

Imperial College London
Department of Chemical Engineering

**Impact of the ionoSolv process on cellulose quality and cellulose-derived
materials**

Wei-Chien Tu

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Declaration of Originality

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Acknowledgement

This journey down the unknowable long winding river Acheron was not one that I took on my own. Through this heart of darkness fraught with danger, distress, intrigue, self-doubt, and self-realization; shamans and guides squeezing blood from a stone. However, no life can grow from blood alone and as this story took on a life of its own; an existence that became real in my mind, I can't help but be thankful that I was not drifting alone. Sometimes this trip seemed like a funny dream and memory is a funny thing. A trip that seemed to start in the jungle, passed through limbo, to lead to a destination that is yet unknown. A learned experience that will endure long past the duration of this expedition that I can only hope will give me the skills and ability, to direct my wheel and find my way through the darkness.

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Publications

- Gschwend F., Brandt A., Chambon C., Tu WC., Weigand L., Hallett J., *Pretreatment of Lignocellulosic Biomass with Low-cost Ionic Liquids*. 2016, Journal of Visualized Experiments.
- Lancefield C., Rashid G., Bouxin F., Wasak A., Tu WC., Hallett J., Zein S., Rodriguez J., Jackson D., Westwood N., Bugg D.H., *Investigation of the Chemocatalytic and Biocatalytic Valorization of a Range of Different Lignin Preparations: The Importance of β -O-4 Content*. ACS Sustainable Chemistry & Engineering, 4(12), 6921-6930, 2016.
- Clarke C., Tu WC., Lever O., Brohl A., Hallet J., *Green and Sustainable Solvents in Chemical Processes*. Chemical Reviews, 118(2), 747-800, 2018.
- Clarke C., Tu WC., Weigand L., Brandt A., Hallet J., *Solvation Behavior of Ionic Liquids and Their Role in the Production of Lignocellulosic Biofuels and Sustainable Chemical Feedstocks*. Advanced Green Chemistry. Series on Chemistry, Energy and the Environment, Vol. 0, 77-134, 2018.
- Tu WC., Hallett J., *Ionic Liquids for Biomass Processing*. 2019, (10), Sustainable Catalysis in Ionic Liquids

Abstract

This thesis is concerned with the valorization of the cellulose pulps produced using the ionoSolv pretreatment process. Experiments using both fresh and recycled ionic liquid, specifically triethylammonium hydrogen sulfate [TEA][HSO₄], were conducted. Characterization of the isolated pulps was used to demonstrate the chemical and morphological effects of the ionoSolv pretreatment process on cellulose. Recycled ionic liquid contains solutes that impact the mechanism for lignocellulose biomass fractionation affecting the optimal pretreatment conditions. Pulp yields were consistently lower (demonstrating better pretreatment extraction, the pulps have a higher glucan content) and saccharification results were better when using recycled ionic liquid, demonstrating that the presence of these solute improved lignin extraction. In addition, it is demonstrated that protic ionic liquid pretreatment will eventually generate humins and pseudolignin, which are undesirable by-products of carbohydrate conversion which reduce saccharification yields and pulp purity. Experiments were conducted by doping the ionic liquid with ¹³C labelled and unlabeled sugars and their derivatives to ascertain the role of these solutes in the formation or prevention of pseudo-lignin formation. The re-deposition of these compounds onto the pulp is directly impacted by the purity of the pulp surface. The use of recycled ionic liquid in combination with additives such as HMF demonstrated a moderating effect on the formation of pseudolignin and humic compounds by increasing their solubility in the ionic liquid, preventing their re-deposition onto the surface of the pulp during pretreatment. In addition, lignin recovered from the pretreatment suggests the incorporation of carbohydrates, with lignin produced using recycled ionic liquid having higher molecular weights. The pulps produced during the pretreatment were submitted to subsequent processing to demonstrate additional potential value streams. Pulps were bleached to produce cellulose fibers and nanocellulose crystals. Fiber analysis suggests the pulps are of sufficient quality for materials production and various other industrial applications.

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List of Abbreviations

[AMIM]Cl	1-allyl-3-methylimidazolium
[BMIM]Cl	1-butyl-3-methylimidazolium chloride
[BMIM]dmpSe	1-butyl-3-methylimidazolium-O,De-dimethylphoroselenoate
[BMIM][HSO ₄]	1-butyl-3-methylimidazolium hydrogen sulfate

[DBNH][OAc]	1,5-diazabicyclo(4.3.0)non-5-enium
[EMIM][OAc]	1-thyl-3-methylimidazoliumacetate
[EMIM][OTf]	1-ethyl-3-methylimidazolium trifluoromethanesulfate
[HBIM][HSO ₄]	1-butylimidazolium hydrogen sulfate
[NMMO]	N-methylmorpholine-N-oxide
[TEA][HSO ₄]	Triethylammonium hydrogen sulfate
12-MPA	12-molybdophosphoric acid
AFEX	Ammonium fiber explosion
AIL	Acid insoluble lignin
AIR	Acid insoluble residue
ASL	Acid soluble lignin
ATR	Attenuated total reflection
BET	Brunauer Emmett Teller
BTX	Benzene, Toluene, and Xylene
CI	Crystallinity index
CNMR	Carbon-13 nuclear magnetic resonance
COSMO RS	Conductor-like screening model for realistic solvents
CSF	Combined severity factor
Cu-PMO	Copper porous metal oxide catalyst
DAP	Dilute acid pretreatment
DHE	2,5-dioxohex-3-enal
DHH	2,5-dioxo-6-hydroxy-hexanal
DI	De-ionized
DLS	Dynamic Light Scattering
DMA-LiCl	N,N-dimethylacetamide containing lithium chloride
DMF	2,5-dimethylfuran
DMSO	Dimethyl sulfoxide
DP	Degree of polymerization
DTF-D	Dispersion-corrected density functional theory
FDCA	2,5-furan dicarboxylic acid
FHMK	Furylhydroxymethyl ketone
FIL	Fresh ionic liquid
FT-IR	Fourier transform infrared spectroscopy
FWHM	Full width at half maximum

GC-MS	Gas chromatography-mass spectrometry
GHG	Greenhouse gas
GPC	Gel permeation chromatography
HHV	Higher heat value
HMF	5-hydroxymethylfurfural
HNMR	Proton nuclear magnetic resonance
HPLC	High-performance liquid chromatography
HSQC	Heteronuclear single quantum correlation
KI	Kink index
IL	Ionic liquid
LUMO	Lowest unoccupied molecular orbital
MCC	Microcrystalline cellulose
MIBK	Methyl isobutyl ketone
MJ	Megajoules
mg	Milligram
ml	Milliliter
MO	Molecular orbital
MFIL	<i>Miscanthus</i> fresh ionic liquid
MHMFIL	<i>Miscanthus</i> HMF fresh ionic liquid
MHRIL	<i>Miscanthus</i> HMF recycled ionic liquid
MRIL	<i>Miscanthus</i> recycled ionic liquid
MXFIL	<i>Miscanthus</i> xylose fresh ionic liquid
MXRIL	<i>Miscanthus</i> xylose recycled ionic liquid
MSW	Municipal solid waste
NCC	Nanocrystalline cellulose
NMR	Nuclear magnetic resonance
LA	Levulinic acid
LC	Liquid chromatography
LC-MS	Liquid chromatography mass spectrometry
NREL	National Renewable Energy Laboratory
OECD	Organization for Economic Cooperation and Development
PBT-PLA	Poly(butylene-adipate-co-terephthalate)-polylactide
PCA	<i>p</i> -coumaric acid
PHS	Post hydrolysis solids

RBF	Round bottom flask
RF	Radio frequency
RI detector	Reactive index detector
RIL	Recycled ionic liquid
SAPO	Silicoaluminophosphates
SEM	Scanning Electron Microscopy
ssNMR	Solid state NMR
TB	Trihydroxybenzen
US DOE	United States Department of Energy
UV-VIS	Ultraviolet-visible spectroscopy
XRD	X-ray diffraction

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Chapter 1 Overview to lignocellulosic biomass, pretreatment, platform chemicals, and humins

1.1 Introduction: lignocellulosic biomass

Continually increasing energy and materials consumption pushes today's research to focus on meeting the needs of future generations for sustainable energy, chemicals, and materials solutions. It is critical for the view of sustainability to encompass environmental, social and economic aspects. Solar, wind, and biomass (to name a few) have been proposed as sustainable/renewable alternatives with different advantages and trade-offs to traditional petroleum resources. However, it is biomass (specifically lignocellulosic biomass) that has the flexibility to provide sustainable energy (liquid fuel) as well as chemical and materials production from a sustainable and renewable resource.¹ This study explores various aspects of the use of lignocellulosic biomass in the sustainable production of fuel, industrially important materials, and chemical feedstocks.

There are several major categories of biomass (see **Figure 1**). Bio-ethanol is produced using first generation biomass by directly fermenting starchy food crops (such as corn) or sugary food crops (sugar cane or beets) to ethanol. While first generation ethanol production has become established with 25 billion gallons produced in 2016, it is not without controversy.²

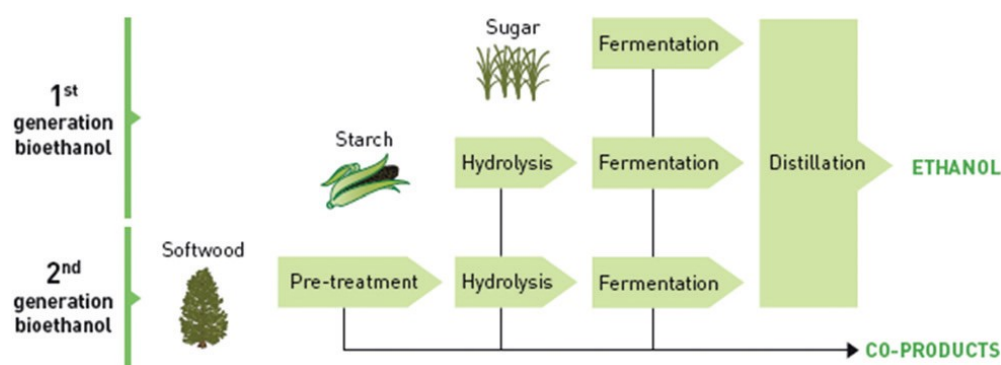


Figure 1 Second generation biomass require and additional pretreatment step in order to produce biofuels.

The production of fuel, using arable land, may lead to competition with food production, and ultimately resulting in consumer food prices increases.³ Second generation biofuels are derived from land-based biomass that are not used for human consumption, thereby eliminating the controversy of using food for energy production. Second generation biomass, known as lignocellulosic biomass, is composed of three major components: cellulose, hemicellulose, and

lignin; organic biopolymers found in the cell walls of terrestrial plants that provide structural support for the plants and defence against microbial attacks. The components of lignocellulosic biomass are attractive to research efforts due to their potential beyond biofuel production, including the production of plastics, materials, and chemical feedstocks.^{4,5}

Lignocellulosic biomass sources are numerous and can be harvested from existing industrial processes and byproducts such as agricultural waste and residues, food waste, energy crops, and municipal waste. Lignocellulosic production globally exceeds 2.0×10^{11} tons per year. These second generation biomass feedstocks are the most abundant sustainable renewable resource on Earth and are generally classified into three categories: hardwoods, softwoods, and grasses (see Table 1).⁶

Table 1 Examples of Common Types of Lignocellulosic Biomass

Common types of Hardwoods, Softwoods, and Grasses		
Hardwood	Softwood	Grasses
Beech	Cedar	Miscanthus
Hickory	Fir	Corn
Mahogany	Pine	Wheat
Oak	Spruce	Bamboo
Willow	Cypress	Rice

An additional and significant advantage, beyond renewability, is that the production of lignocellulosic biomass is available in a range of climates. This means that energy and materials solutions could be provided locally throughout the world. Most global regions already possess the basic industry to supply ample lignocellulosic biomass for biorefineries, from wood – paper industries, agricultural industries, energy crops, and municipal/food waste collection facilities.⁷ However, this process is not without complications. There are basic logistical challenges such as harvesting and transporting the biomass feedstocks for processing that must be acknowledged and accounted for. In addition, second generation biomass requires a pretreatment step that is not required in first generation fuel production to fractionate the composite. Hardwood, softwood, and grasses vary in their composition of the three principal biopolymers that compose lignocellulosic biomass, with variations even occurring within the same species of plant due to differences in growing conditions. This means that each type of biomass can provide a different potential for biofuel and feedstock production, but it also means that each requires different procedures for optimal pretreatment.

One of the main types of biomass used for the production of energy and potential materials production are energy crops. These types of plants are grown and harvested for the

express purpose of producing fuels. One such lignocellulosic biomass is *Miscanthus giganteus* (which is used throughout the bulk of this project) is a fast growing perennial grass that has the ability to grown on marginal land, is high in water efficiency, and has high carbon sequestration.⁸ Their low input requirements, reliance to disease and pests, and moisture content during harvest make them an attractive source for lignocellulose biomass refining

Pretreatment on second generation biomass is necessary due to the recalcitrance of lignin. Pretreatment can be used to remove lignin or hemicellulose and or reduce cellulose crystallinity. Lignin, its structure, and cross linkages hinders the saccharification (hydrolysis of polysaccharides) of carbohydrates to their monomers for fermentation to bioethanol.⁹ A variety of different pretreatment methods exist and can be categorized into biological, chemical, physical, and physiochemical processes.¹⁰ Many pretreatment processes use a combination of these methods. Though lignocellulosic biomass is readily available, valorization of its use in fuel and platform chemical production will depend mostly on the energy/cost for the pretreatment process. The production of additional value streams such as chemical feedstocks and materials could significantly reduce the cost of pre-treatment. Developing a biorefinery process that incorporates elements of both pretreatment for biofuel production and additional chemical feedstock value streams, will significantly help lignocellulosic biomass compete with other fuel/energy, chemical, and material production processes.

Estimates place the pretreatment process at \$0.08/litre and the production of a litre of bioethanol from corn to be between \$0.35-0.48 per litre.^{9,11} The current production is not competitive to fossil fuels production without substantial subsidies. Early feasibility studies had suggested that ethanol produced from lignocellulosic biomass was on average 40% more expensive than corn ethanol.¹² However, advances in research and technology are closing the gap by increasing ethanol production though better sugar saccharification yields. For example, comparative production estimates of ethanol from corn stover were 405 litres per dry ton vs. 435 litres per ton for corn in the year 2000.¹² By 2013, ethanol produced from lignocellulosic biomass was selling for approximately \$0.58 per litre and still not competitive with fossil fuels. However, more recent studies began taking a more holistic approach when considering biofuel production, taking in to account environmental benefits and carbon savings not just the raw cost of fuel production. These studies showed that the cost of producing bioethanol from lignocellulosic biomass created abatement cost savings in carbon credits as much as a \$48-\$375/Mg⁻¹ in greenhouse gas (GHG) compared to gasoline.¹³ Further improvements in

efficiency, yield, and the potential for adding additional value streams are required to bring lignocellulosic biomass into the competitive market place on a global level. Heat and power sectors can be supplied with a variety of renewable sources, namely wind, solar, hydropower and biomass. The transportation sector has a much more limited choice, however. Currently, biomass is the only resource that can provide renewable liquid fuels. Beyond the transportation sector, biomass is also a promising feedstock for use in the chemical industry, with the potential to replace non-renewable petroleum derived chemicals.

1.1.1 Introduction: Lignocellulosic Biomass Composition

Lignocellulosic biomass is not a new material or energy source. It has been used for millennia, as building material, burned for energy to heat our homes and cook our meals. However, for advancement in its efficiency as a source of energy, its composition and structure must be understood. The main components of terrestrial plant cell walls, that will be discussed, are cellulose, hemicellulose, and lignin (see Figure 2). Plant pectin, lipids and phenolics are also present in the cell wall, however their combined total is typically less than 5% of the cell wall composition.¹⁴

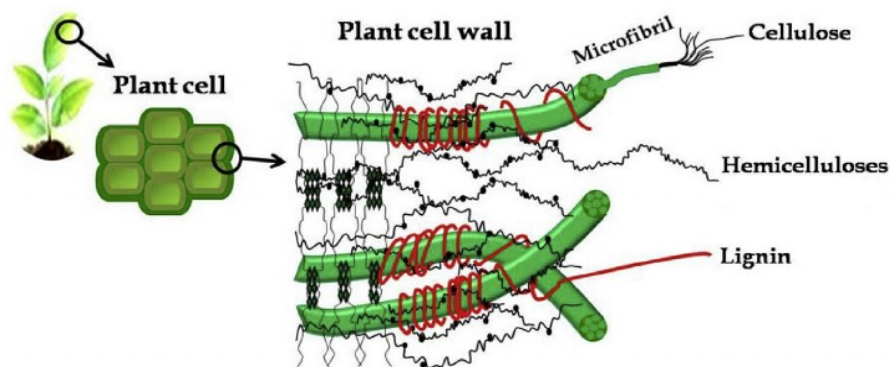


Figure 2 Structure of terrestrial plant cell wall.¹⁵

The quantities of these three components (cellulose, hemicellulose, and lignin) within plant cell walls are dependent on plant species, genotype expression, growing conditions, as well as the plants stage in development.¹⁶ Hardwood and softwood species compositionally differ, with hardwoods containing between 40-50% cellulose, 24-40% hemicellulose, and 18-25% lignin, while softwoods cell wall compositions range between 45-50% cellulose, 25-35% hemicellulose, and 25-35% lignin. Grass composition varies from 25-40% cellulose, 35-50% hemicellulose, and 10-30% lignin.^{17,18} The lamella and primary cell wall are flexible and connect each cell to its adjacent neighbour. Recalcitrance to pretreatment is mainly caused by

the components of the secondary cell wall (see Figure 3).¹⁹ Crystalline cellulose interacts via hydrogen bonding to the surrounding hemicellulose which in turn is covalently bound to lignin. This insulating structure provides structural support to the plant, protects it from microbial attacks, and unfortunately, also from pretreatment methods. Variations in the composition of biomass varieties create both advantages and challenges. Optimal feedstock can be selected and produced for specific purposes, but the impact of this flexibility is that procedures for the pretreatment of the different biomasses will vary. Research investigating the effect of genetically engineering biomass to optimize feedstock components for various applications and reduce recalcitrance is ongoing.^{16,19}

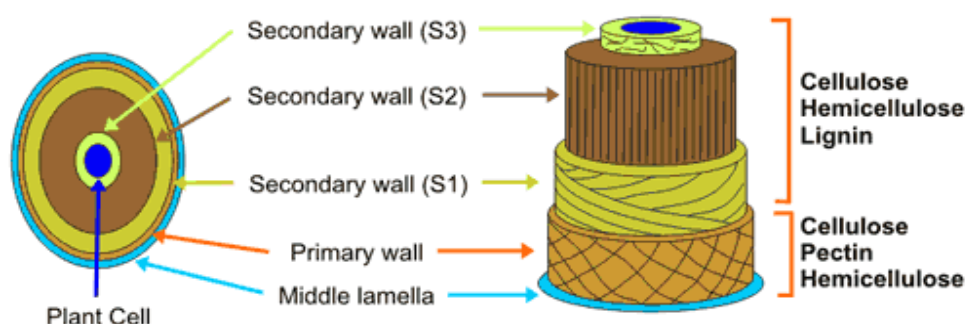


Figure 3 Cross section of plant cells detailing the location of the main organic components found in each section. Cellulose and hemicellulose are found in the primary and secondary walls, with lignin found mainly in the secondary wall of the plant.²⁰

1.1.2 Cellulose

Cellulose is more abundant than any other natural polymer on earth and is produced by plants as a by-product of photosynthesis for structural support. Cellulose is also produced in certain types of bacteria.²¹ Human civilization has been using cellulose for centuries producing textiles (for example from cotton) as well as materials (such as paper). Beyond these physical material applications, cellulose can be chemically transformed to produce fibres as well as platform chemicals such as 5-hydroxymethylfurfural (HMF) and levulinic acid (LA). These chemicals are considered “Top Value Added Chemicals From Biomass” per the United States Department of Energy.²²

Cellulose is a polysaccharide of D-glucose sugars, where the hydroxyl groups are located in the equatorial positions (lowest free energy conformation). The glucose units are connected via $\beta(1-4)$ linkages forming long chains that can easily stack on top of one another, resulting in the formation of cellulose fibrils.²³ Glucose is a monomer and in small units up to 4-5 oligomers is water soluble. However, cellulose chains, which consist of 10,000+ linked

glucose units, form flat, tightly packed, inflexible sheets that are not soluble in water or most organic solvents. Hydrogen bonding and Van der Waals forces connecting sheets of cellulose further reduce solubility (see Figure 4).^{24–26}

There are several known polymorphs of cellulose which differ in the location of the intermolecular hydrogen bonds. Cellulose I and cellulose II are the most common polymorphs seen in biomass pretreatments.²⁷ Cellulose I is native but contains two allomorphs: cellulose

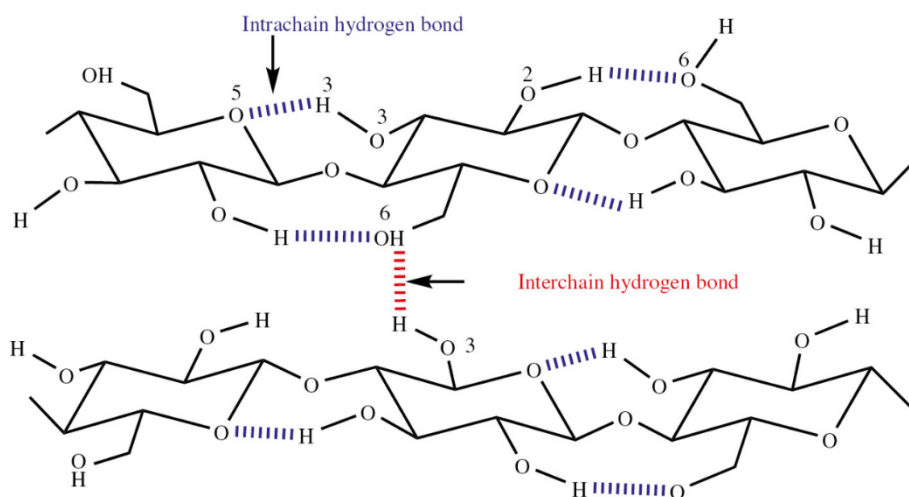


Figure 2 Intra and inter molecular hydrogen bonds in a cellulose chain.

I α and cellulose I β .²⁸ Cellulose I α is found in bacteria and algae while cellulose I β is the most common allomorph found in terrestrial plants. The main difference between cellulose I and cellulose II is the way in which the crystalline structures are stacked (see Figure 5).^{27,29} Cellulose I chains run parallel to each other while cellulose II chains run antiparallel. Differences also manifest in their intermolecular and intramolecular hydrogen bond patterns with the 3 $\rightarrow$ 5 intramolecular bond increasing the rigidity and the 3 $\rightarrow$ 6 intermolecular bond keeping the sheets together in cellulose I. In cellulose II the 3 $\rightarrow$ 5 bond is still present, however rather than a 3 $\rightarrow$ 6 intermolecular a 2 $\rightarrow$ 6 intramolecular bond exists.

Cellulose II is more thermodynamically stable and is the result of either the regeneration or mercerization of cellulose I.³⁰ Solvents such as cupriethylenediamine, N-methylmorpholine-N-oxide (NMMO), and ionic liquids such as 1-ethyl-3-methylimidazolium acetate [EMIM][OAc] can be used to dissolve cellulose, though the regeneration of that cellulose often results in its conversion from cellulose I to cellulose II. The second method for the transformation from cellulose I to cellulose II is via mercerization. Mercerization can be accomplished by soaking cellulose in high concentration alkali solutions, such as sodium

hydroxide (above 12% wt). This process swells the native cellulose. Removal of the solution results in the transformation to cellulose II. This is commonly used in the textile industry to

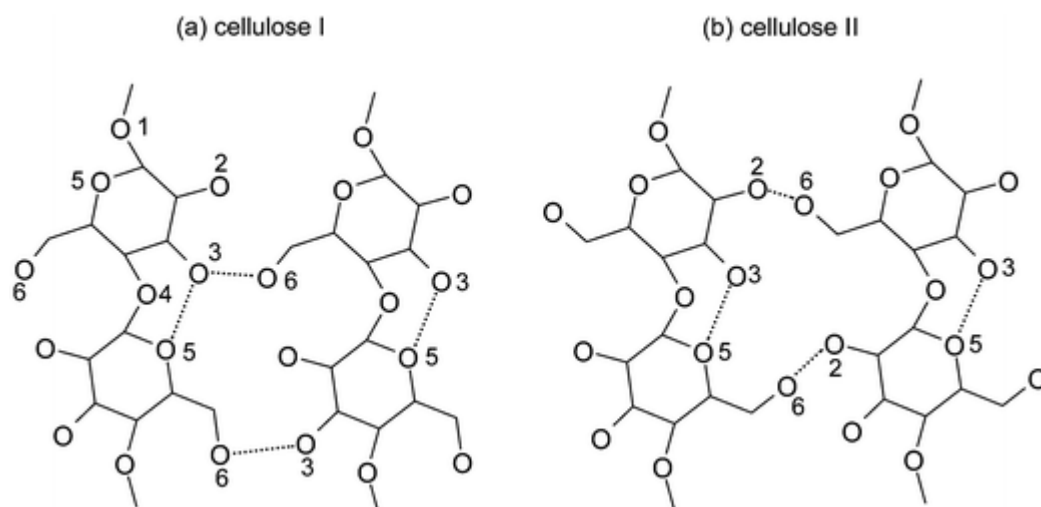


Figure 5 Cellulose polymorph structures. Cellulose I contains intermolecular interaction between the $O3-H \rightarrow O6'$ and $O6-H \rightarrow O3'$. Cellulose II contains intermolecular interactions between the $O2-H \rightarrow O6'$ and the $O6-H \rightarrow O2'$.³¹

add lustre, improve fibre strength, and increase shrink resistance in cottons fabrics. The change from cellulose I to cellulose II has been confirmed via X-ray diffraction (XRD). XRD has also demonstrated that cellulose crystalline formation is imperfect; that less ordered areas or amorphous regions of cellulose also exist.^{4,32,33} These amorphous areas are the first to be hydrolysed during pretreatment.^{34–36}

1.1.3 Hemicellulose

Unlike cellulose, hemicellulose is a heteropolymer comprised of five major linked carbohydrates monomers: xylose, glucose, galactose, mannose, and arabinose (see Figure 6).^{17,37} Xylose and arabinose are pentose carbohydrates and galactose, mannose, and glucose are hexose sugars. The backbone for the hemicellulose chains are made of mannose, xylose, and glucose with equatorial configurations connected via $\beta(1-4)$ linkages; this being one of the factors that differentiate hemicellulose from pectin.

Compared with cellulose the β linkages are much shorter ranging from 50 up to 3000 units. These chains are comprised of both hydrophilic and hydrophobic areas creating a much less rigid and amorphous structure.^{38,39}

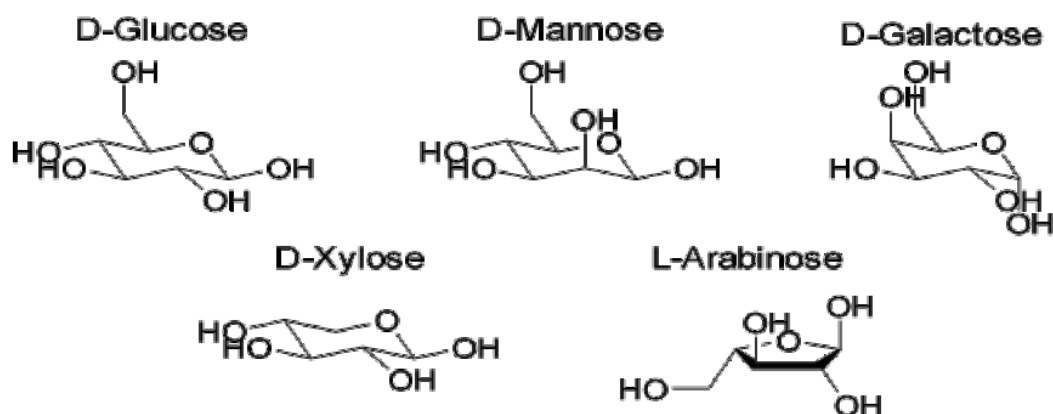


Figure 6 Hemicellulose carbohydrate monomers.⁴⁰

Xylans are the most common form of hemicellulose found in hardwood and grasses, and the second most common hemicellulose in softwoods. Xylan hemicellulose backbones are made exclusively from xylose units (see Figure 7). Mannose, arabinose, and glucuronic acid are common branches found in xylans. These appendages vary depending on the type of lignocellulosic biomass. Hardwood biomass have a greater amount of glucuronic acid appendages while softwoods and grasses are comprised of arabinoglucuronoxylan. Grasses and grains also contain ferulic acid esters residues which protect the cell wall from enzymatic attacks.⁴⁰ Galactoglucomannan is the major hemicellulose found in softwoods. These hemicellulose chains contain a mannose backbone (which can also be acetylated) with glucose or galactose appendages.

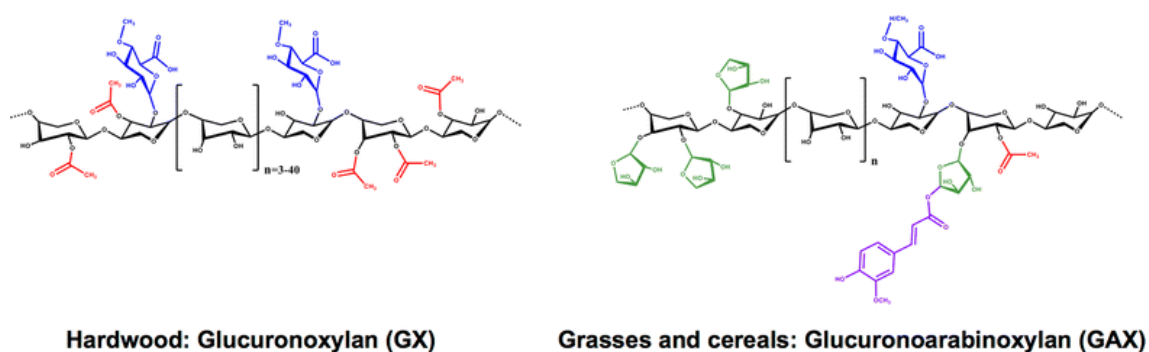


Figure 3 Common xylan structures found in secondary plant cell walls. Acetylated xylose backbone (red), glucuronic acid appendages (blue), arabinan (green) and ferulic acid (purple).

Hemicellulose is not covalently bonded to cellulose. It is less rigid and instead, interacts with cellulose via hydrogen bonds. Hemicellulose is less structured and crystalline than cellulose making it easier to depolymerize. Unlike cellulose, hemicellulose can be dissolved or depolymerized in alkali or acidic aqueous solutions, organic solvents, and other solvents (such as ionic liquids). Hemicellulose has the potential to be used in multiple added value streams

for biorefining including additional sugars for fermentation, food additives, and platform chemical production.⁴¹

1.1.4 Lignin

An aromatic biopolymer, lignin's main function is structural reinforcement in plants while providing water proofing and resisting intrusion from microbes. An additional function for lignin in plants is to facilitate the transport of water through the cell walls. The other components of cell walls, chiefly pectin, hemicellulose and cellulose, are largely hydrophilic (though they do contain some hydrophobic domains).⁴² Lignin is hydrophobic making it impermeable to water allowing for proper conduction through the plants vascular tissues.⁴³ Since lignin represents approximately 1/3 of the lignocellulose composite, over 50 million tons of it are produced annually during common pulping or pretreatment processes such as the Kraft paper making process. Today, most of the lignin produced in the Kraft process is burned for energy recovery.⁴⁴ However, lignin has great potential to replace petroleum-based materials like plastics, paints and other aromatic compounds that are currently being sourced using fossil fuels.

The formation of lignin is a complex mechanism involving oxidative coupling of phenylpropanoids.⁴⁵ The way in which plants control this radical mechanism is not yet fully understood. Amino acids tyrosine and phenylalanine are the starting materials requiring a host of oxidative enzymes to produce the predominant monomers: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (see Figure 8 a). These subunits are incorporated into the building of the cell walls with the most common condensation occurring at the β position. Though lignification is often thought of as an uncontrolled or random process, this is no the case. It has

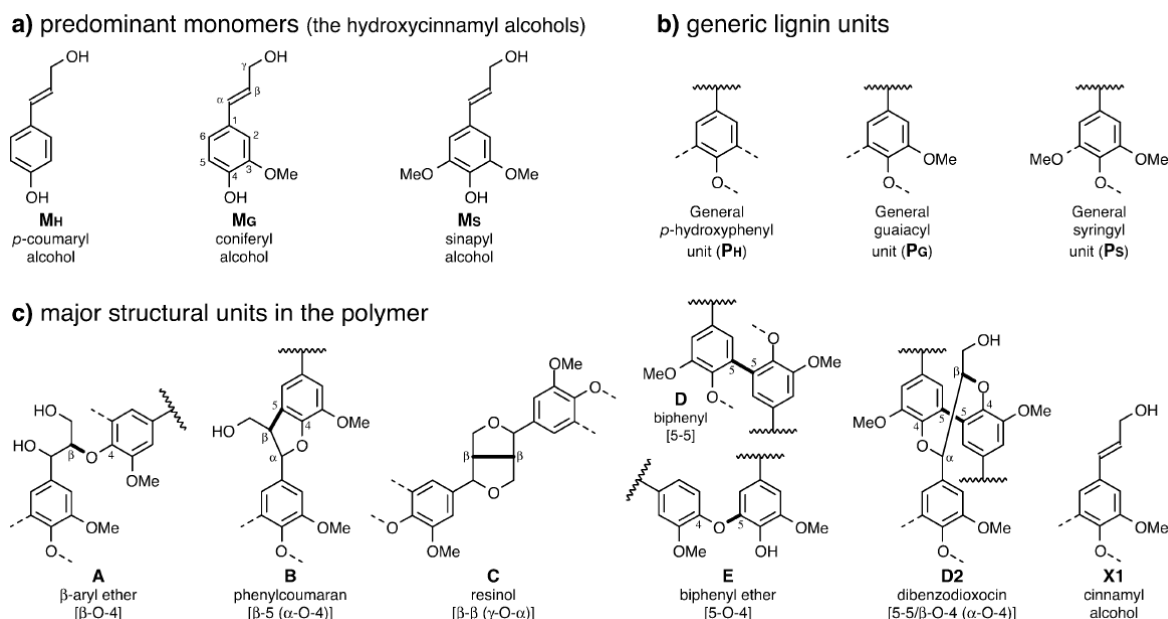


Figure 8 Amino acids are converted into three major subunits of lignin: *p*-coumaryl, coniferyl, and sinapyl alcohols (a), these are represented in lignin as *p*-hydroxyphenyl, guaiacyl, and syringyl units (b) and the 6 most common condensation motifs in lignin structure.⁴⁵

been observed that dehydrodimerization of the monolignols have a higher β -O-4 dimer concentration in native lignin than in synthetic lignin. The cross-coupling of monolignols to the growing lignin polymer does not proceed in the same as dimerization, with the β -O-4 linkage favoured. (see appendix 1-A). Full understanding the structure of native lignin is still incomplete.

Both, species and growing environment, can impact the ratio of the lignin units. Specifically, guaiacyl subunits (Figure 8b) may condense to form carbon-carbon [5-5] linkages (Figure 8C-D) which are higher in energy and more difficult to break, contributing to recalcitrance. Plant types, that have a higher guaiacyl concentration, usually require harsher pretreatment conditions to remove/depolymerize the lignin. This makes softwoods, which are composed mainly of guaiacyl sub-units, difficult to delignify as neither acidic or alkali pretreatments are able to break these bonds. Hardwood lignin contains a mix of guaiacyl and syringyl units and grasses a mix of all three sub-units (which includes the *p*-hydroxyphenyl subunit). However, hardwoods (60%), softwoods (45-50%) and grasses (74%-84%) contain β -O-4 bonds which are responsible for the elongation of the lignin polymer creating an opportunity for more efficient pretreatment.¹⁹ Fractionating the carbon-carbon linkages is extremely energy intensive, but much easier at the β -O-4 locations where most of the depolymerization occurs. Instead of using native hardwoods and softwoods, saccharification of biomass can be enhanced from genetically modified plants that produce less lignin. This

may also mean that if lignin could be valorized, modifications to produce biomass with more lignin content could also be possible for chemical processes designed to develop platform chemicals from lignin.⁴⁶

1.2 Pretreatment

Pretreatment is a preliminary step performed to make subsequent processing of lignocellulosic biomass more efficient and/or effective. Pretreatment is arguably one of the most energy intensive and costly aspects of biorefining. However, without pretreatment, the impact of recalcitrance renders lignocellulosic bio-fuel production economically infeasible.⁴⁷ The sensitivity of commercial viability to energy costs and solvent requirements makes the optimization of separation, and the depolymerization and conversion of biomass extremely important.²⁵

Pretreatment is a method used for the fractionating of biomass to primarily allow for enzymatic access to the cellulose fibrils which are surrounded and protected by hemicellulose and lignin.¹⁰ Cellulose that has been separated from hemicellulose and lignin may proceed to milder saccharification (hydrolysis of sugars to their monomers, in this case cellulose to glucose, for fermentation)(see Figure 9). Though the focus of most pretreatment is the production of cellulose pulp for fermentation to bio-ethanol, pretreatment can also be a means

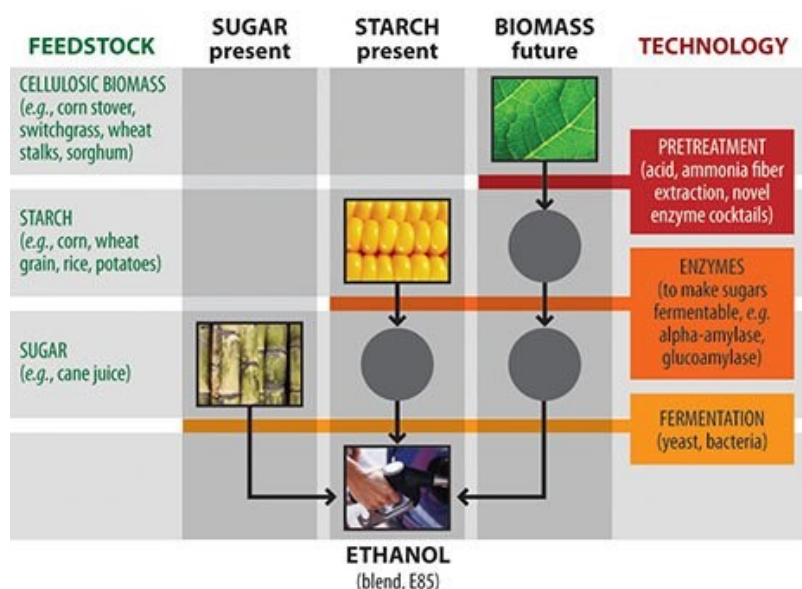


Figure 9 Steps required for various biomass types to produce bio-ethanol.¹⁰

for producing other value add products (for example, aromatics such as xylenes from lignin, and cellulose fibres).

1.2.1 Methods of Pretreatment

Variations in lignocellulosic biomass types has led to the development of a variety of pretreatment technologies, each with different optimal conditions and applications.¹⁰ Pretreatment methods can be placed into four general categories: physical, chemical, hybrid-physicochemical, and biological (see Figure 10).⁴⁸ Capital and operational costs will also vary and may be minimized according to several factors such as: the necessity of biomass size reduction, production of degradation products from the pretreatment which may impact production yield, the preservation of the lignin or hemicellulose fractions for potential use in high value products, the possibility of recycling of solvents and or catalysts, and the compatibility of products produced from the pretreatment with downstream processes.⁴⁹ Most pretreatment processes use a combination of these techniques to maximize results. We will

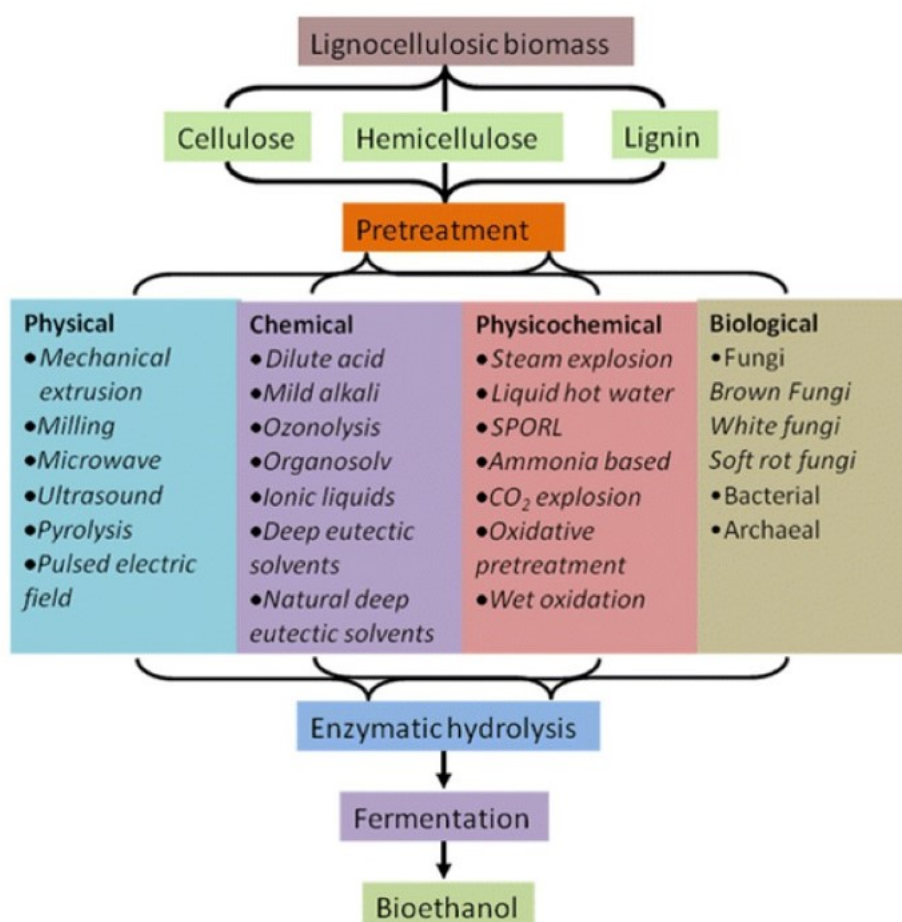


Figure 4 Categories of various pretreatments used for lignocellulosic biomass

briefly discuss important methods of pretreatment across multiple methodologies. Physical pretreatments for discussion include mechanical extrusion, milling, and pyrolysis. Chemical pretreatments will include: dilute acid, Kraft alkali, organosolv, and ionoSolv processing. Additionally, discussion will include physiochemical processes such as steam explosion and ammonium fiber explosion (AFEX). The majority of these pretreatment methods were originally conceived for either paper or bioethanol production. However, some of the processes such as the organosolv and ionoSolv have greater versatility with the potential to produce additional value streams such as platform chemicals and materials.

1.2.2 Physical and Physiochemical pretreatments

Mechanical extrusion technique for pretreatment employs rotating screw blades to chop and grind the biomass usually at elevated temperatures, while simultaneously forcing it through a die. Upon exiting from the die the sudden decrease in temperature and pressure causes the biomass to suddenly expand (explode), thereby fracturing the biomass composite (see Figure 11). The shearing motions generated by the blades disrupt the composite crystalline cellulose,



Figure 11 Various feedstocks pretreated using a single screw extruder.⁵²

improving the efficiency of downstream processes.⁴⁹ Mechanical extrusion is often insufficient for lignocellulosic biomass pretreatment though combining it with other pretreatments (such as acid or alkali pretreatments) has been shown to improve carbohydrate yields.¹⁰ There are some advantages to mechanical extrusion including low carbohydrate degradation and the ability to easily monitor and change system settings, which is helpful if changes in biomass feedstock occur.⁵⁰ These variables include temperature, residence time, and pressure. Additionally, this process can be completed via a continuous reactor which can also allow for easy adaptations and modifications to the process.⁵¹

Milling grinds and shears the biomass to disrupt the crystallinity of the cellulose in similar fashion to mechanical extrusion thereby facilitating enzymatic hydrolysis. Multiple milling techniques have been developed including: planetary ball, hammer, and vibratory milling.⁵³ The biomass feedstock is usually ground to approximately 0.2 mm though there seems to be no significant improvement in enzymatic hydrolysis past 0.4mm.¹⁰ Yields improve with the addition of acid or alkali pretreatments, however energy consumption for this process is largely what prevents its commercialization.⁵⁴

Unlike most of the pretreatments mentioned, pyrolysis is used for the production of bio-oils. The pyrolysis process raises the temperature of biomass to between 400-800 °C while simultaneously depriving the reaction of oxygen (or any halogens). Within these conditions, cellulose rapidly decomposes and reduces to gaseous compounds including methane, hydrogen, carbon monoxide, bio-oils, and charcoal.¹⁰ This pretreatment technique is not biomass specific though results are impacted by feedstock used and heating parameters. Separate processes exist for both Slow and Fast pyrolysis. Slow pyrolysis is the more conventional method and gradually heats the biomass to approximately 500 °C forming charcoal. Fast pyrolysis more rapidly exceeds 600 °C producing gaseous products and bio-oils.

Steam explosion is a physiochemical process that places the biomass into a chamber with a screw loading valve. Temperatures between 180-250 °C with pressures up to 3.5 Mpa are used with resident times of approximately 2 minutes. The biomass is injected with steam which enters the crevices of the biomass composite.⁴⁹ Similar to extrusion methods, as the biomass exits the chamber, the sudden decompression causes the rapid expansion of the trapped steam to rupture the cellulose fibers, improving enzyme access and subsequent hydrolysis. In addition, partial hydrolysis of the hemicellulose occurs during the steam explosion process due to the production of acetic acid from the acetyl groups on the hemicellulose.¹⁰ Steam explosion

was first used in industrial applications in the 1980's. One of its distinct advantages is the versatility to run either via batch reactor or continuous flow.^{55,56} Additionally, steam explosion uses relatively little solvent but additional research in reactor design and energy consumption may improve its feasibility on a larger scale.

1.2.3 Chemical pretreatments

Low and high temperature (less than 120 °C and above 180 °C) Dilute Acid Pretreatment (DAP) vary significantly by runtimes.⁵⁷ High temperature treatments have a duration of 1-5 minutes while low temperature treatments may extend to 90 minutes.¹⁰ The acid catalyses the breaking of ether bonds in lignin (such as the β -O-4 linkages) and depolymerizes the lignin (see Figure 12).⁵⁸ The acid also aids in hydrolysing some carbohydrates, increasing saccharification yield. The most commonly used acids include: sulfuric, oxalic, and maleic acids with a concentration between 1-4% w/v. DAP is attractive due to relatively low cost, and the acids used are readily available. However, one of the disadvantages of DAP is that acid pretreatments often generate by-products such as humins which inhibit yeast and other microorganisms which may negatively impact downstream processing. The acids themselves are toxic to the yeast, inhibiting fermentation yield, and therefore extensive washing steps are required. Additional considerations when using DAP include the design and cost for reactor vessels which must be able to withstand long term exposure to acid corrosion while maintaining efficiency of the DAP.⁵⁹

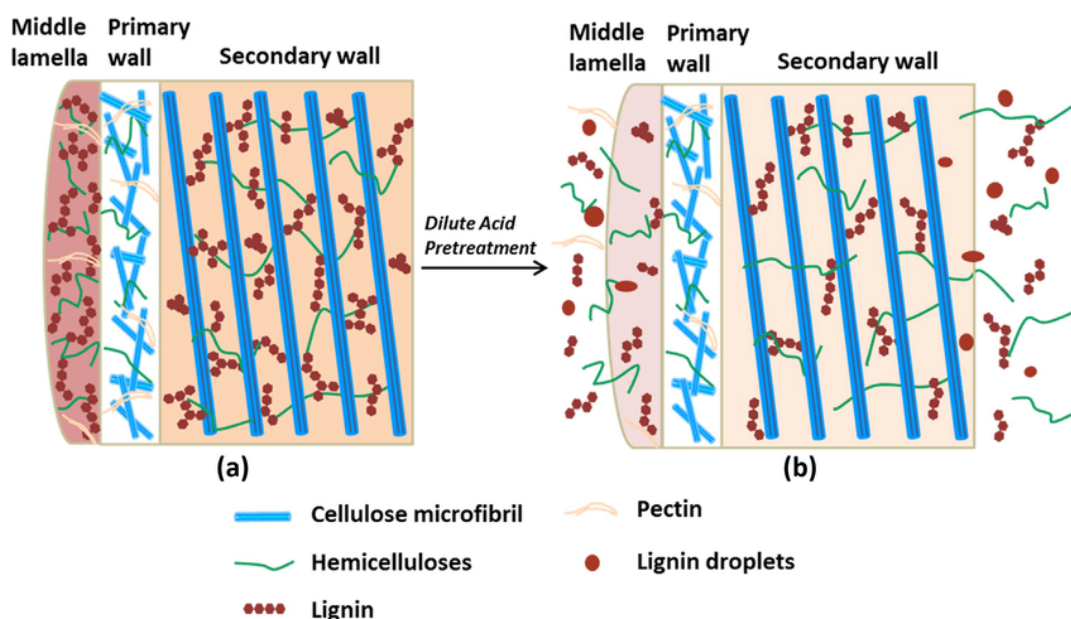


Figure 12 Diagram of dilute acid pretreatment. DAP causes lignin and hemicellulose disruption and depolymerization.⁵⁷

The Kraft process, also known as Kraft pulping, was first developed in the late 1870s and remains the most common method of wood pulp production in the world. Sodium hydroxide, sodium sulphide, and raw material are heated under pressure to approximately 170 °C.⁷ This alkali catalysed reaction cleaves ether bonds, depolymerizes lignin, and deprotonates hydroxyl groups which improves lignin solubility.^{60,61} Today, the recovered pulps have been used for bioethanol production though the original Kraft process was intended to produce bleached and pressed pulps for paper production. Kraft processing faces the same challenges as DAP. In order to increase saccharification yields, extensive water washes are necessary to clean the pulp of acid and other inhibitory products. This additional step substantially adds to the economic and energy cost of the process.⁶²

The organosolv process was originally developed as a pulping method for paper production. Organic solvents such as ethanol, acetone, ethylene glycol, formic and acetic acid are used to fractionate the lignin and hemicellulose of lignocellulosic biomass. Organosolv requires temperatures between 160-200 °C, pressures between 5-30 bar and runtimes of 30-90 minutes. The drive for cleaner energy has driven commercial development of the organosolv process beyond papermaking for bioethanol production.⁶³ Inorganic or organic acids can be added to catalyse the deconstruction of the biomass, though inhibitory by-products, and corrosion of the equipment are issues for this pretreatment method as well.⁶⁴ It is an advantage that solvents can be recycled and reused and, when compared to the Kraft process, produces less air and water pollution.⁶⁵ However, as the name implies, organosolv utilizes a large quantity of organic, highly flammable and potentially toxic solvents at elevated temperatures and pressures presenting concern for safety.⁶⁶

Ionic liquids are neoteric solvents that have demonstrated interesting applications for lignocellulosic biomass pretreatment as well as a diverse range of additional uses. They can be used in extraction chemistry processes in multiple ways including as solvents and catalysts potentially performing chemical modifications and transformations of lignocellulosic biomass into other platform chemicals and materials. Pretreatment applications of ionic liquids are numerous and adaptable to different feedstocks and processes but can be generally divided into dissolution or solubilizing processes. Dissolution of biomass feedstock is the most widely studied and employed technique where the goal is the regeneration and recovery of the cellulose for fermentation.⁶⁷ The ability to dissolve cellulose is also important in allowing for further chemical processing and transformation of cellulose to other desired compounds. Solubilizing lignin is the second method and disrupts the lignocellulose composite rather than

dissolving it completely.⁶⁸ As with other pretreatment methods, optimization is required for different feedstocks, conditions and different ionic liquids.

1.3 Ionic liquids

Ionic liquids are salts that have a low melting point, usually below 100 °C where the irregular shape of poorly coordinated cation and anion pairs prevents the formation of a crystalline structure.⁶⁹ Ionic liquids also have low vapour pressures and are electrically conductive, and in some cases can be recycled and reused. Because of these exploitable properties, ionic liquids have generated a great deal of interest (see Figure 13). The seemingly limitless number of anion and cation pairings allow for customization and flexible applications in various industries. Ionic liquids have been used for catalysis, synthesis, electrochemistry,

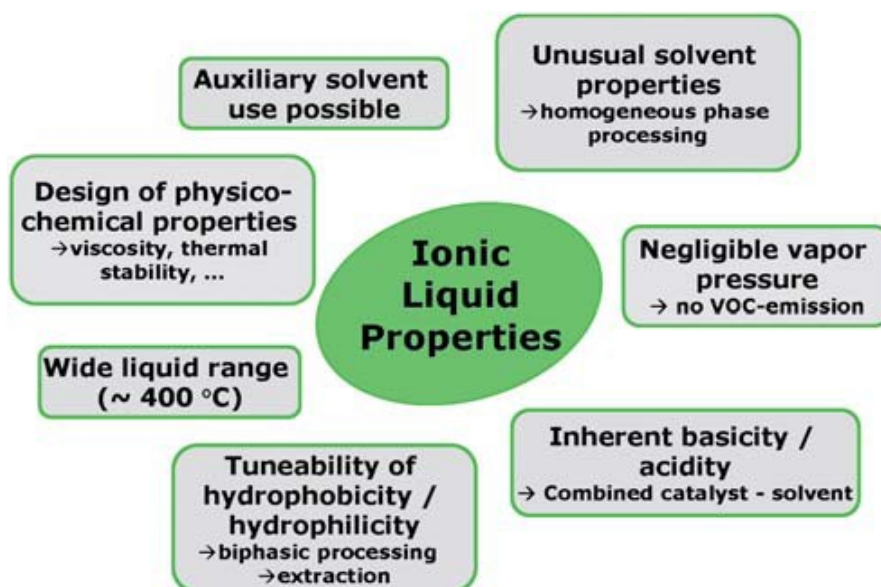


Figure 13 Common properties of ionic liquids.⁷⁰

as well as biorefining.^{71,72} Low vapour pressures and excellent solvating abilities have made ionic liquids attractive as a recyclable replacement to traditional organic solvents.⁷³ However, they have yet to be broadly adopted for commercial use. One of several hurdles to commercialization of ionic liquids is their production cost, along with environmental safety hazards, and toxicity. If ionic liquids are ever to have a feasible commercial/industrial presence or if they are to be considered as replacements to current chemical processes, then health and safety issues must be addressed. Low vapour pressure and high thermal stability make ionic

liquids extremely attractive during a reaction but, these properties become a disadvantage when the time comes to dispose of the ionic liquid. Because there are so many potential ionic liquid cation anion pairings, only nascent attempts at generalizing toxicity and environmental impacts have been made.⁷⁴ Less than successful attempts at generalisation make it clear that there are no set guidelines and protocols for assessing the hazards. Disposal of ionic liquids have implication on aquatic environments as many ionic liquids are soluble in water.^{75,76} Some of these generalizations use hydrophobicity/hydrophilicity and anion/cation functionalities to generalize toxicity. For example:

- Protic ionic liquids are less toxic, more biodegradable than their aprotic counterparts
- Hydrophobic ionic liquids have higher toxicities than hydrophilic ionic liquids due to their propensity to interfere with the phospholipid bi-layer of cell membranes.^{74,77}
- The structure of the cation has a greater impact on cytotoxicity, with branched cations being more dangerous, while the introduction of a polar group on the cation reduces toxicity.

