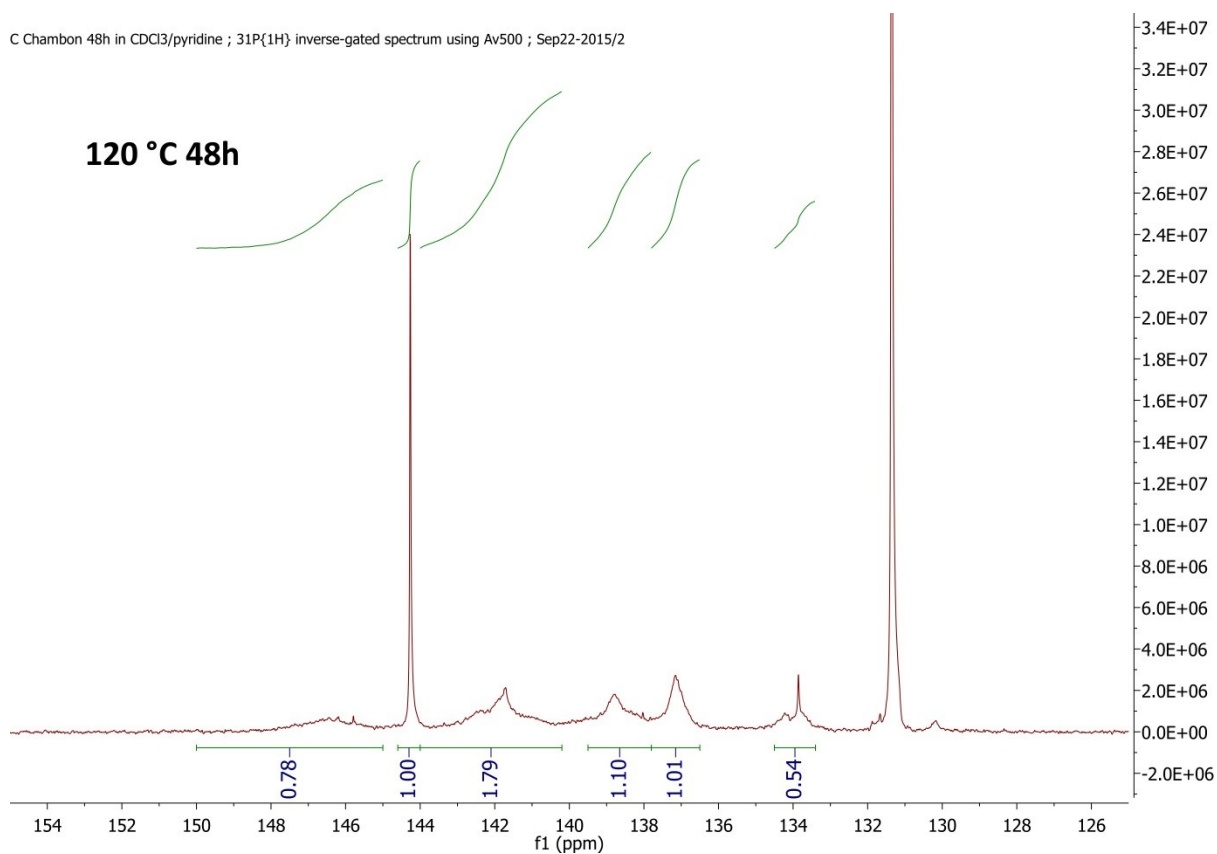
^{31}P NMR spectra (bagasse lignins, Chapter 4)