Several different tests have been used to determine ecotoxicity (see Figure 14). Though these tests can give some insight into the potential impacts they individually are incomplete.

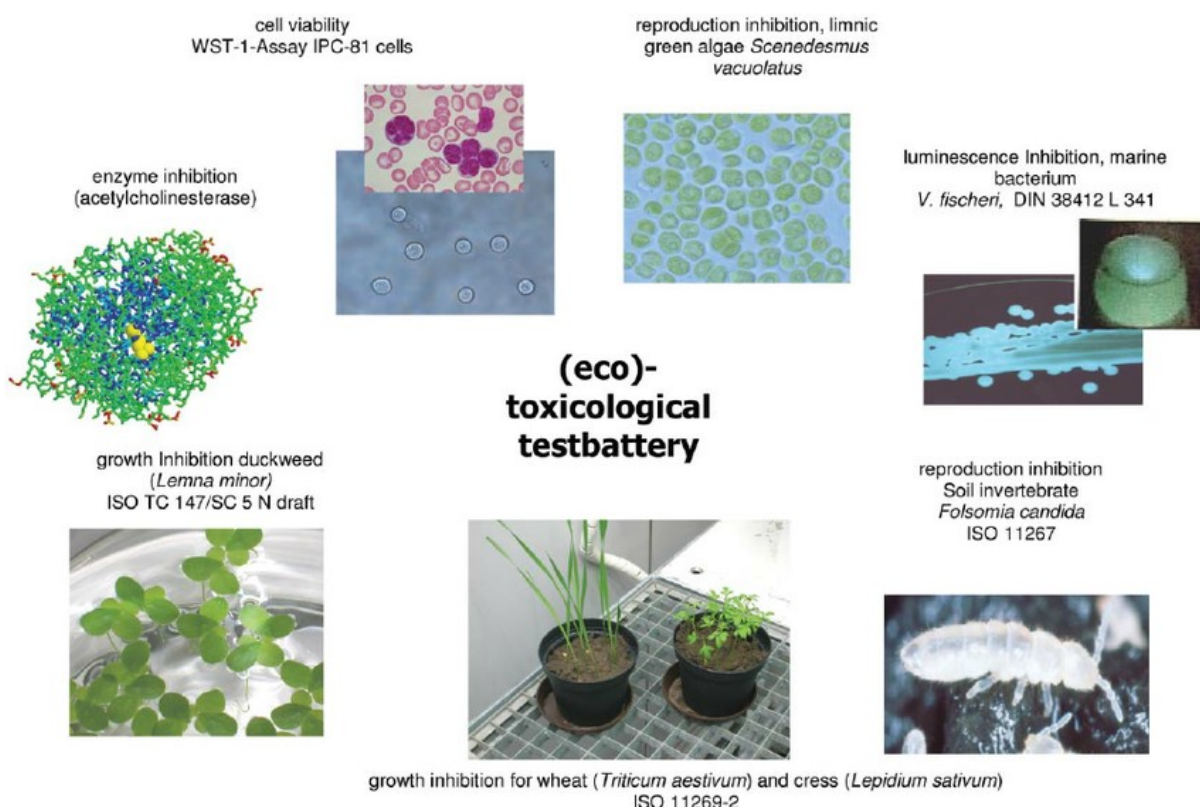


Figure 5 Various tests that can be used to assess ecotoxicity. Growth rate as well as inhibition behaviours are used to measure toxicity.

Different organisms have varying metabolic pathways and what may appear to be a “safe” result for one organism is not the case for another. In addition, damage to an organism may not only occur on the cellular level. Hormonal, growth, and behavioural changes may also be a side effect of exposure and may have impacts to the species beyond the foreseeable future. A complete understanding on the bio-persistence or bio-accumulation of ionic liquids in the environment has yet to be developed.⁷⁸ Studies to date has been short and conducted on a lab scale with small aquatic organisms such as zebrafish or *Daphnia magna* and are not representative of real life scenarios.^{79,80}

In one study, ionic liquids were created using biodegradable starting materials that paired biodegradable anions with biodegradable cations.⁸¹ The study hypothesised that these ionic liquids would be easily digestible by microorganisms based on their original constituents.⁸² However, these ionic liquids failed to meet the Organization for Economic Cooperation and Development (OECD) standards for ready biodegradability. In addition, depending on the ionic liquid and the microorganism used, by-products produced by the bacteria’s metabolism was not always benign.⁸³ Despite the unknown aspects of the use of ionic liquids, significant research continues to understand their ability to provide industrial efficiencies while reducing or removing the environmental impact created by traditional organic solvents.

1.4 Ionic liquids and lignocellulosic biomass

Ionic liquids can be used for a variety of applications during biorefining of lignocellulosic biomass (see Figure 15) and it is critical to understand which ionic liquids are best suited for each task. Studies have demonstrated that different ionic liquids are better suited for interaction and dissolution of certain components of the lignocellulosic composite. Models have been used to predict which ionic liquids would be the most promising for a chemical process depending on the properties of that ionic liquid. These dissolution studies have been conducted on individual components (cellulose, hemicellulos, or lignin).⁶⁷ However, lignocellulosic biomass is a significantly more complex composite with many different types of inter/intramolecular interactions. An ionic liquid which demonstrated effective dissolution of cellulose may not have the same level of success when applied to the entire biomass composite.⁸⁴ Similarly, conclusions drawn from these studies on the independent biopolymers are valuable but cannot be used to predict the success on the entire lignocellulosic biomass composite. Other aspects of pretreatment can impact the outcome of the process. Particle size, moisture content of the biomass and the ionic liquid, as well as the type of biomass used all

can impact final results of the process.⁸⁵ In addition to these variables, methods to reduce particle sizes of the feedstock may also disrupt the native structures of the biomass and further impact the results of the chemical pretreatment.⁸⁶ Therefore, optimizing an ionic liquid for any chemical process requires study of multiple aspects of the lignocellulosic biomass and in particular, how each may impact biorefining feasibility.

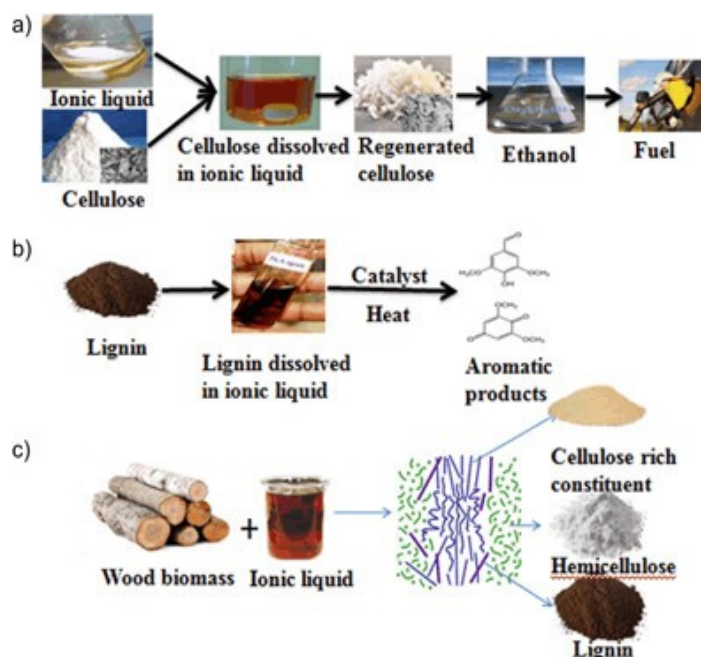


Figure 6 Various methods in which ionic liquids can be used for lignocellulosic biomass processing

1.4.1 Ionic Liquids and Cellulose

Solvents with the ability to solvate cellulose are important to the modification and production of platform chemicals from cellulose, but broadly applicable methods remain elusive. Ionic liquids' customizability and environmental benefits make them strong candidates to replace other solvents by both improving performance and reducing impact on the environment or improving safety. There are few organic/aqueous solvents that can accomplish dissolution with the exceptions of N-methylmorpholine-N-oxide (NMO) (which is used in the production of Lyocell, a type of rayon fabric) and concentrated phosphoric acid.^{87–89} These two solvents are currently limited by incompatibility with downstream processes in a biorefinery. Modification and increase in solubility have also been achieved using an alkali solution with the addition of carbon disulphide to produce Viscose (another type of synthetic

fibre, which can be produced from plants like bamboo and grasses). However, this process to produce sodium cellulose xanthate uses flammable and toxic reagents.⁹⁰ Ionic liquids have become an attractive alternative to other solvation methods because of their low volatility, relatively good thermal stability, and recyclability. Ionic liquids like 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) have demonstrated the ability to dissolve up to 25 wt% cellulose.⁹¹ Ionic liquids could be used to replace other solvents to improve safety, sustainability, as well as lower costs for existing materials like Viscose. Other industrial processes like Ioncell-F are already researching the use of ionic liquids to sustainably transform old textiles, pulps, and newspapers into biodegradable fibers.

Ionic liquids such as [BMIM]Cl or 1-ethyl-3-methylimidazolium acetate [EMIM]OAc cause swelling of the lignocellulosic biomass.⁹² Upon addition of an antisolvent, the native cellulose I fibrils are transformed into a thermodynamically more stable cellulose II. Cellulose II is easier to hydrolyse to monomeric glucose due to significant reduction in crystallinity and increase of amorphous areas during conversion from native cellulose I. Ionic liquids that contain imidazolium cations with strong hydrogen bonding paired with halide, acetate, dialkylphosphates, and trialkylphosphonates can disrupt the hydrogen bonds of the glycosidic bonds at the equatorial regions of the cellulose chain and thereby increase the cellulose's solubility (see Figure 16).^{91,93}

Computer modelling using various experimental parameters like Kamlet-Taft polarity and quantum mechanical COSMO-RS (Conductor-like Screening Model for Realistic

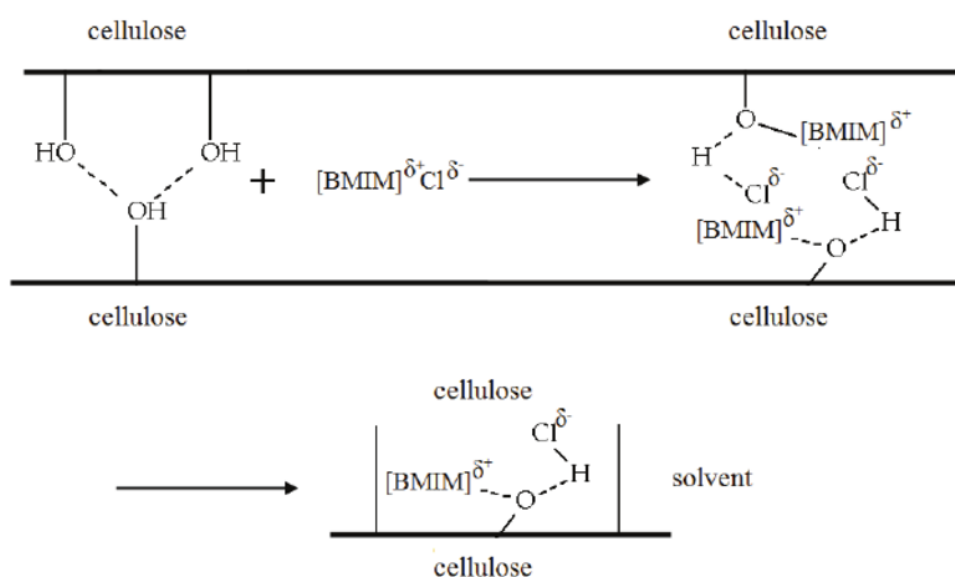


Figure 16 Proposed mechanism for the dissolution of cellulose with [BMIM]Cl involving the disruption of hydrogen bonds.⁹⁵

Solvents) have been used for screening and predicting ionic liquid performance. Kamlet-taft parameters are composed of three measurements: α (hydrogen bond donor) or acidity measurement, β (hydrogen bond acceptor) or basicity measurement, and π^* (polarizability). When looking at solubility of cellulose in an ionic liquid the focus is on the anion or β parameter and ionic liquids that can dissolve cellulose generally have a high β value, however size and geometry of the anion must also be considered.⁹⁴ For example, [BMIM]Cl has a $\beta = 0.80$ is effective at dissolving up to 25 wt% cellulose, while 1-butyl-3-methylimidazolium-O,S-dimethylphosphoroselenoate ([BMIM]dmpSe) which has a $\beta = 0.82$ does not dissolve cellulose. COSMOS-RS predictive modelling can be used to predict solubility by also examining the added influence of the cation (α), suggesting that solubility can also be linked to the differences between these two parameters.

Cations may disrupt the glycosidic and hydroxyl oxygens via dispersion forces and hydrogen bonding at the axial positions of the cellulose fiber.⁹⁶ Correlations have been found between the acidity of the cation and the solubility of cellulose.⁹⁷ Functional groups attached to the cation will impact cellulose solubility while the addition of functional groups like hydroxyl and ether groups decreasing cellulose solubility. The ability of the cation to aid in cellulose dissolution is linked to its' ability to hydrogen bond to hydroxyl and ether oxygen bonds of cellulose, thus increasing the length of the alkyl chains will also reduce cellulose solubility.⁹⁸ Though these interactions with the cation can aid in cellulose dissolution, the anion (β) remains the significantly greater factor in determining cellulose solubility, and is by far a greater contributor to cellulose solubility.⁹⁸

While the use of these ionic liquids to dissolve and regenerate the native cellulose to cellulose II may solve many of the problems for downstream bioethanol and platform chemical production, further research is required to overcome the obstacles required for broader commercial use of ionic liquids. The most significant issues for using these ionic liquids for cellulose dissolution at an industrial level are its production cost and the requirement for dry conditions for most ionic liquids.⁹⁹ Additionally, they have relatively low thermal stabilities compared with other ionic liquids that can be used for pretreatment.¹⁰⁰ This would mean that to utilize these ionic liquids a great deal of cost and energy would be necessary for the drying and recycling process.

1.4.2 Ionic Liquids and Hemicellulose

The focus of current research concerning solubility of lignocellulosic biomass has mainly been focused on cellulose leaving the questions of the solubility of hemicellulose less explored. The structure of hemicellulose is not as ordered as cellulose and is not crystalline with fewer hydrogen bonds and lower molecular weights making it easier to depolymerize. Though cellulose generally requires a high hydrogen bond basicity value for dissolution this is not that case for hemicellulose. In fact, ionic liquids that can dissolve cellulose are also effective for hemicellulose dissolution, although generally, hydrophilic ionic liquids exhibit better performance with hemicellulose than hydrophobic solvents.¹⁰¹ The number of hydroxyl groups will also impact solubility. Xylan with two hydroxyl groups is relatively less soluble in the same ionic liquid as dextrin which has three hydroxyl groups. Solubilities from 3-25% have been achieved with 1-allyl-3-methylimidazolium chloride ([AMIM]Cl).¹⁰² Lowering the β (below 0.85), often with the addition of water, resulted in the selective extraction of hemicellulose from delignified pulp.^{103,104}

1.4.3 Ionic Liquids and Lignin

Exploring the solubility of lignin in ionic liquids is significantly more complicated than the study of either cellulose or hemicellulose. Extracted lignin is a ready source for study (frequently conducted on Kraft or organosolv lignin) but is structurally different than native lignin and thus solubility tests do not actually represent the solubility of native lignin.^{19,67} However, if a reaction incorporating the entire lignocellulosic biomass is used to determine the solubility and extraction yield, the other components of the biomass will impact the results. This makes lignin the most difficult of lignocellulosic composite components to characterize. The configuration of the ionic liquid, the choice of feedstock, and pretreatment conditions are all variables that will impact the dissolution of the native lignin.⁶⁷

Lignin dissolution has been shown to be dependent on both the cation and anion. Varying the cation of an ionic liquid, and especially aromatic cations, can have great effects on the solubility of lignin.¹⁰⁵ The addition of functional groups on the ring of the cation seems to impact the interaction of the cation with the lignin. Additions of methyl groups, for example, will cause the delocalized electrons to congregate above and below the ring. This can cause competition between the anion and cation for access to the lignin.¹⁰⁵ Dispersion-corrected

density functional theory (DFT-D) modelling of ionic liquids suggests that imidazolium ionic liquids may be the most effective for use in lignin dissolution due to π - π stacking and hydrogen bond interactions. The ionic liquid basicity value required to solvate lignin is much lower than that of cellulose. Ionic liquids such as 1-ethyl-3-methylimidazolium trifluoromethanesulfonate ([EMIM][OTf]), which has a $\beta = 0.50$, has been demonstrated to dissolve lignin up to 55 wt%.⁹⁴ Protic ionic liquids have been successful in depolymerizing lignin via acid catalysed cleavage of β -O-4 bonds (see Figure 17).¹⁰⁶ There are several protic ionic liquids that are cost competitive and would have the potential to be used on an industrial level, making them attractive for the use in a biorefinery.¹⁰⁷ However, the protic ionic liquid pretreatment of biomass can lead to the production of various degradation products that are detrimental to downstream processes, reducing saccharification yields.¹⁰⁸ Further examination of the intermediates and their interactions is required to successfully adapt ionic liquids for broader use.

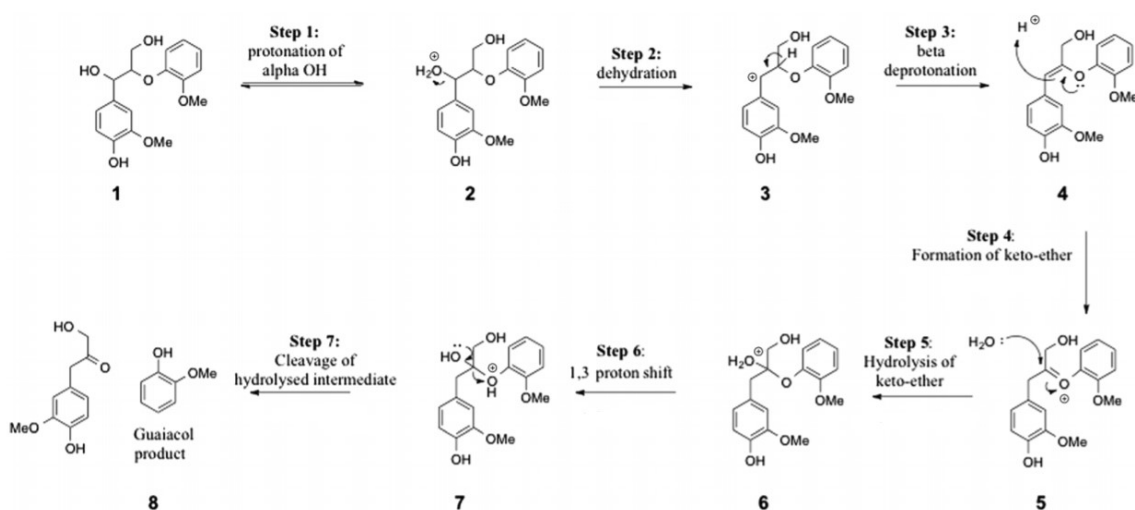


Figure 17 Proposed mechanism for depolymerization of lignin via a HSO_4^- anion ionic liquid.¹⁰⁹

1.5 Lignin Depolymerization Methodologies

Understanding some of the lignin depolymerization methods can potentially shed light on the lignin fragments produced during pretreatment. In organosolv processing, the depolymerization of lignin using a copper porous metal oxide catalyst (Cu-PMO) can provide clues to the types of aromatic compounds or fragments that occur during lignin depolymerization.¹¹⁰ In these trials lignin from candlenut nutshells was extracted via a methanol extraction. The extracted lignin was placed on to the Cu-PMO in the presence of H_2 which resulted in lower molecular weight compounds. The copper minimized the formation of char. Four major 9 carbon catechols (4-propyl)catechol, 4-(3-hydroxypropyl) catechol, 2,3-

dihydro-1H-indene5,6-diol, 4-(3-methoxypropyl)-catechol) were formed during this depolymerization (see Figure 18). Though this process is quite different than the pretreatment using ionic liquids, these trials do suggest that give an idea of possible lignin fragments that may be present in the liquor.

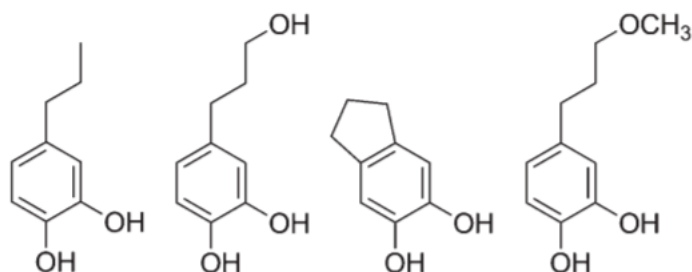


Figure 7 Four major catechols produced from the depolymerization of lignin using the organosolv method (in this case, solvent used was methanol) confirmed via GPC as well as NMR.

Dilute acid pretreatment (DAP) may provide a similar insight into lignin fragments that can be produced. This is of special interest since this project will be utilizing a protic ionic liquid which may produce similar lignin fragments. Investigations have been conducted into the deconstruction of the lignin polymer during DAP from poplar and switchgrass. DAP lignin tends to coalesce into aggregates and relocate on the surface of the biomass, this is not entirely surprising as lignin itself is not soluble in aqueous media and the reaction temperatures are generally above the glass transition point of lignin. DAP drastically reduces the molecular weight of the lignin as the acid cleaves the major linkages. NMR shows a decrease in β -O-4, β -5/ α -O-4 phenylcoumaran, resinol(β - β) units.¹¹¹ There is also an increase in aliphatic and phenolic groups which is attributed to the cleavage of aryl ether bonds which results in the increase in low molecular weight lignin that is soluble in an aqueous environment. Depolymerization, then repolymerization reactions result in an increase in condensed lignin (see Figure 19). As the pretreatment times progressed, there was an increase in

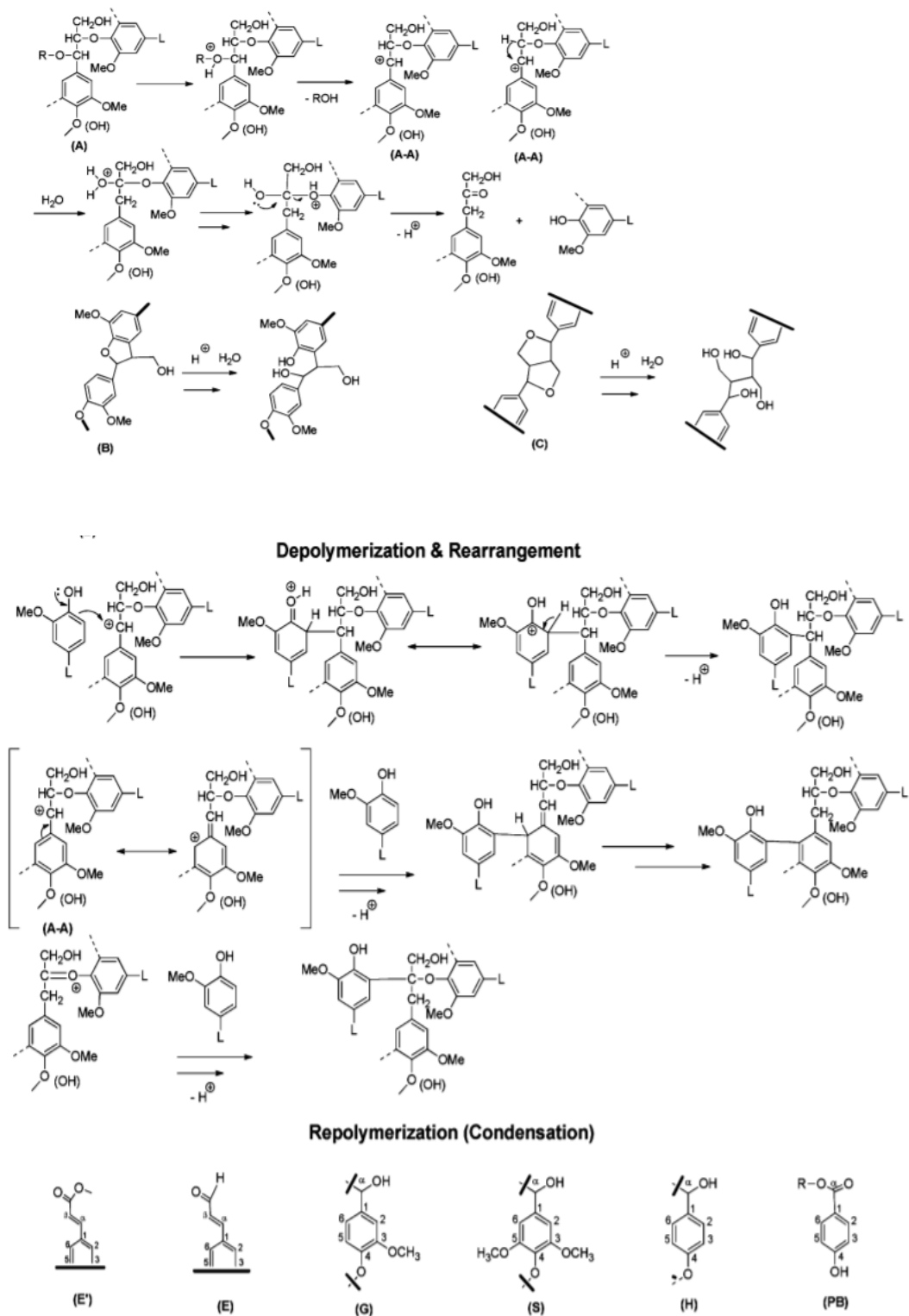


Figure 8 Possible mechanisms for depolymerization of lignin during dilute acid pretreatment (top). Depolymerization and rearrangement of lignin (middle) and the potential condensation motifs from repolymerization (bottom). (A) β -O-4 ether; (B) phenylcoumaran; (C) resinol; (G) guaiacyl; (S) syringyl; (H) *p*-hydroxyphenyl; (PB) *p*-hydroxybenzoate; (E) cinnamaldehyde; (E') *pcoumarate* unit.

repolymerization/condensation reactions, resulting in an overall decrease in the aliphatic hydroxyl groups, total phenolics, and G unit lignin fragments.

A severity factor parameter for pretreatment was originally introduced to reduce the number of experiments needed to determine optimum conditions. The parameter combined temperature, pretreatment time, and in some cases factored the addition of pH into the calculation.¹¹² The combined severity factor (CSF) is calculated as follows:

Where (*t*) is time and (T) is temperature in Kelvins.

$$CSF = \text{Log}_{10} \left[t \times \exp \left(\frac{T - 100}{14.75} \right) \right] - pH$$

The increasing of the CSF leads to more condensation reactions and the production of humic substances during carbohydrate pretreatment.¹¹²

In the depolymerization of lignin, phenylpropane type monomers known as Hibbert ketones have been demonstrated to form and may be an important component in pseudolignin formation. There are two major depolymerization pathways, one that results in an aldehyde and another in the formation of a ketone. As mentioned, the rate of acid-catalysed depolymerization is dependent on the acid used with HBr>HCl>H₂SO₄. HBr and HCl are more likely to produce the ketone version, while H₂SO₄ results in the creation of the aldehyde version as well as condensation product with other lignin fragments.¹¹³

The reason that these two pathways exist is because there are two potential routes for the depolymerization of lignin at the β-O-4 position. One is the conversion of the starting compound to the enol ether and then subsequent cleavage to the phenol and Hibbert ketone compound. Another possibility is the direct cleavage of the starting material to the two end products. One of the generally accepted mechanisms for the depolymerisation of lignin in acidic conditions occurring at the β-O-4 position involves the formation of a benzyl cation and an enol ether structure (see Figure 20 – II and III). The enol structure can be hydrolyzed into a Hibbert ketone (Figure 20 -- V). A potential competing route would generate a formaldehyde (Figure 20 – VII). It has been suggested that the rate determining step is the enol formation step.

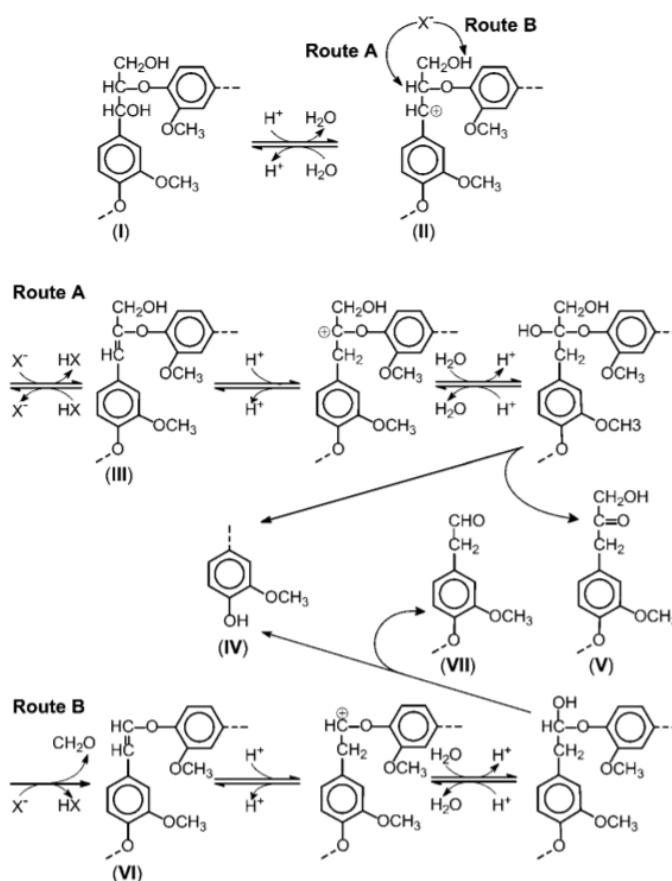


Figure 20 Potential mechanisms for the depolymerization of lignin at the B-O-4 position.¹¹⁴

To investigate the mechanism of cleavage model compounds: 2-(2-methoxyphenoxy)-1-(3,4-dimethoxyphenyl)ethanol (VIII), 1-(2-methoxyphenoxy)-2-(3,4-dimethoxyphenyl)ethylene (IX), 2-methoxyphenol (X), and 3,4-dimethoxyphenylacetaldehyde (XI) have been used to mimic lignin depolymerization (see Figure 21). Acid hydrolysis of (VIII) resulted in the formation of (X) and (XI) in almost equal quantities. When the same reaction was conducted with compound (IX) there was no detectable presence of (VIII) which also resulted

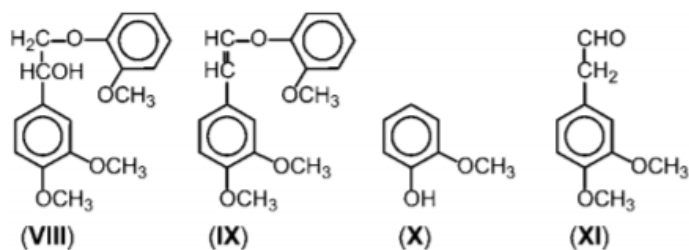


Figure 9 Starting model compounds used to investigate the depolymerization of lignin

in the formation of (X) and (XI). This demonstrated that the enol does not revert to the β -O-4 compound prior to undergoing hydrolysis. Kinetic analysis revealed that compound (VIII) formed the enol intermediate prior to cleavage into the phenol and Hibbert ketone product. Acid type is an important variable in the dissolution process. Deuterated acids were used to

explain the dependence of the depolymerization rate on the type of acid used. The protonation of the alpha hydroxyl group followed by the release of water creating a benzyl carbocation determined the rate of dissolution. Abstraction of the β -proton which forms the enol ether compound is an irreversible rate determining step.¹¹⁵

1.6 Biorefining: Materials and Platform Chemicals

There is great interest and potential for lignocellulosic biomass to significantly reduce the environmental cost and impact of many industrial processes. Materials, fuel, and platform chemicals which are currently derived almost entirely from petroleum refining could potentially be replaced by lignocellulosic biomass.¹¹⁶ Ionic liquids' customisability and ability to interact with the components of the biomass composite make them the subject of significant research for biorefining (see Figure 22). This versatility allows for multiple value-added streams in a single ionic liquid pretreatment biorefinery plant with the production of valuable platform chemicals from some of the waste products of bio ethanol production. Hexose

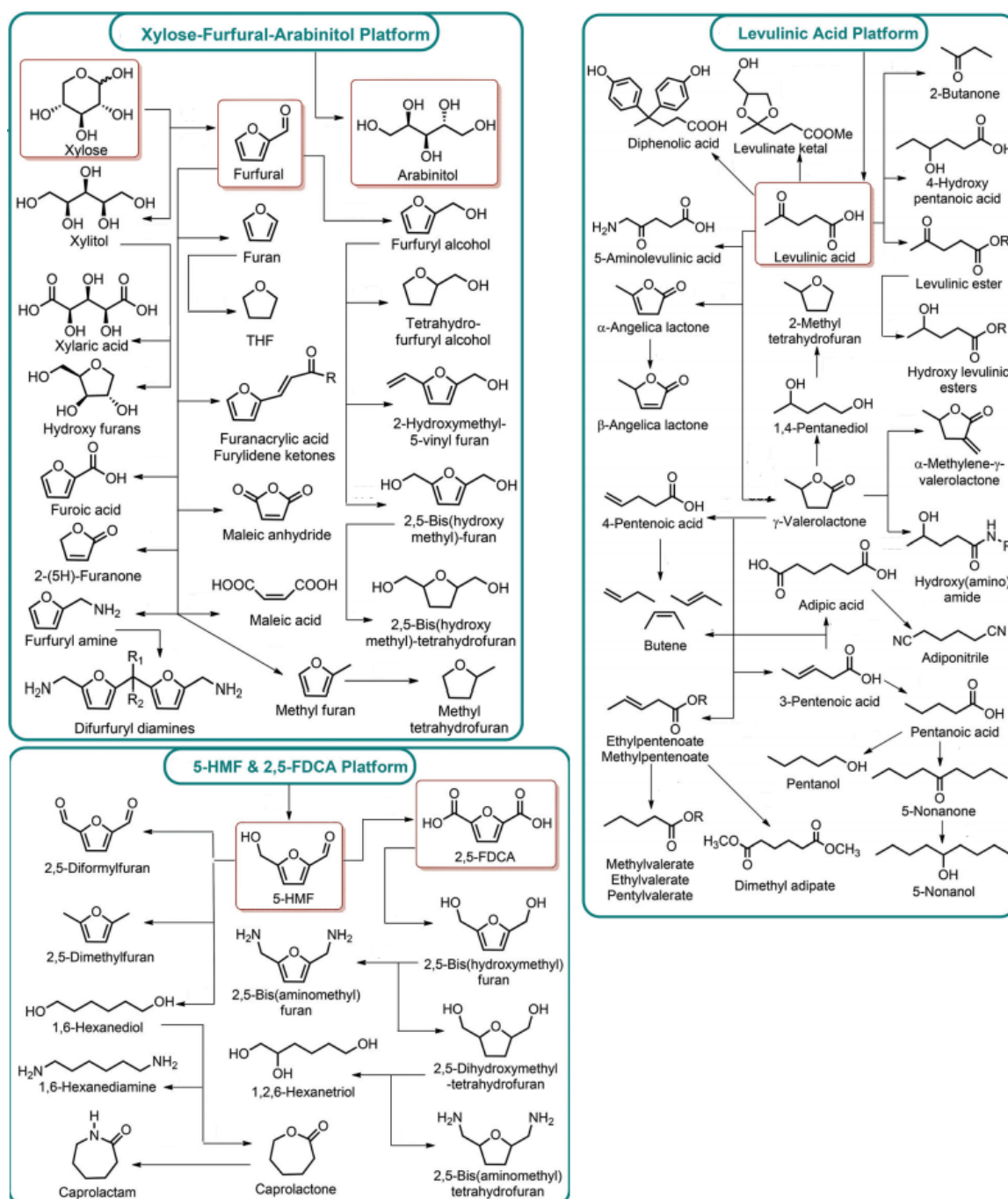


Figure 10 Various platform chemicals that can be derived from lignocellulosic biomass carbohydrates. Some uses for these products include: fuel additive and polymeric building blocks

carbohydrates (galactose, mannose, and glucose) from lignocellulosic biomass can be transformed to 5- hydroxymethylfurfural (HMF), levulinic acid (LA), 2,5-dimethylfuran (DMF), sorbitol, and 2,5-furan dicarboxylic acid (FDCA). All of these chemicals have been listed by the US Department of Energy (DOE) as “top value-added chemicals from biomass”.²² Pentose sugars such as xylose and arabinose can be converted to furfural and xylitol.

Projected values for these compounds demonstrate a clear trend in growth and demand for these platform chemicals (table 2). Levulinic acid and furfural is projected to realize an

almost 50% growth in the next 3-5 years. It is clear that sustainable and environmentally friendly methods for producing energy and materials will be needed. Lignocellulosic biomass has the flexibility to meet these challenges.

Table 2 Current and projected market value of platform chemicals that could be produced from lignocellulosic biomass

	Current Market Value	Projected Growth
Bioethanol	\$5.6 billion USD as of 2015	\$9.5 billion USD by 2022 ¹
HMF	\$120 million USD as of 2017	\$145 million USD by 2022 ²
Furfural	\$663 million as of 2015	\$1.43 billion USD by 2022 ³
Levulinic Acid	\$130 million in 2019	\$250 million USD by 2024 ⁴
Lignin	\$782 million in 2014	\$985 million USD by 2023 ⁵
Toluene	\$ 16 billion in 2016	\$23.4 billion USD by 2023 ⁶
m-Xylenes	n/a	\$1.34 billion USD by 2023 ⁷

1.6.1 Ionic liquids – Cellulose materials and composites

The role of cellulose in plant structure is to provide strength and durability.¹¹⁷ These mechanical properties are what make cellulose attractive for materials production. In addition, cellulose is biocompatible, non-toxic, biodegradable, and the most abundant naturally occurring polymer on earth.¹¹⁸ Though cellulose is not soluble in most organic or aqueous solvent, some ionic liquids have demonstrated the ability to not only dissolve but provide a method to aid in modification of the biopolymer for production of cellulosic materials. From explorations of solubility above, we have shown that imidazolium chloride/acetate ionic liquid have relatively high cellulose solvating abilities. By solvating the cellulose one-pot synthesis, chemical modification of the polymer, and production of platform chemicals may be possible.¹¹⁹ Ionic liquids can be used to effect multiple surface modifications such as grafting, esterification, and amination (see Figure 23). These surface modifications can change the

1. Source: <https://www.alliedmarketresearch.com/bioethanol-market>;
2. Source: <https://www.reuters.com/brandfeatures/venture-capital/article?id=70025>
3. Source: <https://www.alliedmarketresearch.com/furfural-market>
4. Source: <https://www.marketwatch.com/press-release/levulinic-acid-market-2019-global-industry-insights-by-global-players-regional-segmentation-growth-applications-major-drivers-value-and-foreseen-till-2024-2019-04-03>
5. Source: <https://www.globenewswire.com/news-release/2019/04/05/1797716/0/en/Lignin-Market-to-attain-US-985-5-Mn-valuation-by-2023-Product-Innovation-to-Stimulate-the-Growth-TMR.html>
6. Source: <https://www.alliedmarketresearch.com/toluene-market>
7. Source: https://www.researchandmarkets.com/research/ww8khv/2018_metaxylene?w=12

hydrophobicity/hydrophilicity in cellulose fibres as well as prevent aggregation and improve dispersion of nanocellulose particles in solutions.¹²⁰

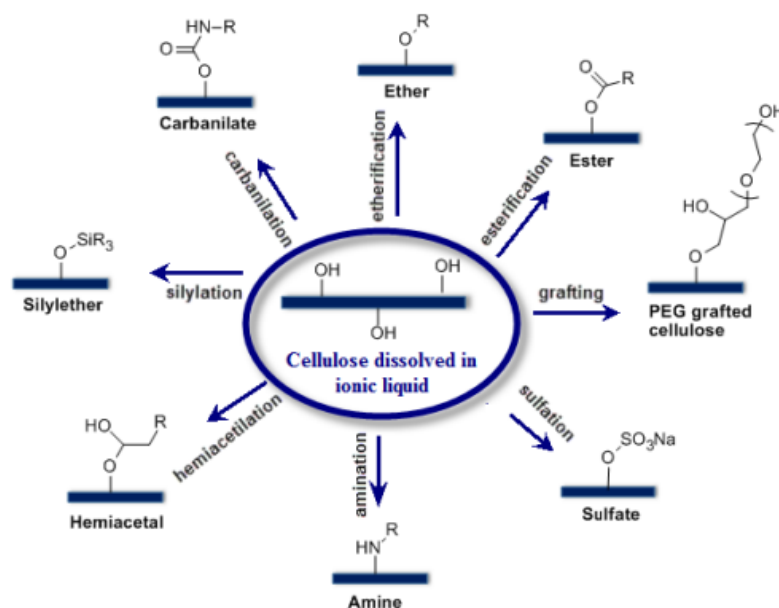


Figure 11 Various modifications that can be performed using ionic liquids to the surface of cellulose to alter its properties such as hydrophobicity/hydrophilicity.

Ionic liquids' ability to modify polymers could be an important enabler for cellulosic materials to have a significant impact on many industries. Some of the industry and materials/chemicals possibilities include: the pharmaceutical/medical industry as a method for biocompatible/non-toxic drug delivery that are responsive to thermal cues.¹²¹ Cellulose based bio adhesives and drug delivery applications are other opportunities.¹²² In building and construction, the production of cellulose-based composites provide improved strength and durability.^{123,124} Production of textiles from an increased range of biomass sources have the potential for higher thermal stability, mechanical strength, and chemical resistance.¹¹⁹

Another opportunity for ionic liquids to replace current protocols is in the production of nanocrystalline cellulose (NCC) and microcrystalline cellulose (MCC), where acid hydrolysis is most commonly used.^{120,125} It has been demonstrated that acidic ionic liquids can produce NCC from microcrystalline cellulose.¹²⁶ These cellulose fibres have high aspect ratios and have lengths up to 1000 nm and width between 10-20 nm. Controlled surface modification of the NCC can occur during hydrolysis altering the hydrophobicity of the NCC. In this case, acetylation of the NCC surface occurred with the addition of acetic anhydride.¹²⁷ Grafting of polyisoprene using 1-allyl-3-methylimidazolium chloride [AMIM]Cl demonstrated the ability

to use renewable resources to produce compounds that were previously derived via petroleum feedstocks.¹²⁸

1.6.2 Ionic liquids- lignin materials and composites

The most abundant, naturally occurring aromatic polymer, lignin, is currently regarded as a waste by product of biomass processing and burned for energy recovery.¹²⁹ However, anticoagulants, adhesives, and emulsifiers are all potential uses for lignin. Additionally, fuel and platform chemicals are achievable via pyrolysis and gasification.^{130–132} There is an increase interest in research to upgrade this aromatic biopolymer to produce materials and chemical compounds. Ionic liquids can be used to perform these chemical modifications. Ionic liquids can also be used to dissolve and or extract the lignin from biomass for use for further downstream processing.

Some of the materials that have been produced from lignin include high-strength carbon fibres where Ionic liquid 1,5-diazabicyclo[4.3.0]non-5-enium acetate [DBNH][OAc] was used to combine lignin and pretreated pulp. The quantity of lignin impacted the tensile strength, thermal stability, and elasticity of these fibres.¹³³ Lignin with the combination of amino acid based ionic liquids have demonstrated the ability to withstand shearing and frictional forces. This blended material can be used in lubricants with less corrosivity than conventional lubricants.¹³⁴ Other ionic liquids enable lignin blends to function as a reinforcing agent improving tensile strength and flexibility by improving mechanical and water vapor permeability of other polymers such as poly(butylene-adipate-co-terephthalate)–polylactide (PBTA-PLA).¹³⁵ The extensive list of example materials underscores the interest in ionic liquids research with the aim of developing better materials and chemicals with reduced cost to the environment by displacing fossil fuel sources.

1.6.3 Lignocellulosic biomass – The production of platform chemicals with ionic liquids

Lignocellulosic biorefining can produce value-add platform chemicals beyond bio-ethanol. The carbohydrate components of biomass have the potential to produce HMF and furfurals which are platforms for the synthesis of other valuable compounds, for polymer creation, and organic solvent replacements for industrial processes. Acid catalysed conversion is commonly used to produce these sugar dehydration products; however, these reactions suffer from low yield and the production of humins and other by-products.¹³⁶ The viability of producing platform chemicals from lignocellulosic biomass is dependent on high yields and

efficient production, while ensuring improvements on the environmental impact over existing methods.

The objective of yield improvement in the production of HMF or furfural from biomass carbohydrates requires the hydrolysis of the cellulose/hemicellulose polymer into their respective monomers. Catalysts, like chromium, iron, or aluminium salts have been shown to facilitate the isomerization of glucose to fructose.¹³⁷ In these reactions, acidity, water content, and temperature can increase the rate of transformation but also lead to the production of other sugar degradation products.^{138,139} Degradation products of pentose sugars are likely to impact secondary reactions and potentially reduce yields in some cases.¹³⁹

1.6.4 Platform Chemical: HMF

HMF is a dehydration product of glucose and can be used as a building block for a wide range of polymers and plastics. HMF can be produced in high yields from fructose and with limited yields (without a catalyst) from glucose. This suggests that the isomerization of glucose to fructose is a necessary step to efficiently produce HMF, however this also means it is not necessarily the only pathway to produce HMF (see Figure 24). Conversion to HMF using ionic liquids from fructose have produced yields up to 96% with no by-product or humin production detected. Extraction of the HMF can be performed using a biphasic system or resin which also aids in pushing the reaction towards product.^{140,141} This method of producing HMF is a promising alternative, but has not yet achieved feasibility due to the cost of fructose.

Conversion of glucose to HMF has been demonstrated using 1-ethyl-3-methylimidazolium chloride [EMIM]Cl.¹⁴² This ionic liquid, which is capable of dissolving cellulose, effectively dissolves glucose and when combined with a catalyst to facilitate the isomerization of glucose to fructose, produces HMF in reasonable yields (near 70%).¹⁴³ Without a catalyst, by-product humin formation occurs and HMF yields are predictably low

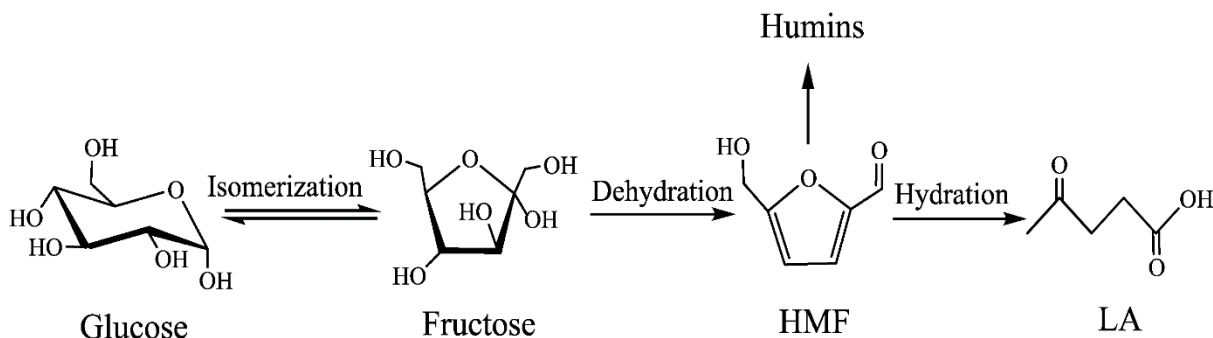


Figure 12 A proposed mechanism for the conversion of glucose to HMF and levulinic acid showing the isomerization of glucose to fructose.

(approximately 28%). Though isolated furanic compounds such as HMF and furfural are stable in ionic liquids, the presence of the other sugar degradation products frequently produced in these processes causes polymerisation resulting in humin formation.¹⁴⁴ The aim of minimising these other by-products to maximise HMF production has shown that the choice of ionic liquids will impact both yield, and humin formation.¹⁴⁵ Hydrogen sulphate ionic liquids produce good yields, when converting fructose to HMF, but lead to humin production when used for glucose conversion. The use of 1-butyl-3-methylimidazolium chloride [BMIM]Cl has produced near quantitative conversion with a chromium catalyst (90% and a 7 mol% catalyst loading). There were no detectable by-products in these trials.

Producing HMF from sugar oligomers has proven to be more difficult. When the same conditions were applied to cellobiose, conversion dropped from 90% to 50%.¹⁴⁶ This suggests that hydrolysis of cellulose to monomeric units would be necessary, adding an additional step/requirement for the transformation to HMF from lignocellulosic biomass.¹⁴⁷ Using water to perform the hydrolysis has been considered along with copper and chromium catalysts to attempt one pot conversion to HMF has been met with some degree of success (56% yield).¹⁴⁸ Additional attempts to hydrolyse cellulose have used acidic ionic liquids as both catalyst and solvent.¹⁴⁹ Low yield, sugar degradation, and humin formation were also results of these attempts to produce HMF from cellulose as well.¹⁴⁸

Once the reactions are completed, additional considerations impact the feasibility of the HMF production process. Recycling the ionic liquid and catalyst are a critical aspect though few publications are available on this topic. Some have suggested that purification and extraction can be performed using supercritical carbon dioxide or a biphasic extraction of the HMF from the ionic liquid.¹⁵⁰ Methyl isobutyl ketone (MIBK) has been used to form an ionic liquid/solvent biphasic system to extract HMF.¹⁵¹ Extracted HMF is better stored as a crystal than a liquid. Liquid HMF has been shown to rapidly degrade.¹⁵² The sustainability, production, and yields of HMF from these recycled systems remain important aspects of the process to be investigated.

1.6.5 Platform Chemical: Furfural

The production of furfural during pretreatment of lignocellulose biomass is a result of the dehydration of pentose sugars (see Figure 25). Xylose is abundant in all types of biomass and its dehydration produces various platform chemicals: ketones, acids, and aldehydes (furfural). Furfural has the potential for resin, polymer, and renewable solvent

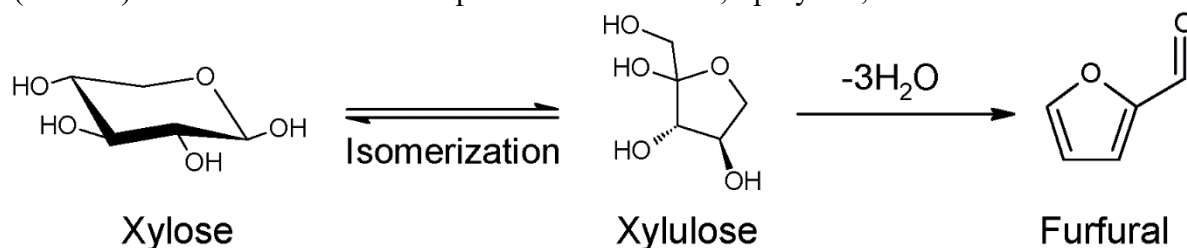


Figure 13 Proposed mechanism for the conversion of xylose to furfural. Similar to the production of HMF, an isomerization to xylulose is believed to be necessary step for conversion.

production.^{153,154} There are however, several challenges with furfural production. Furfural can self-polymerize and when produced in the presence of other compounds from biomass pretreatment, often results in the production of humins.¹⁵⁵

As with HMF production it is believed that an isomerization step of xylose to xylulose is required for furfural production, though other pathways have also been proposed (see Figure 26). Most metal catalyst that can successfully aid in conversion of glucose to HMF have similar

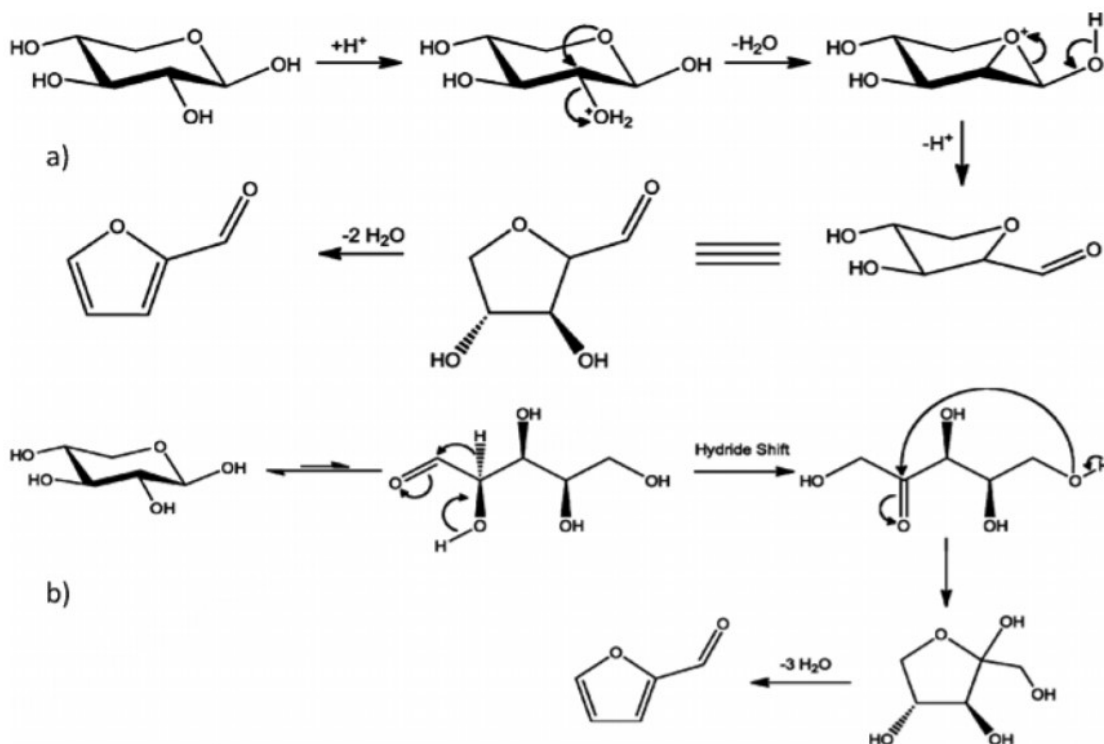


Figure 14 Possible pathways for the dehydration of xylose to furfural (a) via cyclic transformation and (b) acyclic mechanism which goes through xylulose.

success in aiding the transformation of xylose to furfural most likely due to their similar structures. Ionic liquid conversion of xylose to furfural with metal catalysts similar to HMF studies using microwave assisted reactions have been successful.¹⁵⁶ Also similar to HMF production, Acidic ionic liquids such as 1-butyl-3-methylimidazolium hydrogen sulfate [BMIM][HSO₄] have filled the role of catalyst and solvent for the production of furfural from xylose.¹⁵⁷ Though the conversion of xylose in this acidic ionic liquid was near quantitative, the actual yield of furfural was low due to the production of by-products and humin formation. Efforts to modify the cation of the ionic liquid have attempted to prevent humin formation. The thermal stability of the cation in the catalysing conversion of xylose to furfural was achieved through the addition of a sulfonic acid group¹⁵⁸. The use of a biphasic system to extract furfural as it was being produced has also seen success in improving furfural yields and reducing humin formation.¹⁵⁹

Research is currently being conducted on the separation, purification of product, and recycling of solvents for sustainable production of furfural from lignocellulosic biomass. However, HMF and furfural production directly from lignocellulosic biomass is not economically feasible. By-product/humin production, interactions with other components of biomass make purification and separation of product difficult. Achieving adequate quality of product, necessitates a multi-step innovative process design which includes both recycling and sustainability.¹⁶⁰ In addition to energy consumption issues, acidic ionic liquids would require industrial process equipment that can resist corrosion.¹⁶¹ Operational expenditures should factor in ongoing replacement costs.

1.6.6 Platform Chemical: Levulinic Acid

Levulinic acid is produced by the dehydration of HMF through a proposed intermediate 2-5-dioxohex-3-enal (DHE) (see Figure 27). Levulinic acid has a variety of different applications. It can be used as a building block for polymers, functionalized hydrocarbons for platform chemical production, and its dehydration product γ -valerolactone (GVL) can potentially sustainably replace some bulk solvent usages in industry.⁷⁸ Catalysed conversions of levulinic acid from cellulose is possible, though a lack of selectivity during the reaction process results in low yields (34.2%).¹⁶² In addition, separation and purification of product and catalyst can be another issue that add prohibitive cost to the process.¹⁶³

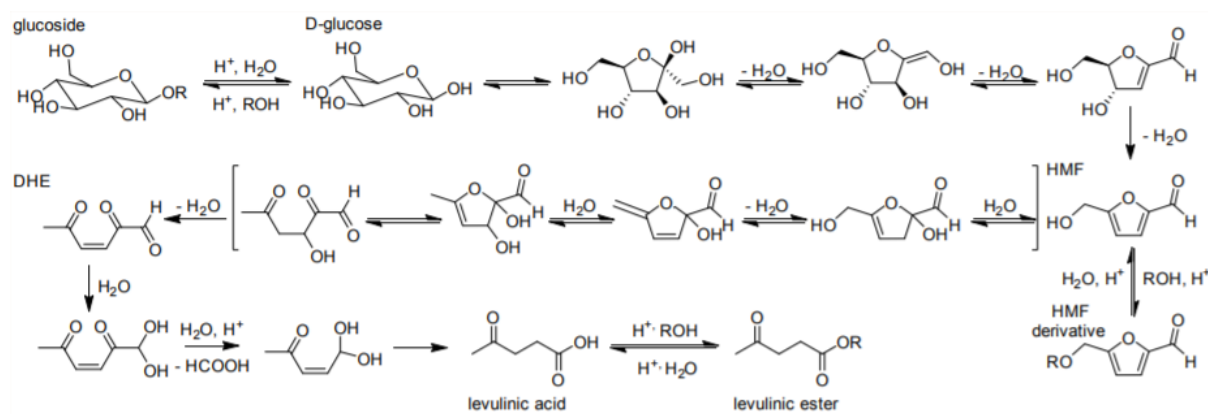


Figure 15 Proposed mechanism for the production of levulinic acid and levulinic ester from cellulose.

Levulinic acid forms within the ideal temperature range between 120 -160° C. At temperatures below 120° C the glucose will not convert and at temperatures above 160° C by-products and humin formation become prohibitively abundant.¹⁶⁴ Functionalization of the ionic liquids (such as with SO_3H) are usually needed to improve selectivity. The added benefit of using these ionic liquids is that they can often be used as both catalyst and solvent. In addition, levulinic esters can be produced with the addition of ethanol/water mixtures though water content of the reaction will also impact the viscosity and kinetics.¹⁶⁵

One pot synthesis of levulinic acid seems to be the goal of many researchers, using mineral acids and or functionalized ionic liquids to facilitate the transformation.^{166,167} Temperature, water content (viscosity), and catalysts (acidity) need to be balanced to optimize the reaction. However, even with efforts to optimise these variables, acidic reactions have resulted in the production of by-products such as glucose, HMF, furfural, lactic acid, formic acid, acetic acid and humins. Efforts to mitigate these some of effects added DMSO to stabilize HMF production and prevent humin production, however this resulted in the lowering of the levulinic acid yields.¹⁶⁸

1.6.7 Platform Chemicals: from Lignin

Lignin's status as the most abundant naturally produced aromatic compound on earth leads researchers to continually search for ways in which lignin maybe transformed to replace various organic solvents and petroleum-based products (see Figure 28). Research has initially focused on organic solvents like dioxane and pyridine for the production of platform chemicals from lignin.^{169,170} Ionic liquids (due to their ability solvate lignin) may potentially be used as a replacement for organic solvents when performing up-scaling lignin

transformation chemistry. Yields may improve through genetic modification of feedstocks with increased β -O-4 bonds, ratios of preferred subunits, and motifs which can be used to ease depolymerization.¹⁹ Tests have generally been conducted using imidazolium based ionic liquids and either kraft or organosolv lignins.

The majority of these studies have focused on transforming lignin monomers into aromatic compounds related to BTX (benzene, toluene, and xylene) (see Figure 28).¹⁷¹ These important petroleum refining derived chemicals are extensively consumed in global materials production. Ionic liquids can play many roles in producing these compounds depending on the

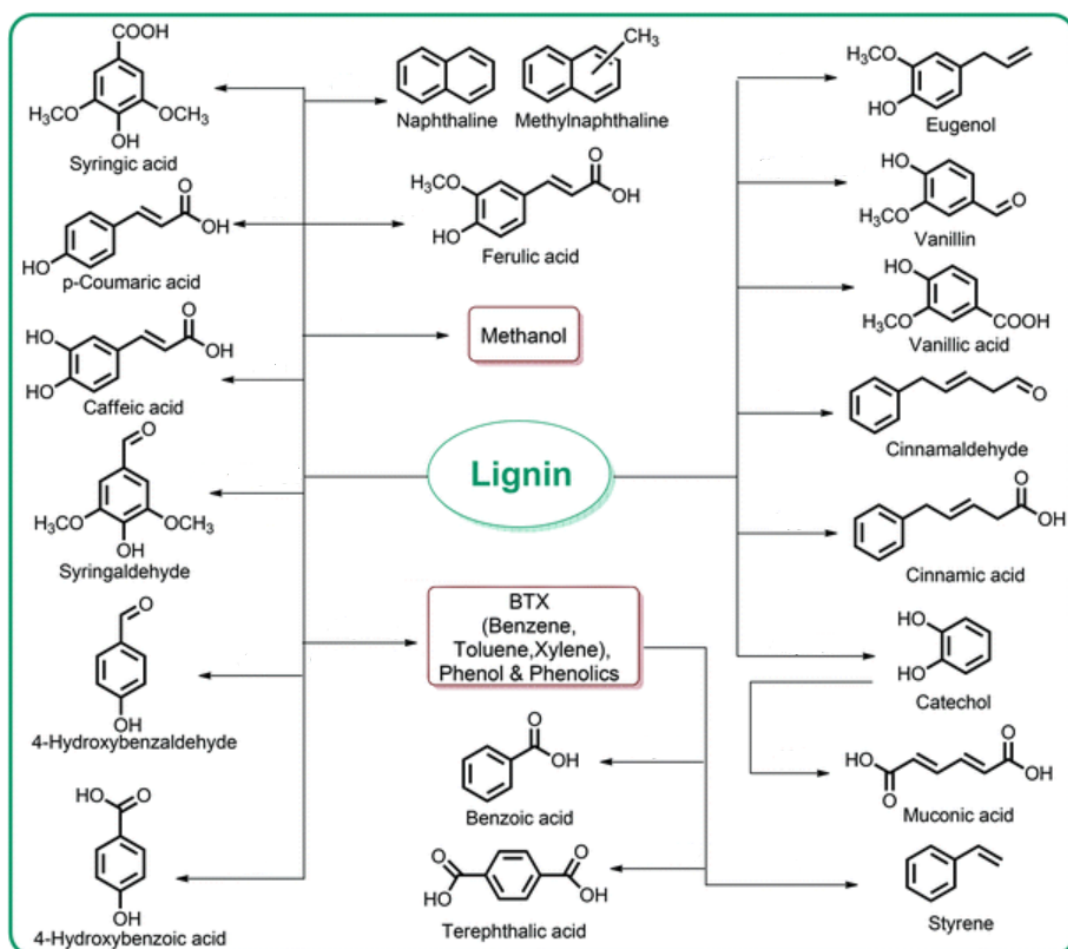


Figure 28 Lignin could be used to produce BTX (benzene, toluene, and xylene) which are currently produced as a part of the petroleum refining process.¹⁷²

reaction. One potential impact of ionic liquids is the stabilization of catalysts which may help facilitate the recovery and recycling of the catalyst. Products of the depolymerization of lignin are dependent on the feedstock and conditions used for the reaction and process specific optimization is required. Syringaldehyde, vanillin, and p-hydroxybenzaldehyde, syringol, sinapinic acid and syringic acid, catechols, and 2,6-dimethoxy-1,4-benzoquinone, are some of the aromatic compounds produced in addition to low molecular weight aromatic oils (see Figure 28). This is ideal if all of these are all desired products (separation and purification

steps would be required), however if they are not desired products the production of these products may reduce yield, generate waste and even contribute to unwanted side reactions.^{172,173}

Research combining ionic liquid depolymerization and simultaneous oxidation has also been investigated. The ability to oxidize lignin would be extremely valuable in providing renewable aromatic feedstocks. The anion cation pairing of the ionic liquid affects the outcome of the lignin depolymerization where cation choice impacts the lignin degradation while anion choice influences the extraction during H₂O oxidative depolymerization reactions.¹⁷⁴ The multiple motifs in lignin interact with imidazolium ionic liquids improving β -O-4 depolymerization depending on the imidazolium ionic liquid used. Free radicals also result from interactions with these ionic liquids aiding conversion of lignin to product.¹⁷⁵ Additional product customisation can be achieved through changing ionic liquids resulting in different molecular weights, polydispersity, and reaction depolymerization rates.¹⁷⁶ Adding catalysts further facilitate and direct selectivity of these reactions.¹⁷⁷

1.7 Humins

Humins as a classification cover a wide range of organic compounds. They are solid, brown, non-homogeneous substances that are typically discussed in soil science and carbohydrate chemistry.¹⁷⁸ For our purposes, the discussion on humins will focus on those produced via carbohydrate chemistry. Even within the study of sugars, the definition of humins remains vague. Rather than describing a specific substance formed in a specific reaction, the term humins is often used to describe aggregations of intermediates and their derivatives from various carbohydrate reactions. The lack of clarity in definition and formation increases the difficulty in understanding the precise impact that humins have in carbohydrate reactions. However, if we are able to have some understanding of humins, we will potentially have the ability to prevent their formation through optimization or transform the humin formation reaction into something valuable.¹⁷⁹ Several studies have attempted to valorize humins to produce composite and materials, potentially adding to the biorefinery value stream (see Figure 29).

Broadly, humins are of particular interest in the study of the dissolution of cellulose and its derivatives due to their ability to significantly reduce yields of cellulose, platform chemicals, or materials production. The complexity in understanding humins and their role in these

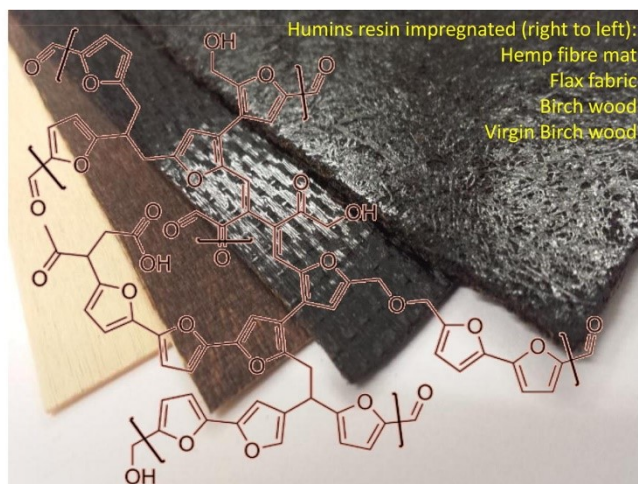


Figure 29 Humins produced via acidic pretreatment of biomass carbohydrates were used to form composite materials. These materials demonstrated good stability and performance as fillers and adhesives.

processes is that they can likely be formed through consecutive dehydration steps from carbohydrates, their intermediates, HMF, and furfural. Further, HMF may degrade further and react separately with the intermediates of any of the others. Humin structure, origin and impact have yet to be thoroughly explored. In an effort to understand these details we will examine several different sugar degradations and the role humins play in an effort to develop a clearer picture of humins and their effects.

1.7.1 Humin composition

As a baseline, elemental analysis on humins reveal that they are composed of 55-65% carbon, 4-5% hydrogen, and 30-40% oxygen, though these ratios are highly variable depending on formation conditions.¹⁸⁰ Humin production in the dissolution of lignocellulosic biomass or its components is extremely varied depending on inputs, reaction parameters, temperature, time, and solvent/catalyst choice. Degradation compound formation is significantly more complicated when other components of biomass are a part of the reaction which increases the difficulty in clearly delineating the process of humin formation. Though glucose can be converted to HMF and subsequently to levulinic acid and formic acid at elevated temperatures, other lignocellulosic biomass compounds such as aldehydes, aliphatics, and aromatics also potentially play a part in humin production and enzymatic inhibition.

Hydrothermal pretreatments that cleave acetyl motifs in xylans, galacto-glucomannans, and lignin lead to the formation of acetic acid. It is proposed that pseudolignin is formed from 3,8-dihydroxy-2-methylchromone and 1,2,4-benzenetriol derived from furfural and HMF respectively.¹⁸¹ However, it is unclear if these are key intermediaries and how other compounds which may be related to humins may interact and contribute to pseudolignin

formation. Compounds such as 3,8-dihydroxy-2-methylchromone have not been isolated from furfural degradation though thermodynamically possible in acidic conditions. Another suggestion is that humins come from the hydration of HMF via 2,5-dioxo-6-hydroxy-hexanal (DHH) which can polymerize with glucose as well as HMF (see Figure 30). The degradation products of glucose and xylose vary depending on the hydroxyl group that is protonated, suggesting multiple pathways to HMF and furfural production are possible (see appendix 1-B). There is evidence that suggests glucose can isomerize to fructose without a catalyst, though there is no conclusive evidence to whether it is via a cyclic or acyclic pathway and if that would impact the formation of humins/pseudolignin (see appendix 1-C).¹⁸¹

DHH is thought to be highly motivating in the formation of humins though it has never been isolated. Studies of the formation of DHH hypothesize it to be highly reactive and would actively interact with aldehydes or ketones (see figure 30).¹⁸² In these reactions, the carbonyl would be consumed and in the presence of HMF it could create a more stable species (see appendix 4-A). There is some support that this compound would still contain three additional carbonyl groups that could continue reacting and polymerizing. This also means that other compounds like benzaldehyde which is commonly present in pretreatment could also be incorporated into the growing humin polymer.¹⁸⁰

During acid catalyzed conversion of fructose HPLC analysis found very low concentrations of several unidentifiable species with only HMF, levulinic acid, and formic acid

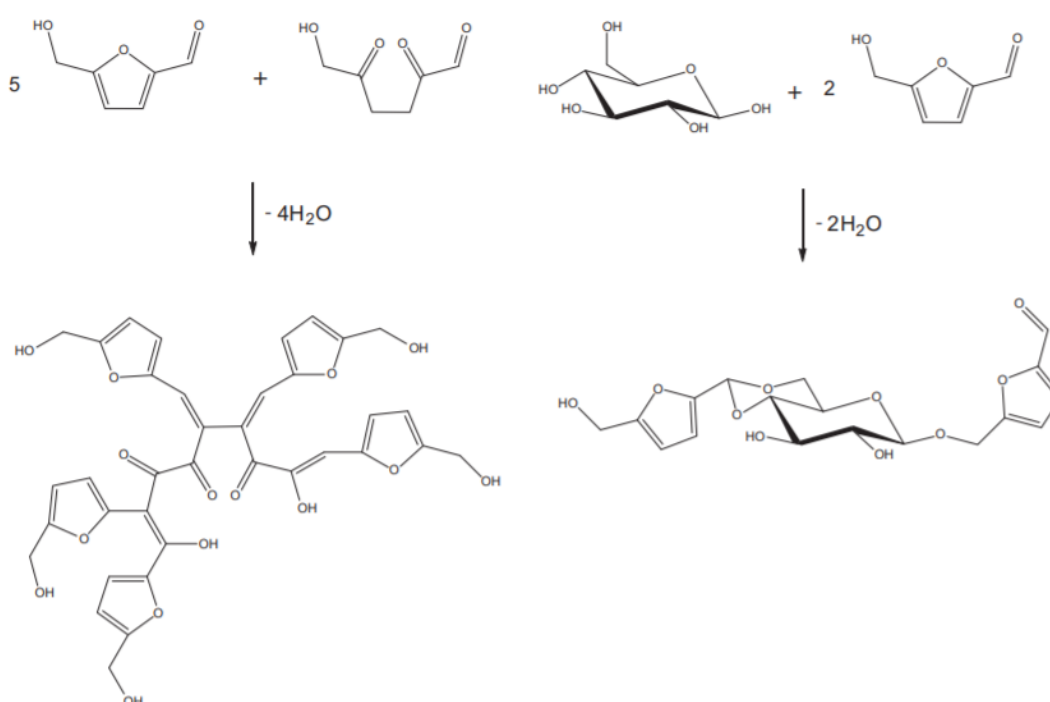


Figure 16 Potential combinations of humin formation from HMF with DHH (left) and HMF with glucose (right).¹⁸¹

appearing in any appreciable amounts. For glucose conversion the only compounds detected were glucose, levulinic acid, and formic acid. FT-IR of humins formed from fructose, glucose and HMF suggest the presence of furan rings.^{182,182} Another possible hypothesis for the formation of humins is that the sugars first form HMF and then DHH. Acidic conversion of cellobiose to HMF/humins seems to undergo the same process as that of glucose. First hydrolysis to glucose monomers, then dehydration of glucose to HMF, then the formation of DHH and then subsequent humin formation. IR spectrometry does not support the idea of HMF self-polymerization for humin formation, the only result would be a dimer. FT-IR also does not support the polymerization of glucose. Sorbitol which is similar in structure to glucose under the same acidic conditions does not form humins. This suggests that neither alcohol condensation nor carbonyl formation is involved in humin formation. In addition, this type of reaction would not be able to incorporate furan rings which are present in FT-IR. It has been demonstrated that the addition of aldehyde compounds like benzaldehyde can be incorporated into humins.

Variability of reaction parameters may also impact humin production. In studies using hydrothermal treatments (absent of an acid catalyst), increasing of processing temperatures correlated with a decrease in oxygen:carbon and hydrogen:carbon ratio suggesting carbonization and aromatization.¹⁸³ This suggests a difference in the surface vs the core of these humin particles and a possible hypothesis is a hydrophobic core and a less dense hydrophobic shell.

Different carbohydrates also lead to different humin particles sizes and compositions. Xylose produced a lower O/C and H/C ratio than other starting materials and lower amounts of aliphatic groups.¹⁸³ Trihydroxybenzene (TB) formed during hydrothermal pretreatments has also been implicated in the formation of larger humin droplets that are more oxygenated. Some studies have reported that humins are made from carbohydrates and consist of approximately of 60% furan rings and 20% aliphatic linkers.¹⁸⁰ Humin yield in wt% was directly related to temperature and concentration of sulphuric acid in this equation:

$$\text{Humin yield} = -120 + 1.507 T - 63.58 [\text{H}_2\text{SO}_4] - 3.56 \cdot 10^{-3} T^2$$

Xylose and fructose seem to convert more readily than glucose.¹⁸³ IR spectra of humins from glucose and fructose are similar, with broad OH peaks for the alcohols and weak aliphatic C-H stretches at 2900 cm^{-1} , and carbonyl peaks at 1700 cm^{-1} . Certain stretches attributed to furans are also detected (1530 cm^{-1} for C-C double bonds and 1020 cm^{-1} for C-O as well as peaks at 800 cm^{-1} and 765 cm^{-1} for substituted furans).

1.8 Glucose, fructose, and HMF to humins

In studies focusing on glucose, decomposition is a function of temperature and acidity. In acidic solutions D-glucose exists in equilibrium with its anomeric forms α -D-glucopyranose and β -D-glucopyranoses. Both of these forms can lead to other side reactions some of which have been detected using LC-MS in the initial stages of the reaction but not later on once glucose conversion was completed.¹⁸⁴ Further studies examining glucose focused on limiting humin production and provide more direct testing of their role.¹⁸⁵ Methods of reducing humin production has been a less common focus for research but is likely to increase as more of the formation of humins are understood. Limiter-additives such as acetonitrile were tested for humin suppression in glucose reaction. Researchers using heteropoly acid 12-MPA (12-molybdophosphoric acid) with [EMIM]Cl demonstrate near quantitative conversion with high selectivity for HMF while reducing humin production. It is believed that the heteropoly acids stabilize the 1,2-enediol and other intermediates involved in the dehydration of glucose avoiding the 2,3-enediol that leads to furylhydroxymethyl ketone (FHMK). It remains unclear how acetonitrile prevents the formation of humins. One hypothesis is the addition of acetonitrile reduces the viscosity of the ionic liquid limiting humin formation. Another is that the acetonitrile interacts with the acid catalyst modifying the proton on the catalyst to reduce humin formation.

The case of fructose dissolution also has been shown to produce humins. Confirming that isomerisation of glucose does not prevent humin formation. Again, additives have been explored in fructose reactions. Dilute acid was shown to accelerate the transformation of fructose to HMF in water as well as ionic liquids. Similar to studies of glucose, the addition of acetonitrile limits humin formation. The same hypothesis applies where the addition of acetonitrile reduces the viscosity of the ionic liquid and decrease the pH. The addition of acetonitrile seems to enhance the protonation abilities of the acid. This leads to the supposition that the strength and concentration of acid could be optimized for the formation of HMF without promoting levulinic acid or humin formation.¹⁴⁵ There is a large body of evidence that connects humin formation to reactions involving HMF, both in reactions where HMF is present and reactions where HMF is formed. Just as in other reactions, conditions and parameters impact humin formation, however levulinic acid and formic acid produced in HMF dissolution may not actively react to create humins¹⁸⁶. FTIR have demonstrated that glucose and fructose humins are not only similar but seem to have HMF incorporation (see Figure 31). Glucose,

fructose, and HMF are converted to DHH (assuming that a ring opening of HMF occurs). DHH is not detected but its peaks would also overlap in the regions filled by C=C, aromatic, and carbonyl regions ($1665\text{--}1760\text{ cm}^{-1}$). As the reaction proceeds there is an increase in 1702 cm^{-1} peak and decrease in 1668 cm^{-1} which could correspond participation of the aldehyde group in HMF in a condensation with DHH. There is also a subsequent decrease in the 758 cm^{-1} peak which is believed to be the reduction due to potential reactions of the hydrogen atoms attached to furanic rings. The increase intensity between $1100\text{--}1300\text{ cm}^{-1}$ and specifically at 1160 cm^{-1} could arise from the increase presence of ether bonds. There seems to be a correlation between aliphatic carbons and C-O bonds. These bonds could be a result of the presence of alcohols, ethers, or acetal bonds meaning that humin formation may involve multiple parallel reaction pathways. Addition reactions, aldol condensations and nucleophilic attacks are other factors that could impact humin formation. Picking the right solvent or co-solvent may limit these types of reactions thereby reducing the formation of humins.

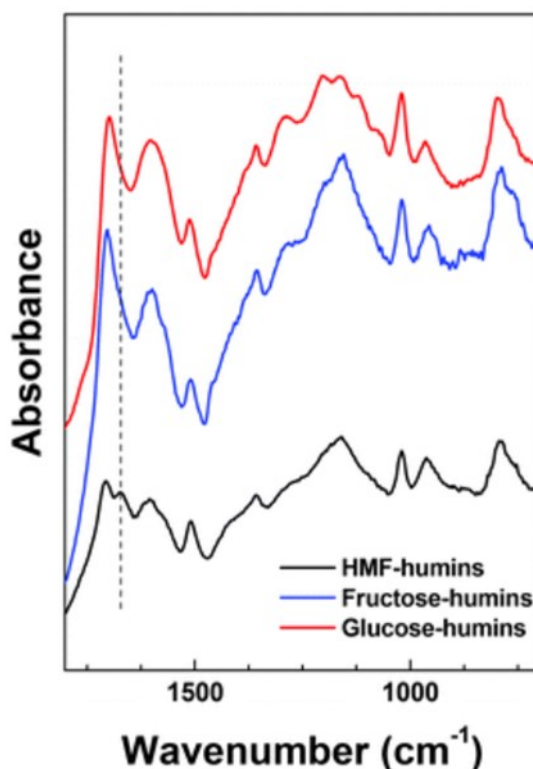


Figure 31 AT-IR of humins produced from HMF, fructose and glucose using mineral acid 0.1M HCl at $140\text{ }^{\circ}\text{C}$ ¹⁸⁶

Different reaction environments will not only affect the formation of HMF itself but may have further impact on any humins that result.¹⁸⁷ HMF formation has been more successful in non-aqueous environments in solutions like DMSO, acetone, dioxane, and ionic liquids. This is a predictable result given glucose or fructose transformation to HMF require several

dehydration steps, an environment with an abundance of water would not be conducive to its formation. In solvents such as DMSO, it has been suggested that the sulfur is electrophilic, the oxygen is nucleophilic and these interactions aid in the dehydration of fructose to HMF. With ionic liquids, the cation and anion act as the electrophile/nucleophile aiding the transformation, though there is no detail descriptions of these mechanisms found in literature.¹⁸⁷

The humins generated from HMF degradation were found to precipitate upon the addition of water or water/DMSO (Dimethyl sulfoxide) mixtures. However, these humins were found to be quite soluble in pure DMSO. This means that the humins have sufficient surface charge to prevent aggregation and precipitation. FT-IR of humins formed with a mixture of DMSO and water were compared with those prepared with just pure water. The peak at 758 cm^{-1} of the humins produced by the water/DMSO mixture is more pronounced.¹⁸⁶ DMSO seemed to prevent the nucleophilic attack of the furan ring to the carbonyl group of the HMF. Using molecular orbital (MO) theory calculations have suggested that DMSO interacts with the carbonyl of HMF and increases its lowest unoccupied molecular orbital (LUMO) energy stabilizing it from nucleophilic attacks. This means that the addition of a polar aprotic co-solvent would aid in minimizing humin formation.

Estimations of the molecular weight of humins may shed further light on these reactions. The average molecular weight of humins has been suggested to be between 300-500 g/mol. These estimations indicate that the humin precursors are relatively small and when considering HMF itself has a weight of 126.11 g/mol, the precipitation of humic substances requires only 3-4 units and even less if sugars such as glucose or xylose (180.15 g/mol and 150.13 g/mol) are incorporated.¹⁸⁰ This suggests that the solubility of these oligomers is greatly reduced. In comparison, the average weight of xylose derived products was higher meaning that solubility can be impacted by the feedstock that generated the humin.

Addition of compounds like trihydroxybenzene leads to faster humin formation and has implications for lignocellulosic biomass pretreatment. Humins may form from sugars and their degradation products but the presence of other aromatic compounds (such as lignin) may facilitate their formation. Other experiments have also shown the potential for formation of humins from compounds that contain the aldehyde functional group. With both of these results in mind, we can examine other degradation reactions to establish the potential for a relationship between them.¹⁸⁰

Hydrothermal conversion of fructose produced three main products at temperatures between 290-350 °C with residence times of 5-15 minutes: HMF, 1,2,4-benzenetriol, and

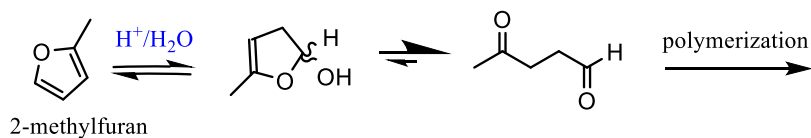
furfural. Depending on reaction conditions, HMF could undergo further transformation to levulinic acid, α -angelica lactone, furfural, or 5-methyl-2-furaldehyde.¹⁸⁸

Water quantity in a reaction also effects the conversion of fructose to HMF. Experiments conducted at 95 °C found that less water increases the dehydration rate to HMF, but also resulted in an overall decrease for the transformation of HMF to levulinic acid and formic acid. When water concentration was controlled via the addition of polyethyleneglycol-600, humin solubility increased and at 70 vol% no humins were formed. However, when water was added to precipitate the humins, the resulting precipitate included polyethyleneglycol-600. Fructose conversion to HMF is faster than HMF conversion to levulinic acid and formic acid as polyethyleneglycol-600 concentrations are increased in these cases.

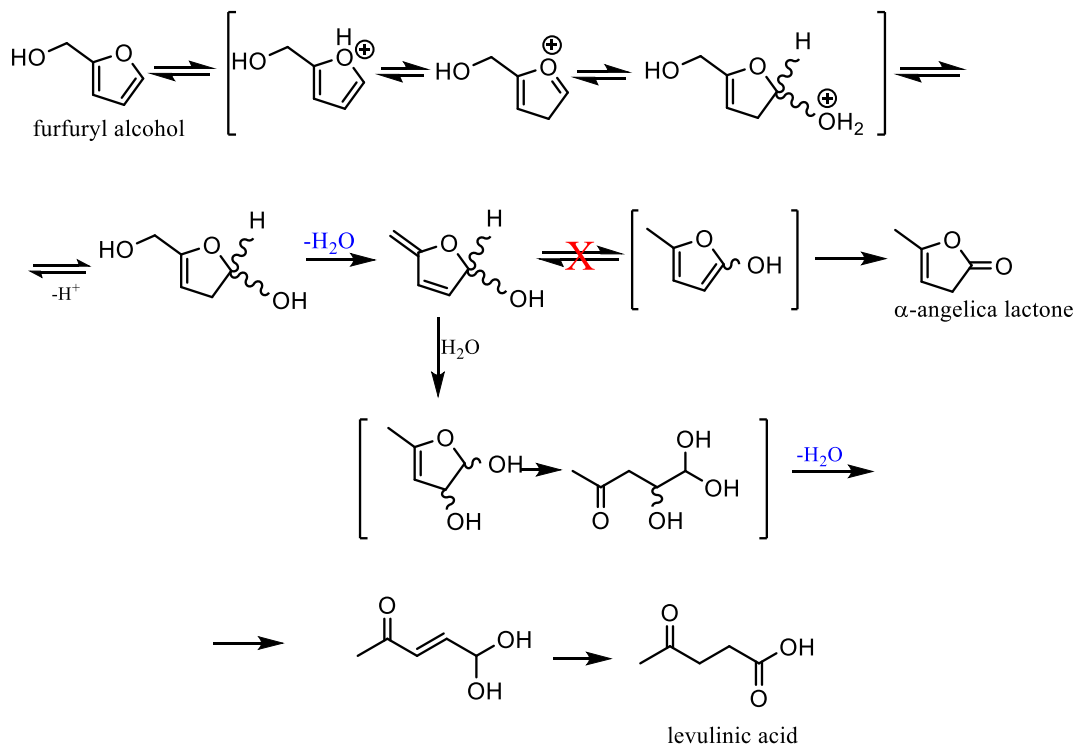
Further aqueous experiments using furfuryl alcohol, 2-methylfuran, and HMF in hydrochloric acid at room temperature were conducted to elucidate the mechanisms of degradation. NMR was used to aid in the determination of the intermediate steps of the reaction.¹⁸⁹ Two major intermediates were observed, hemiacetal 2-methylfuran and 4-oxopentanal (from ring opening of the furan), which then self-polymerized (see Figure 32A). Furfural alcohol under acidic conditions produced an unsaturated hemiacetal intermediate that dehydrated to an unsaturated cyclic compound that, when heated to 70 °C, converts to levulinic acid (see Figure 32B). There was no evidence of α -angelica lactone nor the enolic form suggesting that α -angelica lactone is not an intermediate in levulinic acid formation from furfuryl alcohol. Acid hydrolysis of HMF occurred at higher temperatures (above 100 °C) than furfural. The position of the addition of water produces several intermediates (see Figure 32C) where adding water in the 2,3 positions forms the intermediate 2,5, dioxo-6-hydroxy-hexanal (DHH). It has been hypothesized that DHH is one of the main intermediaries for humin

formation, but evidence remains elusive.¹⁸¹

A.



B.



C.

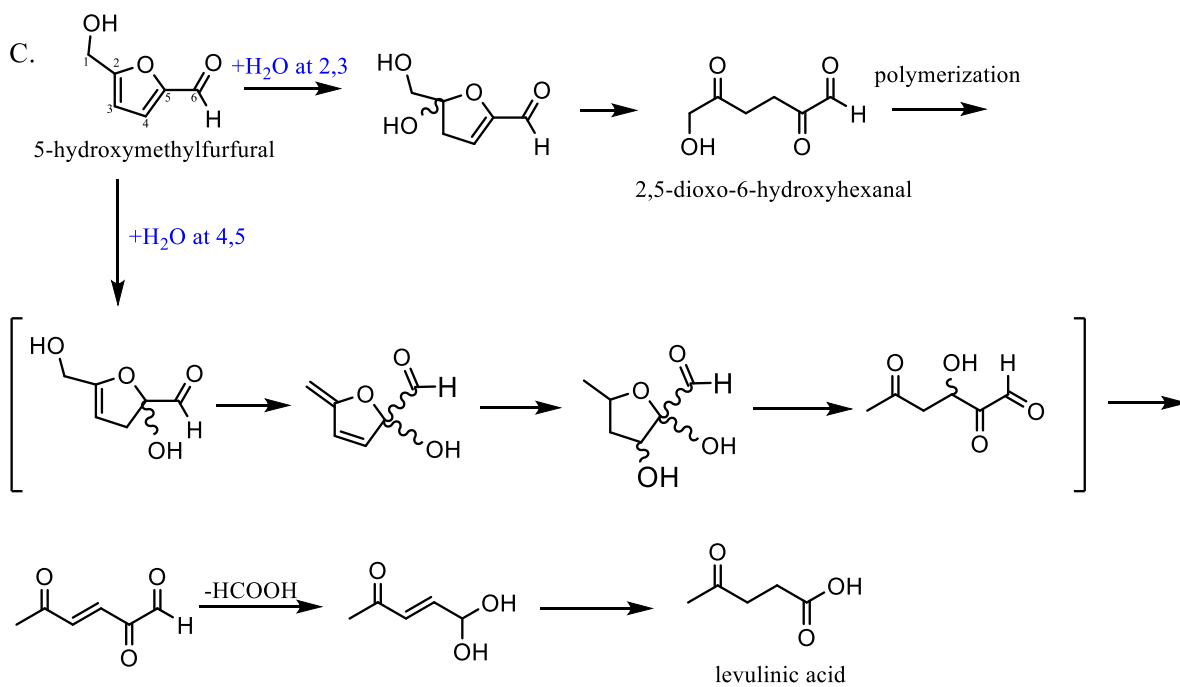


Figure 32 Proposed intermediates for the mechanism of levulinic acid formation from (A) 2-methylfuran, (B) furfuryl alcohol, and (C) 5-hydroxymethylfurfural (HMF). Compounds found in the brackets have not been spectroscopically isolated.

Other studies that examined the conversion of HMF in acid found that the formation of humins during mineral acid pretreatments was due to aldol addition and condensation of various intermediaries including DHH. DHH if produced would be in relatively small quantities and its self-polymerization is unlikely to be the sole cause for humin formation, meaning a more abundant species such as HMF is likely involved (see Figure 33). Modelling has predicted that DHH would form an enol and perform an aldol condensation with HMF. FT-IR evidence shows the retention of the furan rings but a decrease in the HMF carbonyl suggesting that is the site of reaction. The aldol reaction would cease after HMF conversion was completed.¹⁸⁰

In the presence of aldehyde containing compounds such as benzaldehyde, hydroxybenzaldehyde, formaldehyde, and acetaldehyde, humins have been shown to form (see Figure 33). This suggests that under acidic conditions and elevated temperatures (these experiments were conducted at 135 °C) HMF may react with aldehyde containing compounds which are formed during biomass pretreatment. Lignin dissolution is also known to form aldehydes, potentially linking the production of humins and pseudolignin formation.¹¹³

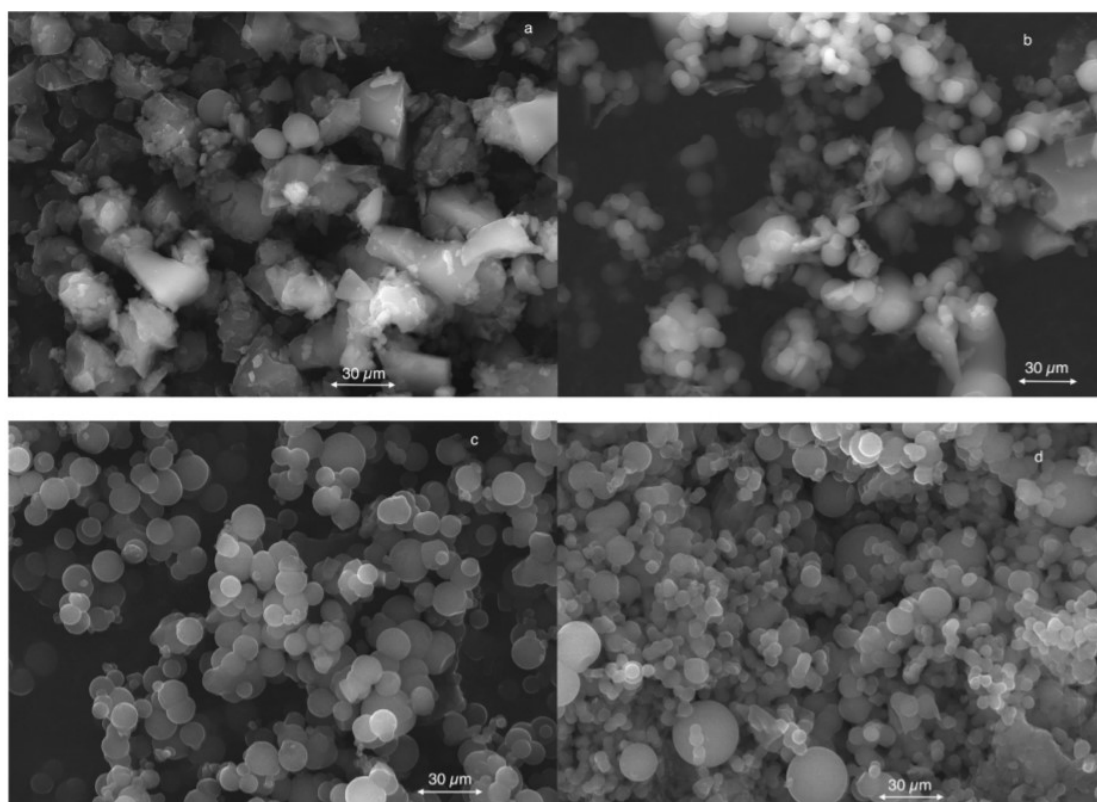


Figure 33 Formation of humic substances via acid catalysed reactions combining HMF with (a) benzaldehyde, (b) hydroxybenzaldehyde, (c) formaldehyde, and (d) acetaldehyde.¹⁸⁰

1.8.1 Levulinic Acid to Humins

Examining the decomposition of HMF into levulinic through an acid screen further supports the importance of HMF in humin formation while demonstrating that levulinic acid is inert in the reaction. An acid screen has shown that hydrochloric acid and sulphuric acid gave the best HMF conversion results, but all acid trials resulted in the production of humins.¹⁸⁴ Not only does the choice of acid matter but the temperature and the concentration affect the conversion rate of HMF to levulinic acid. The conversion rate is only loosely tied to the initial concentration of HMF suggesting a 1st order reaction, however the initial concentration of HMF greatly impacts the levulinic acid yield with lower initial concentrations producing higher levulinic acid yields. In addition, levulinic acid and formic acid do not catalyze the decomposition of HMF under these conditions. Since levulinic acid and formic acid are stable in these acidic conditions, humic substances are entirely dependent on HMF and or the intermediates produced during the reaction to produce levulinic acid.

To test the reactivity of levulinic acid, HMF was produced in an acidic aqueous environment. Levulinic acid and formic acids were stable in solutions of 0.1M sulphuric acid. Though it has been hypothesized that DHH is very reactive, it does not seem to react with the ketones of levulinic acid. Further, levulinic acid and formic acid did not react with each other or form humins, nor was formation α -angelica lactone detected. The addition of HMF to a solution of levulinic acid at 125 °C 0.1 M sulphuric acid also does not form humins or any condensation products. In acidic conditions the majority of levulinic acid is protonated forming the thermodynamically favored cyclic protonated compound dihydro-5,5-methyl-2(3H)-furanone.¹⁹⁰

1.8.2 Xylose and Furfural to Humins

Experiments on xylose using a protic ionic liquid mainly 1-butyl-3-methylimidazolium hydrogen sulfate as both solvent and catalyst have demonstrated that xylose can be quantitatively converted at temperatures above 120 °C.¹⁵⁷ However, the yield of furfural was poor (36.7%) due to decomposition or secondary reactions forming humins in the furfural formation process. The decrease in xylose concentrations does not correspond with the quantity of furfural production suggesting the presence of multiple intermediaries and the existence of both a cyclic and acyclic mechanism. Metal catalysts, which are believed to facilitate the isomerization to xylulose, have been used to improve yield and reduce humin

formation. Further reduction of humins was also achieved by use of a biphasic system via the addition of an organic solvent to extract furfural during the reaction. Organic solvents toluene, methyl-isobutyl ketone, and dioxane all significantly improved furfural yields (up to 82%), extracting the furfural before it could be consumed inside reactions.

Similarly, N,N-dimethylacetamide containing lithium chloride (DMA-LiCl) has been used to convert glucose to HMF as well as both xylose and xylans to furfural. Ionic liquid was added along with metal chromium salts to aid in conversion. The contrast between the furfural yields using acid compared with those using a chromium catalyst suggests that there may be alternative mechanisms for the conversion of xylose to furfural when using a salt. There is a first order dependence on xylose concentration and half order dependence on chromium (II) concentration. This indicates that the chromium is directly involved in the conversion mechanism. It is proposed that perhaps the chromium is aiding in the isomerization to facilitate the evolution or acting as a Lewis acid converting the xylulose into oxocarbenium ion. Fructose seems to produce better yield and converts easily to HMF, xylulose is more likely to form furfural meaning the transformation of xylose to xylulose would be preferential in furfural production. Direct conversion of xylan to furfural using DMA-LiCl with an ionic liquid additive and metal catalyst demonstrated poor results as a one pot system. The depolymerisation to xylose in this system was not effective.¹⁹¹ Comparative studies also address humin formation in the discussion of the solubility of cellulose and hemicellulose in ionic liquids such as 1-butyl-3-methylimidazolium chloride [BMIM][Cl].¹³⁹ Mineral acids are commonly added as a catalyst for the transformation of glucose to HMF or xylose to furfural. In [BMIM]Cl pentoses sugars convert faster than aldohexoses; xylose consumption was faster than glucose and mannose. Xylose conversion to furfural demonstrated higher yields in the presence of water which suggests that water is important for the reaction pathway to furfural formation. However, humin formation is much greater for xylose than the glucosic case. Glucose humin formation increased linearly with time (0th order reaction) while that of xylose increased then decreased over time indicating a positive reaction order. Under these reaction conditions even in the presence of a mineral acid, HMF and furfural were stable and did not result in formation of humins or additional by products. When glucose was combined with HMF and xylose with furfural, the conversion of glucose to HMF and xylose to furfural was significantly increased along with the formation of humins. HMF and furfural are consumed in reactions with other reactants but on their own did not self-polymerize. Extracting HMF or furfural during pretreatment may aid in the prevention of humic substances.

1.8.3 Polysaccharide and Biomass to Humins

Examining the specific processes of the degradation of individual sugars is valuable for providing information about factors that may impact humin formation, however it does not address the complexity of the inter-relationships between the intermediates in full biomass pretreatment. Examining studies more similar to what may occur in the biorefinery are critical to understand the role of humins.

The combination of cellulose and hemicellulose produce different conditions for the formation of humins. One pot conversion of sugars and their polysaccharides to furan compounds have produced some limited success. 1-alkyl-3-methylimidazolium chloride [BMIM]Cl was used to dehydrate glucose with a CrCl_2 catalyst. This resulted in a 70% HMF yield and 90% conversion of glucose, however when the same reaction was attempted with an acidic ionic liquid with glucose, cellobiose, and cellulose the formation of humins resulted in poor product yields.¹⁴⁶ When 1-ethyl-3-methylimidazolium hydrogen sulfate [EMIM][HSO_4] was added to the [BMIM]Cl for the conversion of xylose, over 80% yield could be achieved if a biphasic system was used (toluene in this instance) to constantly extract the furfural. Without the addition of the acid-bearing ionic liquid the reaction did not occur. Very little yield occurred with dimers or oligomers of xylose. This makes one pot conversion of pretreated biomass to furans unlikely. Acetonitrile was added to ionic liquid liquor and the dark coloured humins extracted. Elemental analysis suggested that these residues contained some ionic liquid or sugar ionic liquid derivative. These results suggest that these sugar degradation products may have the ability to react with the ionic liquid.

Studies on the dissolution of cellulose provide further information on the formation of humins in these inter-related reactions. Using 1-ethyl-3-methylimidazolium chloride [EMIM]Cl to dissolve glucose, cellobiose, and cellulose with the addition of an acid catalyst has revealed that hydrolysis and conversion of the sugars are not the same.¹³⁸ Sugars at the ends are hydrolyzed irreversibly and with greater speed than glucan in the middle of the polymer which are depolymerized reversibly most likely due to slow diffusion. This means that during the reaction the formation of glucose and cellobiose are produced at different rates reaching maximum production at different times during the reaction. A potential mechanism for the formation of humins was proposed. What is different about this mechanism from other studies is that it incorporates HMF, glucose, as well as various intermediate states into humin formation (see Figure 34). What this study noted was that glucose and cellobiose are the

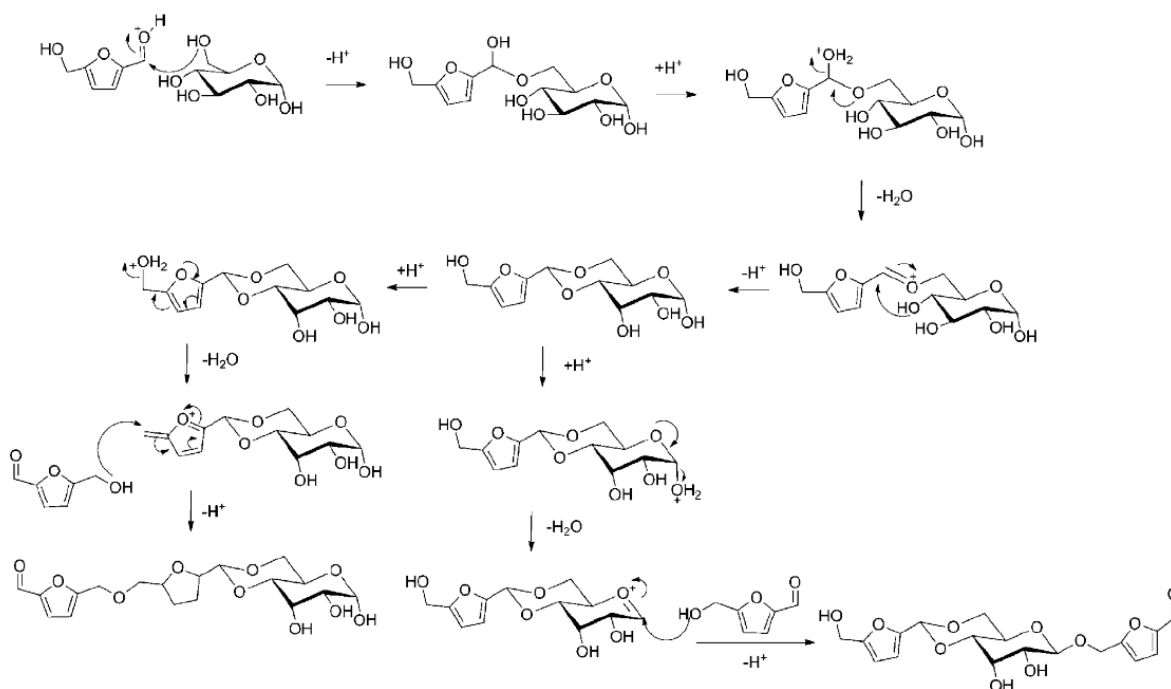


Figure 34 Proposed mechanism for formation of humins which incorporate HMF, glucose, and various intermediate states.¹³⁸

dominant product during initial part of the experiment (first 30 min). Cellobiose increases and reaches a max (between 30 -100 min) while the amount of glucose continues to increase. Glucose reaches a max at 90 min at which time humins are visible. By 100 min into the reaction the humin yield has outpaced the production of HMF. The amount of water in the reaction system also impacted the formation of humins. The addition of water would increase glucose yield and limit the formation of HMF and humins to a point. Addition of water to the later part of the reaction can reduce humin formation. Fructose was not isolated during this reaction. Clearly the landscape of the reaction conditions change as the reaction proceeds adding to the complexity in understanding the exact parameters of humin formation.

There are few studies that provides an example of dissolution reactions using the whole biomass. In this case, hot compressed water depolymerized and solubilized the hemicellulose of eucalyptus wood leaving a solid pulp that is rich in lignin and cellulose. This hot water was collected and used to produce furfural. Ionic liquid 1-butyl-3-methylimidazolium hydrogen sulfate [BMIM][HSO₄] and dioxane were used to pretreat the dehydrated hemicellulose fraction at 160 °C. Practical yields were lower than expected (59.1% conversion) due to side reactions with the furfural generated however still significantly better than without the dioxane. This demonstrates that a biphasic system where extraction of furfural results in better production yields because it prevents the furfural from participating in humin formation.¹⁶⁰

Other experiments with wood confirm that sugars can undergo multistep transformations to form furan compounds which undergo further condensation to form humins. It is also suggested that lignin may also participate in the formation of these acid insoluble compounds.¹⁹² It is believed that these humins form from the reactions of pentose sugars (like xylose) and the secondary transformations of furan compounds (like furfural) (see Figure 35 pathway C and E). This is due to the observation that prolonged heating in DAP will result in

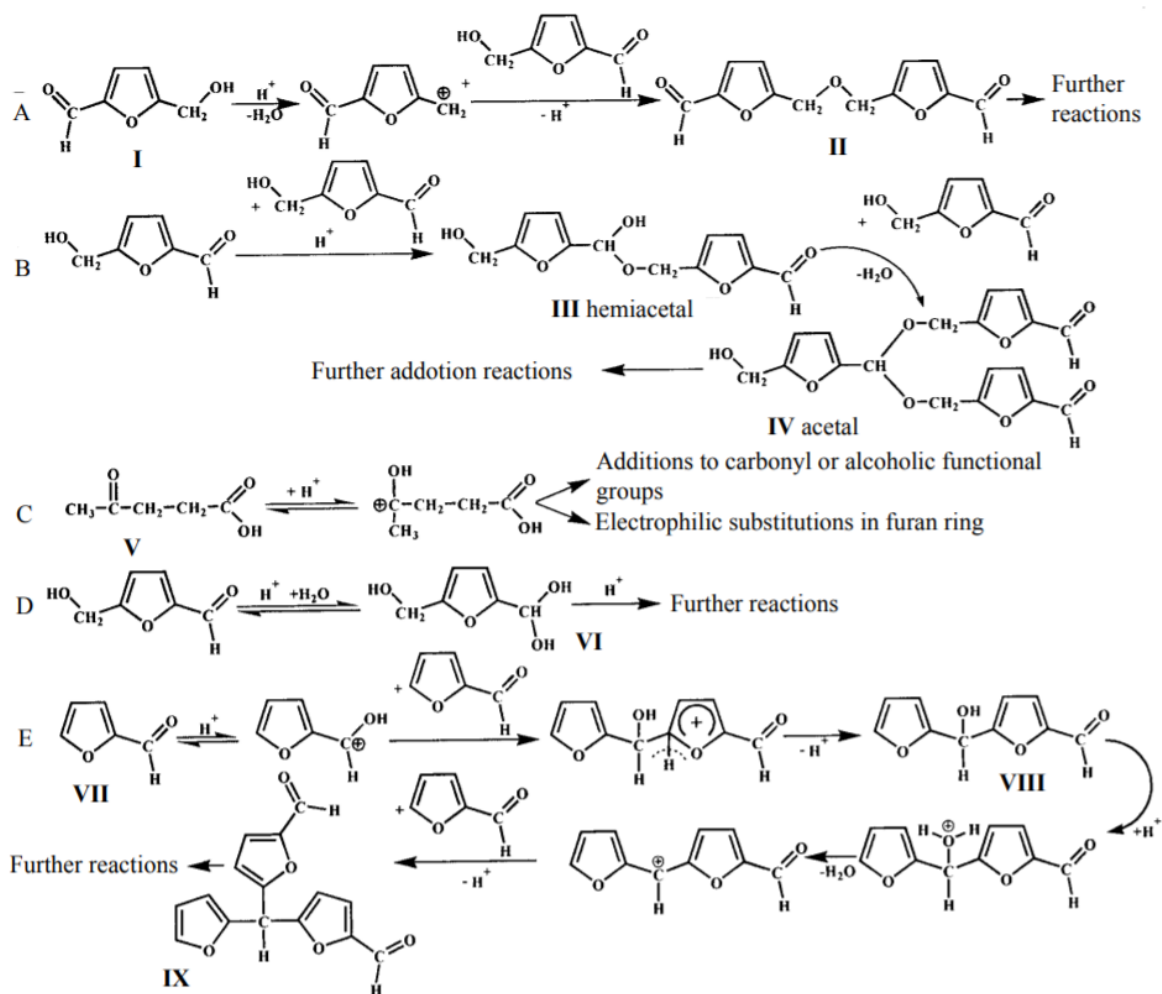


Figure 175 Suggested formation of humins from HMF (A,B, and D), from furfural (E) and from pentose sugars (C)

the decomposition of furfural to formic acid. It was noted that intermediates of pentose sugars polymerize without going through the formation of furfural. HMF on the other hand when heated during DAP decomposes to levulinic acid and formic acid and did not condense to form humins, this providing further evidence that in acidic conditions levulinic acid and formic acid do not participate in humin formation. While similar to glucose, cellobiose under these conditions also forms humins. Elemental analysis and pyrolysis GC-MS suggest that the main components of these humic substances are furan and phenyl rings (up to 45-60%). These humic substances were divided into three groups: water soluble, soluble in organic solvents, and not

soluble in either water or organic solvents. Their solubility is directly linked to how condensed the humins are.¹⁸⁰

Experiments utilizing ionic liquids that can dissolve lignocellulosic biomass, such as 1-butyl-3-methylimidazolium chloride [BMIM]Cl, under relatively mild conditions (120-150 °C) result in the conversion of carbohydrates in to water soluble products such as monosaccharides, oligosaccharides, furfural, and HMF. At 150° after an hour HMF in the liquor reached its peak after which there was a gradual decline as HMF is consumed in other reactions including formations of humins. At lower temperatures (100°C) there is no initial HMF formation, suggesting that HMF is a secondary product. The amount of water is important and necessary for hydrolysis and conversion of sugars to furfural and HMF. Though high conversion can be achieved, there are various monosaccharides, oligosaccharides along with other degradation products that can be found in the reaction solution. Fructose is also not detected during these reactions.