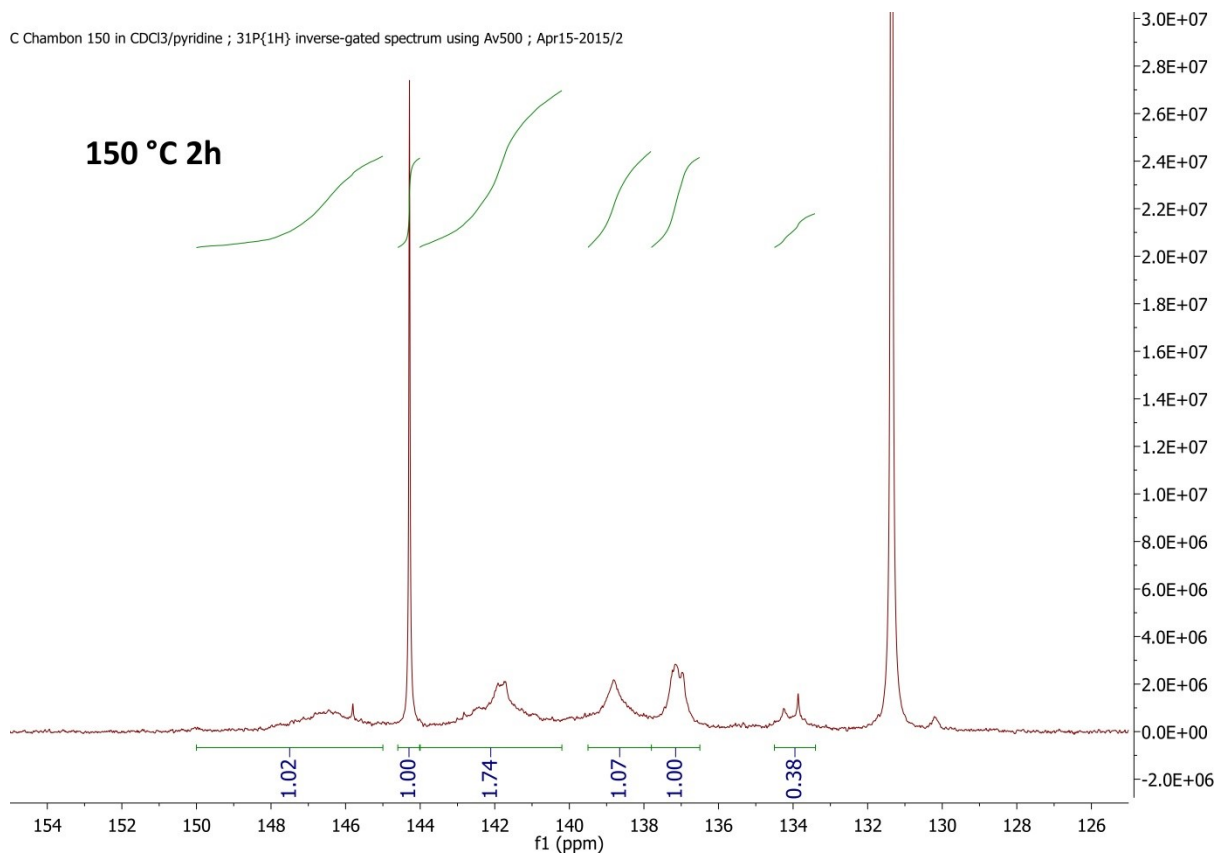




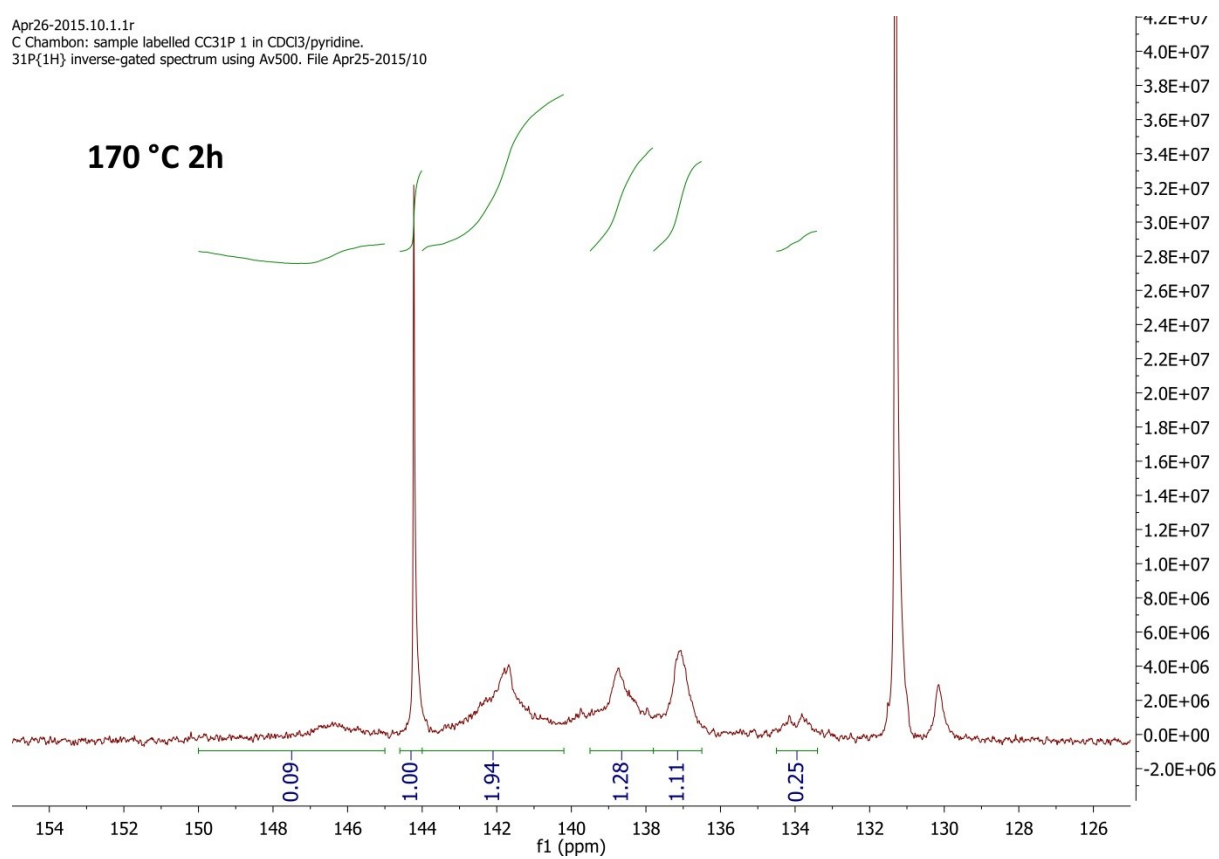
C Chambon 48h in CDCl₃/pyridine ; 31P{1H} inverse-gated spectrum using Av500 ; Sep22-2015/2



C Chambon 150 in CDCl₃/pyridine ; 31P{1H} inverse-gated spectrum using Av500 ; Apr15-2015/2