1.8.4 Lignin to humins/pseudolignin

In these examples of the dissolution of full biomass, the formation of humins may be impacted not only from sugars and their intermediates, but also the lignin abundance in the biomass. The amount of lignin and the types of lignin fragments will impact the condensation of other compounds that form during pretreatment. Prolonged treatment of lignin with mineral acids can lead to fragmentation and substantial quantities of insoluble materials with low monomer yields. These fragments are a result of cleavage at the β -O-4 bonds that occurs easily in acid at elevated temperatures. There are two main types of aromatic fragments: Hibbert ketones and formaldehyde fragments. Using model compounds, it was demonstrated that upwards of 50% of fragments produced generate aldehyde compounds (by following path B Figure 36), while the remaining producing aromatic ketones (Hibbert ketones) (following path

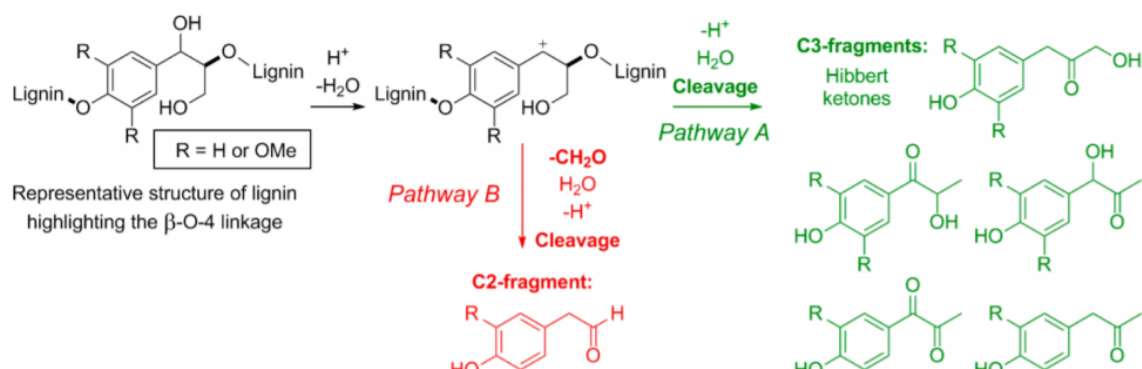


Figure 36 Products that can result from the acid hydrolysis of lignin at the β -O-4 position. There are two major pathways. One produces Hibbert ketones and the other an aldehyde fragment.

A Figure 36).¹⁹³ HMF only studies mentioned above also demonstrated an aldehyde interaction.¹⁸⁰ Lignin derived aldehydes are a potentially fruitful topic of exploration both due to the implication here of an interaction point between humins and lignin but also as an useful tool in materials production.

Indeed, some efforts have recognized aldehyde lignin reactions as a method to employ lignin as a possible replacement for phenol in the creation of resins.¹⁹⁴ The method demonstrated reactivity between lignin and formaldehyde in the resin production. This also has implication during biomass pretreatment and the formation of pseudolignin via the condensation of lignin fragments with aldehyde compounds. Experiments with lignin demonstrated the ability of lignin to affect the resin properties (such as the resin solubility in solvents). Viscosity also increased of the phenol-lignin-formaldehyde product. Resins were produced at 160 °C for approximately 2 hours. The temperature and time parameters described here are similar to those of our own study, detailed below.

Pseudolignin has been an important question in biomass pretreatment where acidic pretreatments result in higher Klason lignin yield than the original biomass. Pseudolignins can inhibit enzymatic activity in two major ways: non-productive binding of the pseudolignins to the cellulolytic enzymes and blocking of enzymes' access to the cellulose for hydrolysis. Additionally, the production of pseudolignin, seemingly from carbohydrate intermediates, consumes material and limits reaction yield.^{195,196} ¹³C NMR shows an increase in methoxy groups as pretreatment severity increases. There are also increases in substituted aromatic carbons around 148 ppm. C-O ether bonds from cyclic compounds are found in almost all pseudolignins and in the most severe trials the presence of ketones is also detected via FT-IR. There is also an increase in peaks at approximately 835 cm⁻¹ attributed to the C-H out of plane stretching that arises from substituted benzene rings.¹⁹⁶

Sugar combinations have shown to produce pseudolignin as well. Dilute acid pretreatments (DAP) have demonstrated successful extraction of hemicellulose from biomass. However, DAP does not delignify the biomass and compositional analysis reveals that often the lignin content is higher than in the original biomass. Experiments were conducted on cellulose and hollocellulose. It is believed that these depolymerized sugars have degraded and re-polymerized to form pseudolignin, negatively impacting saccharification yields.¹⁹⁷ During gravimetric compositional analysis pseudolignin can be seen in the increase in Klason solids (acid insoluble solids). Molecular weight of pseudolignin is much lower than that of milled wood lignin. It has been reported that FT-IR of pseudo-lignin consists of hydroxyl, carbonyl, and aromatic structures. This suggests a dehydration and aromatization reaction. There is some

evidence of a formation of a trisubstituted benzene ring compound. CNMR show ketone, aldehydes as well as carboxylic acid in pseudolignin formed from cellulose and hollocellulose. 1,2,4-benzenetriol and 3,8-dihydroxy-2-methylchromone have been produced from HMF and xylose respectively and may be key intermediaries to pseudolignin formation during DAP where lignin droplets irreversibly bind to enzyme through hydrophobic interactions. They also act as physical barriers to the enzyme reducing saccharification yields.

1.9 A roadmap for conversion of lignocellulosic biomass to chemicals and fuels

These numerous studies on the interactions of sugars, their intermediates, as well as pretreatment of the full biomass presents something of a dilemma for pretreatment science. Simultaneous processing of all carbohydrates offers simplicity of operations, but fractionation of hemicellulose and cellulose allows the process to be tailored and to take advantage of the different chemical/physical properties of these fractions.¹⁹⁸ Hemicellulose is typically hydrolysed faster than cellulose in acidic aqueous conditions. However, acid catalyzed dehydration of glucose to HMF and xylose to furfural often results in humin formation due to the ease in which these compounds can react with themselves and other intermediates. Furfural and HMF are gateway platform chemicals which can be used in oil refining, pharmaceuticals, plastics and agrochemicals. One possible method to prevent the formation of humins using solid acid catalysts such as silicoaluminophosphates, specifically SAPO-5 or SAPO-11 with DMSO, which may be a means to suppress side reactions. These types of catalysts could also be easily recovered and reused. Another solution is not to avoid humins at all but to investigate their valorization.

1.10 Conclusion

The focus of this study will be to examine sugar dissolution using the ionoSolv processes (details and protocol to be discussed in the next chapter) developed at Imperial College London to explore the formation of humins and pseudolignin and their impact on saccharification. We will be using the low-cost ionic liquid [TEA][HSO₄] investigating the impact that pretreatment has on biomass and in this case specifically *Miscanthus giganteus*. Fresh ionic liquid is not realistic for the biorefinery and we explore the impact of recycled ionic liquid on different sugars. Comparison to fresh ionic liquid aids in identifying optimized conditions. Additives will be investigated and used to further optimize recycled liquid reactions to produce a cellulose

rich pulp. Importantly, pure cellulose pulp may have distinct opportunities for materials production. Additional chemical processing will be applied to these pretreated pulps to examine the potential for cellulose fibers and nanocellulose production. Finally, we explore ionoSolv may be used to provide additional value streams with experiments demonstrating its use to extract useable sugars from post hydrolysis solids.

1.10.1 Chapter 1 References

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2 Chapter 2: Materials and Methods

2.1 Ionic liquid Synthesis

Ionic liquids synthesized for this project had a 1:1 cation anion ratio for triethylammonium hydrogen sulphate [TEA][HSO₄] with a 20% water weight. The synthesis workflow can be seen in Figure 1. Reactor synthesis of [TEA][HSO₄] employed a reactor which mechanically and simultaneously fed in the reagents (5M sulfuric acid with 99% triethylamine), stirred, and cooled the contents. The reactor was connected to a computer that monitored the progress of the reaction via conductivity of the output; however, water content was not controlled during this process and evaporation of the water in the synthesized ionic liquid was required. Once synthesis was completed, the ionic liquid was transferred from the

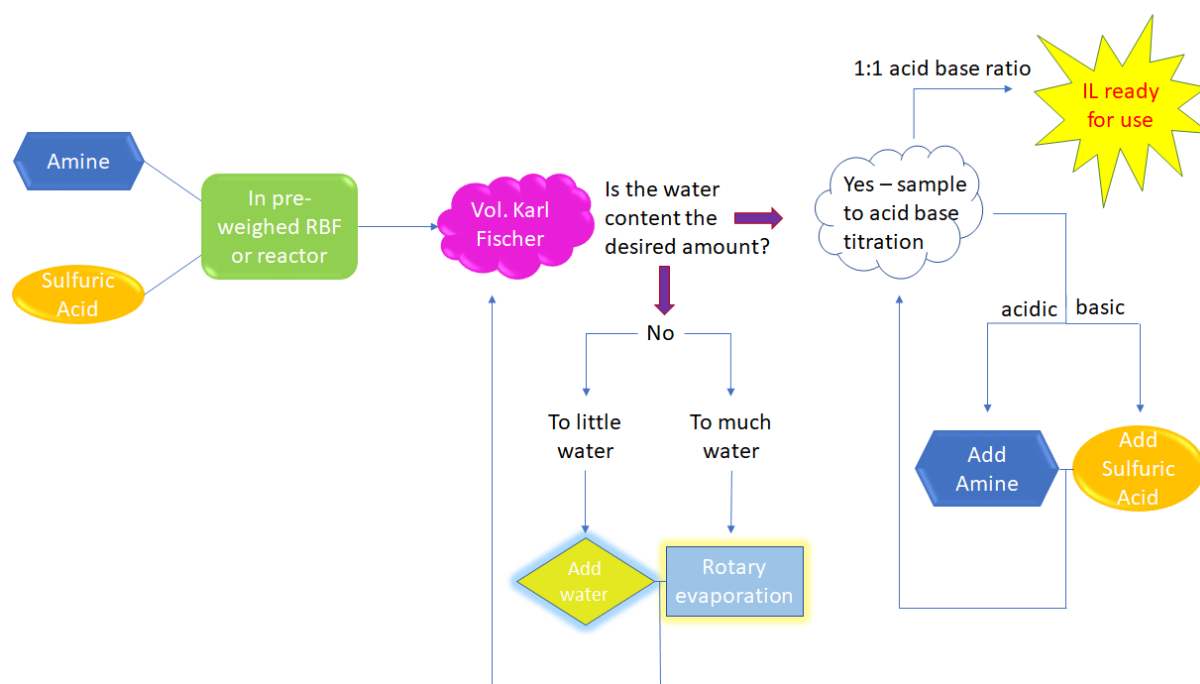


Figure 1 General scheme for triethylammonium hydrogen sulphate ionic liquid synthesis.

reactor to a pre-weighed container. Manual production of [TEA][HSO₄] required a calculated quantity (as well as known weight) of 5 M sulphuric acid placed into a separatory funnel. The procedure required the placement of triethylamine into a 1000 mL round bottom flask (RBF) with a stir bar. The weight of the flask and magnetic stir bar was recorded prior to its placement in a bowl containing ice to aid in cooling of the exothermic reaction. This ice bowl with RBF was placed on a magnetic stirrer. The separatory funnel containing the acid was hung over the opening of the RBF containing the amine allowing the acid to slowly drip into the amine. The entire process was conducted in a fume hood. Once the acid had finished dripping, distilled

water rinses of the separatory funnel ensured all the acid was flushed into the RBF. The ionic liquid was left to stir for several hours in the ice bath. Whether synthesized manually or *via* the ionic liquid reactor, a syringe extracted 0.1 mL of the sample for Karl Fischer volumetric titration to determine water content of the sample. A rotary evaporator eliminated water from the IL and titration measurements were taken. The evaporation process was repeated until the [TEA][HSO₄] was at 20% water weight. A 0.1 mL sample was extracted and submitted for titration using the compact titrator to determine acid base ratio. If the sample was too acidic, the appropriate amount of amine was added to bring the ratio to balance and if the sample was too basic the appropriate amount of sulfuric acid was calculated and added. The sample was then titrated again, and adjustments made until the 1:1 ratio is achieved.

2.2 Karl Fischer Titrations

This procedure used three distinct types of titration devices, coulometric, volumetric, and compact titrators. The coulometric and the volumetric Karl Fischer were used to determine water content of the ionic liquids. The coulometric is more sensitive than the volumetric and was used on samples that were believed to contain less than 5% water. Water reacts with the reagents inside the cell inducing an electrical current. The amount of current (2 moles of electrons per mole of water) required to release enough reagent to transform water into a non-conductive compound determined the moisture content of the sample. Volumetric titration operates with the same principle except the solvent and reagent were not contained in the same cell. The ionic liquid samples were added and the titrator added the reagent to the solution, titrating against the water content of the ionic liquid. The moisture content was calculated using the volume of the reagent. Determining water content for the ionic liquids required a 1 mL syringe to extract approximately 0.2 mL of ionic liquid. The syringe was placed on the balance for taring. Approximately 3-4 drops of the sample were added into either the coulometric or volumetric titrator, and the syringe returned to the balance. The difference in weight was input to the titrator then the water content was recorded. Each sample measurement was repeated three times.

The compact titrator determined the acid base ratio of the ionic liquids. The titrator uses a known concentration of sodium hydroxide (NaOH) solution for titration against protic acids (and in this case a protic ionic liquid). Approximately 50 mg of ionic liquid was placed into the sample container after moisture content had been determined. 50 mL of deionized water was added to the container and submitted to the compact titrator. The compact titrator reported

the amount used in mmols of sodium hydroxide. We calculated the resulting acid base ratio (γ) via the following equation.

$$\gamma \approx \frac{mass_{dry-IL}(gr) + mol_{NaOH} * MW_{base}}{2 * mass_{dry-IL}(gr) - mol_{NaOH} * MW_{acid}}$$

The molecular weight for TEA is 101.19 g/mol and the molecular weight of the sulfuric acid was 98.08 g/mol. This equation combines the mass balance, charge balance, and equilibrium equations for sulfuric acid as well as TEA (see Figure 2). It was generated via Matlab by a colleague, Francisco Maleret, by inputting various masses of ionic liquids that contain different acid base ratios, titrating to equivalence, and plotting the results (publication in progress).

Mass Balance:

$$[H_2SO_4]^0 = [H_2SO_4] + [HSO_4^-] + [SO_4^{2-}]$$

$$[TEA]^0 = [TEAH^+] + [TEA]$$

Charge Balance

$$[HSO_4^-] + 2[SO_4^{2-}] + [OH^-] = [TEAH^+] + [H^+]$$

Equilibrium Equations

$$H_2SO_4 \leftrightarrow HSO_4^- + H^+ \quad K_{a1} = [H^+][HSO_4^-]/[H_2SO_4]$$

$$HSO_4^- \leftrightarrow SO_4^{2-} + H^+ \quad K_{a2} = [H^+][SO_4^{2-}]/[HSO_4^-]$$

$$TEAH^+ \leftrightarrow TEA + H^+ \quad K_{a3} = [H^+][TEA]/[TEAH^+]$$

$$H_2O \leftrightarrow H^+ + OH^- \quad K_w = [H^+][OH^-]$$

Figure 2 System of equations entered into MATLAB by Francisco Maleret to solve for acid base ratio.

2.3 Biomass preparation and moisture content determination

Miscanthus x giganteus, as the primary biomass used in this study, is a fast-growing grass that demonstrates a high photosynthetic efficiency, has the ability to grow in cooler climates, and has a low water consumption. It displays minor variation across multiple soil types and fertilization methods and does not compete with food sources making it an appropriate choice for biorefining.^{1,2} The *Miscanthus x giganteus* for this project originates from Silwood Park.

Milling of the dried *M. giganteus* uses the Wiley Mill Retsch SM 2000 before sieving and collecting particles between 180-850 μm in size for pretreatment.

To determine the moisture content of the *M. giganteus*, a piece of aluminum foil was weighed and twered on the balance. Then a sample of approximately 0.100 g of *M. giganteus* was placed on the foil and the weight recorded. The folded foil containing the biomass was placed in an oven at 105 °C to dry overnight. Once removed, the sample was placed in a desiccator to cool for 15 minutes prior to weighing. The equation which determined moisture content is here:

$$\text{moisture}_{\text{ovendried}}(\%) = \frac{m_{\text{airdried}} - m_{\text{ovendried}}}{m_{\text{ovendried}}} * 100\%$$

This procedure is in accord with the National Renewable Energy Laboratory (NREL) protocols.³

2.4 Protocol for Pre-treatment of Biomass

Pretreatments were conducted with the ionoSolv process which is designed to fractionate biomass into three distinct fractions (see figure 3). The hemicellulose and lignin were dissolved into the ionic liquid leaving a cellulose rich pulp, which can be utilized for further downstream applications. Lignin can be recovered from the ionic liquid with the addition of water as the antisolvent. In the biorefining process, this lignin could potentially be burned for energy recovery, or converted to other aromatic platform chemicals or materials. The pre-treatment process for biomass samples follows the Hallett Lab's standard operating procedure for ionoSolv fractionation (additional details can be found at doi:10.3791/54246) (also see Figure 3 for a scheme of the pretreatment process).

During this project a 10% wt. loading of *Miscanthus* was used unless otherwise specified. Approximately one gram (based on oven dry weight) of *Miscanthus* was placed into a 15 mL pressure tube with approximately 10 grams of [TEA][HSO₄]. Each tube was shaken using the VWR vortex mixer until the biomass and ionic liquid were thoroughly mixed. Each sample was conducted in triplicate and all experiments conducted under the following procedure unless otherwise noted.

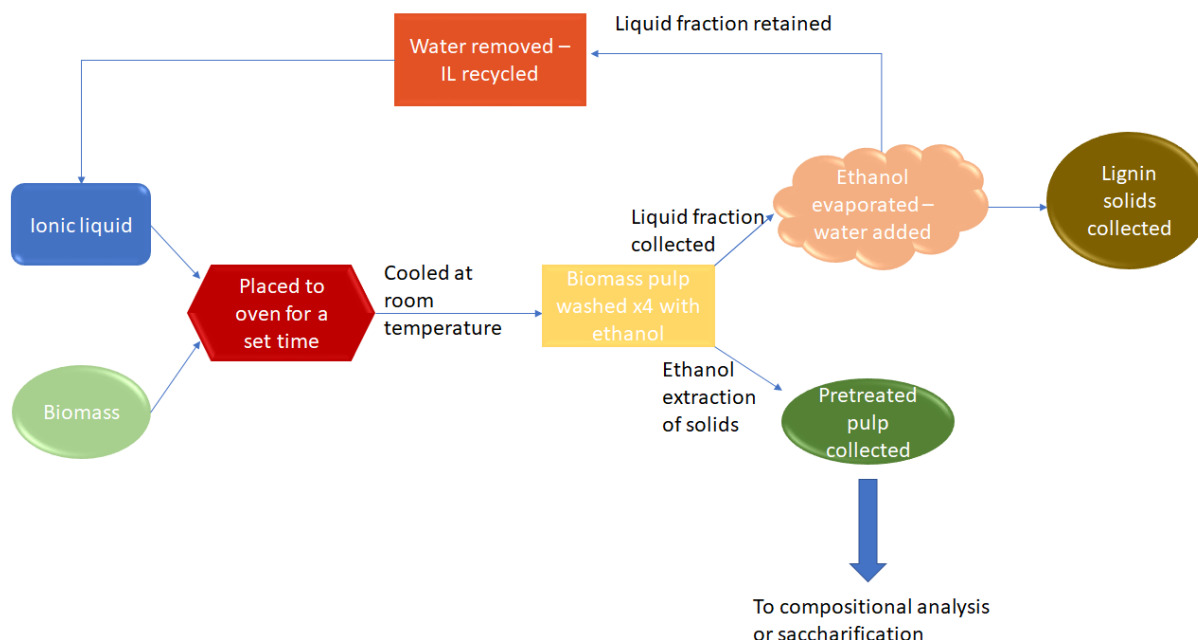


Figure 3 Scheme for the ionic liquid pretreatment process.

The 15 ml pressure tubes containing the ionic liquid biomass mixture were placed in the oven at 150°C for 1 hour. Once removed from the oven, they were left for approximately 15-20 minutes to cool to room temperature. The contents of the tubes emptied and washed with 40 mL of absolute ethanol into a 50 mL falcon tube. The contents were shaken using the vortex mixer and allowed to rest at room temperature for an hour. The falcon tube was shaken once more prior to being placed in the VWR Mega Star 3.0 centrifuge for 50 minutes at 3000 rpm. Once centrifugation is completed, the liquor (liquid fraction) was decanted into a 250 mL round bottom flask (RBF). This wash process was repeated another 3 times for a total of 4 washes. The RBF with the decanted liquor was placed on the parallel evaporator at 40°C under vacuum and the ethanol removed. The solid pulp fraction which remained at the bottom of the falcon tubes after the centrifuged 4 ethanol washes was transferred to a Whatman cellulose thimble for soxhlet extraction with 150 ml of absolute ethanol. The soxhlet extraction of the pulp was conducted for 20 hours at 135°C in the fume hood. The ethanol from the soxhlet extraction was transferred into the RBF that contained the decanted liquor from the ethanol washes and placed back on the parallel evaporator for removal of the ethanol. Once the Soxhlet extraction was completed, the thimbles that contain the pulp were placed on plastic weighing boats allowed to air-dry in the fume hoods overnight. Once dried, the pulps were weighed and moisture content on the pulp determined and overall dry weight and pulp recovery yield calculated.

Water was added to the ethanol evaporated liquor to precipitate the lignin dissolved in the ionic liquid. 30 mL of water was added to the dry liquor from the parallel evaporator and transferred to another 50 mL falcon tube. The contents were shaken and left to stand at room

temperature for 1 hour. Lignin suspensions were visible in the solution. After an hour, the tubes were placed into the centrifuge for 50 minutes at 3000 RPM. The liquid mixture containing water and ionic liquid were decanted into another pre-weighed 250 ml RBF containing a stir bar. This process of washing the lignin was repeated for a total of 3 water washes. The falcon tubes were closed, and the lids pierced with holes and placed into the freezer overnight or placed into a bath of liquid nitrogen for faster cooling. Once frozen, the falcon tubes were placed on the freeze dryer until fully dried (usually 24-48 hours) before removing and recording the weight of the lignin. The decanted water washes were placed on the rotary evaporator to recover the ionic liquid. Karl Fisher titration was used to confirm water content. The recycled ionic liquid was returned to 20% wt. water by following the general scheme shown in Figure 1.

2.5 Compositional analysis

Compositional analysis was used to determine sugar and lignin content of the untreated *M. giganteus* and the pretreated pulps from the experiments conducted during the course of this project. This procedure followed the NREL guidelines outlined by Sluiter et al. 2008 the general flow is shown in Figure 4. All compositional analysis was conducted in triplicate.

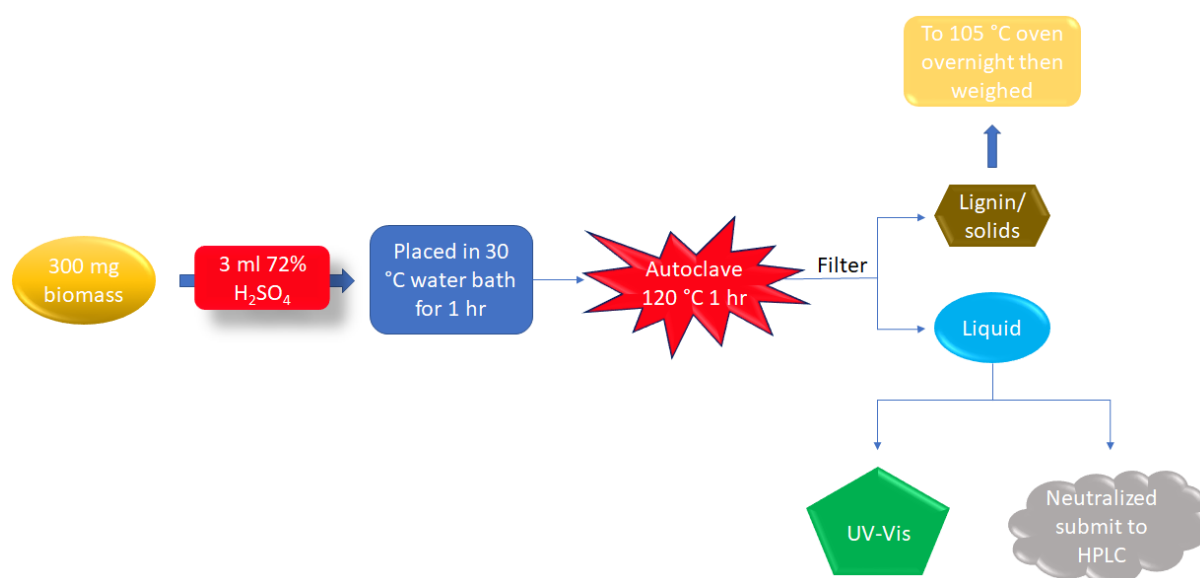


Figure 18 General scheme for the compositional analysis protocol used by the National Renewable Energy Laboratories (NREL).

In preparation for analysis, medium porosity (15 μm pore size) 25 mL filter crucibles were placed in the muffle oven overnight and heated to 575 $^{\circ}\text{C}$. Once removed, the crucibles were

placed in a desiccator and allowed to cool for 15 minutes. Their individual weights were recorded, and the crucibles placed back into the muffle oven. This process was repeated until the crucible's recorded weights were constant. A filtration apparatus for each sample was assembled which included a 250 mL conical filtration flask, two rubber adaptors to ensure a good seal with the filtration flask, the glass funnel, and the crucible at the top. A total of 6 filtration flasks were set up at a time and linked to a vacuum pump. Approximately 500 mL of distilled water is placed in the oven for warm washes for after the initial filtration and collection of the sample aliquots.

After measuring the moisture content of the biomass for compositional analysis (see Biomass preparation and moisture content determination section), 0.300 g of dry weight biomass/pulp was placed into a 100 mL pressure tubes and 3 mL of 72% w/w sulfuric acid added to the tube via pipette. A Teflon stirring rod was inserted into each tube and rack of tubes placed into a 30° C water bath for an hour with stirring occurring every 15 minutes. 84 mL of water was added to each tube, sealed, and placed in the autoclave for 60 minutes at 120 °C. Once the autoclave completed its cycle, the pressure tubes were removed and allowed to cool until warm but easy to handle. The contents of the 100 ml pressure tube were filtered through the filtration apparatus and collected in the flask. Approximately 45 mL of the filtered solution was transferred to falcon tubes for ultraviolet-visible spectroscopy (UV-vis) determination of acid soluble lignin. Another 20 mL was transferred to another falcon tube for the High-Performance Liquid Chromatography (HPLC) for sugar determination.

After running a baseline with deionized water, the UV-vis was calibrated for readings at 240 nm. Target absorbances was set between 0.500-1.000. Dilution of some samples may have been required to fall within that range. Dilutions were noted and taken into account when calculating acid soluble lignin content. The following equation was used to determine ASL (acid soluble lignin) content.

$$\%ASL = \frac{UVabs * Volume_{filtrate} * Dilution}{\epsilon * ODW_{sample} * Pathlength} * 100$$

Samples submitted for Shidmadzu HPLC analysis were first neutralize to a pH between 5-6 with the addition of calcium carbonate. Once neutralized the samples were filtered through a 0.2-µm filter and approximately 1 ml of the aliquot is placed in a 1.5 ml HPLC glass vial for analysis in the HPLC. In addition, previously prepared frozen mixed sugar standards were removed from the freezer, thawed and placed in the HPLC for carbohydrate concentration

determination. Concentrations prepared for standards were 0.1, 1, 2, 4, and 8 mg/ml samples containing glucose, xylose, mannose, arabinose, and galactose. HPLC compositional analysis samples have 25-minute run time per sample.

Warm distilled water was used to rinse and clean out any residual solids from the pressure tubes after removing the aliquot samples for the UV-Vis and HPLC. This rinse water was added by pouring into the porous crucibles. Once filtration was completed, the crucible was placed into the 105°C oven over night for drying. The next day, the crucible was placed into the desiccator to cool for 15 minutes before weighing. The difference between the dry weight of the crucible was calculated to determine the mass of lignin and ash recovered. The crucibles were returned to the muffle oven overnight and heated to 575 °C to burn off residual organic matter. The next day the crucibles were placed into the desiccator cooled for 15 min and weighed to determine the ash content.

2.6 Analysis using HPLC of the sugars and ionic liquid

HPLC (high-performance liquid chromatography) was used to separate, identifies, and quantifies sugars and their degradation products from the pretreatment of biomass. Two different Aminex columns were utilized in our trials, the Aminex HPX-87P and the HPX-87H column produced by Bio-Rad. Each of these columns were packed with a polymer-based matrix (polystyrene divinylbenzene). The Aminex HPX-87P was a lead form column, 300 x 7.8 mm column used mainly for the separation of carbohydrates such as those found in biomass: cellobiose, glucose, xylose, galactose, arabinose, and mannose. It can also provide analysis for other sugar derivatives such as sucrose, lactose, and fructose. The Aminex HPX-87H column is a hydrogen-form, 300 x 7.8 mm column used for analysis of carbohydrates in solution with carboxylic acids, volatile fatty acids, short chain fatty acids, alcohols, ketones, and neutral metabolic by-products. Most often used to analyse organic acids, and in this case the separation and quantification of glucose, xylose, formic acid, levulinic acid, acetic acid, furfural, and HMF. This column can also be useful for fermentation monitoring, biological fluid analysis, and acetylated amino sugar separations. The HPX-87P was used for compositional analysis and saccharification samples for sugar concentration determination (sample preparation is described in the compositional analysis protocol section), while the HPX-87H column was used on ionic liquid samples for sugar and sugar degradation

products/acid concentrations. In both cases, samples were moved through the columns at a 0.6 ml/min flow rate with HPX-87P samples heated to 85 °C and HPX-87H samples at 55 °C.

Preparation of recycled ionic liquid samples for analysis in the HPX-87H column followed the following protocol. Approximately 200 mg of the recycled ionic liquid was placed into a 1 ml microcentrifuge tube and 600 microliters of de-ionised (DI) water was added and the sample vortexed. The sample was allowed to rest at room temperature for 1 hour and then centrifuged. 200 microliters of the supernatant was pipetted into an HPLC vial (containing an insert). The samples were placed into the HPLC to determine the presence and concentration of the five main known sugar degradation products (formic acid, acetic acid, levulinic acid, HMF and furfural). Standards containing 0.1, 1.0, 2.0, 4.0 mg/ml of glucose, xylose, formic acid, acetic acid, levulinic acid, HMF and furfural which were previously prepared and frozen were thawed to room temperature and run in the HPLC. The supernatant was analysed by HPLC (Shimadzu Prominence) equipped with a UV detector (SPD-M20A) as well as a RI detector (RID-10A). The RI detector was used to determine glucose and xylose concentrations while the UV detector was used to determine formic, acetic, levulinic acids, HMF, and furfural as these compounds were UV active.

2.7 Saccharification

In preparation for saccharification, moisture content for untreated and pretreated *M. giganteus* samples were determined. 100 mg dry weight of each sample was placed into a 15 ml sterile plastic vial. All samples were prepared and run in triplicate. The following reagents were prepared for saccharification by creating a master mix, so that each vial contains the following quantities:

- 5 ml Sodium citrate buffer 0.1M at pH 4.8
- 40 µl Tetracycline antibiotic (10mg/mL in 70% ethanol)
- 30 µl Cycloheximide antibiotic (10mg/mL in purified water)
- 4.81 ml Purified water
- 20 µl Enzymes Novozyme

In addition, three vials containing no pulp, and only the above mixture was incubated as the control which was included in the HPLC HPX-87P for analysis to account for the baseline level of sugars. All samples were placed into the Stuart Orbital Incubator at 50° C at 250 RPM. If time points samples were taken, they were taken at 2, 4, 6, 24 hours, 3 and 7 day intervals, otherwise the standard 7 day results were examined. Samples after saccharification were prepared in the following manner. The saccharification vials were removed from the incubator and placed on ice. 500 µl from each sample was place into a micro centrifugation tube and centrifuge at 4 °C for 10 minutes at 13.3 G. Once centrifugation was completed, 200 µl of the aliquot was pipetted into a HPLC vial containing an insert and place into the HPLC machine for analysis.

2.8 FT-IR of lignin and pulp samples

Fourier-transform infrared spectroscopy (FT-IR) measures the ability of a sample to absorb radiation across a spectrum of different wavelengths. The vibrational frequency of the bonds in a compound can provide useful information concerning the functional groups found in a compound and can be used for substance identification. FT-IR can be useful to monitor the changes to a sample which has been treated under varying conditions. It is a nondestructive method of analysis and requires minimal quantities of sample. This project used the PerkinElmer Spectrum 100 FT-IR spectrometer with a diamond germanium ATR single reflection crystal on lignin, feedstock, and pretreated pulps. Background scans were taken prior to sample analysis. Approximately 25 mg of each sample was placed on the diamond germanium surface. Recorded spectra were between 500 cm⁻¹ and 4000 cm⁻¹ with a resolution of 2 cm⁻¹. Each sample was scanned 8 times. A general FT-IR spectroscopy table is shown in Figure 5.

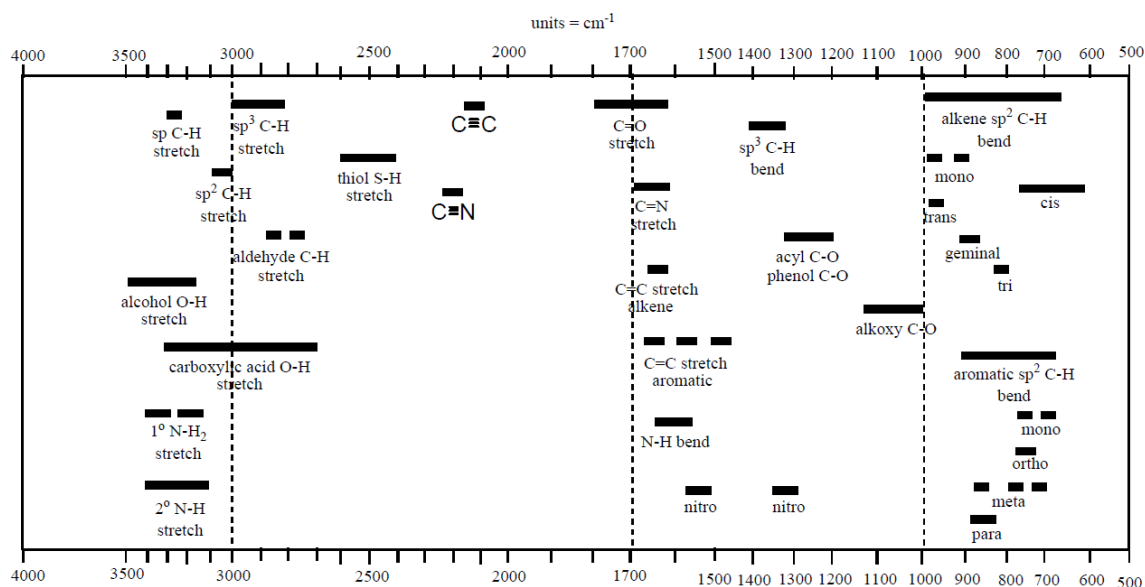


Figure 5 The general range in wavenumber where functional group substituents can be found in the FT-IR spectra.⁴

2.9 Bleaching

Bleaching of pretreated pulps with an alkali and hydrogen peroxide solution process was used to remove residual lignin from pretreated pulps (see Figure 6). The bleaching process

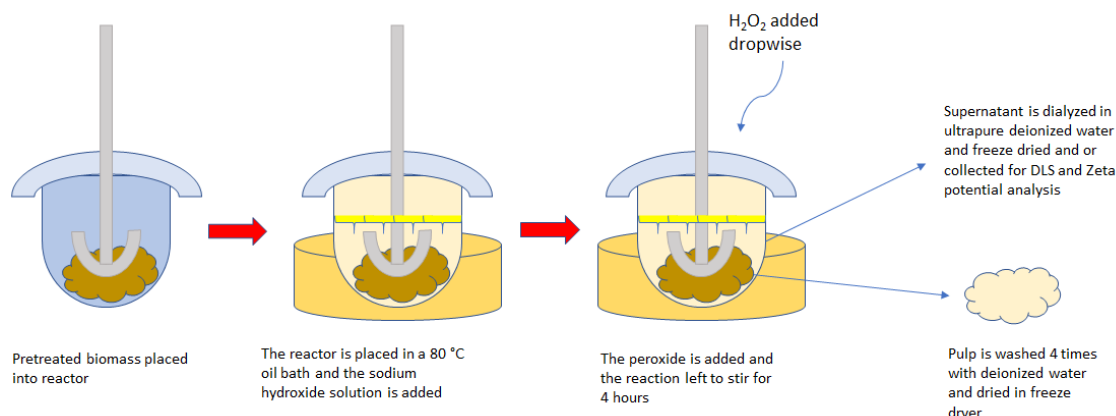


Figure 6 Process for bleaching of pretreated biomass.

in this project was modified from procedures used in the production of fabrics and various other textiles. For the bleaching, a Sigma Aldrich 30% wt. hydrogen peroxide solution was procured, and a separately prepared hydroxide solution was used. The 10% wt. alkali solution prepared used sodium hydroxide pellets from Sigma Aldrich combined with deionized water. 5 grams dry weight biomass was placed into a 100 ml reactor. An external mechanical metal anchor stirring rod was attached and placed into the reactor. The reactor was placed into an oil bath at 80° C. Approximately 25 ml of the hydroxide solution was added to the reactor containing the

biomass and allowed to equilibrate for 10 minutes with constant stirring. Then 25 ml of peroxide was gradually added over a period of 30-45 minutes and constantly monitored to ensure that the reaction did not bubble over. Once the addition of peroxide was completed, the reaction was stirred for 4 hours. After 4 hours, the pulp was split into five 50 mL Falcon tubes. Approximately 40 ml of deionized water was added to the falcon tubes and vortexed. After leaving the tubes for an hour, they were placed into the centrifuge at 3000 RPM for 45 minutes. The supernatant was decanted into a 7000 Dalton medicell dialysis membrane. Four deionized water washes on the pulps were completed on the pulp and the supernatant was collected and submitted to dialysis after each centrifugation. The dry cellulose pulp was placed on the freeze dryer until dry (24-48 hours) and the weight was recorded. The deionized water used on the dialysis of the supernatant was changed twice a day. Dialysis was performed for 48 hours. Samples from dialysis were submitted to dynamic light scattering (DLS) to ascertain size and possible formation of nanocellulose. Samples were also submitted for zeta potential measurements to determine the stability of cellulose particle dispersion in an aqueous environment.

2.10 Dynamic light scattering (DLS), Mastersizer, and Zeta Potential measurements

Particle size analysis is important to various applications in material development and industry. Particle size can impact flow, aerosol, gel, and emulsion properties. Smaller particles, generally, stay in suspension better than larger particles which may flocculate. Dynamic light scattering (DLS) is a helpful tool in determining the size of particles suspended in liquid environments. When light is directed at the sample, photons that hit suspended particles will scatter. Larger particles will have smaller diffusion constants as they tend to move more slowly through the solution, while smaller particles will have a larger diffusion constant. Diameter of the particles is determined via the Stokes-Einstein equation:

$$d = \frac{K_b T}{3\pi D \eta_0}$$

Where k_b is the Boltzmann's constant, T is the temperature, D is the diffusion constant and η_0 is the viscosity so the solution. 100 μ l of the samples prepared from the supernatant generated during bleaching were placed into a glass vial and 900 μ l of deionized water was added. This glass vial which contains the sample was sonicated at a 50 hz frequency for 20 minutes and measured in the Malvern Zetasizer μ V right after sonication (due to potential flocculation

concerns). The chamber in the Malvern Zetasizer was maintained at 20 °C. All samples were run in triplicate.

The Malvern Mastersizer 2000 is a laser diffraction particle size analyser which uses low angle static light scattering to measure particle size distributions. While DLS is often used for small particles (usually in the nanometre scales – less than 1 μm) the Mastersizer can be used for particles from 2 μm to 2mm. Light that comes into contact with smaller particles will have a larger scattering angle than larger particles. The recorded scatter pattern is recorded, and Mie theory is applied to determine the particle size. Samples were prepared by combining the biomass or pretreated pulps with approximately 50 ml of deionized water. The samples were poured into the Mastersizer sample circulator and pumped through the machine at 300 RPMs. The size distribution was recorded using the Mastersizer 2000 software version 5.60. Approximately 500 ml of deionized water was used to rinse in between each sample. All samples were run in triplicate.

ZetaPALS zeta potential analyser uses light scatter to measure size and zeta potential of colloids and nanoparticles suspensions. Zeta potential is the potential difference between the surface of the particles in a solution and the solution itself (see Figure 7). The area closest to the surface of the particle is called the Stern layer which is where charge is relatively tightly attached to the surface of the particle. The zeta potential analysis records the net electrical charges of the slipping plane which is between the Stern layer and the bulk solution. This analysis provides a view on the stability of the suspension in solution and how likely the

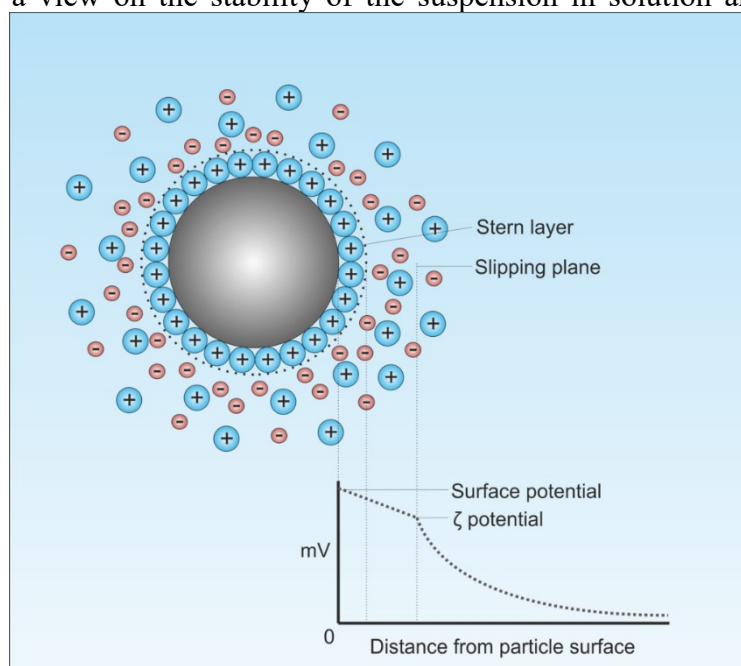


Figure 7 Zeta potential sample distribution of charges around the surface of a particle assuming the particle has a negative surface charge.

solution is to flocculate. The greater the potential the more stable the particles will be in solution. Samples for zeta potential were prepared in the same manner as for DLS. The only difference was the insertion of the zeta potential probe directly into the cuvette during measurements.

2.11 Elemental Analysis

Elemental analysis has multiple uses. It is one way to determine the composition (usually in percentages of elements) of a compound and can also be used to determine the purity of a substance. The most common method for elemental analysis is via combustion. Combustion analysis is a destructive technique that burns the sample in the presence of oxygen. The resulting products of carbon dioxide, water, and nitric oxide were measured to determine the carbon, oxygen, hydrogen, and nitrogen content of the sample. Elemental analysis was completed by London Metropolitan University. Approximately 50 mg of each sample was mailed to their labs for analysis. Results were provided in percentage amounts of carbon, nitrogen, oxygen, and hydrogen of the samples provided.

2.12 Calculating Higher Heating Values (HHV)

The higher heating value (HHV) is the amount of heat generated during the combustion of a compound starting and ending at 25 °C. This value is important for comparison of various fuels and their efficiencies. HHV calculations and estimations were based off the equation provided by Jablonsky et al.:

$$\text{HHV} = 0.40659 \cdot C$$

Where C is the carbon content of the compound.⁵

2.13 NMR/HSQC and solid-state NMR

Nuclear magnetic resonance (NMR) spectroscopy can be useful in providing information regarding functional groups and chemical structure of a compound. General ¹H NMR and ¹³C

NMR values were provided in Figure 8. NMR applies a magnetic field to the sample causing the protons to align with that magnetic field. A radio-frequency (RF) pulse is used to perturb the alignment and the resulting decay is recorded. These samples work on elements that have

deshielding side = less electron rich

Typical ^1H and ^{13}C NMR chemical shift values.

shielding side = more electron rich

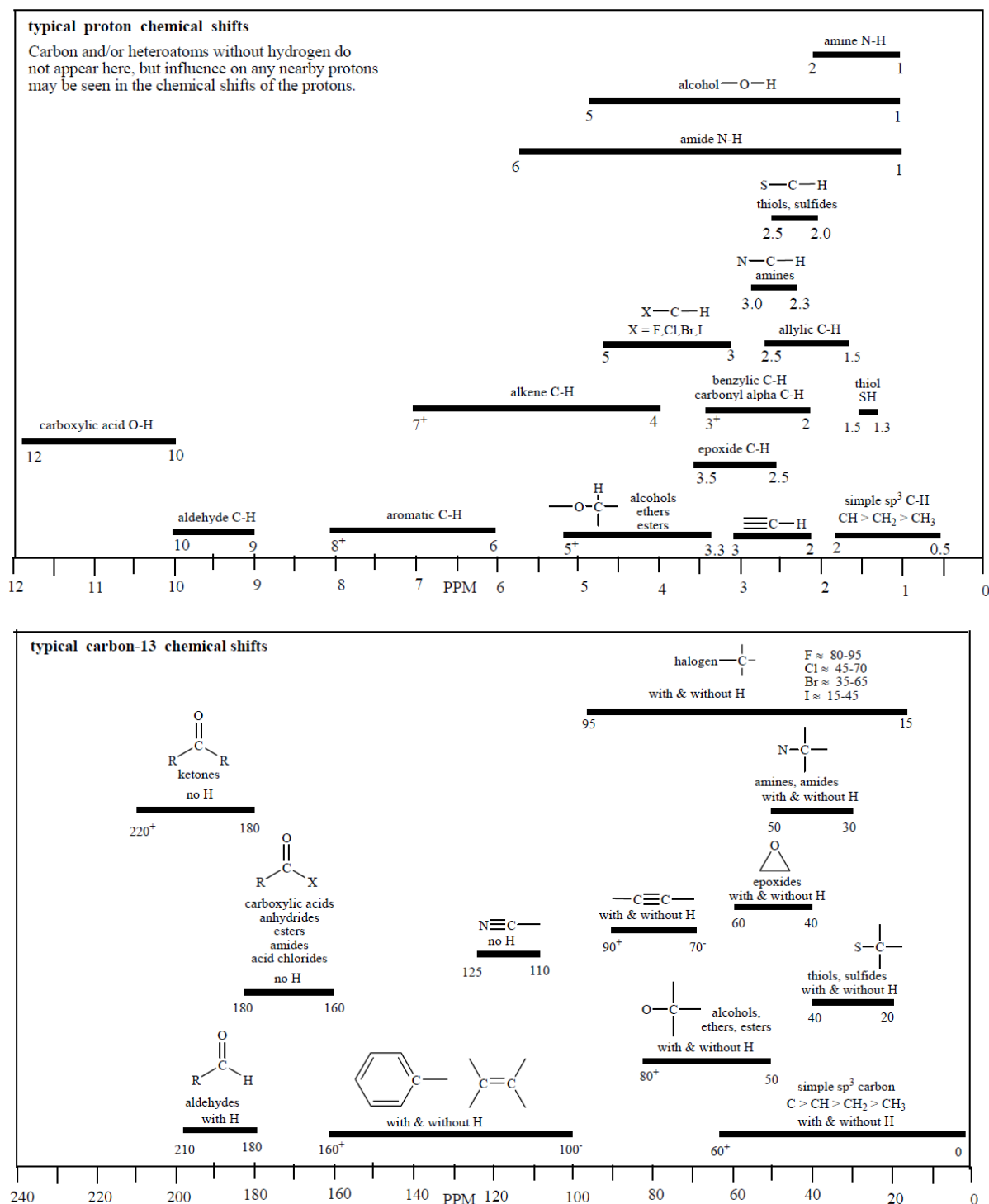


Figure 8 Range of structural features for ^1H NMR (top). Whereas of a compound where the proton has higher electron coverage will be found at lower ppm while more deshielded protons will be found at higher ranges. Carbons that do not have a hydrogen attached will not appear in the ^1H NMR however will appear in the ^{13}C NMR (bottom - table of structural groups).

Figure 8 Range of structural features for H NMR (top). Whereas of a compound where the proton has higher electron coverage will be found at lower ppm while more deshielded protons will be found at higher ranges. Carbons that do not have a hydrogen attached will not appear in the HNMR however will appear in the C NMR (bottom - table of structural groups).

“spin” meaning that they have either an odd number of protons and or an odd number of neutrons. Therefore, NMR and heteronuclear single quantum correlation NMR (HSQC – 2D NMR) were used to detect the hydrogen and ^{13}C carbon isotopes found in the samples. HSQC is valuable to determine which carbons were joined with which hydrogens for a better understanding of the chemical structure of the compound. Most NMR samples were prepared via the dissolution of the sample into a solvent such as deuterated water, acetone, or dimethyl sulfoxide (DMSO). Solid state NMR (ssNMR) were performed on cellulose or pulp samples as these samples were not easily dissolved in any solvents. For ssNMR 100 mg of sample was provided to for analysis. Otherwise, samples for ^1H NMR, ^{13}C NMR, and HSQC were prepared by dissolving approximately 25 mg with 0.5 mL of deuterated DMSO. The samples were shaken and vortexed vigorously to ensure dissolution. The mixture is transferred to an NMR tube using a syringe and submitted to either 400 or 600 MHz Bruker NMR machine. Analysis of the results were conducted using MestRaNova NMR softwwere.

2.14 X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) is a non-destructive method used for the determination of the crystal structure (atom arrangements) within a compound. X-rays were directed at a sample and the intensity, angle, and wavelength of the scattered beams were recorded. XRD was used in this project to determine the crystalline structure of *Miscanthus* and its pretreated pulps. Samples were prepared by grinding and sieving the particles 53 μm or less. The PANalytical Empyrean system was operated at 40 KV and 40 mA with a $\text{CuK}\alpha$ radiation source to carry out the measurements. The patterns collected were in the 2θ range of $5\text{--}50^\circ$ with a step size of 0.033° and a scanning rate of 70 seconds per step. A standard Guassian function was applied to the recorded XRD patterns and the calculated the degree of crystallinity for *Miscanthus* and the pulps were generated using the Segal method:

$$CI = \frac{(I_{002} - I_{am})}{I_{am}} \cdot 100$$

I_{002} is the intensity of the peak at position $2\theta = 22.5^\circ$. The peak at 22.5° is commonly assigned to the crystalline cellulose. The I_{am} is the intensity of the peak at position of $2\theta = 18^\circ$ which is attributes to the amorphous portion of biomass (i.e. hemicellulose, lignin, and amorphous cellulose). The crystallite size is calculated from the diffractogram according to the Scherrer equation: $\tau = \frac{k \cdot \lambda}{\beta \cdot \cos \theta}$. τ represents the size of crystallites, k is the constant shape factor of crystallites (here 0.94), λ is the wavelength of the applied X-rays (here 0.1542 nm), β is the line broadening in radians and θ is the Bragg angle measured in degrees.

2.15 Brunauer-Emmett-Teller (BET)

Brunauer–Emmett–Teller (BET) analysis measures how gas molecules physically adsorb on to the surface of solids and allows us to analyse the specific surface area as well as the pore size on the surface of the solid. This technique measures the physical adsorption of a gas on to the surface of a solid where the major interactive force at play were dispersion forces. The most common gas used for BET measurements is nitrogen as it generally not reactive with most solids. Surface area can be determined by measuring the amount of gas adsorbed. Approximately 200 mg of *Miscanthus* and pretreated pulps were degasses overnight at 80 °C with nitrogen. Use the TriStar surface area and porosity analyser (micromeritics). The software package TriStar 3000 V6.07 fits the adsorption and desorption isotherms using the Brunauer-Emmett-Teller model. Measurements were taken in triplicate.

2.16 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) uses beams of electrons to investigate the surface of a solid compound. Conventional light microscopes were limited by the degree of magnification that can be achieved but also by the amount of light (wavelength) that can be used to illuminate samples. These limitations result in poor resolution. Modern SEM can provide resolutions to 20 nm. Most SEM samples were placed under vacuum in order to minimize interference with the electron beam. Electrons were accelerated though a series of lenses and focused on to the surface of a sample. The electrons impact and reflect from the surface of the sample it generates scattering and x ray emissions. These signals were recorded

and were used to generate an image of the sample. SEM conducted on *Miscanthus* and the pretreated pulps using the JEOL JSM 5610 LV scanning electron microscope (SEM). Samples were coated with a thin layer of gold and images were captured from scattering using a 10 kV accelerated voltage.

2.17 Gel permeation chromatography (GPC)

Chromatography is a technique used to separate mixtures. Gel permeation chromatography (GPC) is a method that separates the analyte, in our case cellulose polymers or lignin, by size. Separation occurs due to the porous materials used inside the column. Smaller compounds will spend more time in the porous column and therefore elute later than larger particles. Lignin GPC was prepared by taking 25 mg of sample and dissolving it in 1.5 ml of lithium brominated DMSO. The samples were then filtered through a 20 micron filter and placed into a 1.5 ml sample vial. Samples were submitted to the Agilent 1260 Infinity equipped with a Viscotek column set (AGuard, A6000M and A3000M) at a flow rate of 0.4 ml/min at 60 °C for analysis.

GPC for cellulose samples was completed by the Department of Forest Products Technology at Aalto University, Finland. Untreated *Miscanthus* and pretreated pulps were submitted for molecular weight and degree of polymerization analysis. The following description of the process was provided by Michael Hummel at Aalto University. *Prior to the analysis, the samples were ground to particles smaller than 60 mesh size and delignified by sodium chlorite aqueous solution according to the method of Wise et al. (Wise 1946).⁶ The delignified samples were pre-activated by a water/acetone/ N,N-dimethylacetamide (DMAc) solvent exchange sequence. The samples were then dissolved in a 90 g/L lithium chloride (LiCl)/DMAc mixture at room temperature. The solution was diluted to 9 g/l LiCl/DMAc and filtered through a 0.2 mm syringe filter and analyzed with a Dionex Ultimate 3000 system with refractive index (RI) detection (Shodex RI-101). The degree of polymerization, M_n (number average molecular weight) and M_w (weighted average molecular weight) was calculated using Chromeleon software. Number average molecular weight, weighted average molecular weight, and degree of polymerization information was provided via Microsoft Excel.*

2.18 Fiber Analysis

Fiber analysis was used for cellulose pulp characterization. Like the Mastersizer, it provides length and width information for the cellulose fibers. In addition, fiber analysis also provides curl and fibrillation information for the pretreated fibers. These characteristics provided a deeper understanding of the potential applications of pretreated biomass beyond saccharification for bio-ethanol production. For example, curl and fibrillation have impact on the mechanical properties and shrinkage of paper and other cellulose fiber materials.^{7,8} For this project, we used the Valmet FS5 Fiber Image Analyzer to determine length, width, curl, and fibrillation of pretreated and bleached *Miscanthus* fibers. Sample preparation followed the manufactured suggested methods. Approximately 100 mg of sample was placed into 50 ml of water and submitted for analysis. The FS5 captures images and tabulates, calculates the fiber sample data by the Valmet analyser internal software.

2.19 UV-Vis Spectroscopy

UV-Vis is a form of absorption spectroscopy that measures the absorption of a compound in the visible and ultraviolet range. This is due to the excitation of electrons from their ground state to a higher energy state. Aromatic compounds with π electrons are generally able to absorb more energy, meaning that absorption is more likely to occur in the UV spectrum range. UV-Vis spectroscopy can be used to quantitatively determine the amount of an analyte in a solution. The main advantage it has over using an HPLC with an UV detector is that no specific column for that analyte is needed as samples can be prepared and directly placed into the machine for analysis. Often only a small amount of sample is needed, and dilution is required. The same UV-Vis machine used for compositional analysis was used in this process.

The UV-Vis spectrometer was used to determine phenolic or aromatic amounts that were present in the recycled ionic liquid as well as for acid soluble lignin concentrations during compositional analysis (which is discussed in the compositional analysis section). Standards for UV-Vis with [TEA][HSO₄] as the solvent were prepared using a variety of different compounds. Baseline measurements of pure [TEA][HSO₄] were taken and subtracted from the standards and samples. Standards were prepared via a series of dilutions. Ferulic acid standards in [TEA][HSO₄] were generated from a 0.04 mg/ml of ferulic acid and diluted to

0.02, 0.01, 0.004 mg/ml concentrations. Recycled ionic liquid samples were prepared by taking 10 μ l and diluting with 1990 μ l of [TEA][HSO₄]. Each sample was analysed from 700-200 nm and a regression line was generated from the standards using the three peaks found at 330, 295, and 240 nm which conformed to the maxima found in the recycled ionic liquid. Using the regression line, an approximate concentration of phenolics was calculated. Similar standards were also generated with Kraft alkali lignin. A standard of 1.75 mg/ml was first created and dilute to 0.07, 0.035, 0.0175, and 0.00875 mg/ml. Absorption peaks at 228, 285, and 331 nm were used to generate a regression line to calculate phenolic concentration.

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3 Impact of the ionoSolv process on *Miscanthus* structure

3.1 Introduction

One of the main challenges that faces the use of second generation biomass as a fuel source is the cost associated with the additional pretreatment step required to access the cellulose for subsequent saccharification and fermentation to bioethanol.^{1,2} Pretreatment is arguably the most costly step in the production of bioethanol in terms of both energy and monetary expense. Research into various methods for the deconstruction of lignocellulosic biomass has been a focus for many years with the ultimate goal to increase the competitiveness of green energy sources and materials to the fossil fuel processes.³ A plethora of pretreatment technologies have been developed including steam explosion, dilute acid pretreatment (DAP), organosolv, and ammonia fiber explosion (AFEX).⁴⁻⁸ However, none of these are effective and cost efficient pretreatments for a diverse range of feedstocks, limiting their potential for broader adoption outside of specific chemical processes.³

To this point, the use of ionic liquids (IL) for pretreatment has attracted attention for its potential versatility and cost competitive processing.⁹ Ionic liquids are seen as a class of green solvents due to their negligible vapour pressure and properties such as the ability to be recovered and reused, additionally some have good thermal stability making them attractive for the use in biomass pretreatment.^{10,11} In addition, selective pairing of their cation and anions components fine tune reaction properties, which allows for tailoring of IL to many different chemical processing situations, whether for catalysis, synthesis, or pretreatment for the biorefinery.¹²⁻¹⁶

The principal objectives of IL pretreatments seek either the dissolution of the entire biomass pulp (including cellulose) or the dissolution/partial fragmentation of the surrounding lignin. To date, most of the research focus has been on the dissolution of cellulose followed by a regeneration step.¹⁷⁻¹⁹ In this type of pretreatment, cellulose I, which forms natively in biomass, is dissolved and regenerated to cellulose II which has a higher thermodynamic stability.^{20,21} These processes commonly use an imidazolium cation with a chloride or acetate anion. Some of the most common ionic liquids used for this type of pretreatment include 1-ethyl-3-methylimidazolium acetate [EMIM][OAc] and 1-butyl-3-methylimidazolium chloride [BMIM]Cl; structures are shown in Figure 1. While these ILs are highly effective at dissolving cellulose, these ionic liquids have yet to be adapted for broad or commercial use. They are

costly to produce and suffer from low thermostability, rendering these solvents unfeasible for bulk use.

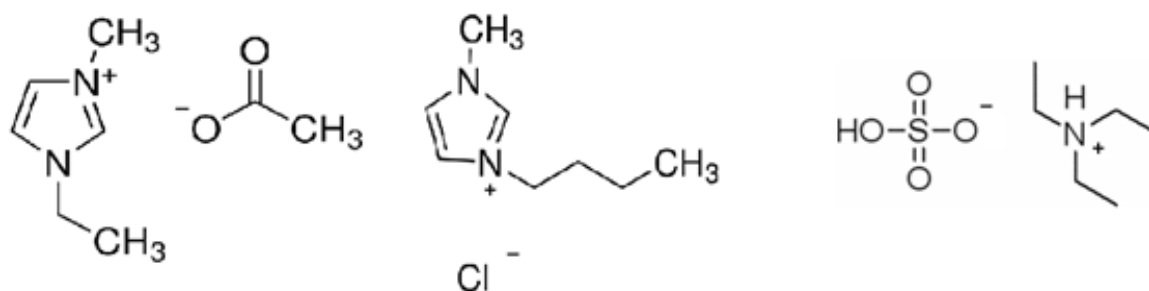


Figure 1 Structures of 1-ethyl-3-methylimidazolium acetate (left) and 1-butyl-3-methylimidazolium chloride (center) which are commonly used for dissolving cellulose. Triethylamine hydrogen sulfate (right) is a protic ionic liquid which is used during this project with the *ionoSolv* protocol.

The second approach focuses mainly on the dissolution or fragmentation of recalcitrant lignin. In this work we present our investigations into the *ionoSolv* process and the impact on lignocellulosic biomass pulp generated after pretreatment. The *ionoSolv* process was developed by the Hallett group at Imperial College and uses low cost and easily synthesised ionic liquids to pretreat biomass for further biorefinery processing.²² This process seeks to improve access to cellulose for enzymatic saccharification via the hydrolysis and partial dissolution of lignin by the use of an acidic IL. In addition, conditions for pretreatment can be optimized and adjusted depending on the type of biomass, whether the feedstock is a hardwood, softwood, or grass.^{23–25} Low cost production and flexibility have the potential to develop more economically scalable methods of using IL in multiple industrial processes.

Cellulose is the major component of the cell wall structure of lignocellulosic biomass. Native cellulose consists of both crystalline and amorphous regions. The success of a pretreatment method has historically been measured by the glucose yield of the enzymatic hydrolysis of biomass pulp, as a precursor to biofuel production via fermentation or chemical conversion.²⁶ Cellulose also has great potential in various industries beyond the fermentation for bioethanol fuels.²⁷ Material properties such as, biodegradability, biocompatibility, high tensile strength, and abundance have made cellulose and its derivatives extremely attractive in industries ranging from pharmaceuticals, to optics and textiles, and the production of composite materials for various functions.^{21,28,29} With cellulose's high hydroxyl group count, modifications and adjustments to properties such as hydrophilicity/hydrophobicity can be achieved depending on application needs.^{30–33} No characterization of the cellulose contained in *ionoSolv* pulps has been completed to date.

In this chapter we explore the impact of the ionoSolv process and specifically the protic ionic liquid [TEA][HSO₄] on the lignocellulosic biomass and cellulose structure (see Figure 1). This study will explore time variance in pretreatment of biomass feedstock, with the aim of understanding which dynamics of the ionoSolv process can be tuned for a more efficient pretreatment. A detailed understanding of the ionoSolv pretreatment impact on cellulose is critical for developing potential material applications of process outputs. *Miscanthus giganteus* (*M. Giganteus*) a fast-growing hardy grass suitable for use as a potential biorefinery feedstock was used. The pretreated cellulose-rich pulps were analysed for polymer molecular weight, material porosity, particle size and cellulose crystallinity. The length of treatment time was varied to assess the impact of increasing pretreatment severity on the properties of the cellulose pulp.

3.2 Material and method

*for additional details on each method please refer to the appropriate section in chapter 2

3.2.1 Selected Biomass feedstock and synthesis with IL

Miscanthus giganteus from the Silwood park campus of Imperial College London was selected as the feedstock and harvested in its senescent state. The grass was prepared by drying in open air, then reduced to particle sizes ranging between 180-850 µm by grinding and sieving. Plastic bags were used to store the biomass at room temperature. Sigma Aldrich was the source of triethylamine and 5 M hydrogen sulphate. No further purification steps were necessary. [TEA][HSO₄] was synthesised in a 1:1 acid to base ratio and dried on a rotary evaporator to 20% wt water, confirmed via Karl Fischer volumetric titration.

3.2.2 Pretreatment via the ionoSolv process

The ionoSolv protocol was followed. Please refer to materials and methods chapter (chapter 2) for details of the protocol.³⁴ In addition, more information can be found here at <http://www.jove.com/video/54246/pretreatment-of-lignocellulosic-biomass-with-low-cost-ionic-liquids>. Approximately 1 gram of *M. giganteus* was mixed with 9 grams of IL. For these series of experiments *M. giganteus* was pretreated at 120° C for 4 hours, 8 hours, and 24 hours.

3.2.3 Compositional Analysis

Compositional Analysis was conducted on the feedstock as well as the resulting pulps following the NREL guidelines.³⁵

3.2.4 X-ray diffraction (XRD)

To determine crystallinity, the biomass was ground and sieved. Particles under 53 microns were utilized. The PANalytical Empyrean system operated at 40KV and 40 mA with a CuK_α radiation source was used to take measurements. The patterns were collected in the 2θ range of $5-50^\circ$ with a step size of 0.033° and a scanning rate of 70 seconds per step. Each sample was scanned 4 times.

3.2.5 Fourier transform infrared spectroscopy (FT-IR)

The procedure utilised a PerkinElmer Spectrum 100 FT-IR spectrometer with a diamond germanium ATR single reflection crystal. Feedstock and pretreated pulps were each examined. 500 cm^{-1} and 4000 cm^{-1} with a resolution of 2 cm^{-1} was the range of measurements.

3.2.6 Malvern Mastersizer 2000/ particle size analysis via microscopy

Approximately 1 gram of sample was placed in to deionized water and run through the Malvern Mastersizer. These results were compared with microscope images of the pretreated biomass using the Nikon Eclipse *Ti* and NiS elements software. Images were measured and collated via ImageJ software.

3.2.7 Scanning electron microscopy (SEM)

Images were captured using a JEOL JSM 5610 LV scanning electron microscope (SEM) of both untreated and pre-treated samples. A thin layer of gold coated samples and images were captured up with 10 Kv accelerated voltage.

3.2.8 Gel permeation chromatography (GPC)

GPC was performed by the Department of Forest Products Technology at Aalto University in Finland.

3.2.9 Brunauer–Emmett–Teller (BET)

Approximately 200mg of samples were degassed with nitrogen at 80 °C overnight and analysed with TriStar 3000 V6.07 software. Triplicate measurements were taken.

3.2.10 Enzymatic Saccharification

Saccharification performed according to the protocol Enzymatic Saccharification of Lignocellulosic Biomass (NREL/TP- 510-42629) published by NREL27 (National Renewable Energy Laboratory).³⁶

3.3 Results and Discussion

3.3.1 Pretreatment and Compositional Analysis

Pretreatment of *Miscanthus* was conducted at 120 °C for 4, 8, and 24 hours. The recovered pulp and lignin yields are detailed in Figure 2. There is a decrease in pulp yield from 4 (51%) to 8 (48%) hours demonstrating that during this time, pretreatment is continually extracting lignin and hemicellulose from *Miscanthus*.

This is corroborated with the compositional analysis discussed below, as well as in previous publication from the group.³⁷ However, at 24 hours there is an increase in the recovered pulp weight (53%) suggesting redeposition of pseudolignin onto the pulp. The lignin recovered also

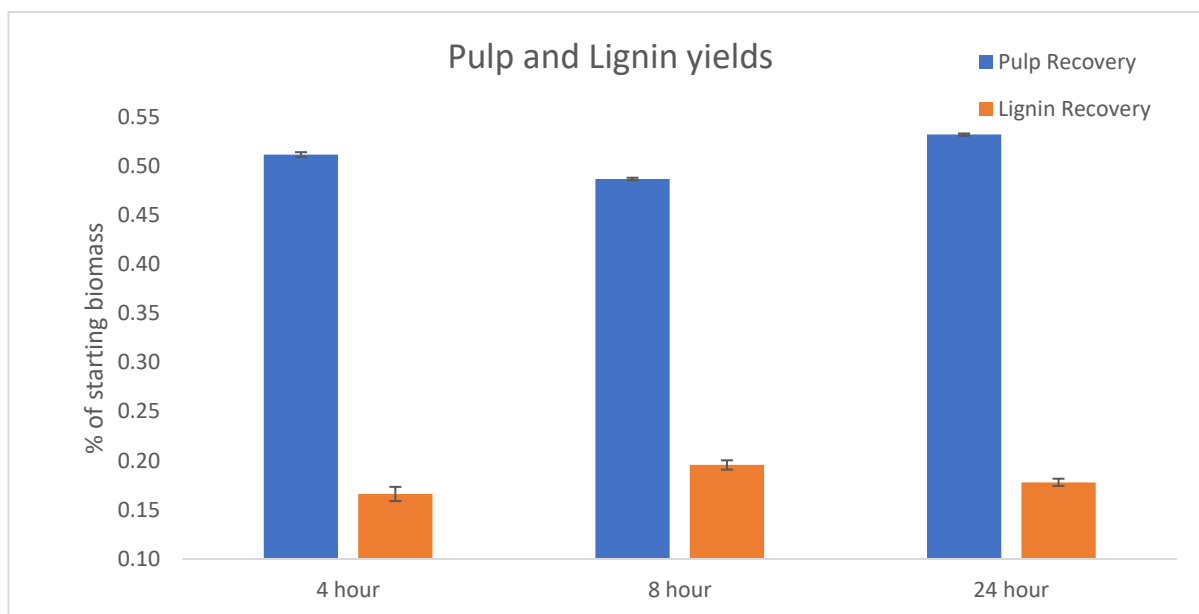


Figure 2 Recovered yields of *Miscanthus* pulp and lignin after pretreatment. Yields are shown as a percentage of the starting dry mass of *Miscanthus*.

increase from 16% to 20% from 4 to 8 hours, but then decreases to 17% at 24 hours. This decrease in recovered lignin corresponds with the increase in pulp recovered. It is hypothesized that lignin fragments may be condensing with sugar intermediates or humins to form pseudolignin. It is possible that this pseudolignin is redepositing onto the surface of the pulp resulting in the increase in the pulp recovered. The effect of the solutes dissolved in the ionic liquid as well as pseudolignin formation during the ionoSolv process will be explored later in this project.

The results of the compositional analysis of *M. giganteus* and the pretreated pulps are detailed in Figure 3 show that the ionoSolv pretreatment is effective at removing and dissolving the majority of the lignin, as well as the hemicellulose component of the biomass.³⁷ A portion of the lignin fraction can later be recovered via water precipitation. As pretreatment time increased from 4 to 8 hours the amount of hemicellulose and lignin removed from the biomass and dissolved into the ionic liquid increased, up to a limit. Lignin in untreated *Miscanthus* represents approximately 27% of the biomass by weight. After 8 hours of pretreatment approximately 5% remains in the pulp (indicating approximately 82% of the total lignin was extracted), while an increase to 11% is observed at the 24-hour pretreatment condition. These

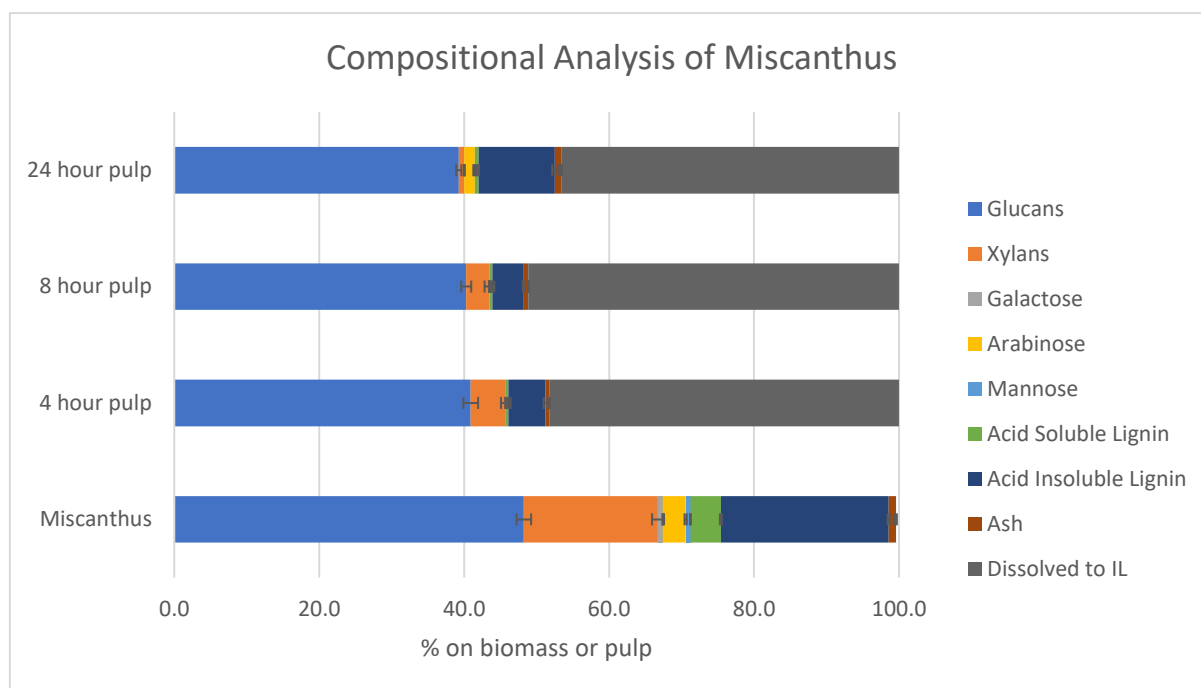


Figure 3 Compositional analysis of untreated *M. Giganteus* and 4, 8, 24 hour pretreated pulps

results demonstrate that over-treating the biomass may result in potential redeposition and condensation of solids onto the pulp as the compositional analysis demonstrates an increase in acid insoluble, or Klason lignin. The amount of hemicellulose decreased from 18% in untreated *Miscanthus* to 3% in the 8-hour pretreatment and less than 2% in the 24-hour trial. The pretreatment also decreases the amount of glucan in the biomass from 48% to 41% representing an 85% glucan recovery. This decrease in glucan content is attributed to the removal of amorphous cellulose from the biomass into the ionic liquid. In all pretreatment time trials, the normalized glucan content after pretreatment remains constant at approximately 40% suggesting that the crystalline cellulose portion of the biomass remains intact. This finding is also supported by the XRD data (presented later in this chapter) which demonstrates an increase in cellulose crystallinity. Pretreatment also gradually reduced the ash content in the biomass. In previous studies conducted by other group members, inorganics and metal have been successfully extracted from the biomass, thus having potential use in industry for heavy metal extraction on waste wood and via phytoremediation.³⁸ The ionoSolv pretreatment seems better suited to lignin removal, removing about 82% of the total lignin compared to the 95% ethanol organosolv with 0.2M HCl pretreatment of *Miscanthus* for the equivalent 8 hours, which removed only 44% of the total lignin. Hemicellulose removal using organosolv is also lower than that achieved with ionoSolv, with approximately 19% xylan extraction at 8 hours.³⁹

3.3.2 XRD

Native cellulose consists of both crystalline and amorphous regions.⁴⁰ Other studies have correlated cellulose crystallinity to glucose yield from enzymatic hydrolysis; those studies have focused on cellulose dissolving ionic liquids, usually using [EMIM][OAc]. However, little is known about the impact of the ionoSolv process and specifically the ionic liquid solution [TEA][HSO₄] on the cellulose structure. Therefore, X-ray diffraction measurements were performed to understand the changes of the cellulose crystallinity and crystallite size as a result of the ionoSolv pretreatment. The X-ray diffractograms of the untreated *Miscanthus* (black) and pretreated pulp (red, blue and green) are shown in Figure 4. The diffractograms

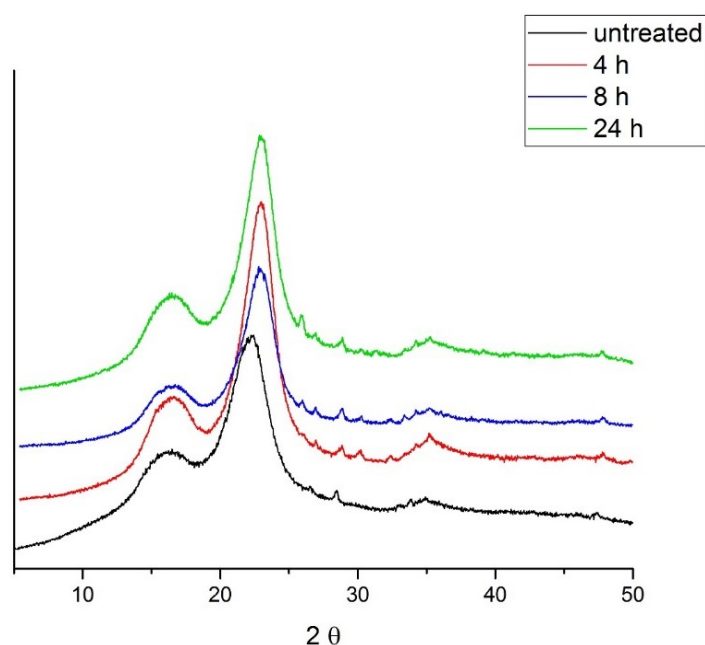


Figure 20 XRD diffractograms of untreated (black) and pretreated *Miscanthus* at 4 hours (red), 8 hours (blue), and 24 hours (green).

were deconvoluted using Gaussian profiles so that crystallinity and crystalline size of the cellulose in the samples could be calculated. In all samples analysed the typical crystalline cellulose polymorph I was present with the 3 major peaks visible at visible at 15-16°, 22° and 35° 2θ. These correspond, respectively, to the combined [110] + [1 $\bar{1}$ 0], [200] and [004] crystallographic planes found in cellulose I. The most intense reflection at 22° 2θ corresponds to the [200] plane of the cellulose. There was a slight shift of the [200] reflection from 22.4° 2θ for untreated biomass to 22.6° for pretreated pulp. This shifted position of the major reflection [200] in the pretreated samples suggest that the cellulose structure has become more crystalline, which agrees with the observed crystallinity index (CI), as detailed in (see Table

1). These results are also supported by the decrease in glucan measured in the compositional analysis. This might be a potential and significant advantage of the ionoSolv process: the dissolution of the amorphous cellulose indicated by the increase in crystallinity, while keeping the crystalline cellulose intact.

The crystallinity index was calculated using the Segal method, which is used to rapidly determine the relative crystallinity of samples (see Table 1).⁴¹ Untreated *Miscanthus* has a crystallinity index of 53.6%. After 4 hours of pretreatment the index increased to 73.0%. There is a noticeable increase in the order of the cellulose present. This increase is the result of the removal of hemicellulose and lignin from the pulp as well as the removal of amorphous regions of cellulose. These results differ from lignocellulosic biomass pretreated with [EMIM][OAc] (and other dissolution pretreatments) where the XRD diffractograms of pretreated biomass displayed significantly less ordered cellulose structures, as compared to the untreated samples.⁴² The main reason for this difference is that the ionoSolv process leaves the cellulose I polymorph intact, while the regeneration of cellulose during the dissolution pretreatments transforms the cellulose into the thermodynamically more stable but less crystalline cellulose II polymorph. There is a slight decrease in the crystallinity index from 70.9 % and 69.4 % for 8 and 24 hours pulps respectively. This decrease in crystallinity is likely due to the redeposition of pseudolignin onto the surface of the pulp, demonstrated by the compositional analysis and corroborated by the FT-IR results (as discussed below). Though it is generally accepted that lower crystallinity will result in better enzymatic glucose release, there are many factors such as pore size, surface area, pretreatment conditions, and lignin removal that contribute to the overall success of the process.⁴³ In addition, the option to produce high crystalline cellulose has various uses in materials chemistry such as the fabrication and applications of cellulose nanoparticle-based polymer composites.⁴⁴

Table 1 Crystallinity Index [CI] and crystallite size of untreated (entry 1) and [TEA][HSO₄] pretreated *Miscanthus* (entry 2-4).

Entry	Pretreatment time [h]	CI [%]	Crystallite size [Å]
1	0	53.6	30.4
2	4	73.0	38.2
3	8	70.9	39.0
4	24	69.4	38.8

Crystallite size of the reflection of plane [200] was calculated from the Scherrer equation based on the full width at half maximum (FWHM) of the diffraction pattern at $22^\circ 2\theta$ and using a K (shape constant) value of 0.94. In addition to the shift of the [200] reflection there is also a change in the width of the diffraction peak for the pretreated samples. There is a decrease of the FWHM of the reflection at $22^\circ 2\theta$. The crystallite size for the [200] lattice planes of cellulose I increases from $3.04 \text{ \AA}$ for raw biomass to $3.82 \text{ \AA}$, $3.90 \text{ \AA}$ and $3.88 \text{ \AA}$ for pulp isolated after 4 hours, 8 hours and 24 hours of pretreatment, respectively. This phenomenon has been observed by others during dilute acid pretreatment which also resulted in an increase in cellulose crystallite size.⁴⁵ Dilute acid pretreatment has been documented to successfully remove hemicellulose but not lignin, while ionic liquid pretreatment using [TEA][HSO₄] successfully removes both. The removal of the barriers between the cellulose crystallites might be causing the crystallites to aggregate and coalesce, thereby increasing the overall size.

3.3.3 FT-IR

FT-IR was used to investigate the effects that the ionic liquid pretreatment process over a course of time using [TEA][HSO₄] (see Figure 5) compared to the untreated *Miscanthus*. The untreated *Miscanthus* shows peaks at 1654 , 1590 , and 1505 cm^{-1} indicative of the carbon

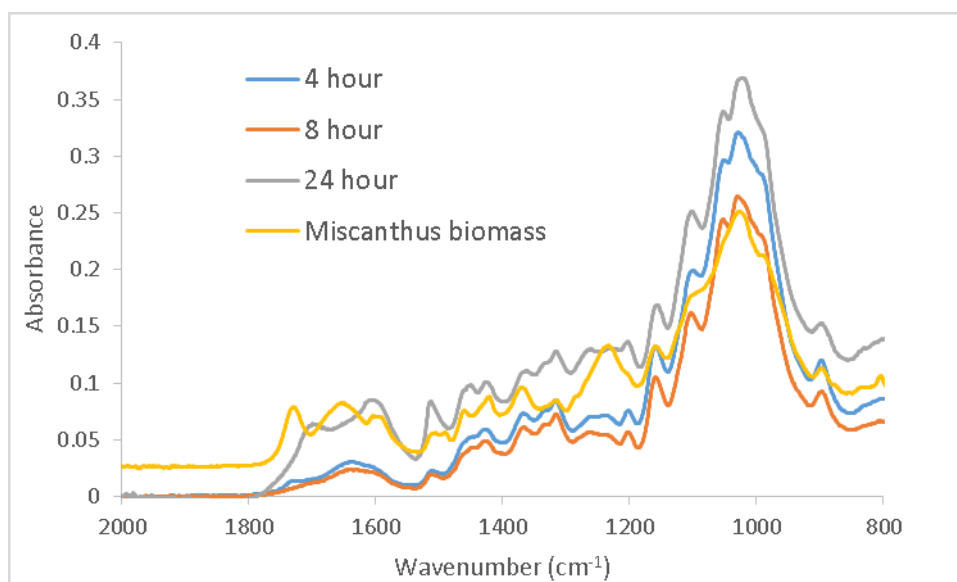


Figure 21 FT-IR of the fingerprint region for untreated and pretreated *Miscanthus* at 4, 8, and 24 hours

carbon aromatics rings in lignin. The peak at 1730 cm^{-1} has been attributed to the various acyl groups (such as carboxylic acids and ester motifs) found in the hemicellulose.^{46–48} Based on

pulp yields, compositional analysis, and XRD, it is expected that much of the lignin and hemicellulose signals would be diminished in the FT-IR spectra. Indeed, after 4 hours of pretreatment, there is a clear decrease in the acyl and aromatics with an even more significant decreases in these peaks at 8 hours corresponding to the removal of the hemicellulose and lignin.

There are several ester bonds in biomass, however it is observed that there is a general decrease at 1230 cm^{-1} (acyl or phenolic carbon oxygen bonds of acetic, ferulic and p-coumaric esters found in lignin) suggesting the cleavage of the β -O-4 bonds found in lignin, this has also been observed and published by others in the group.³⁷ The decrease in the ester bonds confirms the cleavage and depolymerization of the hemicellulose matrix. Each of these peaks would be expected to decrease until the spectra closely resemble that of pure cellulose (supplementary 3-A). There is an interesting appearance of peaks in the acyl and aromatic regions (1692 , 1600 , and 1513 cm^{-1} attributed to aromatics – phenyl and furanic rings) during the 24-hour pretreatment. It is hypothesized that this may be the result of the formation and redeposition of pseudolignin onto the pulp. Others have also shown the development of these peaks in the aromatic region using acidic pretreatments with hollocellulose. The formation of pseudolignin not only increases the apparent lignin content of the pulp, but also reduces enzymatic saccharification (which will be demonstrated later in this chapter). These results demonstrate that FT-IR can potentially be used on pulps to predict the success of saccharification as well as purity of the pulp generated.

3.3.4 BET and SEM

Effects of the time course pretreatment on the surface area and porosity of the *Miscanthus* pulps were analysed using BET analysis (see Figure 6). Information such as

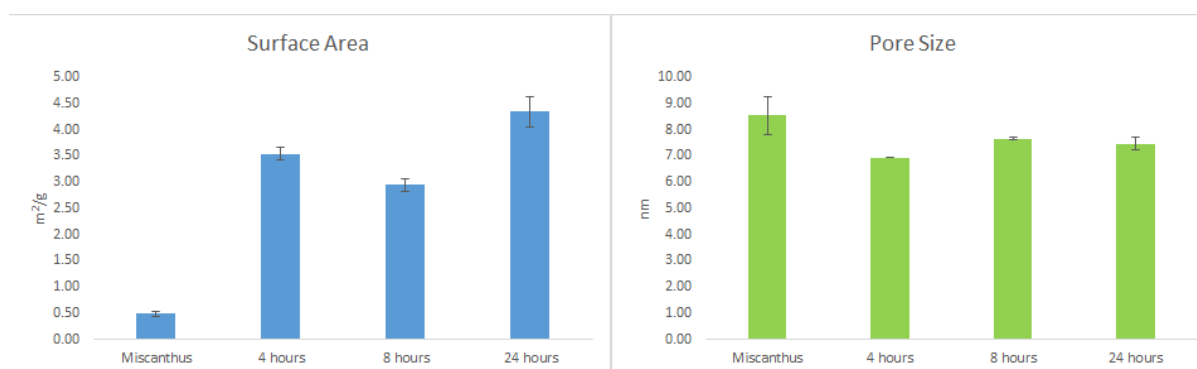


Figure 6 BET surface area and average adsorption pore with of untreated *Miscanthus* compared to pretreated pulp.

surface area and porosity are important for various industrial applications as well as saccharification for eventual bioethanol production. Increased surface area can affect chemical interactions and modifications to the pulp, which in turn alters the properties and characteristics of composites made from the cellulose pulp. Porosity has been correlated to the success of enzymatic saccharification. It is thought that small pore size limits the access of enzymes, reducing enzymatic glucose release.⁴⁹ The combination of increased surface area and either creating new pores or increasing existing pore size should improve enzymatic performance.⁴³ Untreated *Miscanthus* had an average surface area of 0.49 m²/g. After pretreatment, we see a significant increase in the surface area of the biomass to 3.54 m²/g for the 4 hour pretreatment and 2.95 m²/g and 4.34 m²/g for the 8 and 24 hour pretreatment respectively. However, there is also an overall decrease in pore size when compared with the untreated *Miscanthus*. Pore size for untreated *Miscanthus* averaged at 8.53 nm when compared to 6.93, 7.66 and 7.46 for the 4 hour, 8 hour and 24 hour pretreatments. Most likely the removal of lignin and the dissolution of hemicellulose is responsible for the movement of surface materials. One hypothesis is that this disruption also exposed some of the cellulose fibrils which simultaneously increases the surface area of the pulp and opens new, smaller pores.

Structural morphology was further examined via scanning electron microscopy (SEM) (see Figure 7). Significant changes can be observed over the course of pretreatment. Untreated *Miscanthus* which has a small surface area shows a relatively smooth surface. However, after just 4 to 8 hours of pretreatment peeling and fissures in the surface can be seen. By 24 hours the surface texture is significantly broken down demonstrating a clear disruption to the biomass. This disruption may be a result of lignin and hemicellulose removal from the biomass and potential pseudolignin formation, as well as defibrillation of the cellulose fibrils. Though previous studies with hollocellulose have shown the formation of pseudolignin droplets during dilute acid pretreatment, no evidence of droplets was seen on these SEM images at these magnifications.⁵⁰ It is possible that the differences in our pretreatment operating conditions account for the alternate result (temperature was conducted below the glass transition temperature of lignin – which according to simulations is approximately 140 °C, feedstock, pretreatment solvents, and wash up protocols).⁵¹

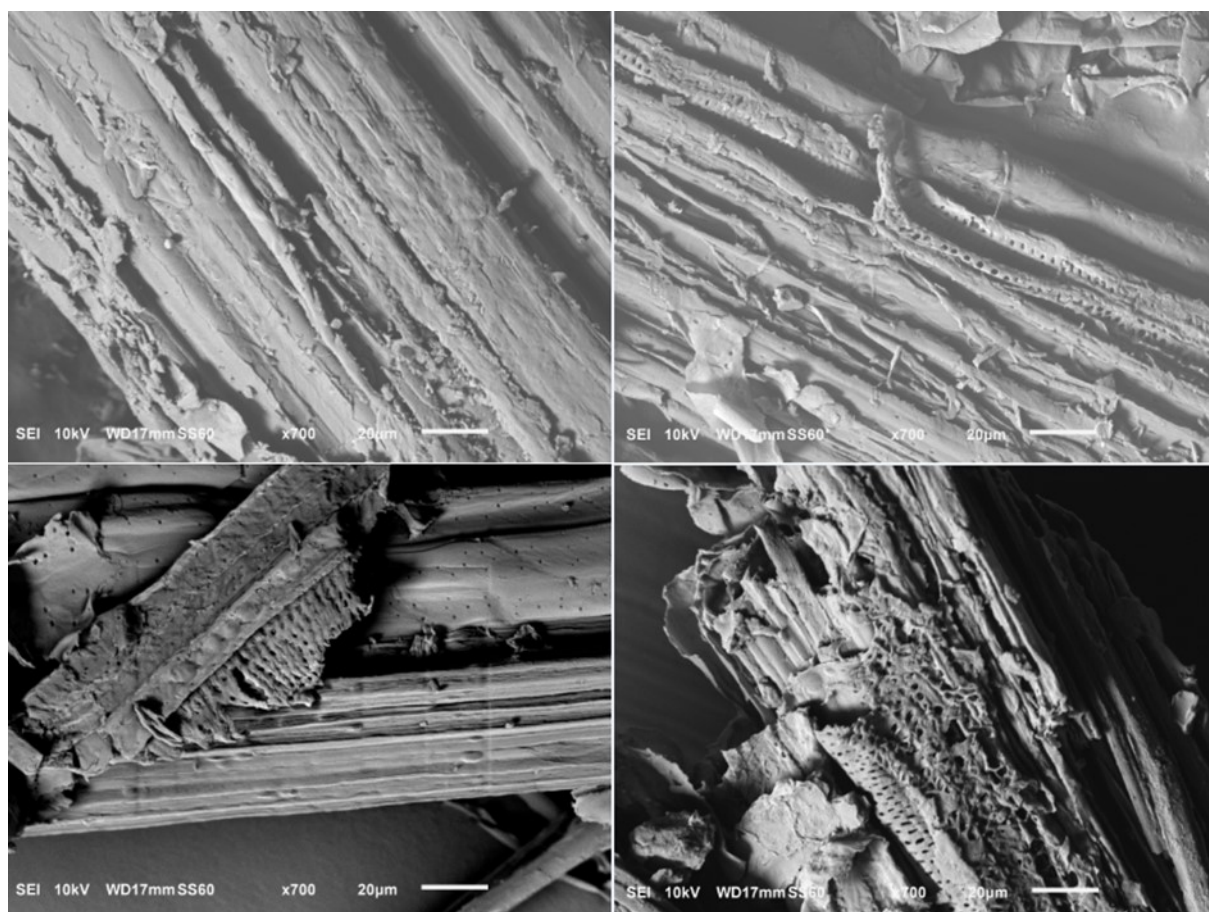


Figure 7 SEM of *Miscanthus* before pretreatment (top left), after 4 hours (top right), 8 hours (bottom left) and 24 hours (bottom right)

3.3.5 Size analysis and GPC

A Malvern Mastersizer was used to determine the influence that pretreatment may have on the particle size of the *Miscanthus*. Microscopy was used to confirm these measurements with results listed in Table 2. Particle size is important for materials applications. For example, in nanocellulose production, size can influence the ability to disperse or flocculate in solution. Similarly, size can impact the abrasive, absorption, and polymerization abilities of microcrystalline cellulose and larger particles.^{52,53} Untreated *Miscanthus* used for pretreatment was ground and sieved to sizes between 850-180 μm , this resulted in an average width of 302 μm and an average length of 1388 μm . After 4 hours of pretreatment the average width did not materially change (310 μm) however there is a significant decrease in the average length of the particles. Significant decrease in the width and length of the particles occurs after 8 hours of pretreatment at 193 μm and 665 μm respectively.

Table 2 Average particle size of *Miscanthus* before and after pretreatment.

Treatment	Average width (μm)	Average Length (μm)
Untreated <i>Miscanthus</i>	302	1388
4 hours	310	803
8 hours	193	665
24 hours	286	769

Comparing the untreated *Miscanthus* average width and length to that which has been subjected to 4 and 8 hours of pretreatment confirms the disruption of the biomass surface and the dissolution of the materials on the surface of the biomass resulting in a decrease in the average overall size of the particles, with significantly shorter particles. At 24 hours, there is a slight increase in the average size and width of biomass to 286 μm and 769 μm . In conjunction with the compositional analysis, XRD, and FT-IR, this evidence suggests that extended pretreatment or over-treatment leads to redistribution of solids/pseudolignins onto the surface the pulp as well as partial delamination of the cellulose fibril bundles.

GPC on all samples demonstrated the impact the IonoSolv process has on the cellulose morphology as well as molecular weight and degree of polymerization (DP) (see Figure 8). Untreated *Miscanthus* has an average weight of approximately 68,000 g/mol with a weighted

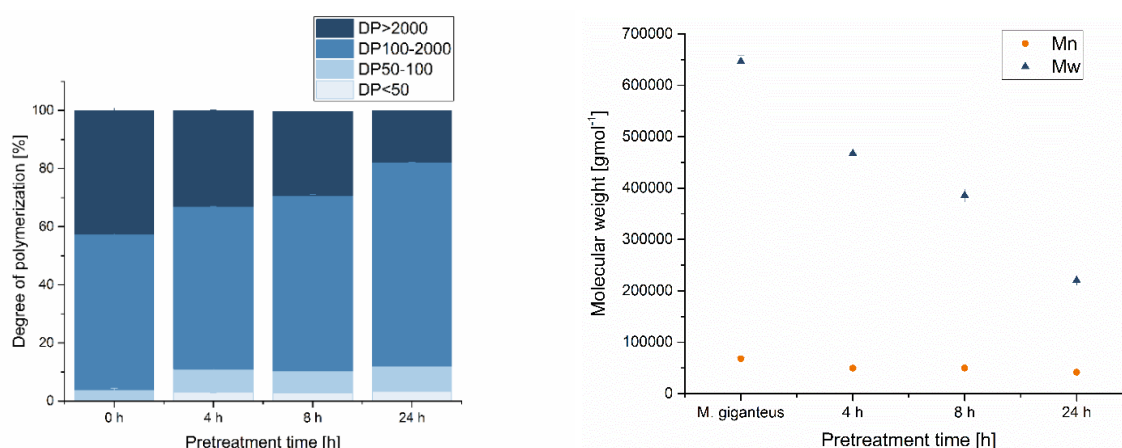


Figure 8 GPC of untreated *Miscanthus*, 4 hour, 8 hour, and 24 hour pretreated with IonoSolv process detailing degree of polymerization (DP) below 50 (DP50), below 100 (DP100) between 100-2000 (DP100-2000), and above 2000 (DP2000) on the left. In addition, molecular weight by average weight (Mn) and by weighted average (Mw) is also displayed on the right.

average of 646,000 g/mol. The means that the majority of the biomass (96%) has a degree of polymerization greater than 100 and approximately 43% over 2000 sugar units. Pretreatment resulted in the reduction of the average molecular weight as well as an overall decrease in the

weighted averages from 467,000 in the 4-hour pretreatment, 234,000 after 8 hours, and 220,000 g/mol in the 24 hour pretreatment. This corresponds to the increase in the cellulose fraction with DP below 2000, with 67% after 4 hours, 71% after 8 hours and 82% after 24 hours of pretreatment. The bulk of the cellulose and greatest increase occurred mainly in the area between 100-2000 with the decrease mainly occurring in the DP of the cellulose above 2000.

Number average degree of polymerization was calculated using the following equation:

$$DP_n = M_n / M_0$$

where M_n is the number-average molecular weight and M_0 is the molecular weight of the glucose monomer unit (180.15 g/mol). The degree of polymerization decreases from 380 for the 4-hour pretreatment to 275 after 8 hours and 230 after 24 hours. These results are similar to results produced from the organosolv pretreatments which have reported cellulose pulp DPs ranging from 150-200.⁵⁴ The hydrolysis of cellulose into smaller sizes may aid in enzymatic digestion and glucose release.

The GPC results demonstrate that the ionoSolv process slowly hydrolyzes the cellulose as well as removing/fractionating lignin and dissolving hemicellulose. This breakdown of the biomass can potentially aid in the saccharification of the pretreated pulp by increasing the surface area for enzyme binding. As mentioned, overtreatment results in cellulose coated with pseudolignin which reduces enzymatic digestion. Results of these trials produced polymerization and molecular weights congruent with microcellulose (DP 150-300) and nanocellulose (DP<150). Molecules of these sizes open further materials uses for size specific processes. Nanocellulose and microcellulose particles produced by the ionoSolv process could be separated after pretreatment for potential applications in drug delivery (pharmaceuticals industry) or composite formation. These pulps can undergo further treatments and modifications allowing for a diverse range of products adding value to the biorefinery.

GPC was performed on the lignin resulting from the ionoSolv process to investigate the effects on the lignin produced (see Figure 9). It should be mentioned that lignin molecular weights can be largely influenced by the feedstock as well as the pretreatment process.⁵⁵ Lignin recovered from 4 h of pretreatment had an average molecular weight of 1340 g/mol and a weighted average of 3570 g/mol. After 8 hours of pretreatment these number slightly decreased

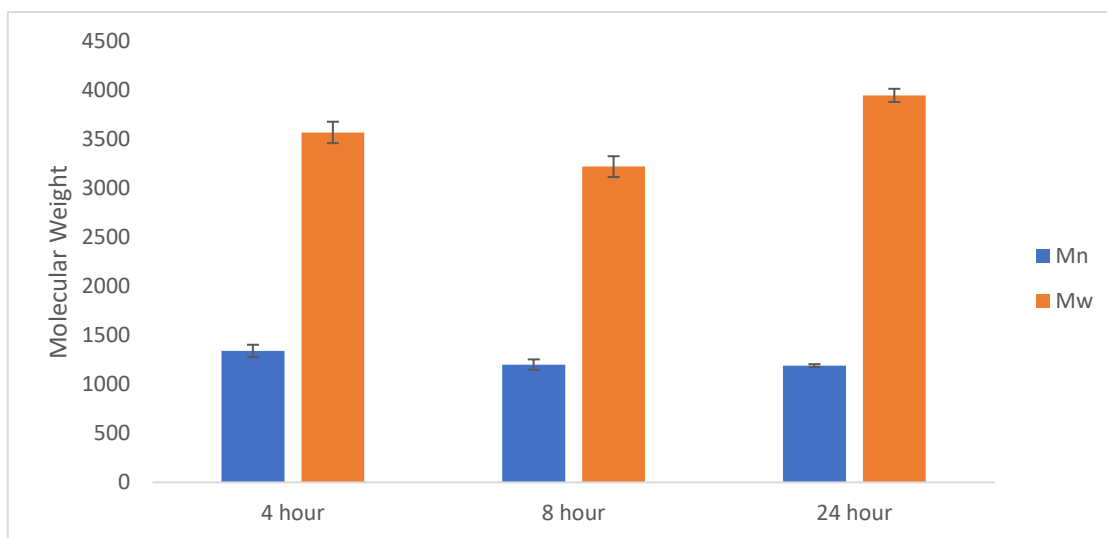


Figure 9 Number average molecular weight (Mn) and weighted average molecular weight (Mw) of lignin precipitate recovered from the pretreatment with [TEA][HSO₄] at 120 °C for 4, 8 and 24 hours.

to 1202 and 3220 g/mol respectively. The increase in the weighted average of the lignin produced after 24 hours of pretreatment (3910 g/mol) suggests an increase number of condensation reactions and possible pseudolignin formation. These figures are in line with lignin produced from alkali pretreatments of bamboo and ethanol organosolv pretreatments of *Miscanthus*.⁵⁵

3.3.6 Saccharification

Based on the previously stated results, the ionoSolv process has a significant impact on the morphology of the biomass. One of the goals of pretreatment is the preparation of biomass for subsequent fermentation for potential production of bio-ethanol. Therefore, saccharification was performed on the *Miscanthus* comparing the results after a 7-day incubation process. Saccharification results demonstrate that lignin removal greatly improved sugar release. Saccharified untreated *Miscanthus* yielded approximately 6%; after 4 hours of pretreatment a 73% theoretical yield was achieved with a similar yield after 8 hours at 72%. However, sugar release was greatly reduced in the 24 hour pretreated *Miscanthus* (35%). Though increased pretreatment time reduced particle size, and DP, in addition to increasing surface area for enzyme access, the solid/pseudolignin formation and deposition on the surface of the pulp ultimately inhibited sugar release (see Figure 10). Correlation between increased Klason content/pseudolignin and reduced glucose release has been reported in dilute acid pretreatment.⁵⁶ Though past studies have suggested that lower cellulose crystallinities demonstrate better enzymatic digestibility, this does not seem to be the case in the ionoSolv process.⁵⁷ Saccharification success is impacted by many factors, including crystallinity, pore

size, surface area, hemicellulose and lignin removal, and pseudolignin formation; all aspects of cellulose accessibility.⁵⁸

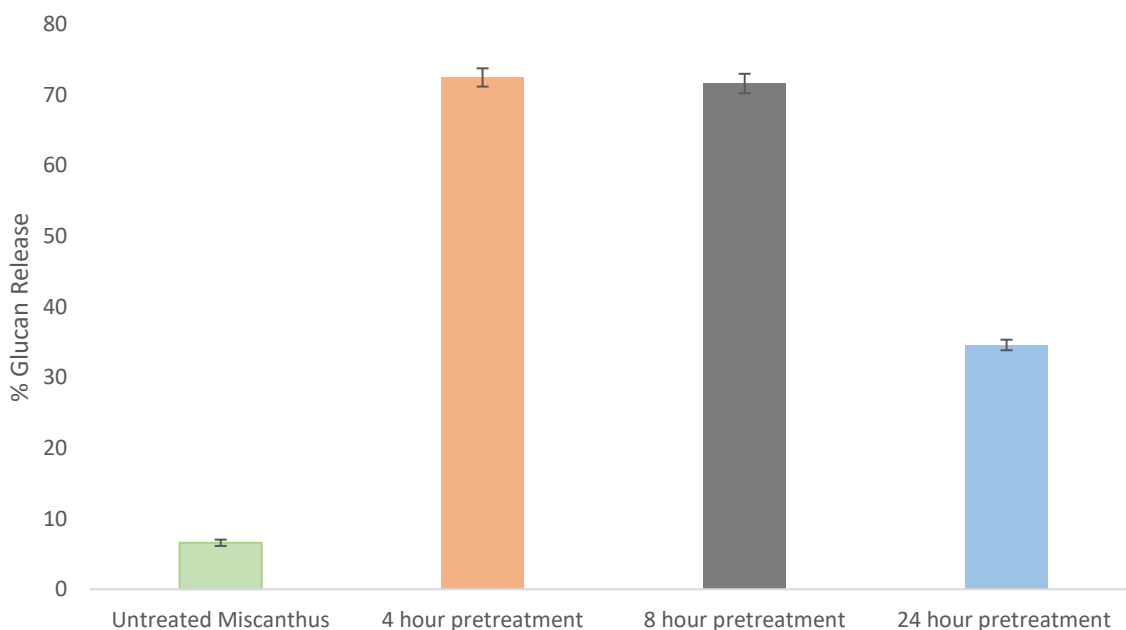


Figure 10 Results of glucan release after 7 days of incubation of Miscanthus and pulps pretreated at 120 °C with [TEA][HSO₄]

3.4 Conclusions

The ionoSolv process is effective at producing a cellulose rich pulp. Lignin and hemicellulose removal is achieved by disrupting the linkages between lignin, hemicellulose and cellulose. The cellulose of the pulp retains the cellulose I structure and has been hydrolysed producing pulps ranging in size allowing for a diverse range of applications. Though other studies have suggested that high crystallinity decreases enzymatic sugar release, this does not seem to be the case with our ionic liquid process, as saccharification is dependent on many factors. Overall pretreatment with [TEA][HSO₄] at 120 °C up to 8 hours, increases surface area, decreases molecular weight and degree of polymerization, increases crystallinity via the removal of amorphous cellulose, lignin, and hemicellulose which greatly improves enzymatic digestion. However, over pretreatment via increase in pretreatment severity leads to a decrease in sugar release, and pseudolignin formation. Ultimately, the ionoSolv process has the potential to be used in biorefinery beyond the production of bioethanol. The pretreatment provides a cellulose rich pulp that can undergo additional modifications for material applications.

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4 Chapter 4: Ionic liquid solutes, carbohydrates and the formation of humins and pseudolignin during the pretreatment process

4.1 Introduction

Economic considerations are critical to assess feasibility of chemical processes employing ionic liquids. Past studies have identified production costs, energy costs, and reusability as potential obstacles to integrating ionic liquids into industrial production. In the case of bioethanol production, the cost of the solvent, energy required for the process, minimization of solvent losses, maximization of biomass loading, processing time (turn over), water usage, and recovery and reuse of the ionic liquid are all opportunities for optimization. Of these optimization considerations the most impactful are those related to the solvent; the ionic liquid per liter is more valuable than the product itself, making recycling obligatory for economic feasibility in any ionic liquid biorefinery. As an example, costs for processes that use [EMIM][OAc] have been estimated to be at least \$20 per kg at large scale. Compounding the raw cost of the ionic liquid, [EMIM][OAc] has low thermal stability and requires dry condition for dissolving cellulose (additional energy for water removal is necessary during recycling). However, the choice of ionic liquid along with process configuration increase efficiency and may significantly improve feasibility.¹⁻³ The ionoSolv process protocol, which is described in chapter 2, uses protic ionic liquid such as [TEA][HSO₄] which has a bulk production cost as low as \$1.24 per kg, sharing a similar price point to other organic solvents on the market such as acetone, ethyl acetate, and toluene.^{4,5}

Production and procurement costs of ionic liquids are often high and thus biorefineries will mainly be operating using the recycled ionic liquid. Prior studies have focused on the economic feasibility of recycling the ionic liquid and its effect on lignin molecular weight.⁶ However, there have been no focused studies on the direct effects of the recycled ionic liquid on the optimization of pretreatment for *Miscanthus* and the formation of humins/pseudolignin in the ionoSolv process. There have been several studies concerning the formation of pseudolignin and its effect on enzymatic saccharification. However, most of these studies have been conducted using the dilute acid pretreatment method (DAP) using cellulose or hollocellulose. These studies do not take in to account the complete biomass composite which

is what is likely to be used in production.⁷ Optimizing an ionic pretreatment process is complicated due to the various chemical interactions that can occur. Interactions between biomass composite, the derivative products produced from carbohydrate transformation, their intermediaries, lignin fragments and the various build-up of solutes dissolved when using a recycled ionic liquid. Pretreatment conditions will also impact the optimization process. Increasing the severity of pretreatment via the increase of acidity, temperature, or processing time impacts the formation of these compounds. Increase severity increases the acid insoluble or Klason content found in the pulps. In both the generation of sugars for fermentation or the production of valuable platform chemical feedstocks, balancing these variables is critical to increase the feasibility of using ionic liquids. Ionic liquid pretreatment that is competitive with other organic solvents has significant potential to reduce the environmental impact for the production of biofuels, materials, and chemical feedstocks. Developing a deeper understanding of the interactions of the components of biomass composite during pretreatment is a critical part of optimizing the process and improving feasibility of using the ionoSolv process for biomass pretreatment.