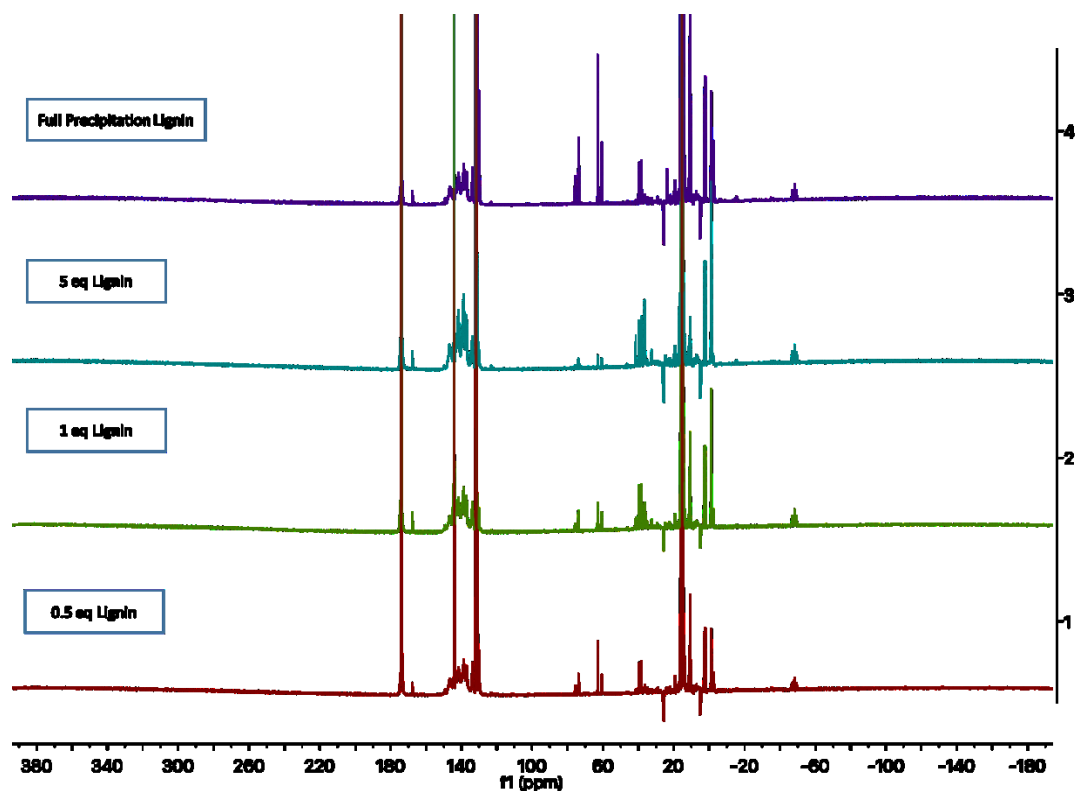


Apr26-2015.10.1.1r
C Chambon: sample labelled CC31P 1 in CDCl₃/pyridine.
31P{1H} inverse-gated spectrum using Av500. File Apr25-2015/10