The aim of this chapter is to determine whether only carbohydrates/carbohydrate intermediates or both carbohydrates/intermediates and lignin fragments are involved in the formation of pseudolignin, as their formation reduces enzymatic saccharification and therefore bioethanol production. The goal of this exploration is a better understanding for the optimization of the pretreatment process using recycled ionic liquid and its impact on the formation of humins and pseudolignin. This chapter will first investigate the solutes present in a recycled ionic liquid to develop a better understanding of the various compounds that might be influencing the production of pseudolignin. Next, it will investigate the formation of humins using the ionoSolv process. Though others have demonstrated the formation of humic substances in acidic ionic liquids, there have been no studies conducted using the ionoSolv process with [TEA][HSO₄] to specifically study humin and pseudolignin formation in conjunction with the entire biomass composite.⁸ Findings presented here will aid in optimization of the ionoSolv process, further increasing the competitiveness of an already less expensive ionic liquid pretreatment by improving efficiency and reducing unwanted side product formation. Other acidic pretreatments have been shown produce humic compounds and it is expected that [TEA][HSO₄] pretreatments are likely to also produce humic substances as well as pseudolignin. The investigation into the formation of humic compounds was conducted by pretreating individual sugars (glucose and xylose) as well as their dehydration

products (HMF and furfural) using [TEA][HSO₄] and comparing the results with the pretreatment using recycled ionic liquid. Lastly, this study explored the effect of doping the *Miscanthus* pretreatments with these sugars and the effects on humin and pseudolignin formation, their impact on pulp and lignin quality, and the effects that doped pretreatments had on saccharification. Humins formed from carbohydrates during these pretreatments will be compared with the lignin/pseudolignin (which may contain lignin fragments) precipitated from biomass pretreatments. Pretreatments were conducted as 150 °C as prior studies which have investigated processing temperature showing that a 50 min pretreatment time is most optimal.⁹ Though similar results can be achieved at 120 °C after 8 hours, this residence time would most likely be too long for an industrial process.⁹

4.2 Materials and methods

(*For additional details please consult the appropriate section of chapter 2)

4.2.1 Synthesis of Ionic Liquid

Triethylamine ($\geq 99\%$) and 5 M sulfuric acid was obtained from Sigma Aldrich and used as received with no additional purification or alteration. [TEA][HSO₄] was synthesized in a 1:1 acid to base ratio following the procedure outlined in chapter 2. In summary, the sulfuric acid was slowly dripped into the triethylamine cooled by an ice bath and stirred until completely mixed. The water content in the ionic liquid solution was reduced using a rotary evaporator and adjusted to 20 wt% water. Water content of the ionic liquid was confirmed by Karl Fischer titration using a V20 volumetric titrator (Mettler Toledo) (for additional information see chapter 2 titration protocol). The recycled ionic liquid was generated from pretreatments using a 10% loading *Miscanthus* at 150 °C for 1 hour. Recycled ionic liquid was also rotary evaporated after lignin water washes to 20 wt% water. Details on recycling can be found in the biomass pretreatment protocol in chapter 2.

4.2.2 Pretreatment of Biomass

Moisture content for *Miscanthus* was determined by placing approximately 500 mg in a foil envelope (preweighed) and leaving the biomass package in a 105 °C oven over night. The foil package and with biomass was reweighed and moisture content of the biomass was

calculated. Approximately 1 gram of dry weight *Miscanthus* was placed into a 15 ml pressure tube with 9 grams of ionic liquid, mixed with a vortex, and placed in the oven 150 °C for 1 hour. The pressure tube was cooled to room temperature and the pretreated slurry was transferred to a 50 mL centrifuge tube, washed with 40 mL absolute ethanol and allowed to sit for 1 h before centrifugation for 50 min. The liquid was decanted, and the ethanol wash repeated three more times. The washed pulp was subjected to 20 h of Soxhlet extraction with absolute ethanol to remove any residual ionic liquid. The resulting pulp was dried for 48 h before being stored in a glass container at room temperature in the dark. Lignin precipitate from the addition of water into the ethanol evaporated ionic liquid was dried on the freeze drier and weighed to determine lignin extraction yield. The recovered liquid fraction was evaporated and the ionic liquid recycled.

4.2.3 Pre-treatment of sugars and furans

Three experiments for the combined sugar pretreatments were carried out to show the formation of humins during ionic liquid pretreatment: (1) glucose and xylose was mixed with fresh IL. The quantity of sugars used was based on the compositional analysis of untreated *Miscanthus* assuming 100% hydrolysis of glucan and xylan to monomers. (2) recycled IL was mixed with the two sugars, and (3) just reheating and processing the recycled IL (RIL), as a control. For the individual sugar experiments approximately 1.0 g of each sugar (glucose or xylose) was dissolved in 10 g of IL, either fresh IL or recycled IL.

The sugar dehydration products trials were conducted with HMF and furfural which were placed into pressure tubes in amounts of approximately 300 mg and 120 mg respectively. These amounts assume the monomerization and dehydration of the glucose and xylose to HMF and furfural. To understand the impact on lignin recovery, xylose, HMF and furfural were also added to pretreatments of *Miscanthus* using fresh IL and recycled IL.

To investigate the potential impact of pseudo-lignin on saccharification, fresh IL and recycled IL were both used to pretreat cellulose (20 µm Sigma cellulose powder was used). For the recycled IL with pulp, the pulp isolated during the generating of the recycled IL solution was used. In all cases approximately, 430 mg grams of cellulose was used. The amounts were calculated from the compositional analysis and represent the amounts of cellulose found in untreated *Miscanthus*.

^{13}C doped glucose and xylose experiments were conducted in the presence of *Miscanthus* to determine and highlight the carbohydrate incorporation into the lignin as well as the pulp produced during pretreatment. In both the case of glucose and xylose 300 mg of each sugar was added to the biomass 15 mg of which were ^{13}C labelled glucose or xylose. Lignin samples and lignin recovered from compositional analysis were submitted to HSQC for analysis.

4.2.4 Pretreatment of Sigma cellulose

These pretreatments were conducted at 30, 60, and 120 min time intervals at 150 °C. The 120 min interval was performed because it would normally be considered a clear over pretreatment of the biomass and should result in the decrease in enzymatic saccharification due to the formation or redeposition of humins or pseudolignin on the surface of the cellulose. It is important to note that the compositional analysis protocol was designed as a method to determine the carbohydrate, lignin, and ash content in a complete biomass composite. Compositional analysis performed on pretreated pulps, and in this case Sigma cellulose, are useful to give us an approximation of constituents with the understanding that this protocol was not designed to give exact figures on modified biomass. The 72% sulfuric acid required to perform the analysis no doubt produces humic substances and may artificially inflate the lignin content.

4.2.5 Compositional analysis of the pulps

Compositional Analysis was conducted on the feedstock as well as the resulting pulps following the NREL guidelines.¹⁰ All were conducted in triplicate. The HPLC used the Aminex HPX-87P column run at 85 °C with a flow rate of 0.6 ml/min.

4.2.6 Saccharification

All saccharification were conducted following the standard group protocol for dry grasses. Saccharification was performed according to the protocol ‘Low Solids Enzymatic Saccharification of Lignocellulosic Biomass’ (NREL/TP- 510-42629) published by the

NREL.¹⁰ 20 μ l of CTEC 2 cellulase enzymes (Novozymes) per 100 mg of lignocellulose or pulp were used. HPLC was used with the same conditions as in compositional analysis.

4.2.7 FT-IR and HSQC

Lignin and pulp samples were submitted to FT-IR and compared. A PerkinElmer Spectrum 100 FT-IR spectrometer with a diamond ATR single reflection crystal was used to analyze the feedstock and the pretreated pulps. Initial background spectra were taken and subtracted from the sample measurement. The spectra were recorded between 500 cm^{-1} and 4000 cm^{-1} with a resolution of 2 cm^{-1} .

HSQC samples were prepared *via* the dissolution of approximately 25 mg of lignin sample into dimethyl sulfoxide (DMSO). The lignin dmso solution was transferred to an NMR tube using a syringe and submit to either 600 MHz Bruker NMR machine. HSQC conducted on lignin samples and analyzed *using* Mestra Nova software.

4.2.8 Analysis of pretreatment and recycled IL – HPLC

Approximately 200 mg of the recycled ionic liquid was placed into a 1 ml microcentrifuge tube and 600 μ l of de-ionized (DI) water was added and the sample vortexed. The sample were allowed to rest at room temperature for 1 hour and then centrifuged. 200 μ l of the supernatant was pipetted into an HPLC vial (containing an insert). The concentration of solutes in the recycled ionic liquid was analyzed using the HPX-87H bio-rad column was used and samples at run 55 $^{\circ}\text{C}$ at 0.6 ml/min flow rate.

4.2.9 UV-Vis spectroscopy

For the analysis of phenolic/aromatics in the ionic liquid standards using ferulic acid as well as precipitates lignin were prepared (please see chapter 2 under UV Vis spectroscopy for additional details on standard production and concentrations). Recycled ionic liquid was analyzed using the Shimadzu UV-2600. Recycled ionic liquid samples were prepared by taking 10 μ l and diluting with 1990 μ l of [TEA][HSO₄]. Each sample was analyzed from 700-200 nm and a linear regression line was generated from the standards using the three peaks found at 330, 295, and 240 nm which conformed to the maxima found in the recycled ionic

liquid. Using the linear regression line determined from the standards, a concentration of phenolics were calculated. Similar standards were also generated with Kraft alkali lignin. Absorption peaks at 228, 285, and 331 nm were used to generate a regression line to calculate phenolic concentration.

4.3 Results and Discussion

4.3.1 Solutes in the Recycled Ionic Liquid

Prior research conducted into the various degradation/intermediate products formed during biomass pretreatments have generally focused on dilute acid or steam explosion pretreatment methods.¹¹ Several of these suggested that HMF and furfural are the main compounds responsible for the formation of humins.^{7,12} However, to date there is still no conclusive evidence of the mechanism, structure, or composition of humic substances or pseudolignin. For the purposes of this project humins, or humic substances, will refer to solids and compounds produced from carbohydrates. Pseudolignin will refer to solids that have been deposited on to the biomass pulp surface (thereby affecting the acid insoluble content) and or solids that may also incorporate lignin fragments.

To investigate the solutes that were present in the recycled ionic liquid a compositional analysis was performed on the untreated *Miscanthus* and compared to the pretreated pulp (Figure 1). Compositional analysis of the pretreated *Miscanthus* found little to no presence of sugars other than xylose and glucose remaining on the pulp. This indicated that all other hemicelluloses sugars were removed and dissolved into the ionic liquid during pretreatment. The amount dissolved into the liquor was calculated by taking the difference between the total

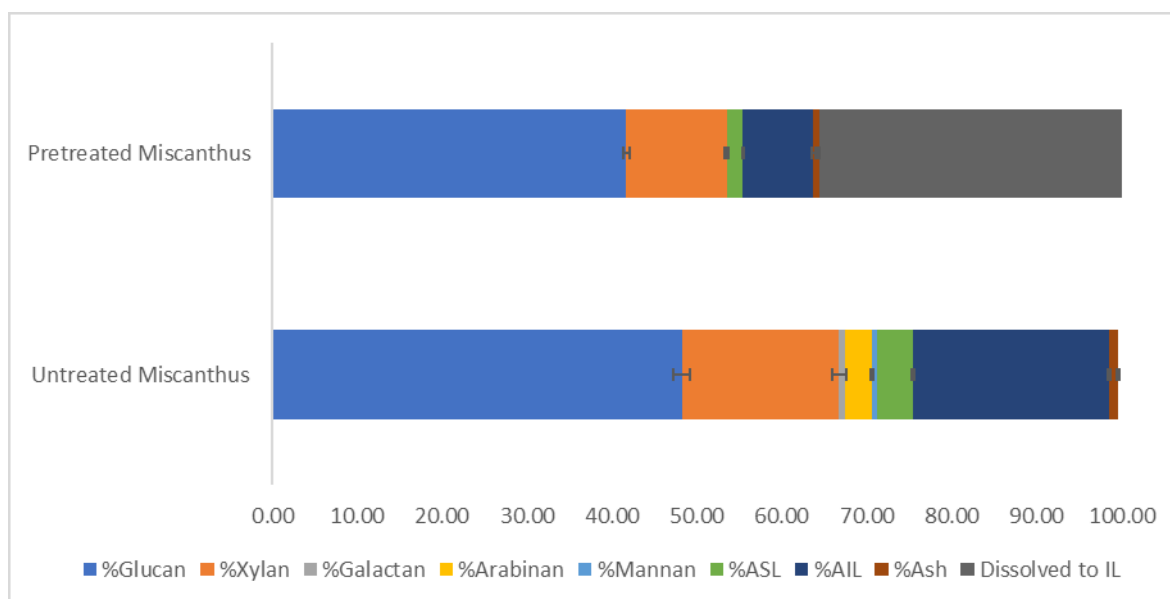


Figure 1 Compositional analysis on untreated *Miscanthus* compared to *Miscanthus* pretreated at 150 °C for 1 hour. Amounts are normalized to the untreated biomass. The total mass loss to ionic liquid is show in grey.

mass of each component of the untreated biomass and subtracting what remained on the pretreated *Miscanthus* pulp (Table 1).

This meant that the solutes in the ionic liquid right after pretreatment would contain a mixture of these carbohydrates, as well as any intermediates, dehydration products, and lignin fragments.

HPLC analysis of the ionic liquid liquor showed over 30 compounds present after pretreatment. Previous studies which have attempted to identify these compounds have demonstrated that the seven compounds with the highest concentrations are glucose, xylose,

Table 7 The difference of each component of untreated *Miscanthus* to 150 °C pretreated *Miscanthus* (left column). HPLC detail of the components and intermediates/degradation products found in the ionic liquid directly after pretreatment (center column). Details of solutes after recycling the ionic liquid (right column). Lignin amounts are calculated after the subtraction of solid lignin precipitation after recycling.

mg removed during pretreatment		mg/ml of solute in ionic liquid after pretreatment		mg/ml of solutes in ionic liquid after recycling	
Glucan	65.3 ±14.0	Glucose	0.25 ±0.02	Glucose	0.28 ±0.04
Xylan	67.1 ±8.1	Xylose	2.86 ±0.22	Xylose	2.78 ±0.41
Galactan	6.4 ±0.1	Formic Acid	0.00 ±0.00	Formic Acid	0.00 ±0.00
Arabinan	31.7 ±1.5	Acetic Acid	2.37 ±0.12	Acetic Acid	1.43 ±.44
Mannan	6.6 ±0.1	Levulinic Acid	0.00 ±0.00	Levulinic Acid	0.00 ±0.00
ASL	22.9 ±1.0	HMF	0.28 ±0.02	HMF	0.26 ±0.04
AIL	148.1 ±1.8	Furfural	2.71 ±0.31	Furfural	0.00 ±0.00
Ash	4 ±2.1	Lignin	14.8 ±1.8	Lignin*	4.8 ±0.02

formic acid, acetic acid, levulinic acid, HMF and furfural.¹³ Though pretreatment conditions and the feedstock involved will greatly impact the intermediates and solutes produced, these

compounds are most likely to be involved in the formation of humin and pseudolignin and therefore was the focus in the experiments conducted.

Analysis of the ionic liquid was conducted at several intervals: immediately after pretreatments, after the ionic liquid was recycled, and after 60 days of storage of the ionic liquid at room temperature. Levulinic acid and formic acid are formed from the hydrolysis of HMF and are expected to be present in a 1:1 molar equivalent. Both levulinic acid and formic acid were not detected via HPLC analysis of the recycled ionic liquid which suggested that HMF was not hydrolysis in the pretreatment process and so no further investigation into their role for pseudolignin formation was pursued (Figure 2). Studies have suggested that levulinic and formic acids are either unlikely to participate in humin formation (such as in our pretreatment conditions with the use of the protic ionic liquid [TEA][HSO₄]), and are likely to be stable in

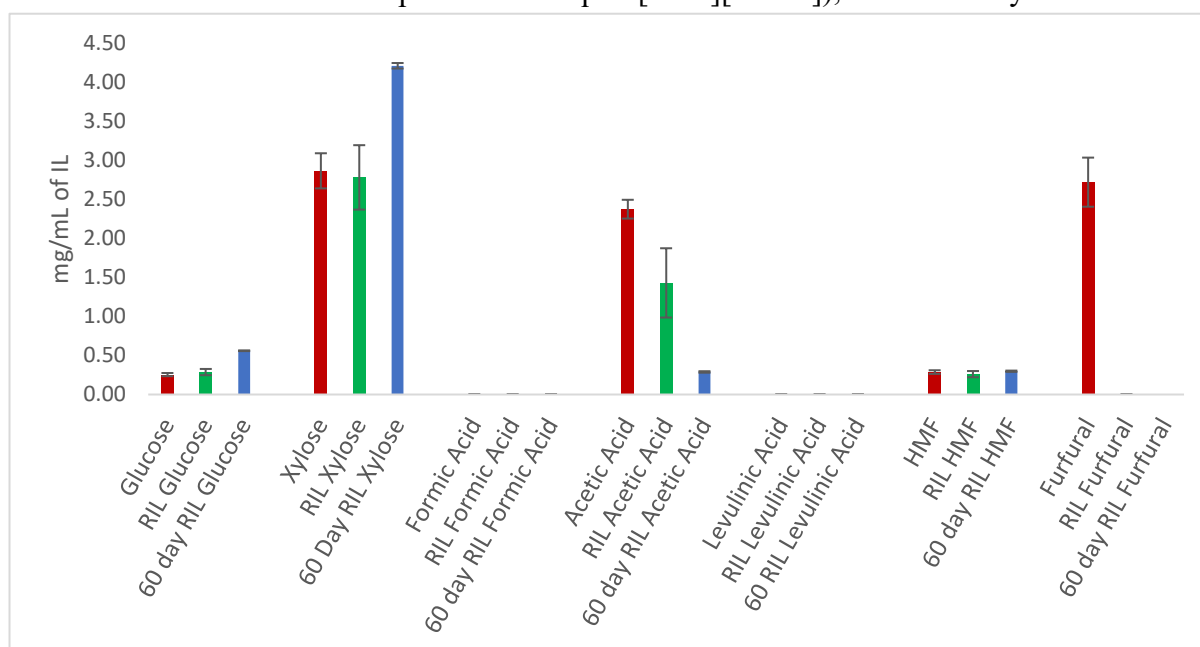


Figure 2 HPLC analysis of the ionic liquid right after pretreatment of *Miscanthus* at 10% wt loading 150°C for 1 hour (red), after recycling (green), and after 60 days of storage at room temperature. Storage of ionic liquid over time changes some of the solute concentrations, though concentrations of HMF remain constant suggesting its stability in the ionic liquid. Oligomers of the glucose and xylose are gradually hydrolyzed as evidenced by the increase in their concentrations.

acidic conditions and would not contribute to humin formation.^{14,15} The stability of HMF has also been demonstrated in other protic ionic liquids such as 1-butyl-3-methylimidazolium hydrogen sulfate [BMIM][HSO₄].⁸ Results of the same study showed no formation of either levulinic or formic acid suggesting the conditions of this pretreatment process are not favorable for their production. One study did suggest that the formation of these products is inversely related to the proportion of humin formation in acidic conditions.¹⁶ This suggests that

intermediates from hexose sugars up to and including HMF are likely culprits for humin and or pseudolignin formation.

Glucose, galactose, and mannose can be responsible for the formation of HMF, though the low levels of HMF found in the liquor suggest that the conditions of this pretreatment are not advantageous to HMF formation from these sugars (Table 1 and Figure 2), this was also found to be true in [BMIM][HSO₄] experiments.⁸ A significantly greater amount of glucose was removed from the *Miscanthus* than the concentration detected in the ionic liquid after pretreatment. The low concentrations of glucose and xylose suggest that they may be present in the liquor as oligomers, dimers, or some other intermediate but not in their monomeric forms. Conversely, there was a more significant presence of furfural detected in the liquor, which would have formed from the pentose sugars: xylose or arabinose. This would mean that conversion from these pentose sugars to furfural occurs readily under these conditions. The lower concentration of xylose detected in the ionic liquid liquor when compared to the amount extracted, is likely related to the conversion of xylose to furfural and other intermediates residing in the ionic liquid liquor.¹⁷ This result was also demonstrated with other protic ionic liquids. The highest concentration of monomeric carbohydrate sugars detected in the liquor were glucose and xylose, which is expected as they have the highest abundance in the biomass composite.

After the lignin was precipitated and the ionic liquid recovered, furfural was removed during the drying process to recover and recycled the ionic liquid (right column of Table 1 and Figure 2). Furfural forms an azeotrope with water and was removed during water evaporation when the ionic liquid was returned to 20% wt. water. In order to approximate the lignin concentration in the recycled ionic liquid the amount of lignin recovered during water precipitation after pretreatment and the amount of residual lignin left on the pulp from the compositional analysis of the pretreated pulp was subtracted from the total amount of lignin determined from the compositional analysis of untreated *Miscanthus* (see Table 1, column 3). This means that after recycling the ionic liquid contains approximately 4.8 mg of lignin per ml of ionic liquid. Compared to the other solutes, lignin fragments have the highest concentration in the recycled ionic liquid.

During the recovery of the ionic liquid there was also a decrease in acetic acid; this was expected due to its relative volatility (see Figure 2). Volatile acetic acid also gradually decreases as the liquor was aged, the amount of acetic acid in a pretreatment has been

demonstrated to impact the structure of biomass.¹⁸ HMF on the other hand remained constant, suggesting that it is stable in this ionic liquid at room temperature. There was an overall increase in glucose and xylose concentrations over time as the sugar oligomers are slowly hydrolyzed under the acidic conditions. No increase in HMF levels are detected indicating that glucose was not converting to HMF, this has also been demonstrated using [BMIM][HSO₄].⁸ Similarly, no furfural was detected, even after 60 days of storage indicating that pentose sugars are also not dehydrating to furfural under these storage conditions.

Assessing recycled ionic liquid and its impact on pretreatment was particularly challenging due to variable solute composition in liquor over time. Not only is time a variable that can impact the concentration of solutes in the ionic liquid, but the composition of the solutes can change even when using the same feedstock depending on the pretreatment conditions. Varying temperature, time, ratio of anion to cation of the ionic liquid are all factors that will impact the concentration of solutes found in the recycled ionic liquid. This was demonstrated when the recovered liquor was analyzed from a pretreatment conducted using a large batch reactor which contained a stirring mechanism (Appendix 4A). The recycled ionic liquid in this batch reactor was also produced using the same *Miscanthus* feedstock at 150 °C for 1 hour. However, the heat profile, stirring rod, as well as time required to ramp the batch reactor to 150 °C, caused a huge difference in the concentration of solutes in the liquor. The complications of managing the solutes (as well as the composition of the biomass during pretreatments) was further demonstrated in lab scale experiments designed to mimic a continuous flow pretreatment of *Miscanthus*. The experiments (Figure 3) maintained the 150 °C, 1-hour pretreatment conditions with a starting biomass of 1 gram (this configuration was the method that was used to prepare the recycled ionic liquid for all recycled ionic liquid

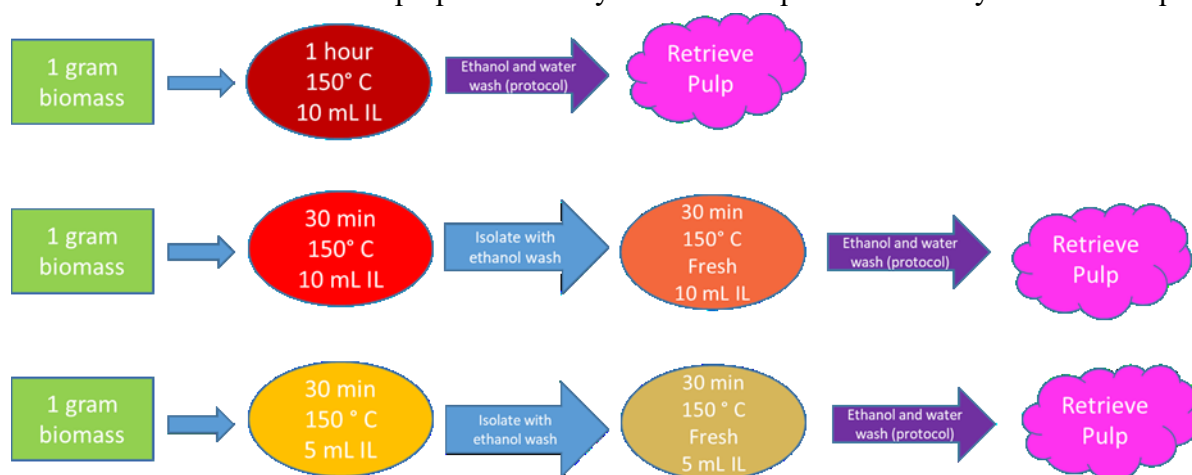


Figure 3 Scheme for lab scale mimic of continuous flow pretreatment of *Miscanthus* at 150 °C for 1 hour.

pretreatments). The pulp retrieved from this reaction condition was compared to pulps generated from pretreatments of the biomass in two separate reactions at 30 min intervals in 10 ml of ionic liquid (30/10) and two 30 min intervals using 5 ml ionic liquid (30/5) during each reaction. The 30 min interval pretreatments each pretreated the same amount of biomass though a fresh aliquot ionic liquid was used for the second 30 min session (using a total of 20 ml of ionic liquid in the first trial Figure 3 middle scheme and 10 ml for the bottom scheme).

The compositional analysis of the pulps demonstrated that how the conditions of 150 °C for 1 hour were applied and how the biomass loading can affect the solutes in the ionic liquid (Figure 4). By completing a 150 °C pretreatment for one hour in two separate reactions it was likely that the reaction spent less time at 150 °C. Research on pretreatment reactions conducted at varying temperatures have demonstrated that the heating profile can have a profound impact on the composition of the biomass, and therefore indirectly the contents of the recycled ionic liquid.⁹ The pretreatment condition of 30/10 provides a pulp that has a higher glucan amount, most likely because the amorphous cellulose areas were left intact due to overall milder heating of the biomass. This reaction condition also contains a lower hemicellulose and residual lignin content on the pulp. However, in terms of the solutes which can be found in the ionic liquid after pretreatment, the recycled ionic liquid produced by the 30/10 pretreatment condition would be expected to have a lower glucose concentration and a higher xylose as well as a higher lignin fragment concentration. This potentially has

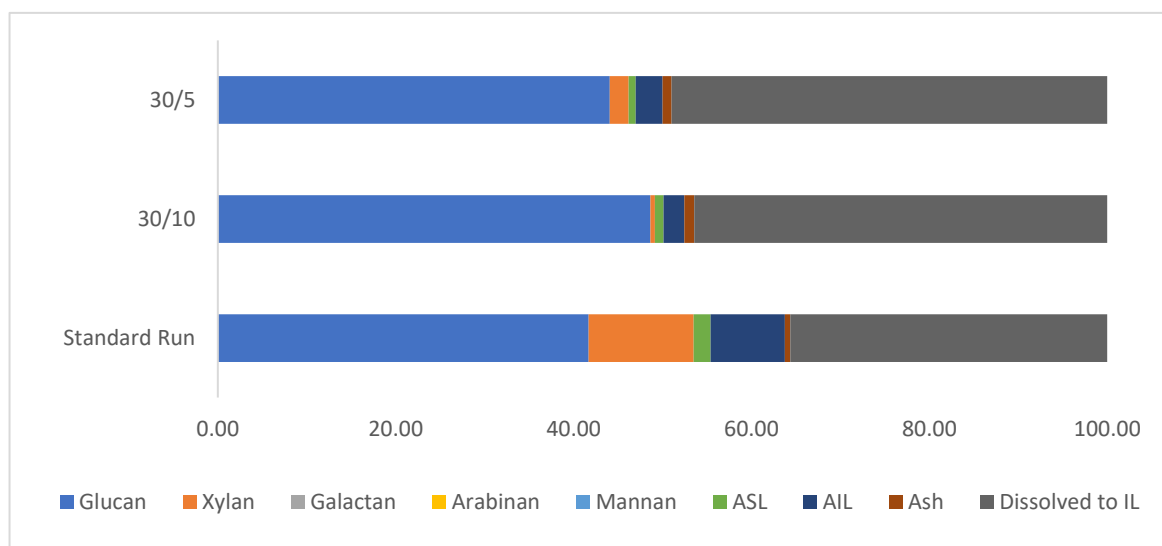


Figure 4 Compositional analysis of “flow reactor” pulps performed at 150 °C for 1 hour using [TEA][HSO₄] running 30 min using 5 ml of ionic liquid twice (top), 30 min with 10 ml of ionic liquid twice (middle), and 60 min with 10 ml (bottom).

implications for the optimization of the pretreatment process, the production/prevention of humins and or pseudolignin, the pulp, and any downstream processes.

If future upscaling of the ionoSolv pretreatment process is focused on pulp composition or humin and pseudolignin formation/prevention, then additional variables that may impact efficiency (like stirring), type of reactor processing, and thermal control of the process will have to be addressed. The solutes and therefore the pretreatment process will change as the ionic liquid is recycled. The pretreatment optimization variables will change over time and age of the recycled ionic liquid due to the changes to the solute concentration. Further recycling studies for batch, as well as continuous flow processing would be necessary to understand the effect of solutes in the recycled ionic liquid over many cycles.

In an attempt to produce a hemicellulose free liquor, it was previously reported that the use of 1-ethyl-3-methylimidazolium acetate [EMIM][OAc] containing 20-40% wt. water would selectively extract hemicellulose.¹⁹ However, compositional analysis of the pulps generated demonstrated that little of the hemicellulose was removed. Subsequent pretreatments of these hemicellulose extracted pulps resulted in high pulp recovery yields due to poor extraction. It has been suggested that this was possible due to hornification of the pulp which poor solvent accessibility.²⁰ Therefore, no further attempt was made to pursue a creation of a hemicellulose free recycled ionic liquid.

4.3.2 Lignin/Phenolics concentrations in Recycled Ionic Liquid

Studies on dilute acid pretreatment by using chromatography-mass spectrometry (GC-MS) and liquid chromatography (LC) have demonstrated that over 100 sugar intermediaries/degradation products can be produced during the pretreatment process.²¹ Different pretreatments will result in varying concentrations of these degradation products, though most of the phenolic and aromatic compounds appear to be present in very small (10-30 μ M) concentrations.¹¹ One study has suggested the formation of phenolic compounds derived from furfural and or HMF during the dilute acid pretreatment process, mainly 3,8-dihydroxy-2-methylchromone and 1,2,4-benzenetriol, are responsible for the formation of pseudolignin.²² Most studies focus on the production of aromatic compounds from carbohydrate degradation, though research that has been conducted on wet oxidation and steam exploded samples have suggested that phenolics can also be formed from the degradation of

lignin.²³ It was also demonstrated that acidic conditions tend to produce greater quantities of phenolics than an alkali process. To investigate the phenolic and other aromatic compound concentration that can be expected during the pretreatment process using [TEA][HSO₄], a sample of the liquor was diluted and analyzed with UV-Vis. Because the potential phenolic compounds in the liquor are unknown, a series of different model compounds were used as standards. Previous research had used lignin derived phenolic compounds for comparison.^{24,25}

Based on a literature review, initial standards were created from ferulic acid.²⁶ Ferulic acid is an aromatic compound commonly found in the cell walls of plants and is one of the aromatic units that is polymerized for lignin formation.²⁷ It has also been shown to be a common aromatic degradation product occurring through hydrothermal and dilute acid pretreatments.^{11,27} The recycled ionic liquid was compared to the standards generated using ferulic acid and an approximate phenolic value was calculated (see Figure 5, and appendix 4A for linear regression equations). Absorption for the recycled ionic liquid at 235 nm, 285 nm, and 331 nm were 1.522, 1.133, and 0.786 respectively indicating a phenolic concentration of 4.0 ± 1.7 mg/ml. This amount was feasible based on the amount of lignin determined from

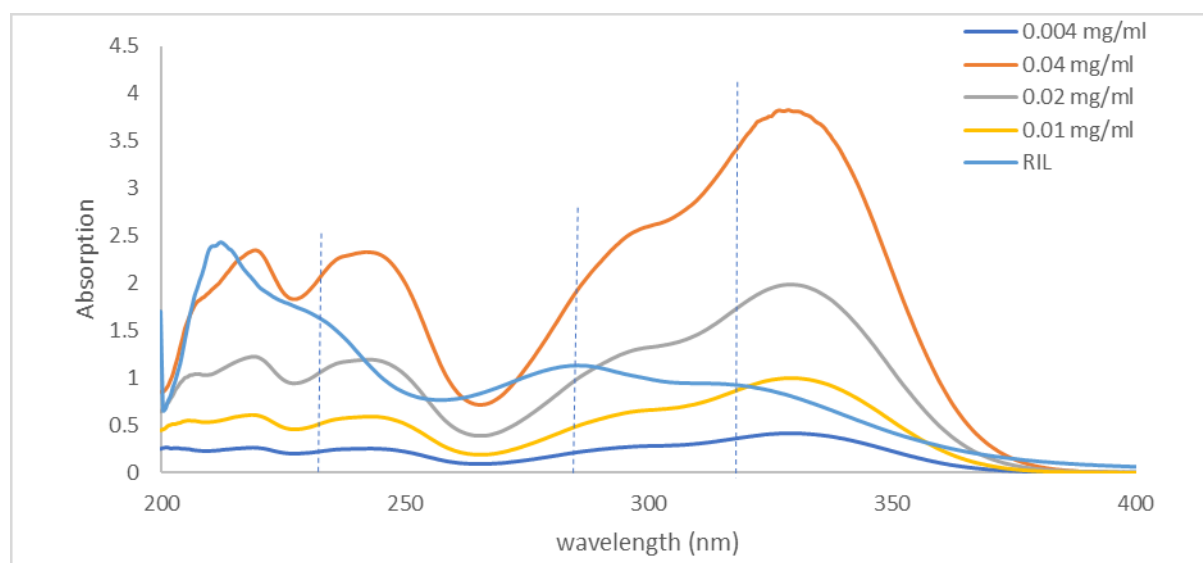


Figure 5 UV-Vis of ferulic acid standards compared with recycled ionic liquid (RIL) from a 1 hour 150 °C pretreatment of *Miscanthus*. Dotted vertical lines are peaks at 235, 285, and 331 nm which were three main peaks found in the recycled ionic liquid.

compositional analysis of the untreated biomass and the amount of lignin calculated to have been dissolved in the ionic liquid. However, it was also clear from the spectra that ferulic acid was not the best standard for comparison as most of the peaks in the ionic liquid do not match closely with ferulic acid. The recycled ionic liquid spectra deviated significantly from that of ferulic acid. The spectra for the recycled ionic liquid would contain phenolics from lignin fragments and based on the literature these peaks appear in the 400-200 nm range due to the

presence of congregated aromatic electrons.²⁵ Though these peaks did not match up well with ferulic acid these measurements can provide a rough estimate. Cinnamic acid and vanillic acid are also two compounds found during lignin production. Comparisons of their UV Vis peaks to the recycled ionic liquid (cinnamic acid which show peaks at 220 and 280 nm and vanillic acid which contain peaks at 225, 260, and 295 nm based on a literature search), also do not match well with the compounds in the recycled ionic liquid, suggesting structural differences of the lignin fragments to these monomeric compounds.²⁸ It was possible that vanillic and cinnamic acids are present, however, the spectra suggests that other aromatics are responsible for the peaks in the liquor.

A second attempt was conducted using Kraft lignin as the standard with the hypothesis that some of the same structural motifs would be present. Though Kraft lignin has been demonstrated to be structurally different from native lignin and the condensed lignin fragments generated during the ionSolv process, Kraft lignin could still potentially give an approximate concentration of aromatic compounds.²⁹ Standards were prepared along with the recycled ionic liquid for UV-Vis analysis detailed in Figure 6 (see appendix 4C for linear regression equation). Though not an exact match to the solutes in the recycled ionic liquid the aromatic peaks at 235 and 285 are the same ranges as the aromatic solutes in the recycled ionic liquid. Calculations using the linear regression of the Kraft lignin as standards suggest a phenolic concentration

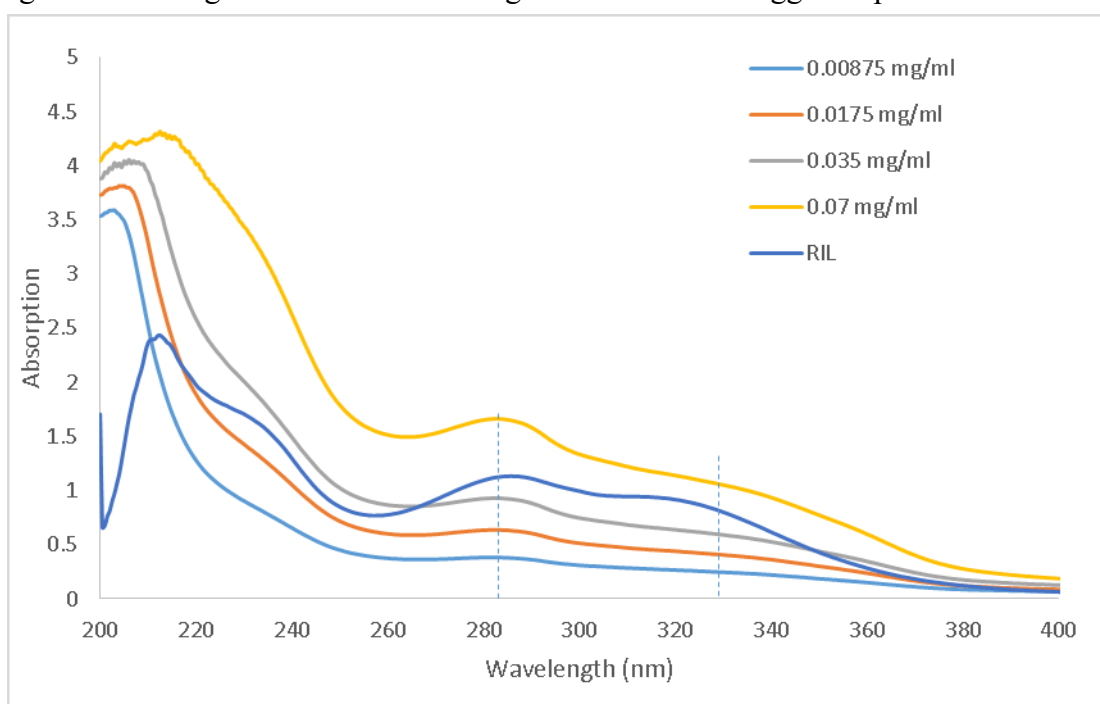


Figure 6 UV spectra of recycled ionic liquid compared with Kraft lignin standards. Dotted lines at 235 and 285 mark the peaks used for comparison.

average of 7.0 ± 2.5 mg/ml. This amount was much higher than the lignin content calculated in Table 1.

One explanation for this higher than expected concentration may be due to the HMF in the liquor (known to have a max absorbance at 283 nm) was being included in this calculation.²⁶ However, even taking this into account, the concentration was still higher than expected. There are several possible reasons for these results. One possibility is the presence of other phenolic compounds in the liquor that are not lignin fragments or HMF. These phenolics are most likely formed from the sugars during the pretreatment process. This seems to be supported by literature and other research which shows the production of aromatics and phenolics during biomass pretreatment (Figure 7).³⁰ It is possible 1,2, 4-benzenetriol, one of the compounds believed to be derived from HMF and has been hypothesized to be responsible for humin production (has an UV absorption at 272 nm) was present in the liquor. A further

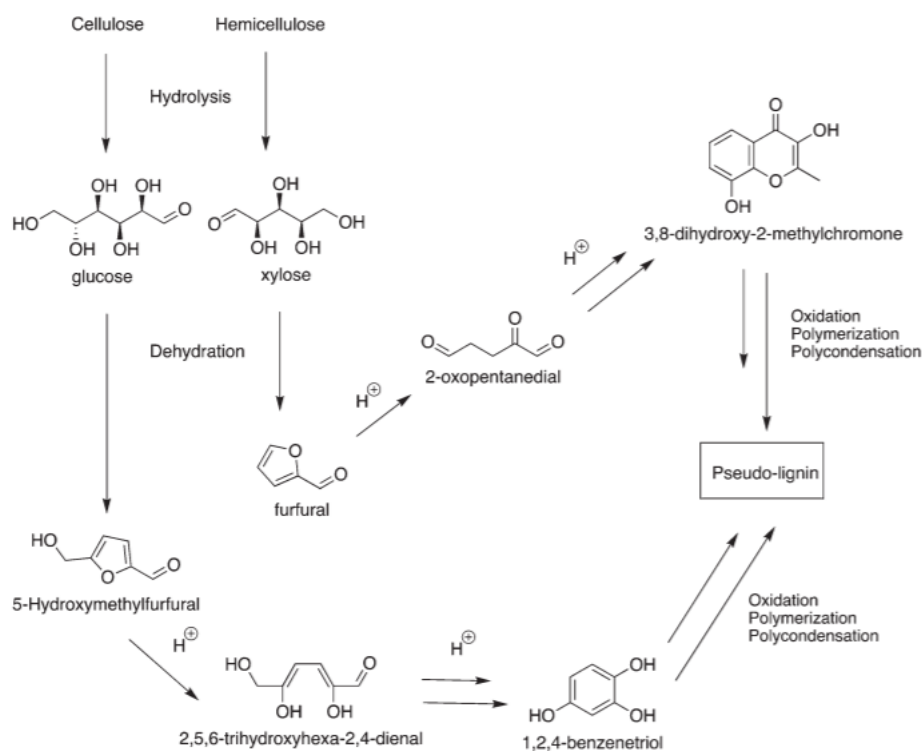


Figure 7 A proposed pathway for the formation of pseudolignin from biomass components which include the formation of 1,2,4-benzenetriol and 3,8-dihydroxy-2-methylchromone.²²

possibility is that 3,8-dihydroxy-2-methylchromone which is believed to be derived from furfural was also present. However, there is no reported UV vis absorption for 3,8-dihydroxy-2-methylchromone.²² The second potential explanation for the higher than expected

concentration is that Kraft lignin was not a good standard to use. It was not conclusive that these peaks are being generated from phenolic motifs. Ultimately a more in-depth study is needed to determine an accurate phenolic and lignin concentration during the pretreatment process. However, this initial investigation has produced a rough though valuable estimate of what is expected and suggests that the compounds within the liquor are a result of pseudolignin formation.

4.3.3 Behaviors of Glucose, Xylose, HMF, furfural, and sigma cellulose using the IonoSolv pretreatment process

There are few direct studies on the formation of humins/pseudolignin in ionic liquids, with most studies examining results from dilute acid or organosolv pretreatments (see Figure 7). Most research on the topic has been ancillary to studies on saccharification or feedstock yields where pseudolignin formation inhibits efficiency. However, both dilute acid pretreatment (DAP) and organosolv pretreatments employ acidic conditions and high temperatures which have been shown to promote the formation of humins and that higher temperatures produce more humic substances.¹⁶ Direct study of humin formation in pretreatment may yield results in understanding how to increase the efficiency of pretreating biomass. Experiments on the behaviors of glucose, xylose, HMF, and furfural during the IonoSolv pretreatment process were explored.

The interaction of pretreatment parameters and degradation products in pseudolignin formation is complex and isolating potential contributors may help focus future research. A series of experiments were conducted using both fresh and recycled ionic liquid with sugars (glucose and xylose). These experiments were used to determine the likelihood of humin/pseudolignin formation using the IonoSolv process and specifically the protic ionic liquid [TEA][HSO₄]. Fresh ionic liquid and recycled [TEA][HSO₄] were used to see how the sugars may interact with the various solutes during pretreatment. These trials were followed by a series of pretreatments that used the individual sugars and their dehydration products. The goal was to gain a better understanding of which constituent maybe most responsible for humin formation during the pretreatment process. HSQC and FT-IR were conducted on the solids precipitated to develop a more detailed understanding of the structure of these humic substances. Time trial experiments were used to investigate the variation of pretreatment

conditions using recycled ionic liquid compared to fresh ionic liquid to examine the formation of pseudolignin and its impact on saccharification. To potentially improve pretreatment pulp purity and saccharification yields by reducing pseudolignin formation, doped experiments were used to investigate the potential suppressing effects of additives. Glucose and xylose ^{13}C labelled sugars were used to aid in tracking the formation of pseudolignin during the pretreatment process.

4.3.4 Combined Glucose and Xylose Trials

Previous studies using acid catalysts in [BMIM]Cl found the combination of glucose with HMF and xylose with furfural seems to increase humin production.³¹ The interactions between these four compounds is not well understood. Further, no studies have been completed on the use of recycled ionic liquid. This study aims to understand the interactions between these compounds, in both fresh and recycled ionic liquid, and their potential to form humic substances in the ionoSolv process. Initially a combination of glucose and xylose was pretreated using both recycled ionic liquid (RIL) and fresh ionic liquid (FIL). Approximately 470 mg of glucose and 185 mg of xylose to simulate the monomerized amounts that are found in *Miscanthus*. In addition, the recycled ionic liquid with no sugars added was reheated and treated with the same wash up protocol used with all other pretreatment samples and the solids recovered are detailed in Figure 8. An average of 22 mg of humic solids were recovered in the fresh ionic liquid pretreatment compared with 83 mg using recycled ionic liquid. Reheating the recycled ionic liquid generated approximately 40 mg of solids. The solids recovered from the fresh ionic liquid with sugar (FIL with sugar) experiment confirms that humins can be formed from the polymerization of the sugars and/or their degradation products during the ionoSolv pretreatment. In addition, the production of solids from reheating the recycled ionic liquid suggests that the solutes dissolved in the ionic liquid from prior pretreatments are

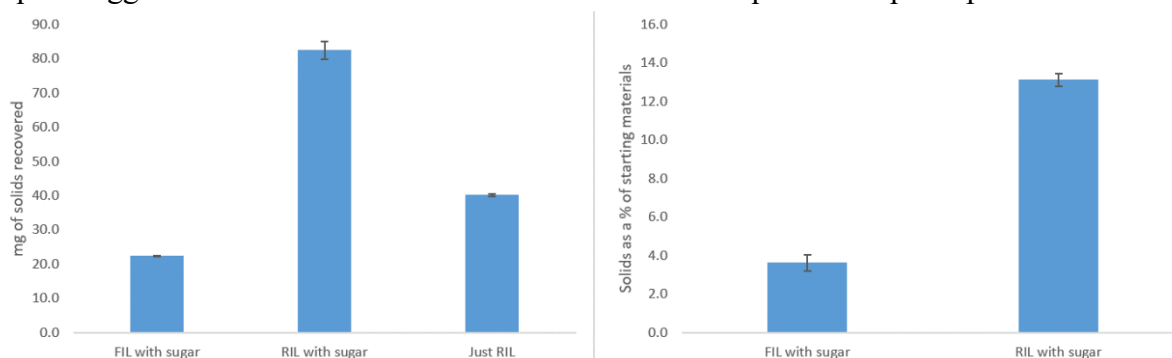


Figure 8 Solid precipitates from combined glucose and xylose pretreatment using [TEA][HSO₄] at 150 °C for 1 hour. Amount of total solid precipitate recovered (left) and represented as percentage of starting materials (right).

reactive during the thermal process and may be a concern for pseudolignin formation during the pretreatment of biomass. If this was the case, the reheating and subsequent precipitation of solids in the recycled ionic liquid may also provide a method to “clean” the recycled ionic liquid for future reuse though reheating further adds to pretreatment cost. The largest amount of humins/pseudolignin was recovered from the recycled ionic liquid and combined sugar trial (RIL+sugar). It was interesting that the combined amount of precipitate from reheating the recycled ionic liquid (RIL) and (FIL with sugar) experiments were significantly less than the solids recovered from the (RIL with sugar) experiments. This result provides evidence that lignin fragments in the recycled ionic liquid may also be contributing to the formation of pseudolignin.

Past investigations into the formation of humic substances have suggested that glucose and xylose dehydration compounds HMF and furfural respectively are the major components in humin formation.³² The research suggests that a combination of HMF with intermediates such as 2,5-dioxo-6-hydroxyhexanal (DHH) aids in the extension and growth of the polymer. Other experiments conducted on the formation of humins and pseudolignin have demonstrated that the presence of any aldehyde compounds would be incorporated into that growing chain.¹² Studies on lignin depolymerization have demonstrated that lignin fractionation results in the formation of aldehyde containing aromatic fragments and these aldehydes fragments are reactive to polymerization. The combination of the results of these studies would suggest that lignin was indeed being incorporated with humins during pretreatment, generating pseudolignin. This may also explain why previous investigations have not detected aldehyde lignin fragments in lignin precipitates.

FT-IR was performed on solids precipitated from pretreated *Miscanthus*, combined glucose and xylose pretreated with fresh ionic liquid and recycled ionic liquid and reheated recycled ionic liquid without additional additives. The analysis was used to investigate their structures and compared their spectra of the lignin precipitated using the IonoSolv process (Figure 9). FTIR spectra of solids precipitated showed peaks at 1600, 1512, and 1453 cm^{-1} indicating C=C stretching from aromatic peaks (dotted lines in Figure 9). These peaks were present in the solids from FIL+sugar (which do not contain any lignin fragments), though their intensity was greatly reduced. These peaks are not present in the FT-IR of pure glucose or xylose, nor do

the humin solid spectra fingerprinting region resemble those of the individual sugars (appendix 4D). All the spectra contain -OH stretch between 3700-3300 cm^{-1} and sp^3 hybridized

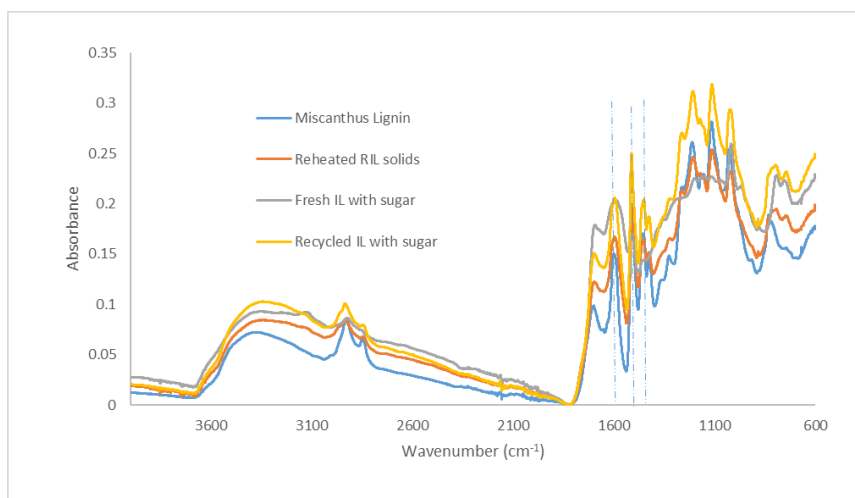


Figure 9 FT-IR of *Miscanthus* lignin precipitated from ionoSolv process compared with humins/pseudolignin of pretreated sugars using [TEA][HSO₄] at 150 °C for 1 hour. Aromatic peaks are marked at 1600, 1512, and 1453 cm^{-1} .

C-H bonds between 2900-2800 cm^{-1} . The (FIL with sugar) precipitate did not have a peak at 1425, 1260, 1210, and 1112 cm^{-1} , which are all present to some extent in the other spectra. These peaks can be attributed to the presence of acyl or phenyl C-O bonds. This would mean that the solids produced from the sugars lack acyl or phenyl carbon oxygen bonds which are commonly found in the lignin structure. The presence of these acyl or phenyl carbon oxygen peaks in the solid precipitated from the recycled ionic liquid is evidence of the condensation and incorporation of lignin fragments with the carbohydrates and their degradation products. The spectra from the recycled ionic liquid solids have the same peaks as those of precipitated lignin from *Miscanthus* as well as the peaks from just sugar pretreatment. Despite the fact that the intensities of these peaks are significantly less than those of pretreated *Miscanthus* lignin, the similarity of the FT-IR spectra suggest that the lignin recovered during the Ionosolv process also contain humic components. All spectra showed peaks at 1030 cm^{-1} for C-O-C bonding, though the peaks for FIL with sugar are much less intense, suggesting that the precipitate from the fresh liquor contained fewer ether and/or ester bonds.

GPC measurements were conducted on the lignin precipitate from the reheated recycled ionic liquid. These results could give an indication as to the approximate minimum molecular weight lignin (and or pseudolignin) would start to precipitate from the ionic liquid. The M_n for the recycled lignin was 731 g/mol and the weighted average of 1650 g/mol was recorded. These macromolecules are not very large and other reported values have suggested that solid precipitates can have molecular weights even lower than this, between 300-500 g/mol.¹⁶ When

the potential precursors are considered (glucose 180.15 g/mol, xylose 150.13 g/mol, HMF 126.11 g/mol, and furfural 96.08 g/mol) the number of units required to initiate precipitation is relatively small, approximately 2-8 units not considering any lignin fragments. However, compared to the other reported values (300-500 g/mol) the recycled ionic liquid was able to retain a higher molecular weight compounds in solution.

4.3.5 Individual Sugar Trials

The combined glucose and xylose sugar trials demonstrated that humic substances may be formed during the ionoSolv process using [TEA][HSO₄]. However, it was not clear how each individual component would behave during pretreatment and which one might be the predominate contributor to humin or pseudolignin formation. To explore these questions, individual sugars (glucose and xylose), and their dehydration products (HMF and furfural) were pretreated and analyzed. The amount of precipitate as a percentage of the starting materials is represented in Figure 10. After removing pretreatment sample from the oven, but prior to water precipitation of “lignin” the sugars, glucose, xylose, HMF, and furfural were completely dissolved in the ionic liquid during the pretreatment process. There were no visible traces of the compounds could be seen with the eye when removed from the oven or after cooling to room temperature. This indicated that any humins or pseudolignin remain soluble in the ionic liquid even after cooling. Solids were precipitated only during the water wash (lignin precipitation step). These results displayed significantly different precipitation rates for each sugar as well as differences between fresh and recycled ionic liquid. Glucose and xylose

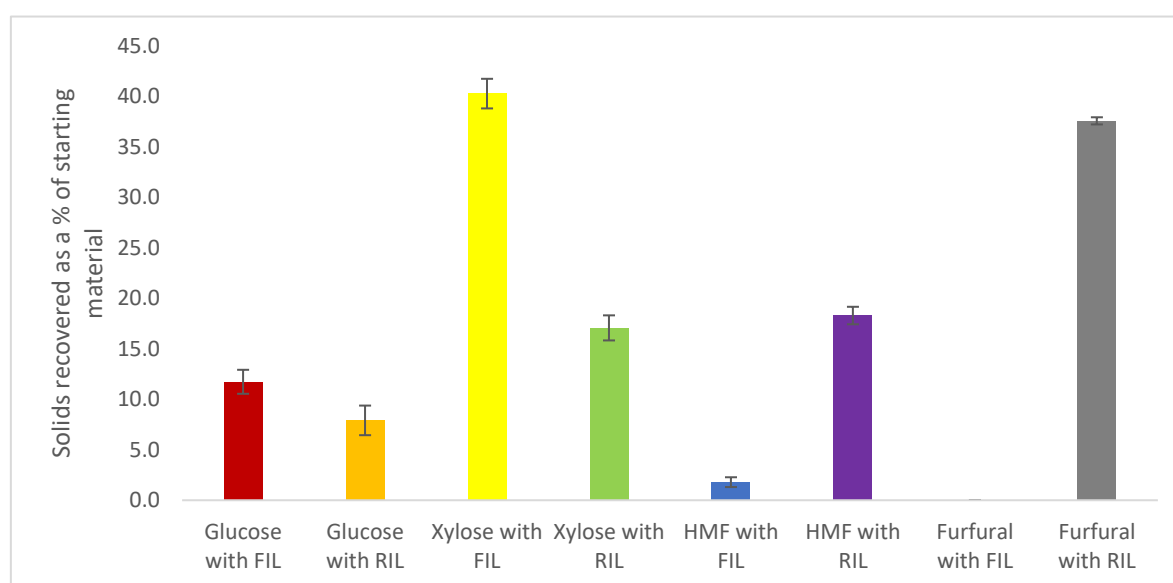


Figure 10 Solid recovery after pretreatment of glucose, xylose, HMF and furfural in [TEA][HSO₄] for 1 hour at 150 °C in fresh ionic liquid (FIL) and recycled ionic liquid (RIL) produced from *Miscanthus*.