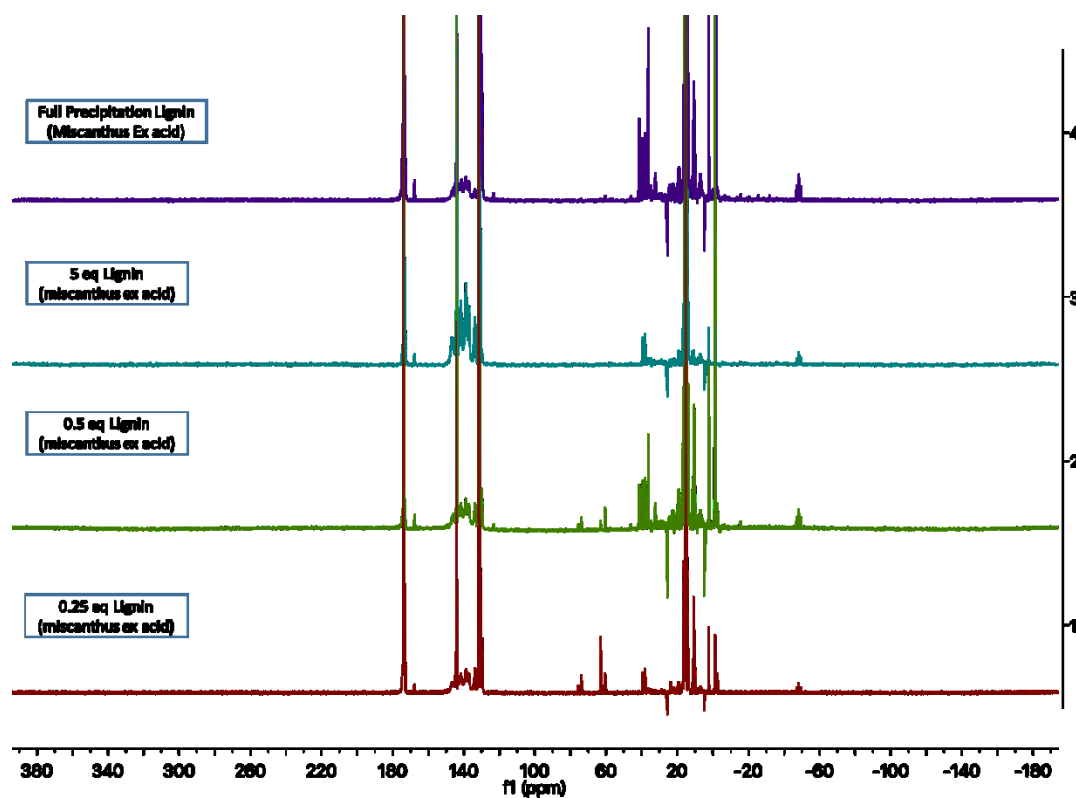


^{31}P NMR spectra (*Miscanthus*, pine and willow lignins, Chapter 4)

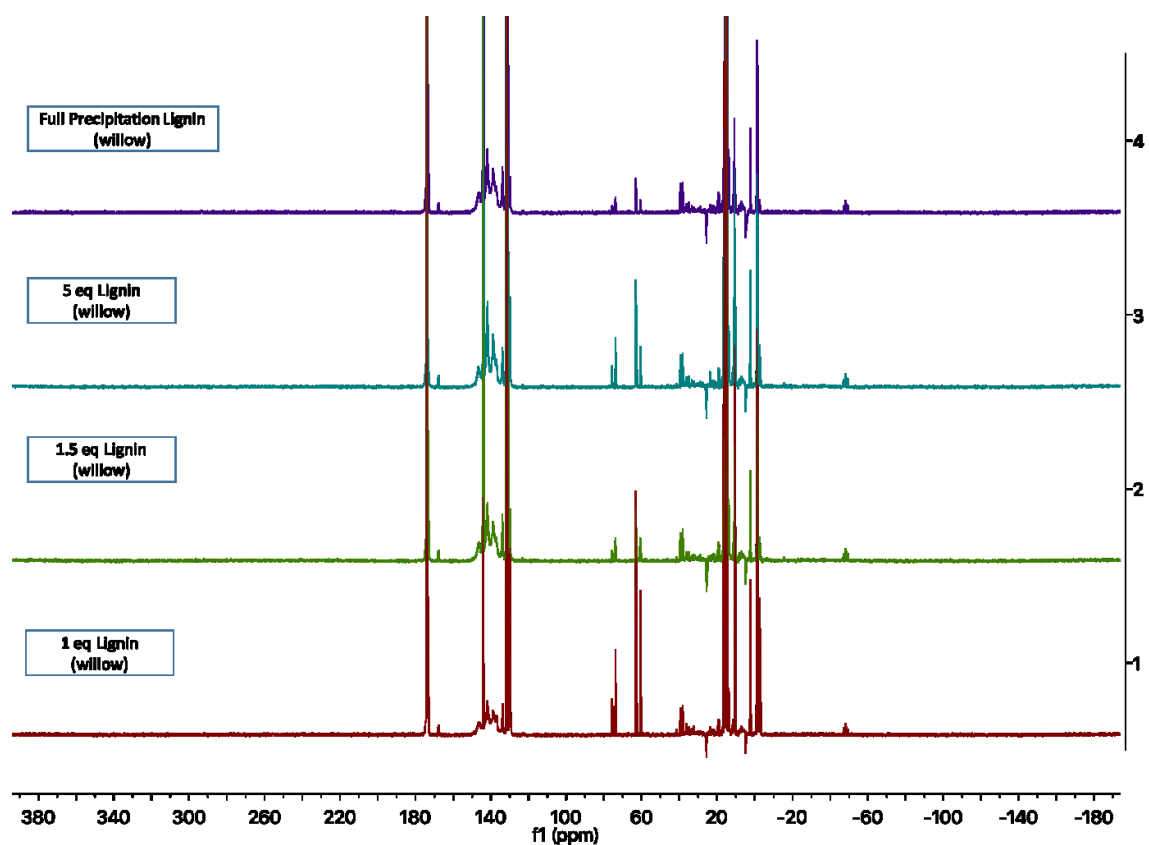
Miscanthus 1:1



Miscanthus Excess Acid



Willow 1:1



Pine 1:1