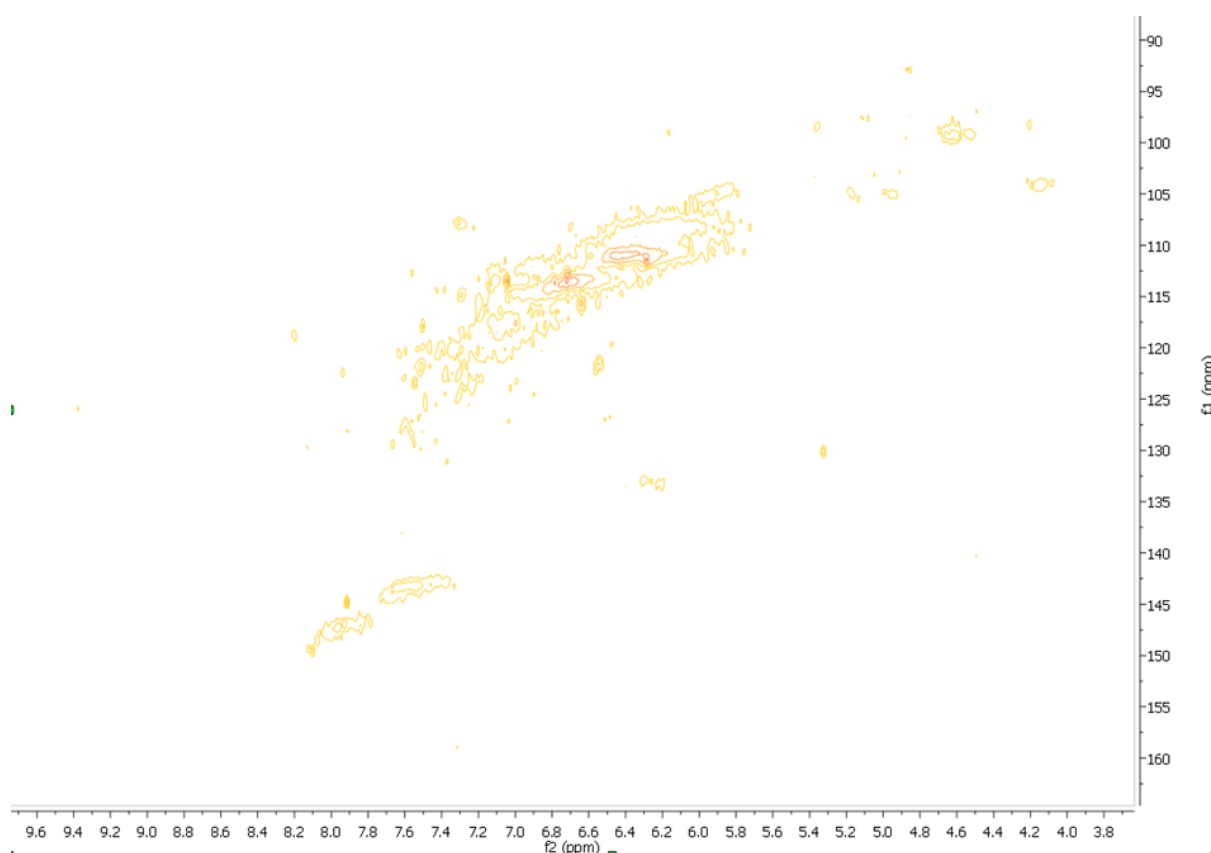
generated more solids with fresh ionic liquid (11.7% and 40.3%) than recycled ionic liquid (7.9% and 17.1% respectively). However, the opposite was true in the case of HMF and furfural (1.8% and 0% in fresh ionic liquid and 18.3% and 37.6% in recycled ionic liquid respectively). An operating biorefinery would likely use RIL almost exclusively and results from RIL are important to consider.

Xylose (recovered over 40% in solids) and its intermediates was far more reactive with itself (in the fresh ionic liquid) than with the solutes in the recycled ionic liquid producing significantly greater percentages of precipitation than glucose. This was in agreement with previous studies that used the non-protic 1-butyl-3-methylimidazolium chloride [BMIM]Cl ionic liquid and demonstrated that xylose was by far more reactive than glucose, though no recycled ionic liquid comparison was provided.³³ The recycled [TEA][HSO₄] using the ionoSolv process in the cases of both the glucose and xylose produced fewer humic substances. This suggests that the solutes in the recycled ionic liquid maybe aiding in maintaining the solubility of the sugar conjugates and or their intermediates. It was possible that the solutes allow for larger compounds to stay soluble as supported by GPC data, thereby reducing the amount of precipitates generated. Another possibility was that the recycled ionic liquid at least partially prevents polymerization of these compounds thereby reducing solid precipitation. The previous experiments which had combined the sugars (see appendix 4E which includes combined sugar – glucose and xylose trials), shows that an interaction between the sugars will also cause humin formation. However, these results demonstrate the presence of xylose leads to significantly greater humin production. Xylose seems to be a major component in humin formation and the management of xylose (and other pentose hemicellulose sugars) and intermediaries would be necessary to improve yield and saccharification of acidic pretreatments.

HMF and furfural displayed different results from those of glucose and xylose, producing far more precipitate with the recycled ionic liquid than with fresh ionic liquid. Despite the large quantity of precipitate recovered the amount of precipitate was only slightly higher than the solids generated from reheating only the recycled ionic liquid. When recycled ionic liquid was reheated 44 mg of solid was recovered. However, when HMF and furfural was added to the RIL (in amounts of 310 mg and 110 mg respectively), the solids recovered were 58 and 44 mg respectively. This indicated that HMF and furfural remained in the ionic liquid and if any polymerization occurred it remained dissolved. The results in fresh ionic liquid also suggest that both furfural and HMF are unlikely to self-polymerize with the ionoSolv process using

[TEA][HSO₄] unless they are in the presence of other biomass components. Previous studies in dilute acid pretreatment demonstrated that combined glucose with HMF and xylose with furfural will result in increased humin production, these experiments confirm that on their own HMF and furfural are unlikely to form pseudolignin.³³ Interestingly, as demonstrated, HMF was significantly less reactive than furfural producing precipitates at half the rate. The high reactivity of furfural to other biomass components may suggest that the removal of furfural during the recycling process maybe one reason that recycled ionic liquid generally performs better than the use of fresh ionic liquid in biomass extraction, and pulps from recycled ionic liquid have a better saccharification yield.

HSQC of the solids precipitated from glucose and xylose does reveal subtle differences between the solids (Figure 11 and appendix 4F for full spectra). It was clear from these spectra that humic substances have an aromatic component. Glucose humins generated several peaks in the aromatic region: (6.3,111.4) and (6.7,113.4). Xylose aromatic peaks can be seen at: (6.3, 110.0) and (6.40, 110.6). These humin solids do have one similar peak with HMF at (6.6, 110.2) and furfural at (6.7, 113.3) (see appendix 4G for HMF and furfural HSQC), however, it was clear that these humins are not HMF or furfural. This information along with the FT-IR



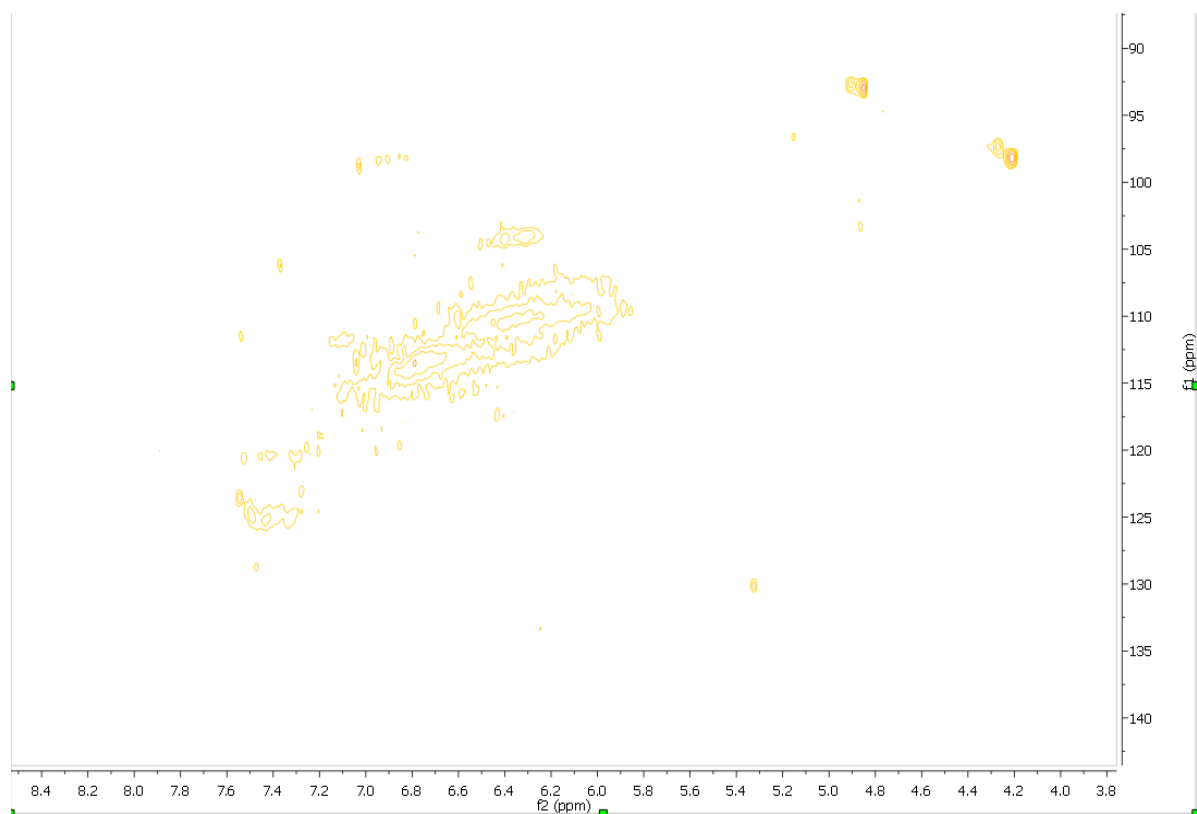


Figure 11 HSQC of solid precipitated from glucose (top) xylose (bottom) from fresh ionic liquid pretreatment at 150 °C for 1 hour.

suggest the possible presence of furan incorporation during the formation of humins during pretreatment, other studies support this as well.³² However, when these two sugars are pretreated with recycled ionic liquid the signals from the solids produced become difficult to clearly distinguish from the signals associated with *Miscanthus* lignin. The peaks where these sugars would normally be present overlap areas where *p*-coumaric acid (PCA) and the guaiacyl

(G5) signals making them difficult to delineate (Figure 12).⁹ Experiments were conducted

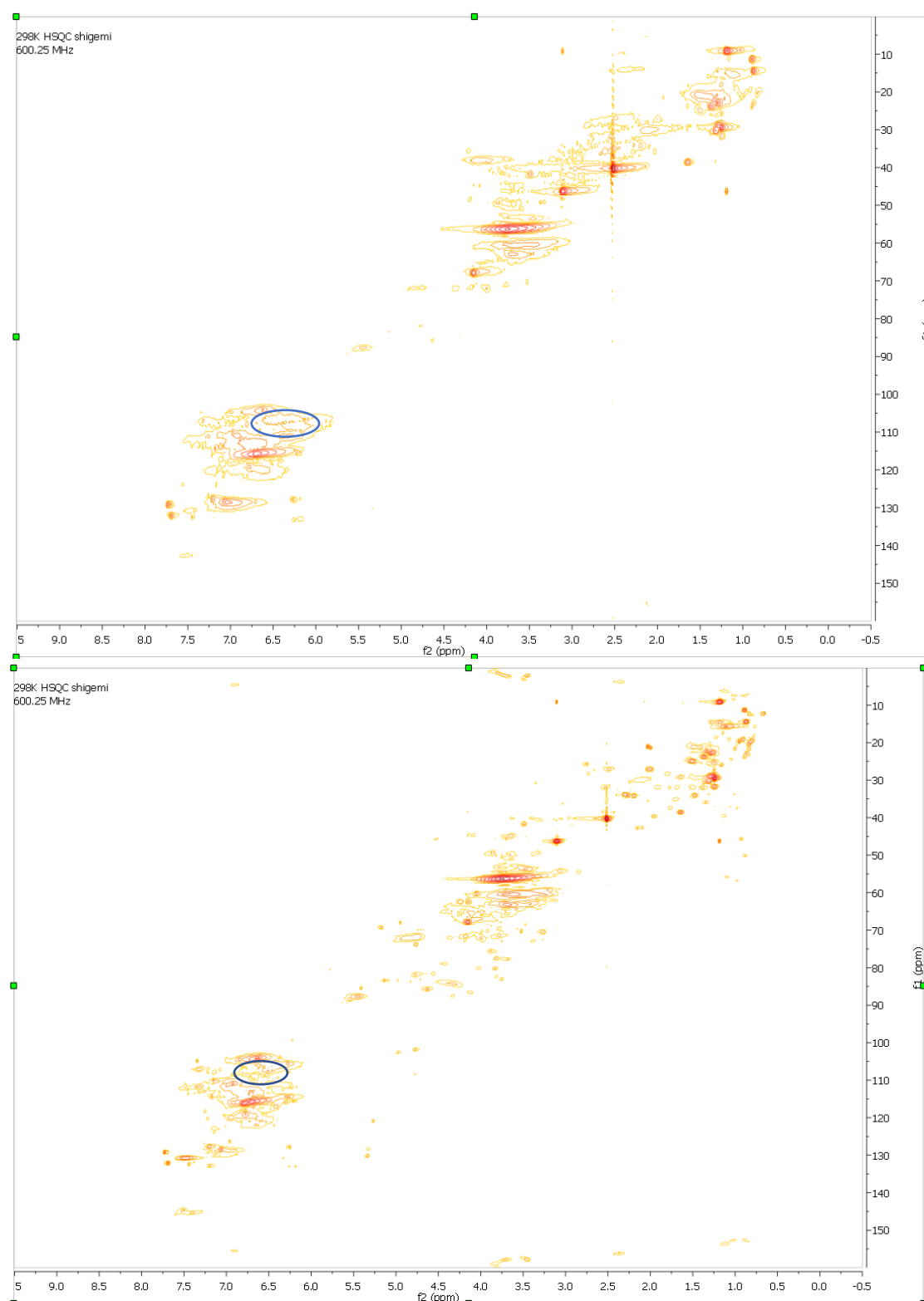


Figure 12 HSQC of glucose and xylose pretreated with recycled ionic liquid (RIL) at 150 °C for 1 hour (top). *Miscanthus* lignin produced under the same conditions using fresh ionic liquid (FIL) (bottom).

using ^{13}C labelled glucose and xylose to determine the presence of sugars and their degradation product incorporation for pseudolignin formation during pretreatment; this will be discussed

later in the chapter. The HSQC results provide strong evidence that lignin recovered during the ionoSolv pretreatment incorporates carbohydrate intermediaries and furanic motifs. This incorporation would mean that there is a loss of sugar yields to lignin recovery, and the lignin produced is not purely lignin condensation products.

HSQC was performed on the humins formed from HMF (there was insufficient solid formation from furfural to perform a HSQC) and is shown in Figure 13 (see appendix 4H for the full HMF spectra). The HSQC of the solids produced from the pretreatment of HMF suggests that much of the structure of HMF remains intact. The main peaks at (6.6, 110.2) and (7.5, 124.8) though shifted, remain present suggesting that these solids are most likely low molecular weight perhaps dimers. The difference in the quantity of solutes recovered from HMF and furfural trials indicates a very different reaction between the two of them with the pseudolignin constituents in the recycled ionic liquid. In these trials, HMF displayed a reactivity in the recycled ionic liquid which caused various sugar conjugates generated from glucose and hemicellulose sugars to remain soluble in the ionic liquid. This led to a hypothesis that perhaps the adjustment of the concentration of these solutes as additives maybe able to reduce pseudolignin production and improve saccharification. Previous studies conducted by other groups have suggested the additives like dimethyl sulfoxide (DMSO) and even

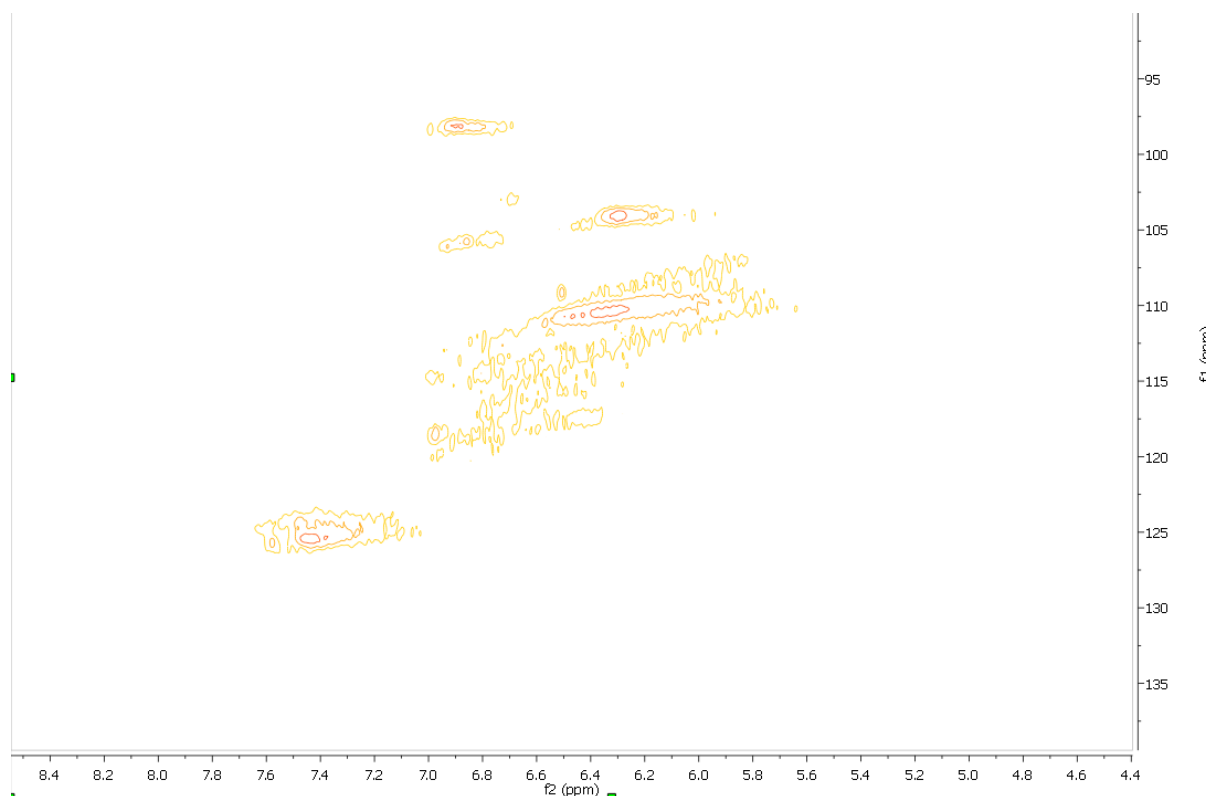


Figure 13 HSQC of humic substances formed from the pretreatment of HMF at 150 °C for 1 hour with [TEA][HSO₄].

acetonitrile as methods for controlling or suppressing the formation of pseudolignin.³⁴ This suppression was attributed to the ability of DMSO to stabilize and solubilize various intermediates preventing the formation of pseudolignin. However, DMSO or acetonitrile would not be feasible reagents for the biorefinery process as high concentrations of either solvent is detrimental for enzymatic saccharification and separation and recycling of these solvents would be costly. Instead, the potential of using additives of components already produced during the pretreatment process was examined in combination with the entire biomass composite to observe their impact on saccharification. In the cases of the sugars we have studied here, aromatic π electrons from furanic compounds may serve to stabilize or scavenge intermediates to prevent redistribution of pseudolignin on the pulp. The addition of additives will be explored later in this chapter within the context of the full biomass compound to include variables more similar to an actual biorefinery.

It is important to note that the production of humins is not necessarily a direct indication of how successful the pretreatment is in terms of saccharification. Humin/pseudolignin production is not desirable because of the reduction in saccharification yield due loss of sugars to side reactions. However, it is possible to produce humins and still retain a high saccharification yield, if the humins are prevented from inhibiting the saccharification process. Humin production itself during the precipitation stages also means that these substances are not redeposited onto the surface of the pulp to inhibit enzymatic saccharification. It is possible that some of the solutes in the recycled ionic liquid, besides acting as scavengers stabilizing intermediates and transition stages, may be condensing with these humins and lignin fragments, improving solubility in the ionic liquid therefore indirectly preventing redeposition of pseudolignin onto the pulp. In addition, there are questions regarding whether the surface of the pulp itself may facilitate the redeposition of pseudolignin. It is possible that the way the biomass was extracted created areas on the surface of the composite that were favorable towards condensation reactions with solutes in the ionic liquid. It seems that once the pretreatment was extended past the optimum time condition, condensation and redeposition of pseudolignin outpace the removal and extraction of lignin and hemicellulose from the pulp. Experiments were conducted to determine if the remaining surface lignin and hemicellulose maybe the reason for the redeposition of pseudolignin back onto the surface. These scenarios will be examined shortly (4.3.6 Trials with Sigma Cellulose).

4.3.6 Trials with Sigma Cellulose

There has been evidence that humins/pseudolignin will redeposit onto the surface of the biomass during dilute acid pretreatment.²² However, it is unclear whether these compounds are forming directly on the biomass or if the humic substances are first forming in the pretreatment solvent and then redepositing onto the surface. It maybe that the surface of the biomass itself may play a role in the attachment or deposition of pseudolignin. To explore this hypothesis, pretreatment was conducted using Sigma cellulose (microcrystalline 20 μm) purchased from Sigma Aldrich. Sigma cellulose has little to no hemicellulose and lignin and pretreatment of the Sigma cellulose would be the closest equivalent to a pretreatment of a pure pulp. The objective was to ascertain if the surface composition of the pulp would impact the redeposition of humins and or pseudolignin. Several possibilities must be considered to explore this question, but in all cases, the use of Sigma cellulose with a protic ionic liquid is expected to dissolve the amorphous section of the cellulose into the pretreatment liquor. The first possibility is that recycled ionic liquid solutes would form humins or pseudolignin and redeposit on the surface of the cellulose thereby decreasing the saccharification yield. The second possibility is that the recycled ionic liquid would still outperform the fresh ionic liquid sample due to the ability of the recycled ionic liquid to scavenge or improve the solubility of pseudolignin components. It is also possible that the release of amorphous cellulose could be responsible for the formation of pseudolignin, especially while using fresh ionic liquid as previously demonstrated and this could result in the generation of humic substances which could redeposit on to the surface of the cellulose and decrease saccharification yield.

Compositional analysis on the cellulose confirmed the release of glucan during the pretreatment. Though there was a slightly lower glucan content in trials with recycled ionic liquid, the difference was not significant (95.7% vs 95.2% fresh ionic liquid vs recycled ionic liquid in the 30 min pretreatment, 97.0 vs 95.9% in the 60 min pretreatments, and 98.4% vs 94.2% in the 120 min pretreatment) (Figure 14) (complete compositional graphs can be found at appendix 4I). Glucan content in all cases remained between 95-98% and acid insoluble lignin content ranged between 1.8-2.2%, again with no significant difference between the recycled or fresh cellulose pulps. Pretreatment with either recycled ionic liquid or fresh ionic liquid resulted in a slight decrease in glucan content. This was likely due to the dissolution of amorphous cellulose regions in the fibers. There was slight variation in the amount of acid

insoluble lignin that was found on the cellulose with a maximum at 60 min (2.2% for the FIL treated cellulose and 2.1% for the RIL). However, the differences in the amount of acid insoluble lignin was not significant. The acid insoluble lignin content could indicate that there was some deposition of pseudolignin onto the pulp or could be a result of acid protocol of the compositional analysis. However, the small differences between fresh and recycled ionic liquid would suggest that the solutes of the recycled ionic liquid do not seem to cause additional deposition. The result suggested that the process of recycling the ionic liquid may be crucial to removal of compounds that may contribute to pseudolignin and humin production. This also seems to be in agreement with previous observations that redeposition of pseudolignin back onto pretreated pulps is linked to the pulp's purity.

The results of the saccharification of the pretreated cellulose after seven days is shown in Figure 15. The saccharification yields do not correlate to the acid soluble lignin content on the pulp. In all causes the recycled ionic liquid provides a better glucan yield than the fresh ionic liquid counterpart, suggesting that the solutes in the recycled ionic liquid generated a pulp that was more favorable to enzyme access. Recycled ionic liquid for 30 minutes yielded the

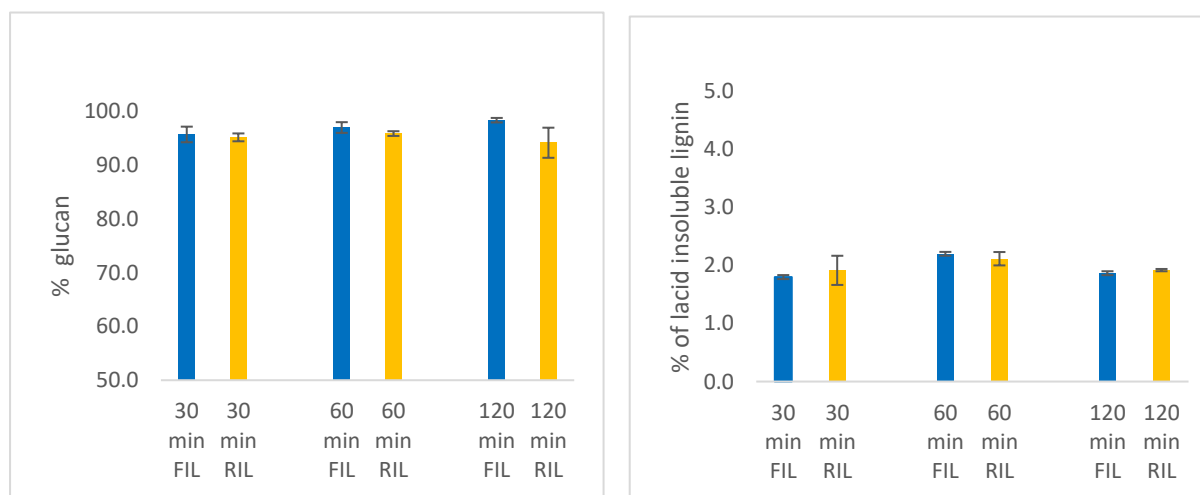


Figure 14 Compositional analysis of pretreated Sigma cellulose glucan content (left) and acid insoluble lignin (right) with [TEA][HSO₄] at 150 °C using fresh ionic liquid (FIL) and recycled ionic liquid (RIL).

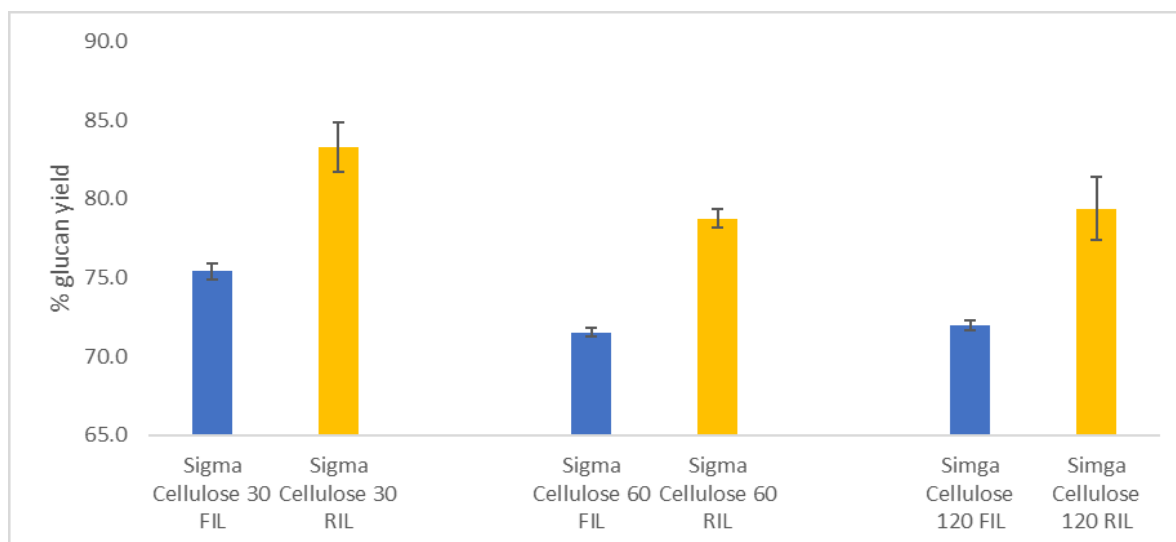


Figure 15 Saccharification yields of pretreated Sigma Cellulose with fresh ionic liquid (FIL) and recycled ionic liquid (RIL).

best results (83% yield). There was a slight increase in the saccharification yield from 60 min to 120 min in both cases (from 71 to 72% in the case of fresh ionic liquid and 78 to 79% in recycled ionic liquid). The decrease from the initial 30-minute pretreatment may be a result of the removal of amorphous cellulose which is more easily hydrolyzed. Other studies have demonstrated that lignin removal, though correlated directly with enzymatic yield, is not the only factor that determines high sugar release.³⁵ In addition, there is a complex interplay between pore size, surface area, crystallinity, and degree of polymerization that can impact saccharification yields. Though this procedure used the ionic liquid to remove lignin and hemicellulose from the biomass composite, the ionic liquid itself can impact the biomass pore size, surface area and crystallinity (as demonstrated in chapter 3). Without the presence of lignin and hemicellulose on the pulp, the recycled ionic liquid still outperformed the fresh ionic liquid saccharification. It was possible that recycled ionic liquid left more of the amorphous cellulose intact or was better at reducing the degree of polymerization of the cellulose fibers, though the latter is unlikely as the pH tested suggested not significant change (1.61 to 1.64). It was considered that perhaps the acidity of the ionic liquid was changed after the recycling process, causing the recycled ionic liquid to be less acidic. Experiments conducted to investigate the impact of pH noticed that at a 10% loading, there was no noticeable difference in pH detected, but at higher biomass pretreatment loadings, pH will increase, and optimization of the process would need to be recalibrated.

However, based on these experiments the surface of the pulp seems to have a great impact on whether pseudolignin will be readily formed/deposited. The purer the pulp the less likely the formation of pseudolignin. Most likely, the process of depolymerizing the lignin and

hemicellulose from the biomass composite leaves polymer ends that may allow for condensation with humic substances and pseudolignin from the liquor. As the severity of the pretreatment was increased, depolymerization was outpaced by these condensation reactions causing the surface of the pulp to be coated. It seemed that neither scenario (the recycled ionic liquid solutes or fresh ionic liquid generating pseudolignins) was responsible for pseudolignin redeposition on to the surface of the cellulose fiber. Rather it would appear that the purity and effectiveness of the extraction of the biomass surface impacts whether recondensation and redeposition will occur.

4.3.7 Pretreatment of *Miscanthus*- using Fresh and Recycled Ionic Liquid

Findings from the sugar trials produced results with interesting differences between fresh and recycled ionic liquids. To further explore these differences, a series of time trials using *Miscanthus* with no addition of additives were completed. Time trials in 10-minute intervals from 40-80 minutes investigated the effect of the recycled ionic liquid on the optimum conditions, its effects on saccharification, as well as pseudolignin formation. Previous investigations have suggested that the ideal pretreatment for *Miscanthus* using fresh ionic liquid [TEA][HSO₄] occurred approximately 50-60 mins at 150 °C.⁹ This will vary with the feedstock as well as the acid:base ratio of the ionic liquid. By extending the pretreatment past the optimum pretreatment time it was expected that there would be a decrease in saccharification and an increase in pseudolignin production.

The fresh ionic liquid trials were compared with the pretreatment results using recycled ionic liquid (it should be noted that multiple recoveries of the ionic liquid can impact performance). Differences in the kinetics of pulp extraction were immediately seen in the pulp recovery yields. The fresh ionic liquid pulp yields decreased to 63% at 70 minutes but then increased to 66% at 80 minutes suggesting that there has been redeposition of pseudolignin back onto the surface of the pulp (Figure 16). However, this trend was not seen in the recycled ionic liquid pulps. Though the rate of biomass extraction has slowed compared with initial extraction with recycled ionic liquid pretreatment, pulp yield after 80 min (60%) was still decreasing. The lower pulp yields found in RIL ionic pulps when compared with the FIL pretreated pulps also demonstrated that the fragments in the ionic liquid play a role in extraction. The difference in extraction demonstrated that the use of fresh ionic liquid and recycled ionic liquid have different kinetics and optimization conditions for pretreatment.

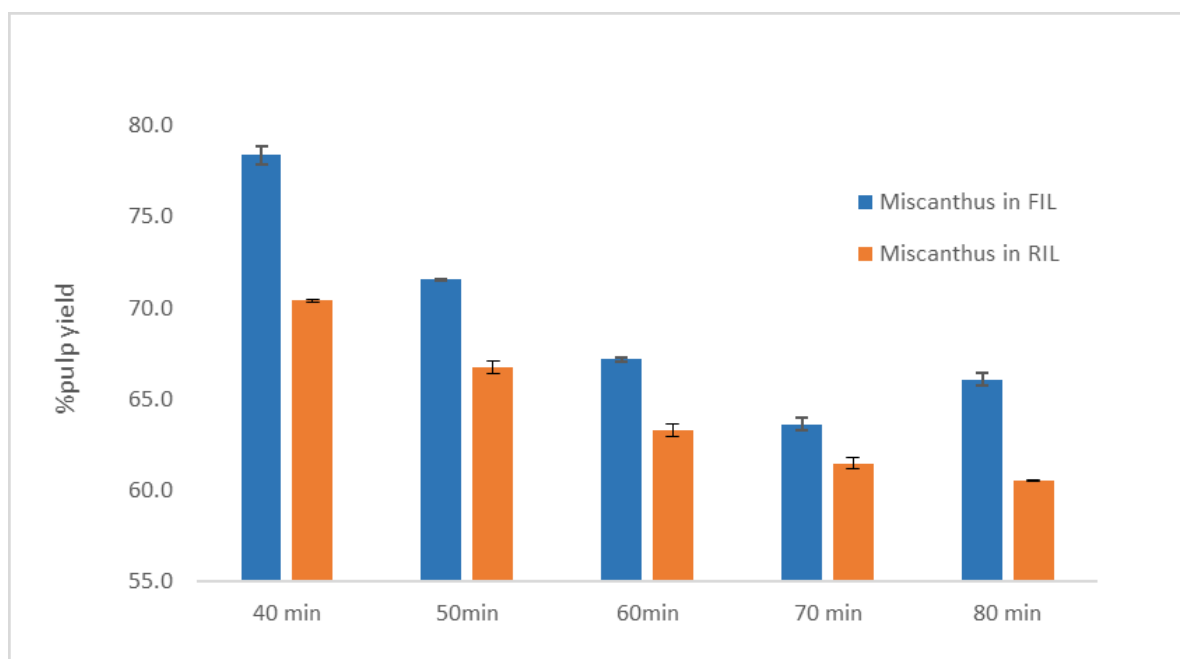


Figure 16 Percentage of pulp recovered after pretreatment at each time interval. Pretreatments were conducted at 150 °C using fresh [TEA][HSO₄] (FIL) as well as recycled [TEA][HSO₄] (RIL).

Future experiments will be needed to investigate the effects on pretreatment optimizations after continuous recycling of ionic liquid to determine if different cycles will impact pulp purity and saccharification differently.

Recycled ionic liquid at each time interval was more effective at dissolving and extracting the biomass composite. Though the recycled ionic liquid extracted more of the biomass, the fresh ionic liquid has a greater rate of extraction between the 40 to 70 min time course (at 40 min there was an 8% difference between FIL and RIL compared with the 70 min pretreatment where there was only a 2% difference in pulp recovered). In all time trial experiments, the pulp yield was lower in the recycled trials when compared with its corresponding fresh ionic liquid pretreatment. This suggested that the use of recycled ionic liquid will generally produce a more cellulose rich pulp than using a fresh ionic liquid under reasonable conditions for biomass pretreatment. It was likely that the solutes in the recycled ionic liquid are aiding in extraction by potentially reacting with and solubilizing the lignin and or carbohydrate conjugates from the biomass, or by potentially reducing lignin condensation reactions.

Compositional analysis was performed to understand the effects of the pretreatment on the cellulose, hemicellulose, and lignin content. The results were normalized against untreated *Miscanthus* (see Figure 17). In all cases there was complete removal of galactans, mannans, and arabinan hemicelluloses. The recycled ionic liquid (RIL) also removed more xylans from the *Miscanthus* than its corresponding fresh ionic liquid (FIL) trials. The glucan content was consistent at around 44-45% regardless of whether the ionic liquid was recycled or not. No detectable ash content was recorded in either case suggesting that recycled ionic liquid could potentially be used for extracting inorganics (metals) as fresh ionic liquid. What was notable was the change in the acid insoluble lignin content. In each case the recycled ionic liquid outperformed the fresh ionic liquid in the removal or extraction of acid insoluble lignin. For example, in the case of the 80 min pretreatment, FIL pulp contained a residual 8.6% lignin compared to 5.6% in the recycled pulp. Interestingly, the residual lignin on the FIL pulp showed an increase from the 7.4% found on the 70 min pretreated pulp (the RIL contained 6.3%). This result corroborates the pulp yield recovery data and the hypothesis that

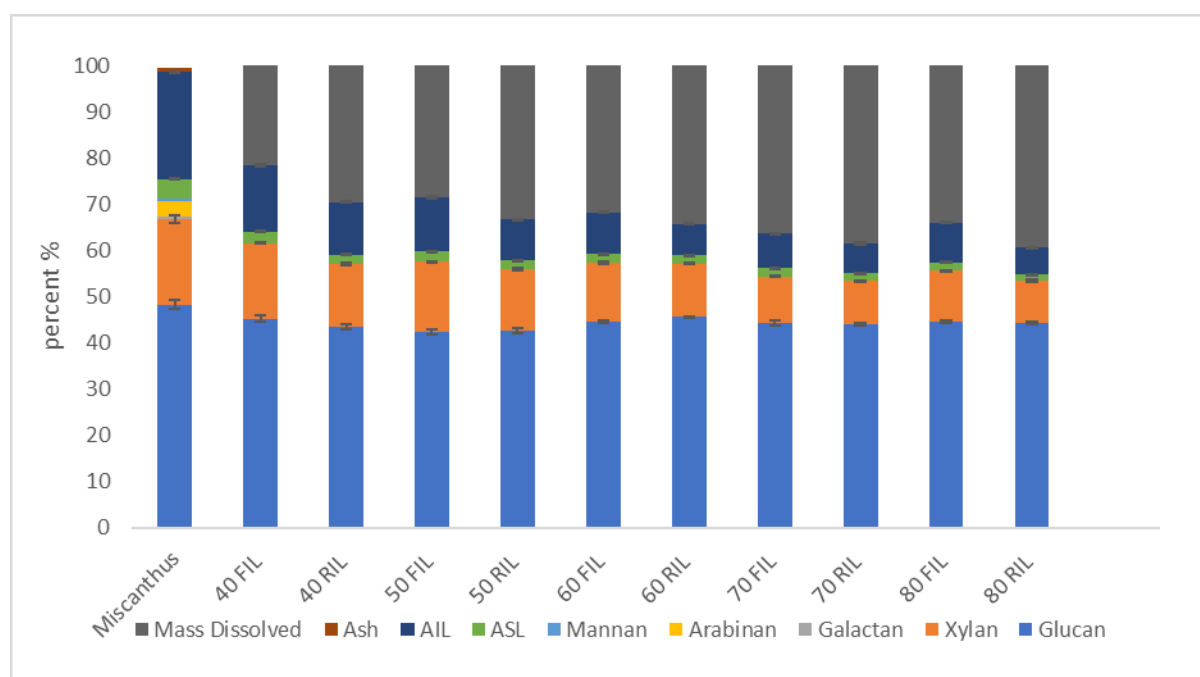


Figure 17 Compositional analysis of untreated *Miscanthus* compared with pretreatments with fresh $[TEA][HSO_4]$ and recycled $[TEA][HSO_4]$ at 150 °C at 40, 50, 60, 70, and 80 minutes.

pseudolignin has redeposited onto the *Miscanthus* pulp. This also suggests that the solutes in the recycled ionic liquid can play an important role in the potential prevention of pseudolignin formation.

FT-IR spectra of the pretreated pulps showed that the fresh ionic liquid and recycled ionic liquid transmissions were different when comparing pulps from the same pretreatment

time frame, confirming the differences in the kinetics of the pretreatment. The FT-IR shown in Figure 18 displays the region between 1200-2000 cm^{-1} for each of the time trials with dotted lines at 1730, 1650, and 1592 cm^{-1} in the carbonyl and aromatic regions of the spectra

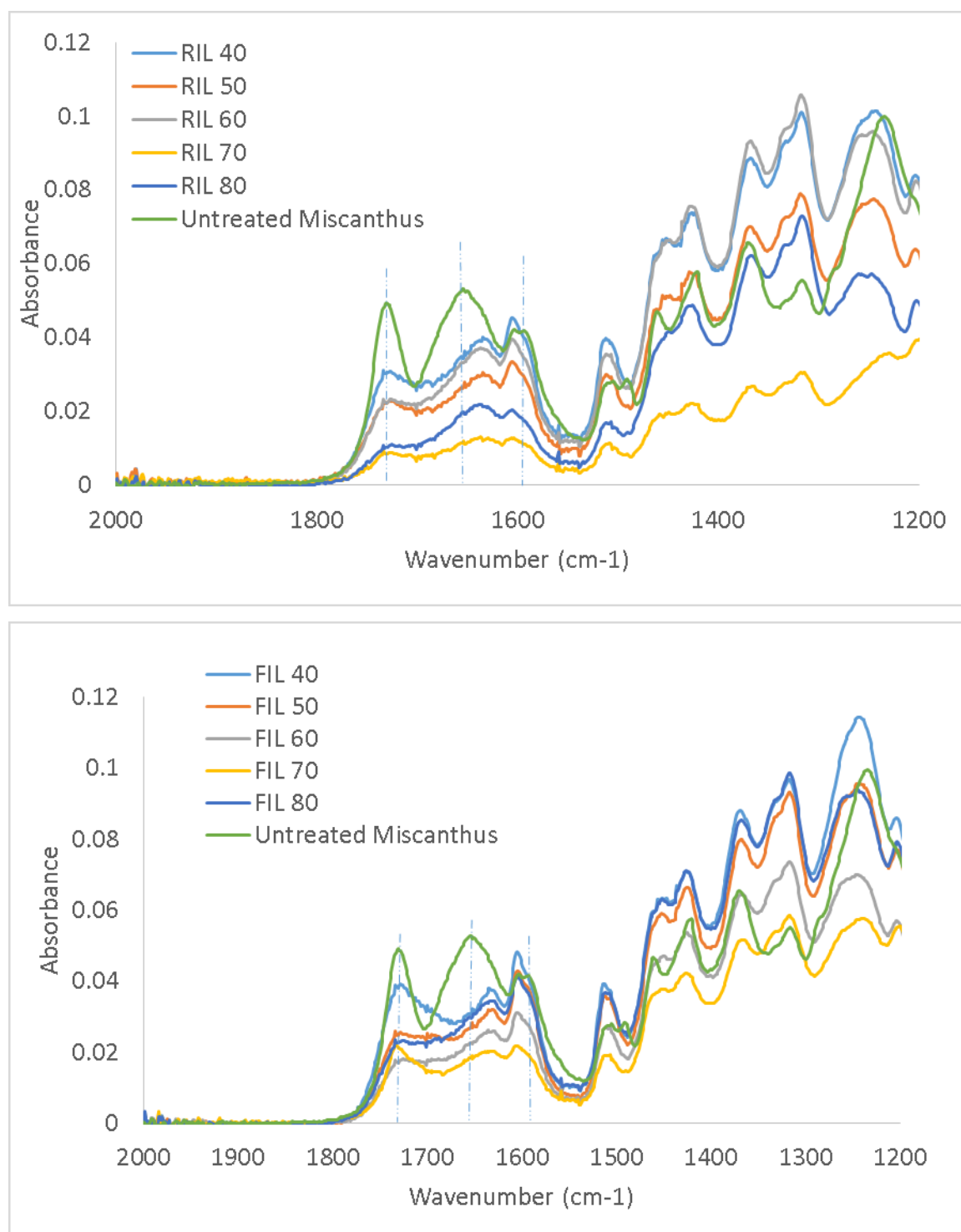


Figure 18 FT-IR between 1200-2000 cm^{-1} of *Miscanthus* pretreated at 40, 50, 60, 70, and 80 min time intervals with [TEA][HSO₄] at 150 °C using fresh ionic liquid (FIL) (bottom) and recycled ionic liquid (RIL) (top) compared with that of untreated *Miscanthus*. Dotted lines at 1730, 1650, and 1592 cm^{-1} have been outlined representing the carbonyl and aromatic regions of the spectra.

compared against that of untreated *Miscanthus*. After 40 min of pretreatment, there was a

decrease in these signals for both the fresh and recycled ionic liquid, which corresponds to the removing of the lignin from the pulp. These signals continue to decrease and flatten in the fresh ionic liquid pretreatment until 60 min into pretreatment, when the peak at 1740 cm^{-1} almost disappears. It seems that the best pretreatment times lies between 60-70 min for fresh ionic liquid pretreatments, which contain the least amount of aromatics on the pulp surface at 1630 , 1600 , and 1520 cm^{-1} . After 70 mins there is an increase in the aromatics on the pulp which have slightly shifted peaks to that of original removed lignin. The recycled ionic liquid sees the formation of these peaks starting at the 70-80 min pretreatment with the 70 min spectra being almost flat in this area. This phenomenon was previously displayed in chapter 3 Figure 5, which shows the increase in these peaks as aromatic compounds are redeposited onto the surface of the pulp as the severity of the pretreatment increases. FT-IR could potentially be used to judge if the pulps are optimized for bioethanol production as these measurements seem to correlate with the saccharification yield; best saccharification yields were obtained with fresh ionic liquid occurring at 60-70 min 70-80 min with recycled ionic liquid (Figure 19).

In each case the glucose release was higher with recycled ionic liquid than its fresh ionic liquid counterpart. The saccharification inversly correlates with the pulp recovery yield (higher pulp recovery with lower saccharification), the FT-IR spectra (better saccharification with less lignin/aromatic compounds), as well as the compositional analysis (less residual acid

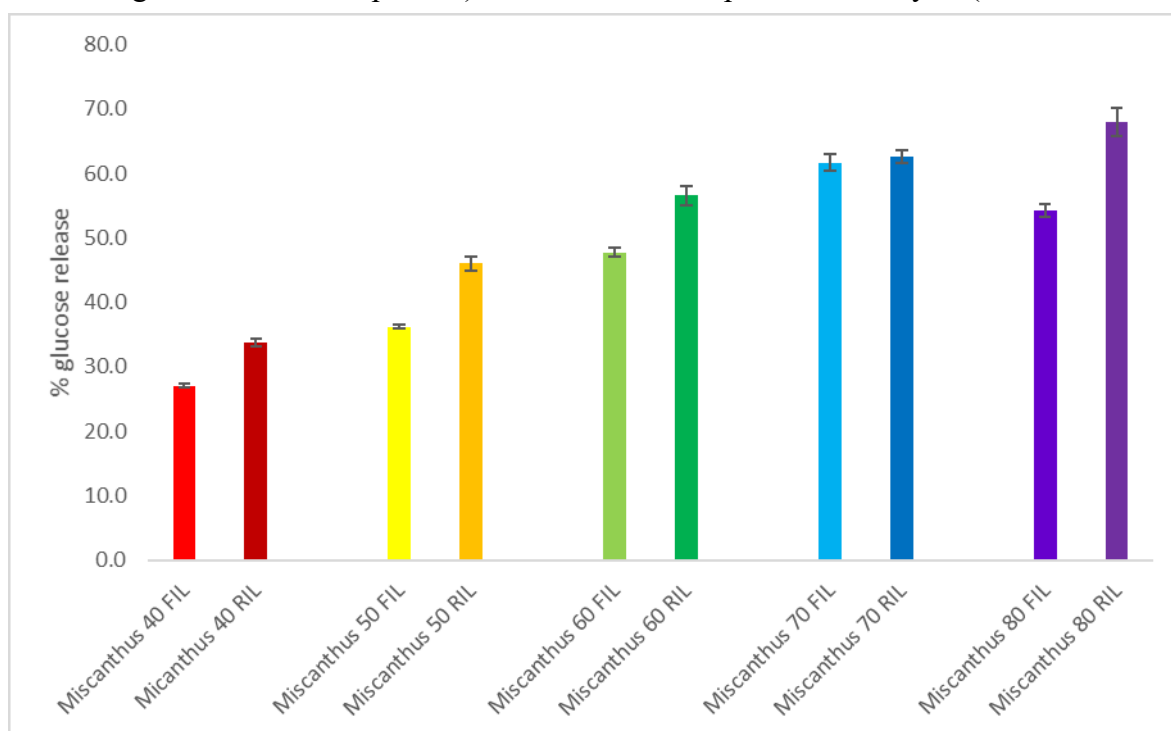


Figure 19 Saccharification for *Miscanthus* pulps pretreated at 40, 50, 60, 70, 80 minutes using fresh ionic liquid (FIL) and recycled ionic liquid (RIL) at $150\text{ }^{\circ}\text{C}$ with $[\text{TEA}][\text{HSO}_4]$

insoluble lignin with higher glucose release). Each suggests that the best lignin and hemicellulose removal occurs between 60 and 70 min for fresh ionic liquid and 70 to 80 min for recycled ionic liquid. The best saccharification yields were found at 70 min for fresh ionic liquid and 80 min for recycled (62% and 68%) respectively. At 80 min not only do we see an increase in the pulp recovered but a decrease in the saccharification for the fresh ionic liquid pretreatment. Based on the FT-IR of the pulp it was likely that the optimal conditions for pretreatment using recycled ionic liquid was at 80 min. At 80 min, recycled ionic liquid had begun to show the formation of peaks in the aromatic regions suggesting the beginnings of pseudolignin formation on the pulps. In addition, both the pulp and saccharification yields were beginning to plateau at 80 min which suggested that the optimum for this condition had been reached.

Examining lignin yield recovered also seemed to indicate the optimal times for fresh ionic liquid and recycled ionic liquid pretreatments are at 70 mins and 80 mins respectively. It was also not surprising to see that in all cases the recycled ionic liquid experiments produced a greater amount of solid precipitate (Figure 20). Similar to the pulp yields, fresh ionic liquid pretreatment has a greater rate of delignification as time progresses based on the lignin recovered during precipitation.³⁶ However, recycled ionic liquid displayed better initial lignin removal as demonstrated by compositional analysis where 14% of lignin remained after 40 min pretreatment with fresh ionic liquid compared with 11% using recycled. At 70 min the lignin

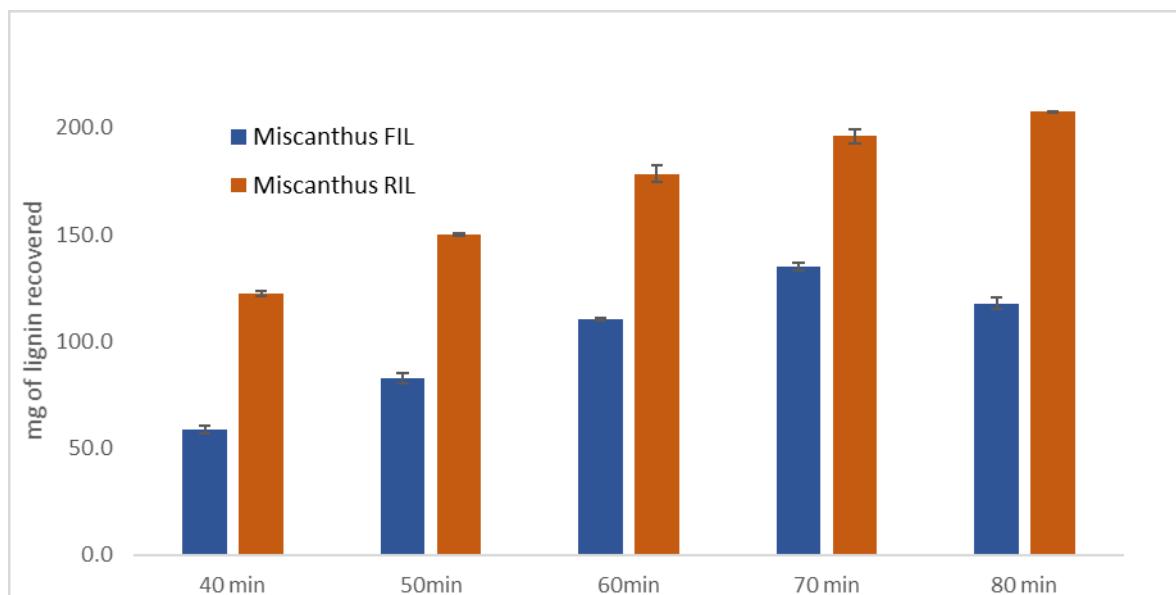


Figure 20 Lignin recovered from pretreatment of 40, 50, 60, 70, and 80 min Miscanthus at 150 °C using fresh (FIL) and recycled (RIL) [TEA][HSO₄].

solids recovered from the fresh ionic liquid pretreatment drops from 135 mg (7% lignin found on the pulp) to 117 mg (8.5% acid insoluble lignin on the pulp) at 80 min. After 80 minutes of

pretreatment this decrease in lignin recovered as solids coincides with the increase in acid insoluble lignin found on the pulp as well as the increase in the aromatic signs in the FT-IR. These results also provided evidence that the lignin produced during the ionoSolv process was likely a combination of lignin fragments as well as sugar degradation products.

The lignin produced from the pretreatment of *Miscanthus* was analyzed via GPC which produced data on the polydispersity of the lignin which may impact its potential for industrial or product applications. Both polydispersity and molecular weight can be impacted by the ionic liquid used to perform the extraction of lignin as well as the feedstock used to produce the lignin. Pretreatment conditions and differences in the severity used for lignin extraction can also impact the rate and degree of lignin condensation. Various feedstocks have different lignin subunit ratios as well as carbohydrate ratios which can impact the lignin recovered. Polydispersity of the Kraft lignin produced under these conditions ranged from 3.4 to 6.06 depending on the ionic liquid used (pyridinium, 1-methylimidazolium, pyrrolidinium acetate), with the original Kraft lignin having a polydispersity of 4.06.³⁶ In comparison to lignin pretreated with other protic ionic liquids, the polydispersity of the lignin produced using [TEA][HSO₄] and the ionoSolv process, was relatively homogeneous with polydispersity ranging between 2.6 and 3.7 (Figure 21).³⁶ The polydispersity of the lignin recovered from the recycled ionic liquid was slightly higher than that of the fresh ionic liquid. Apart from the 70 min pretreatment, the polydispersities of the fresh and recycled lignin are remarkably similar.

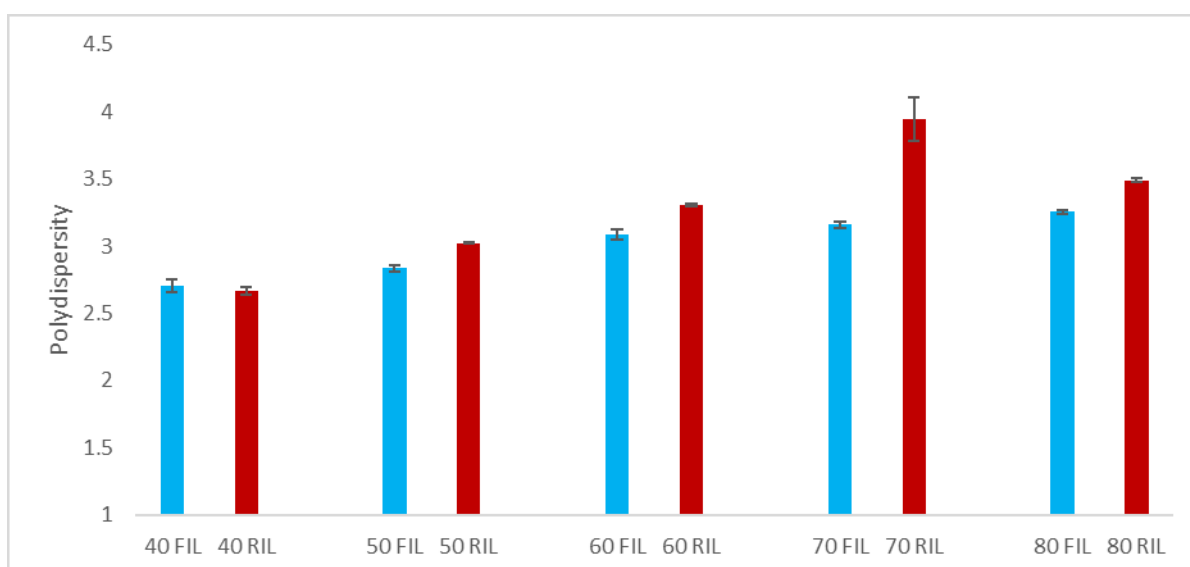


Figure 21 Polydispersity index of lignin produced from pretreatment of *Miscanthus* using fresh and recycled [TEA][HSO₄] at 40, 50, 60, 70, 80 minute intervals.

The average molecular weight, in Figure 22, shows that the lignin polymer initial molecular weight at 3450 g/mol for fresh ionic liquid and 3230 g/mol for recycled ionic liquid.

These molecular weights are within range of Kraft lignin recovered from dissolution in protic ionic liquids, which suggest that the ionicSolv produced lignin could potentially be used for various applications such as carbon fiber production.^{36,37} As the pretreatment time increased the molecular weights also increase to a point. The lignin from the fresh ionic liquid reached a maximum weight after 60 mins (4230 g/mol) after which the average molecular weight began to decrease. This pattern does not occur for the recycled ionic liquid until after 70 mins where the average was 4780 g/mol and decreased to 4120 g/mol after 80 min. This decrease of the molecular weight of the recovered lignin seems to correspond to the increase in lignin content on the pulp. This could suggest that pseudolignin formation occurs after the depolymerization of lignin and carbohydrates but prior to condensation and redeposition on to the surface of the pulp. It was also possible that the depolymerized composites condensing directly on to the surface of the pulp. Similar results have been produced by other research using dilute acid and hydrothermal pretreatments, which saw pseudolignin droplets forming on the surface of the

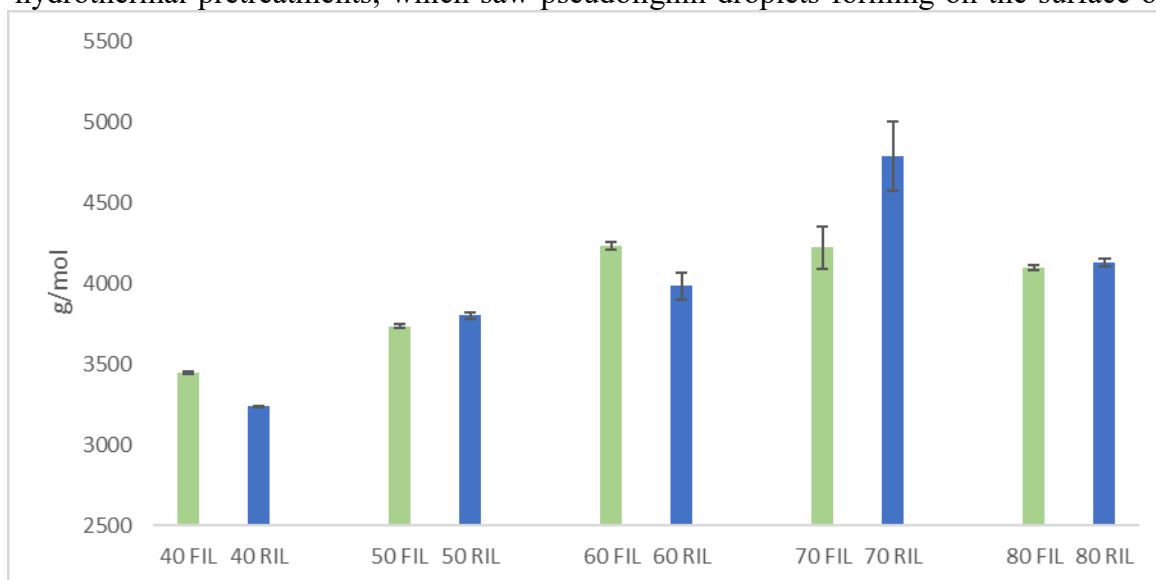


Figure 22 Average molecular weight (M_w) of lignin produced during pretreatment of *Miscanthus* at 40, 50, 60, 70, 80 minute pretreatment intervals using fresh (FIL) and recycled (RIL) [TEA][HSO₄] at 150 °C.

pulp.³⁸ It should be noted that the recycled ionic liquid was able to solubilize a higher molecular weight lignin than that of fresh ionic liquid.

4.3.8 The Use of Additives during Pretreatment of the *Miscanthus* biomass composite

There have been very few studies on the use of compounds produced during the pretreatment process as additives to prevent the formation of pseudolignin. Though several studies have suggested polar aprotic solvents such as DMSO or acetonitrile could extract and dissolve pseudolignin preventing its formation on the pulp, few studies have used additives during the

pretreatment of the entire biomass composite.^{16,39,40} It was hypothesized that these DMSO and acetonitrile interact with the carbonyl groups of compounds such as HMF and prevent nucleophilic attacks suppressing the evolution of humins.³⁴ Biphasic systems have also been tested as a means to extract HMF and prevent its combination with other intermediates to form humins.^{41,42} The uses of these systems could be promising due to the potential value of HMF as a chemical feedstock. However, separation of the HMF from these systems can prove to be difficult. In addition, these biphasic systems frequently use organic solvents which are volatile (potentially hazardous to health and environment), flammable, toxic, corrosive and requiring a costly additional steps to remove the product and recycle the solvent.^{41,43} Neither of these suggested methods (addition of DMSO or organic biphasic solvents) would be suitable for the ionoSolv pretreatment method. The use of DMSO or acetonitrile with ionoSolv would produce difficulties and additional costs during the separation process. DMSO has a high boiling point so would be difficult to evaporate from the ionic liquid during recovery. Acetonitrile is highly flammable and could be potentially dangerous thermal pretreatments. In addition, DMSO and acetonitrile could affect downstream processing, requiring additional water for washing up of the pulp.

It was hypothesized based on these findings in literature that perhaps an additive that was already generated during the pretreatment process could be added to improve pulp purity as well as saccharification results. Since others have had some success in using compounds that are believed to aid in stabilization of reactive intermediates and preventing further condensation, we hypothesized that perhaps the increase in or addition of aromatic compounds such as HMF would improve pretreatment of the *Miscanthus*. HMF has been demonstrated to be stable in our ionic liquid and does not decompose to form levulinic acid or formic acid. When HMF was combined with recycled ionic liquid, even though the quantity of HMF was large in comparison to solutes in the ionic liquid, the precipitation was in line with quantities obtained by just reheating the ionic liquid. In addition, recycled ionic liquid has outperformed its fresh ionic liquid counterpart in all trials. It was hypothesized that under these pretreatment conditions, HMF may be acting as a scavenger and prevent the formation of pseudolignin on the pulp thereby improving pulp purity and saccharification yields. Though other studies have suggested that HMF is integral for the formation of humins and that it was the presence of HMF that causes the growth of the humic polymer, under the conditions of the ionoSolv pretreatment HMF may increase solubility of these humic/pseudolignin polymers via

incorporation in to these polymers.^{15,16,44} By increasing the solubility in the ionic liquid, it maybe preventing their deposition on the surface of the pulp.

To explore the impact of sugars as additives in line with studies listed above, xylose, HMF, and furfural were added to the entire *Miscanthus* composite for pretreatment. Pulp and lignin yields were compared, compositional analysis, and saccharification were conducted on all the resulting pulps. Pulp and lignin recovery yields for each trial is presented in Figure 23. The samples containing a xylose additive resulted in lower pulp recoveries compared with pulps pretreated without, with the exception of xylose. Xylose pulp and lignin yield using recycled ionic liquid matched closely to the no additives recycled ionic liquid pretreatment (xylose added pulp yields 64.5% vs. 64.4% for MRIL and lignin yields of 15.7 and 15.4% respectively). However, fresh ionic liquid extraction with xylose added results in a higher pulp yield and lignin production (xylose pulp yield 69.3% vs. 68.5% for MFIL and lignin yields of 11.2 and 9.6% respectively). Previous sugar experiments detailed earlier in the chapter demonstrated that xylose on its own in fresh ionic liquid can be highly reactive to produce humins. Without the solutes to help stabilizes and potentially scavage the sugar conjugates and lignin frgements to prevent pseudolignin deposition, there was an increase pulp yield and lignin yield when using fresh ionic liquid with a xylose additive.

Miscanthus with HMF pretreated with recycled ionic liquid (MHRIL) has the lowest pulp recovery with the highest lignin yield (62.5 and 22.1% respectively) (see Figure 23). This

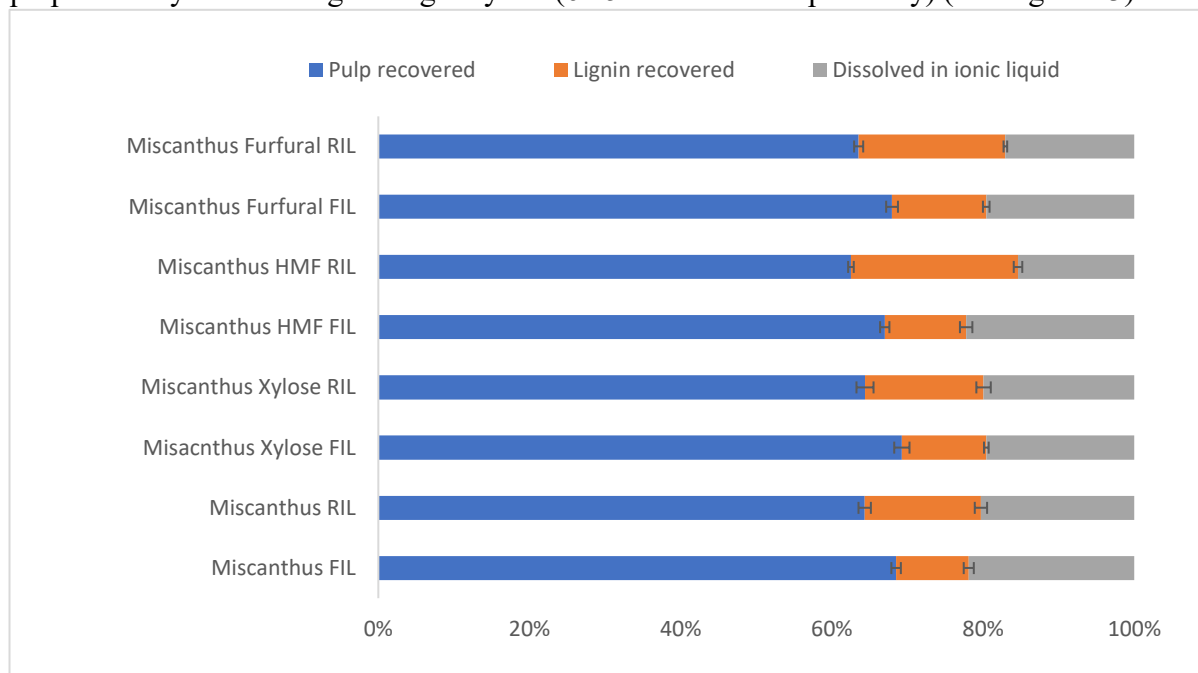


Figure 23 Pulp yield, lignin yield, and mass dissolved into the ionic liquid as a percentage the starting amount of dry *Miscanthus*. All pretreated with the addition of furfural, HMF, xylose, or glucose with [TEA][HSO₄] at 150 °C for 1 hour. No additive *Miscanthus* pretreatment with fresh ionic liquid and recycled ionic liquid (RIL) is also shown for reference.

seems to substantiate the idea that HMF was preventing the deposition of pseudolignin onto the surface of the pulp, resulting in a higher extraction of the biomass composite. It could be that HMF by scavenging or stabilizing intermediates and or polymerizing with the humins and lignin fragments in the liquor, alters and increases their solubility in the ionic liquid. It was perhaps these factors and increased solubility of the pseudolignin that was responsible for the higher lignin recovery yield. These yields are higher than that of the *Miscanthus* RIL (MRIL), *Miscanthus* furfural RIL (MFRIL), and the *Miscanthus* Xylose RIL (MXRIL) samples (which recovered 15.4, 19.4, and 15.7% respectively).

HMF also has the lowest pulp yield (67.0%) when using fresh ionic liquid, though without the already high presences of solutes comparatively less lignin was recovered (10.7%). *Miscanthus* pretreated with fresh ionic liquid had the pulp recovery at 68.5% and low lignin recovery of 9.5%. Both the furfural and xylose spiked pretreatments displayed higher lignin recovery compared to other FIL trials (12.5 and 11.1% respectively) which demonstrates their likelihood for polymerization and formation into pseudolignin. These results seem to be in line with the individual sugar trials. The pulp recovery of 68.0 and 69.3% for furfural and xylose respectively seems to also indicate their hinderance of the extraction process or perhaps the facilitation of deposition of pseudolignin. This suggested that having some solutes in the ionic liquid benefits the extraction, which was demonstrated by the fact that every RIL trial outperformed its FIL trial. But it was also clear that some solutes have a more beneficial effect than others on *Miscanthus* extraction.

Compositional analysis results on the pulp supports the pulp yield results (see table 2) (normalized yields compared to untreated *Miscanthus* detailed in appendix 4L). Similar to the time course pretreatment, with the exception of HMF additive set, every recycled trial contained less hemicellulose and acid insoluble lignin (AIL) than its fresh ionic liquid counterpart. This correlation with a greater lignin removal using recycled ionic liquid suggest that the solutes are either aiding in the extraction of the composites and or increasing their solubility preventing deposition of pseudolignin onto the pulp. It was also clear that none of the additives reacted the same way. Without additives, the pretreated *Miscanthus* using fresh ionic liquid resulted in a pulp containing 67.1 % glucan, 19.0 % xylan, and 13.4% lignin. Based on the individual sugar trials which resulted in xylose producing the greatest amount of humic solids, it was expected that the xylose with fresh ionic liquid contains the highest acid insoluble lignin content at 16.1% (it also has the highest pulp yield of 69.3%, suggesting the deposition of pseudolignin). It also contains the lowest glucan content (62.1 %) meaning that the addition

of xylose could aid the removal of amorphous cellulose and polymerization of the hydrolyzed glucose units. Amorphous cellulose is generally more readily hydrolyzed to its monomers in acidic conditions than its crystalline counterpart.⁴⁵ Therefore, based on the combined xylose and glucose experiments (in the combined glucose and xylose trials section detailed earlier in this chapter) which demonstrated that the interplay of these two sugars and their intermediaries result in humin production, it would be expected that there was an increase in acid insoluble lignin deposited on the pulp. Compared to the MXFIL the MXRIL not only has a significantly lower pulp yield (64.5%) but also lower residual lignin content on the pulp (16.1 vs. 13.6%).

Furfural pretreatments with fresh ionic liquid contain the second highest lignin content on the pulp. Though it has been demonstrated that furfural does not readily polymerize on its own during the ionoSolv process using [TEA][HSO₄], it does readily react with other solutes. This may explain why the furfural with *Miscanthus* FIL trial has the second highest lignin content at 15.5%. It also has pulp recoveries comparable with those of xylose and no additives fresh ionic liquid pretreated *Miscanthus* (68.0% compared with MXFIL at 69.3 and MFIL at 68.5%). These numbers compared with the recycled ionic pretreatments suggest that furfural either hindered the biomass extraction or contributes to the deposition of pseudolignin.

Table 2 Compositional analysis of *Miscanthus* with xylose, HMF, or furfural added. Pretreatments were conducted with fresh ionic liquid (FIL) and recycled ionic liquid (RIL) [TEA][HSO₄] at 150 °C for 1 hour.

	Miscanthus Furfural FIL	Miscanthus Furfural RIL	Miscanthus HMF FIL	Miscanthus HMF RIL	Miscanthus Xylose FIL	Miscanthus Xylose RIL	Miscanthus FIL	Miscanthus RIL
Glucan	42.74 ± 0.83	43.21 ± 0.31	43.23 ± 0.21	41.37 ± 0.38	43.28 ± 0.25	42.80 ± 0.54	41.71 ± 0.41	47.11 ± 0.58
Xylan	12.58 ± 0.68	9.91 ± 0.07	11.20 ± 0.25	12.13 ± 0.89	11.80 ± 0.38	9.88 ± 0.13	11.80 ± 0.17	11.99 ± 0.09
Galactan	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Arabinan	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Mannan	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
ASL	1.93 ± 0.07	1.72 ± 0.05	1.95 ± 0.01	1.52 ± 0.08	2.17 ± 0.06	1.88 ± 0.08	1.91 ± 0.03	1.86 ± 0.03
AIL	10.08 ± 0.22	7.98 ± 0.24	9.65 ± 0.29	6.64 ± 0.21	11.19 ± 0.35	8.85 ± 0.42	8.34 ± 0.28	6.95 ± 0.03
Ash	0.70 ± 0.01	0.77 ± 0.02	1.01 ± 0.01	0.92 ± 0.02	0.87 ± 0.04	1.03 ± 0.02	0.61 ± 0.11	0.69 ± 0.03

Compositional analysis of the pulps with HMF as an additive revealed that using fresh ionic liquid vs. recycled ionic liquid may have a large impact. The pulp using fresh ionic liquid contains 65.1% glucan, 16.9% xylan, and 14.5% lignin compared to the recycled ionic liquid pulp which contains 72.7% glucan, 21.35% xylan, and 11.7% lignin. Though the trial using fresh ionic liquid produced extraction results better than any of the other fresh ionic liquid trials (67.0% pulp recovery), the recycled ionic liquid was much better at extraction with the best pulp recovery of 62.5%. This suggests that HMF alone was not the sole factor for improving biomass extraction, but rather, the combination of the solutes with the increase HMF levels. HMF with recycled ionic liquid left a higher amount of hemicellulose (specifically xylans)

intact. In addition, HMF with recycled ionic liquid resulted in the lowest residual lignin content on the pulp (11.6%). It seems that HMF may slow the rate of xylan hydrolysis, meaning that these sugars would not be able to quickly or readily participate in pseudolignin formation. In addition, the high lignin recovery yield but low residual lignin on the pulp seems to support the idea that the HMF may be acting to increase the solubility of pseudolignin ionic liquid. Previous studies have demonstrated that the depolymerization of lignin can lead to fragments containing aldehyde groups which are highly reactive and can easily polymerize with humic substances from sugar degradation. It would be expected that incorporation and condensation of HMF or furfural with these lignin fragments would occur.^{12,46}

Based on higher lignin removal, or fewer pseudolignin deposits with recycled ionic liquid expectations were to see RIL pulps to outperform FIL pulps during saccharification and specifically for the MHMFRIL trial to have the best results. Seven-day saccharification results are detailed in Figure 24. As predicted in each case the RIL out performs the FIL for amount of glucan released. Though pretreatment of any sort is better than the saccharification of untreated Miscanthus (12.3% glucan release), it also demonstrated that additives may help to enhance the process. It is also very promising for the use of the ionoSolv process for biorefining to see that recycled ionic liquid yields better results both in pulp purity as well as saccharification. The addition of additives such as HMF may improve the extraction of the biomass composite preventing pseudolignin deposition and thereby enhancing saccharification. In this case the best results of 73.6% glucan release was achieved. This was

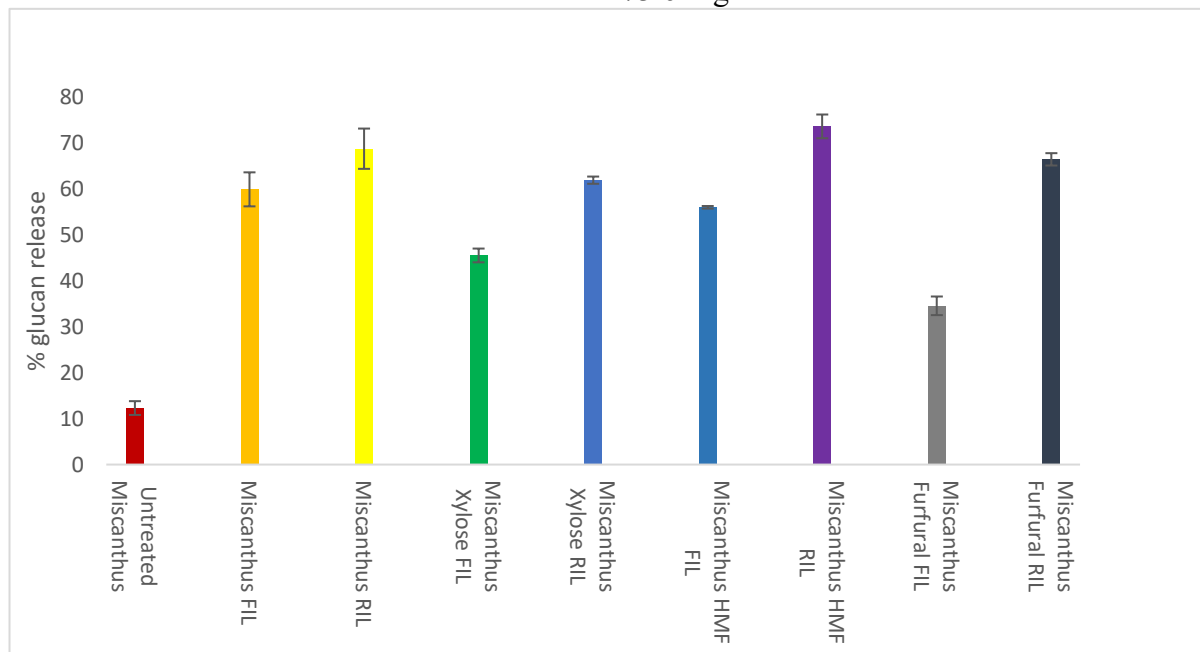


Figure 24 Seven-day saccharification of pulps pretreated with no additives, xylose, furfural, and HMF using fresh ionic liquid (FIL) and recycled ionic liquid (RIL) [TEA][HSO₄]. Pulps were generated with 300 mg states additive and pretreated at 150 °C for 1 hour.

only the case when there are other solutes involved confirming that HMF is not the only compound that aids in the prevention of pseudolignin deposition. With the use of fresh ionic liquid, additives only depressed saccharification yields as they increase the overall lignin (or pseudolignin) residue on the pulp. The second highest yielding results were seen with pretreatment using recycled ionic liquid with no additive (68.7%). As predicted from the individual sugar trials xylose sugar release (45.5 % in the FIL trial and 61.9% with RIL) and furfural (34.6% sugar release with FIL and 66.4% with RIL) seem to inhibit saccharification due to pseudolignin deposition on the surface of the pulp which limits enzymatic access, though the use of recycled ionic liquid can mitigate some of that inhibition.

4.3.9 ^{13}C Labeled Glucose and Xylose Pretreatments

There is general agreement that pseudolignin deposition on the pulp surface will reduce saccharification yields. It does this by binding to the enzyme, inhibiting and blocking enzyme access and activity. This has been demonstrated extensively for the dilute acid pretreatment of various types of biomass.^{7,47} FT-IR of the pulp surface (Figure 18) and gravimetric analysis (Figure 17) has confirmed the deposition of pseudolignin onto the pulp during the ionoSolv pretreatment with [TEA][HSO₄] and its impact on saccharification has also been demonstrated (Figure 19). However, there are no studies that we are aware of that attempt to track these humic compounds or pseudolignin throughout the fractions of the pretreatment process.

To investigate the presence of sugars and their degradation products on the pulp and confirm the condensation of these compounds in the lignin precipitate, *Miscanthus* was pretreated with ^{13}C labelled glucose and ^{13}C labelled xylose. A compositional analysis was completed on this pulp and the resulting acid soluble lignin was submitted to HSQC for structural analysis. However, after the pulp was exposed to the 72% sulphuric acid used to perform the compositional analysis, the resulting acid insoluble lignin were not soluble in solvents required to perform HSQC. The pulps were instead submitted to ssNMR for analysis. The lignin precipitated during pretreatment was processed via the standard HSQC protocol. Though both the pulp and lignin would also produce their own signals in the HSQC, it was hypothesized that the pretreatments doped with ^{13}C labelled isotopes would be significantly more intense providing evidence of their incorporation. Sigma cellulose and untreated *Miscanthus* spectrographs were used as a baseline for comparison. Peaks for carbons contained in cellulose can be seen in both samples, with the cellulose C1 carbon located at 106 ppm, C2,5 at 73 ppm, C3 at 76 ppm, and C4 at 90ppm (5). Untreated *Miscanthus* contains broad peaks in

between 110-170 ppm which are the aromatic peaks assigned to lignin. No signals in the ssNMR for the ^{13}C pretreated xylose and glucose samples were found in this region. This was rather surprising as humin and pseudolignin formation in acidic conditions have been demonstrated. There are several possibilities that could explain why there are no visible peaks generated in the solid-state NMR spectrograph (see figure 25). One possibility was that the samples were not sufficiently doped to produce an adequate signal when using solid-state NMR, which was not as sensitive as solution NMR. Another possibility was that the pretreatment was not severe enough to have generated sufficient pseudolignin deposits to the surface of the pulp for the ssNMR to detect. Increasing the amount of ^{13}C doping as well as severity via time and or temperature would most likely be necessary to produce the visible signals via ssNMR.

^{13}C glucose doped HSQC of the lignin precipitated during pretreatment of *Miscanthus* is shown in Figure 26. Aromatic signals in the fresh ionic liquid are detected at (6.6, 103.5), (6.9, 110.2), (6.8, 115.5), (6.7, 115.0), (6.8, 118.5), (7.5, 130.1), (7.7, 128.5), (7.68, 131.4) and (7.0, 128.8). There are significantly fewer signals in the aromatic region in the recycled ionic liquid experiments (occurring at (6.6, 103.5), (6.9, 110.2), (6.7, 115.0), (6.8, 118.5), (7.5, 130.1), and (7.0, 127.9)). Signals (6.6, 103.5) and (6.9, 110.2), which occur in both the fresh and recycled ionic liquid treated lignin, produce peaks in areas that overlap with lignin units 2,6 position on the syringyl subunit, and G2 of the guaiacyl sub unit, making it difficult for these peaks to be used to identify the presence of pseudolignin.⁴⁸ In addition, if quantitative measurements of lignin are attempted, these signals should be avoided as they may artificially

guaiacyl unit (6.8, 115.0) may cause overlap leaving (6.8, 118.5) and (7.5, 130.1) as better potential indicators of pseudolignin presence.

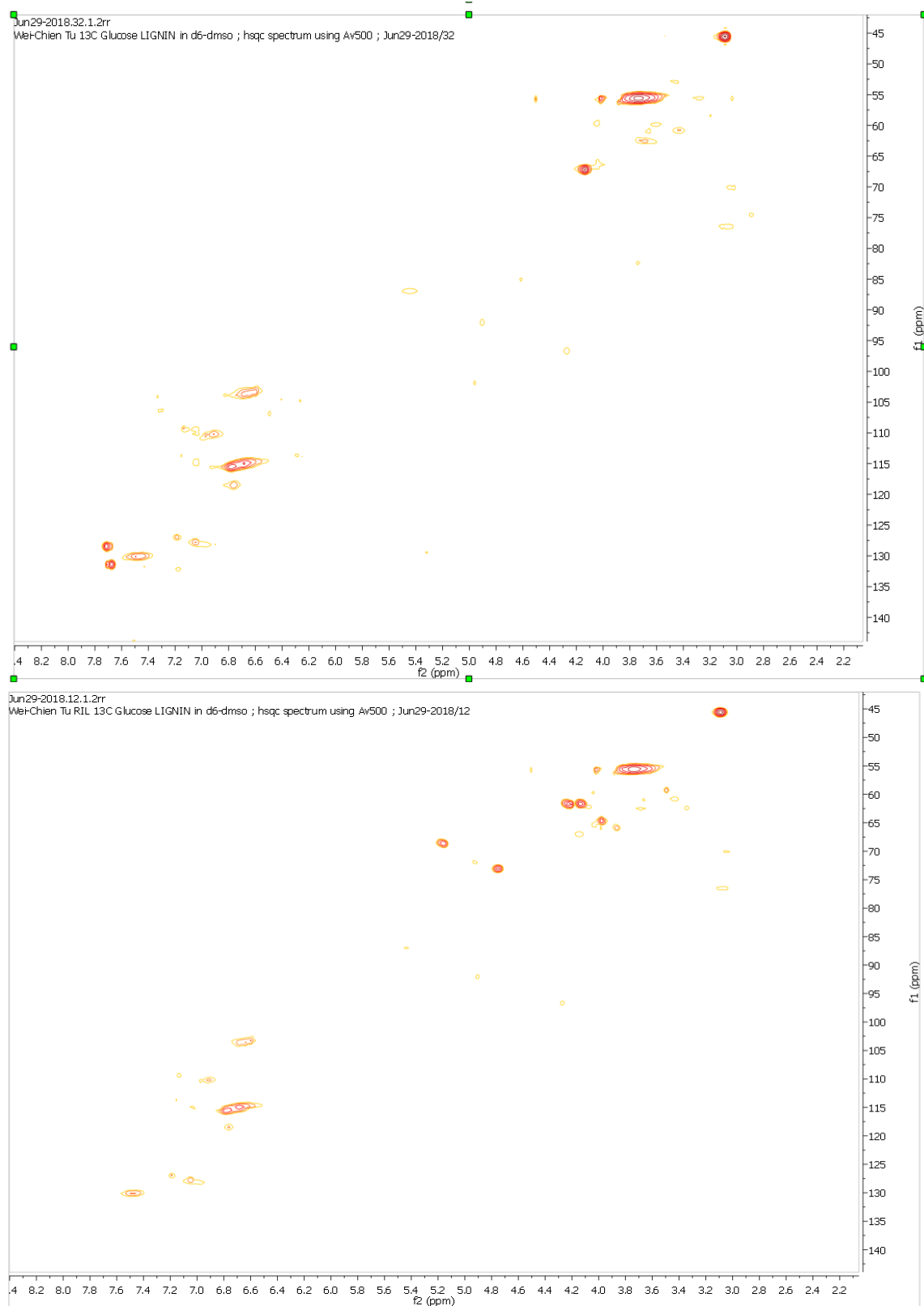


Figure 26 *Miscanthus* lignin recovered from pretreatment with ¹³C labelled glucose with fresh ionic liquid (top) and recycled ionic liquid (bottom) [TEA][HSO₄] for 1 hour at 150 °C.

While more signals were found in the aromatic region in the fresh ionic liquid lignin, the opposite was true in the aliphatic region. This may suggest that the one of the ways that the recycled ionic liquid may change the solubility of pseudolignin was in the form of preventing the conversion of the sugars into aromatic compounds. In the fresh ionic liquid lignin peaks were found at (3.7, 55.6), (4.0, 55.8), and (4.1, 67.2) and in the recycled ionic liquid at (3.7, 55.6), (4.0, 55.6), (4.2, 61.8), (4.1, 61.7), (4.0, 64.8), (5.2, 68.6) and (4.8, 73.1). The signal at (3.7, 55.6) belong to the methoxy groups associated with lignin. In both cases the signal at (4.0, 55.8/ 55.6) may potentially be used as a pseudolignin indication for pseudolignin from produced from glucose degradation. The other signals in the recycled ionic liquid sample do not correspond to either lignin or carbohydrate signals, it is likely that they are intermediaries of the sugars that have been incorporated during the pretreatment process. For processes that use recycled ionic liquid these may serve as potential indicators of incorporation of glucose and its intermediaries.

¹³C labelled xylose doped *Miscanthus* lignin in fresh ionic and recycled ionic liquid are shown in Figure 27 (combined HSQC for comparison can be seen in appendix 4N). The fresh ionic liquid aromatic signals show a greater similarity to the signals seen for general *Miscanthus* lignin and may suggest that the lignin that is recovered from the ionic liquid pretreatment may be a result of condensation of xylose with lignin fragments: signals at (6.6, 103.5), (6.9, 110.2), (6.4, 110.4), (6.8, 115.4), (6.8, 118.6), (7.5, 130.1), and (7.0, 127.8). When compared with those of the recycled ionic liquid the recycled peaks were more defined and less spread: (6.6, 106.6), (6.8, 115.5), (6.7, 114.9), (7.5, 130.1), and (7.0, 128.0). Though all signals in the recycled ionic liquid lignin are also present in the fresh ionic liquid sample, there were two additional signals in the fresh ionic liquid at (6.9, 110.2) and (6.4, 110.4) which are not found in the undoped *Miscanthus* pretreated lignin or in the reheated recycled ionic liquid. These could potentially be used as an indicator for the presence of pseudolignin formation from xylose during fresh ionic liquid pretreatment, though a significant amount of pseudolignin formation may be required in an undoped situation. Signals at (6.6, 106.5) would overlap with the 2,6 position of the syringyl lignin subunits, while the (6.8, 115.5) and (6.7, 114.9) signals would overlap with the G5 guaiacyl subunit, making these signals unreliable for pseudolignin detection. However, the (7.5, 130.1), and (7.0, 128.0) though located in the similar area to p-hydroxyphenyl (7.2, 128.0) could provide another signal as a means for pseudolignin identification. ¹³C xylose doped *Miscanthus* lignin shows very little signal in the aliphatic

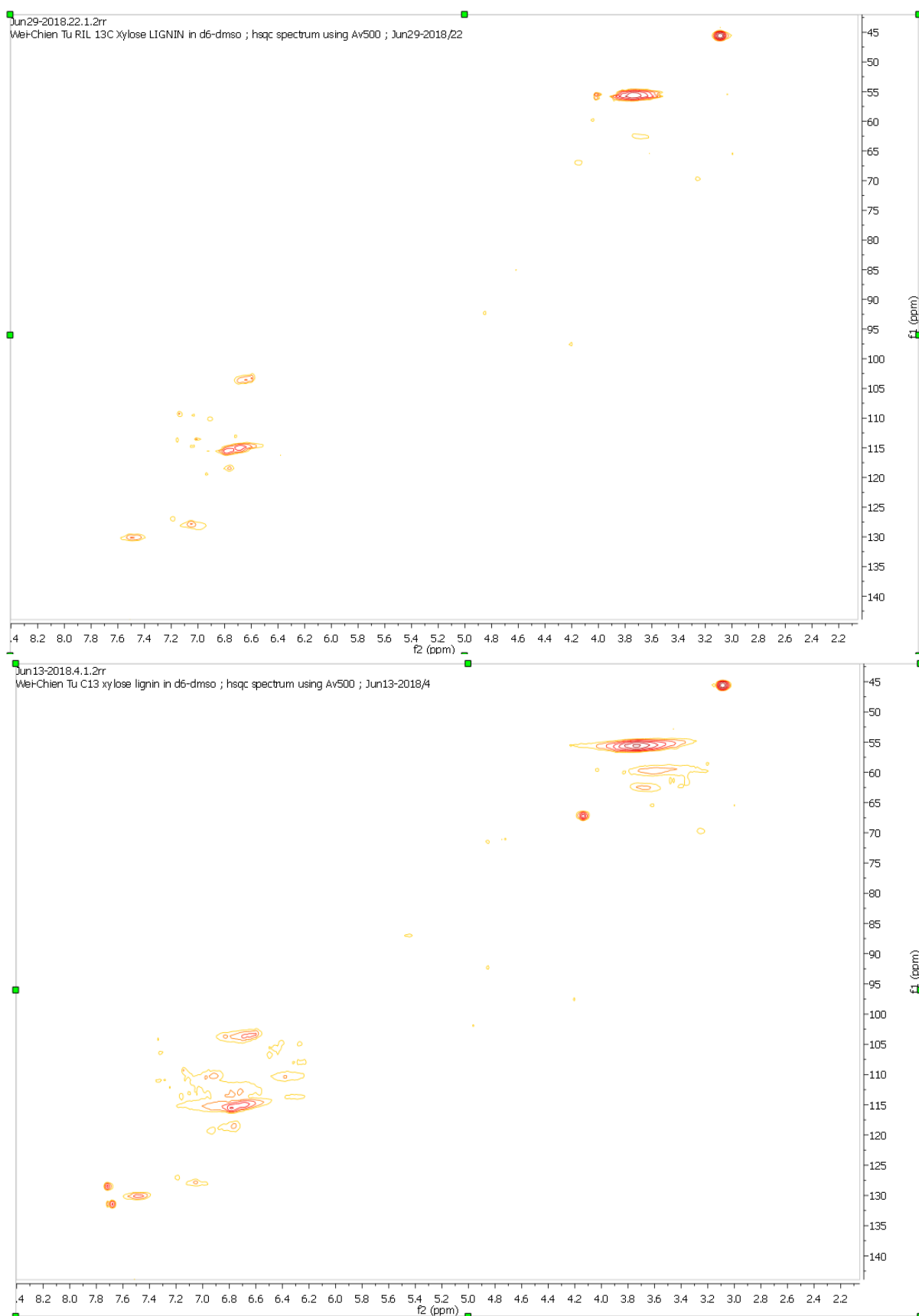


Figure 27 ^{13}C labelled xylose dope *Miscanthus* lignin from pretreatment conducted at 150°C for 1 hour using recycled ionic liquid (top) and fresh ionic liquid (bottom).

regions compared with that of glucose doped lignin. The methoxyl lignin signal at (3.7, 55.6)

was present in both cases though much stronger in the fresh ionic liquid than in the recycled which could suggest that the fresh ionic liquid incorporated a greater amount of aryl methoxy groups from fragmented lignin. This could also explain why there are broader signals in the fresh ionic liquid samples in the aromatic regions. Fresh ionic liquid peaks can be seen at (3.6, 59.7), (3.7, 62.6), and (4.1, 67.2) while recycled ionic liquid only contained two noticeable peaks at (4.0, 55.5) and (3.6, 59.7). (3.6, 59.7) which would overlap with the γ hydrogen of β -O-4 structures and unless severe pretreatment was used to ensure this motif was fractured during pretreatment, this signal would not be a reliable indicator of pseudolignin presence. (3.7, 62.6) and (4.1, 67.2) do not correspond to any known lignin signals or carbohydrate signals and could be a signature of pseudolignin formed from xylose degradation. The peak at (4.0, 55.5) only appears in the recycled ionic liquid spectra and could be used to identify pseudolignin formation for these pretreatments however, its signal would most likely be masked by the larger methoxy signal that was common in non-doped pretreatments.

These doped experiments confirm the hypothesis that sugars and their degradation products are being incorporated into the lignin recovered during pretreatment. None of the signals generated from the either fresh or recycled correspond to signals measure of the solids generated during the pretreatment of glucose or xylose. The signals also do not correspond to those of pure HMF or furfural.

4.4 Conclusion and Future Studies

It is clear that pretreatment parameters like time, temperature, and choice of ionic liquid will affect the quality of the results. Additionally, the ionic liquid used for any biorefining process will inevitably be recovered or recycled, and how the pretreatment process is conducted will determine the solutes that are found in the ionic liquid further impacting the outcome. In the experiments, the use of the batch reactor caused a higher hemicellulose release, resulting in a higher hemicellulose concentration in the recycled ionic liquid. This suggests that the size of the reactor, type of reactor, whether the reaction is stirred, and the heat profile of the reactor will be very important in determining the concentration of the solutes in the recycled ionic liquid. This in turn will affect the optimum condition of the pretreatment. It appears that the recycled ionic liquid optimum, at least on the lab scale, has a longer residence processing time than that of fresh ionic liquid. Though the residence time may be longer, it does appear that

recycled ionic liquid provides better overall pretreatment results when compared with the fresh ionic liquid.

Pseudolignin formation during the ionicSolv pretreatment was due to the condensation of lignin fragment, carbohydrates, and their intermediates. The purity of the pulp or comprehensiveness of the extraction on the surface of the pulp will also determine the likelihood of redeposition and pseudolignin formation onto the surface of the pulp. Though furfural was highly reactive in the presence of other solutes, its removal during the recycling process may aid in the prevention of pseudolignin formation. The addition of additives that are already produced during the pretreatment process may potentially be used as a preventative measure. It appears that solutes and additives can improve humin solubility during pretreatment and act as a potential scavenger to prevent pseudolignin formation onto the pulp. The addition of HMF improved fractionation of the *Miscanthus* biomass and saccharification yields of the pulp produced. HMF displayed the strongest ability to prevent the formation and deposition of pseudolignin on to the surface of the pulp by increasing the solubility of the pseudolignin via its incorporation. ^{13}C NMR shows that the lignin produced during the ionicSolv process are a result of the condensation of sugar intermediates and depolymerized lignin.

Additional studies will be needed to determine the optimal processing conditions during scale up. Tests for solute concentration and phenolic content would need to be determined and correlated with saccharification and materials production results. Studies into the solute change over time and its effects on pretreatment especially with ionic liquid that would be recycled multiple times, especially because biomass loading would affect the pH over each use. HMF optimization will also need to be explored along with how solubility is affected. The possibility of using different ionic liquids to manipulate that solubility to prevent pseudolignin formation will also need to be studied. GPC analysis and additional XRD analysis of the cellulose pulps after pretreatment using recycled ionic liquid would need to be conducted to investigate the impact of the time and recycling on the degree of polymerization, molecular weights of the pulps, and impact on crystallinity because this information may impact the cellulose pulp applications. In addition, the results would need to be compared between batch reactors as well as flow reactors. Time and heat trials with additives would also need to be conducted to determine the optimum. Tests with membranes, zeolites, and other filters could be conducted to examine if the solutes and specific solutes could be removed from the ionic liquid. This might be a means to control and mitigate the formation of pseudolignin, improve processing,

and extraction of various valuable chemical products. Further attempts at pre-extraction of hemicellulose from the biomass and subsequent pretreatment could be examined to see if removal of the hemicellulose sugars would reduce pseudolignin formation and provide a value stream for the hemicellulose sugars. There have been other reports in literature that suggests the potential use of dialkyl phosphate type ionic liquids for hemicellulose extraction.⁴⁹ Studies into other potential additives and combinations that may improve yield and efficiency would also be needed. These results would need to be viewed through a processing and technoeconomic lens to ensure that any additional steps are feasible for biomass biorefining.

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5 IonoSolv Applications with Post Hydrolysis Waste, Waste Paper, Bleaching and Nanocellulose Production

5.1 Introduction

In 2016 more than 2 billion tons of municipal solid waste (MSW) was generated globally.¹ Much of this waste ends up in landfill and dump sites which cause hazardous conditions and environmental problems.² These include potential fires and explosions from methane production and leaching of toxic metals into ground water. However, MSW can also be a potential solution for the increasing global demand for energy. Throughout history, humans have always been producing waste, potentially making MSW a reliable “sustainable” resource, particularly food waste and other organic biomass products (i.e. furniture, textile, boxes and paper waste). The possibility exists to use these types of waste for energy production in a biorefinery through carbohydrate extraction, removing remaining sugars for fermentation to bioethanol and other platform chemical production. Any solids remaining after extraction could be used for biogas production.

Besides the hydrolysis of cellulose to glucose and its fermentation for bioethanol production, the production of a lignin and hemicellulose free cellulose pulp has multiple potential applications across a variety of industries. From drug delivery systems in the pharmaceutical industry, to paper, and textiles, and feedstocks for polymers, cellulose is abundant, and has become a focus for sustainable, biocompatible materials research.^{3–5} Specifically, the research on nanocellulose production has dramatically increased in the past 20 years. The production and potential use of nanocellulose has been published in over 1700 journals just in 2014.⁶ With its mechanical strength, optical properties, electrical conductivity, and its ability to be modified for specific applications, nanocellulose has been viewed as the ‘new’ renewable and sustainable versatile polymer.⁷

Critical to the sustainable use of cellulose in any industrial process is the development of efficient biorefineries that are competitive with fossil fuels. Ionic liquids have demonstrated great versatility in the production of cellulose, but also can have the sustainable benefit of being recyclable, reusable, and therefore may be a good choice for biorefineries. For any type of ionic liquid biorefinery to be successful, multiple value streams are necessary to increase competitiveness. Flexibility and efficient transition between different biorefinery processes provides resilience in various market conditions. Beyond the direct pretreatment of harvested biomass to produce sugars for fermentation to bioethanol (which has been explored in previous chapters), the cellulose rich pulp could be used for materials production. Biorefineries which have expanded capabilities to produce materials may be able to make better use of waste in cellulose treatment or shift production from bioethanol to materials. This flexibility may ultimately increase the competitiveness of biomass pretreatment and displace currently fossil fuel dependent industrial processes. Cellulose and materials produced from cellulose can produce the high value products that make the economics of using ionic liquid for biorefining possible (table 1). The market value and therefor demand for cellulose fibers is anticipated to increase by over 300% in the next 5 years. In this chapter we will be investigating potential applications for the ionoSolv process in developing cellulose materials. Specifically, we will explore the generation of additional value from further fractionation of post hydrolysis solids produced from solid municipal waste as well as extraction of carbohydrates from waste newspaper. Finally, the bleaching of ionoSolv produced *Miscanthus* will be explored for materials and nanocellulose production. Though these are not the only possible value streams that could be added for the ionoSolv process, both of these routes can be easily adapted into current processing methods.

Table 1 Current and project market value for cellulosic materials.

	Current Market Value	Projected Growth
Cellulose (fibers)	\$10 billion USD as of 2015	\$39 billion USD by 2024 ¹
Cellulose (MCC and NCC)	\$885.1 million USD as of 2018	\$1.24 billion USD by 2023 ²
Cellulose Acetate	\$3.81 billion USD as of 2017	\$4.87 billion USD by 2022 ³

1. Source: <https://www.globenewswire.com/news-release/2017/02/07/914547/0/en/Cellulose-Fiber-Market-to-exceed-39-Billion-by-2024-Global-Market-Insights-Inc.html>
2. Source: <https://www.prnewswire.com/news-releases/microcrystalline-cellulose-mcc-market-worth-1-241-4-million-by-2023-890245050.html>
3. Source: <https://www.marketsandmarkets.com/PressReleases/cellulose-acetate.asp>

5.2 Materials and Methods

5.2.1 PHS derived from MSW and The Sun newspaper

Post hydrolysis solids (PHS) (Figure 1 on the left) was received from Fiberight, a company that specializes in the processing of MSW, to investigate the possibility of using ionic liquids processing to further extract the remaining carbohydrate fraction for biofuel production. Different ionic liquids using the IonoSolv pretreatment process (detailed in chapter 2) were used to pretreat these cellulosic waste streams. Ionic liquid 1-butylimidazolium hydrogen sulfate [HBIM][HSO₄] which had been demonstrated to be more successful at delignification than [TEA][HSO₄] was requested to be used by Fiberight to ensure high lignin extraction. Ionic liquid 1-butylimidazolium hydrogen sulfate [HBIM][HSO₄] was produced from the combination of 1-butylimidazole and 5M sulfuric acid following the ionic liquid synthesis protocol detailed in chapter 2. 1-Ethyl-3-methylimidazolium acetate [EMIM][OAc] which was purchased from Sigma Aldrich was used on samples of *The Sun* newspaper as well as the post hydrolysis waste to swell the cellulose portion in the waste stream. Pretreatments with [HBIM][HSO₄] were initially conducted at 150 °C for 1 hour and then again at 120 °C for 6 hours. [EMIM][OAc] trials were conducted at 120 °C for 4 hours.

Two copies of *The Sun* newspaper were purchased and summarily ground using the Wiley mill protocol (see chapter 2 under biomass preparation and moisture content determination) (see Figure 1 on the right).



Figure 1 Samples of the post hydrolysis waste (left) and the ground and sieved 'The Sun' newspaper.

Lignin extracted using [HBIM][HSO₄] was sent to London Metropolitan University for elemental analysis so that caloric content could be determined. Compositional analysis on pretreated pulps follow the NREL protocol detailed in chapter 2.

5.2.2 Bleaching of Miscanthus and nanocellulose production

Bleaching of Miscanthus protocol is detailed in chapter 2. Pulp produced after bleaching was analyzed with the Valmet FS5 Fiber Image Analyzer for particle size, curl, and kink. The supernatant from the bleach pulp washes were analyzed via dynamic light scattering (DLS) for size determination and Zeta Potential measurements were gathered to ascertain the stability of the colloid.

5.3 Results and Discussion

5.3.1 PHS and *The Sun*

Municipal solid waste can be separated and the curbside-type recyclables from food waste, wood products, and other organic substances collected. This waste, which is composed mainly of biomass, is washed and separated into two streams. One stream is used for biogas production and the other, which contains the higher cellulose fraction, is submitted to hydrolysis. The hydrolysate can be used for biofuels and biochemical production, while the remaining solid, the post hydrolysis waste (PHS), is generally burned for energy recovery. There are several challenges to pretreating MSW and specifically PHS. Not only is the waste extremely heterogeneous in carbohydrate content, but it invariably contains inorganics, metals, plastics, and non-ideal components which may impact downstream processing.

There have been few pretreatment methods that have been successfully applied to the pretreatment of MSW. Though there are several different categories for MSW pretreatment: mechanical, thermal, chemical, and biological; the most economically feasible are low temperature thermal pretreatment and two stage anaerobic digestion.⁸ However, few studies have been conducted on the further extraction of the PHS produced from the pretreatment of MSW (PHS provided by Fiberight had previously undergone acid hydrolysis). However, because the PHS still contain a high concentration of glucans, prior to incineration for bio gas or energy production, one further extraction is attempted. In our study we will using the ionicSolv process to pretreated PHS produced from pretreated MSW, first using two different ionic liquids. 1-butylimidazolium hydrogen sulfate [HBIM][HSO₄] has been demonstrated to be highly effective at lignin removal and 1-ethyl-3-methylimidazolium acetate [EMIM][OAc] effective at dissolving cellulose. With [HBIM][HSO₄], the objective is to produce a cellulose rich pulp (or in this case PHS) that can be saccharified, then fermented for bioethanol production. In the second set of trials, [EMIM][OAc] will be used to dissolve the cellulose in the PHS, the cellulose is then regenerated and submitted for saccharification.

A compositional analysis was first conducted on the PHS and *The Sun* newspaper (see Figure 2). Both *The Sun* and PHS have similar amounts of glucan 47.6 and 49.1% respectively. These measured results are similar to expectations for these quantities in untreated biomass, which may indicate that these “sustainable” waste materials could be an option for bioethanol

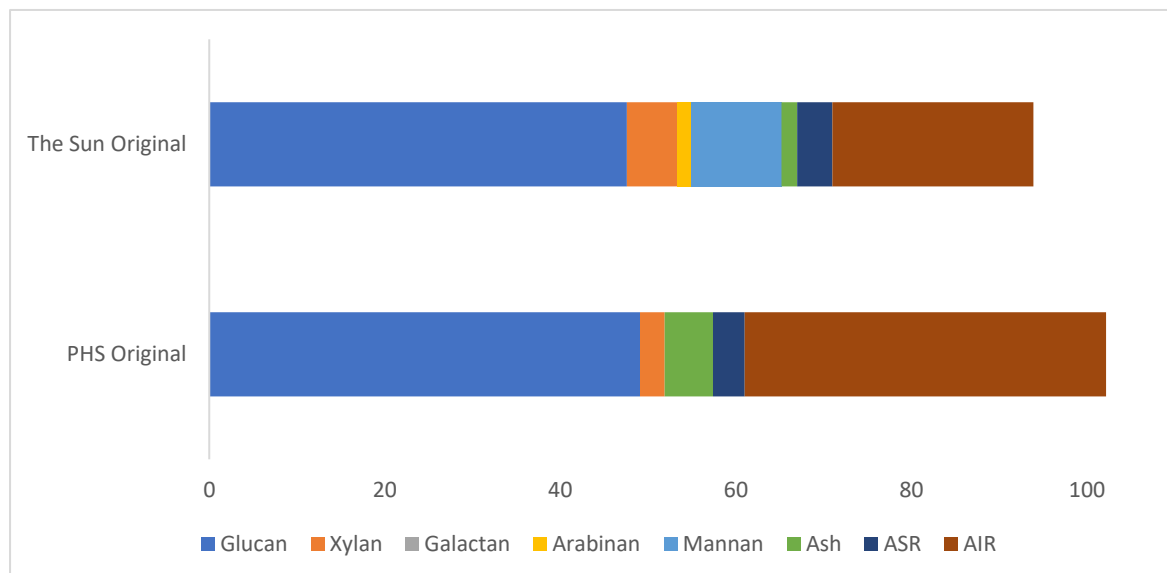


Figure 2 Compositional Analysis of *The Sun* newspaper and PHS provided by Fiberight.

production. *The Sun*'s composition contains a significant quantity of hemicellulose sugars specifically mannose. These results are not surprising as most newspapers and low-grade paper products are generated from softwood biomass.⁹ The greatest difference to untreated biomass in composition can be found in the acid insoluble residue (AIR). *The Sun* contained approximately 23% AIR while PHS contains about 41%. The low hemicellulose content and high AIR content in PHS is likely a result of having been pretreated once already under acidic conditions as well as plastics and other waste compounds that are not a part of any biomass composite.

Compositional analysis was performed on the solid pulp remaining after pretreatment (see Figure 3). Pretreatment with [HBIM][HSO₄] resulted in the extraction and removal of the remaining hemicellulose in *The Sun* and the PHS. After pretreatment samples of *The Sun* contained a greater amount of acid insoluble residue (AIR) suggesting the formation of pseudolignin on the solid pulp during the pretreatment process (increasing from 22.9% in the untreated to 29.1 and 30.3% in the [HBIM] and [EMIM] samples respectively). *The Sun* contained a much higher hemicellulose component and possible a higher amorphous cellulose component, leading to the formation of pseudolignin under these pretreatment conditions,

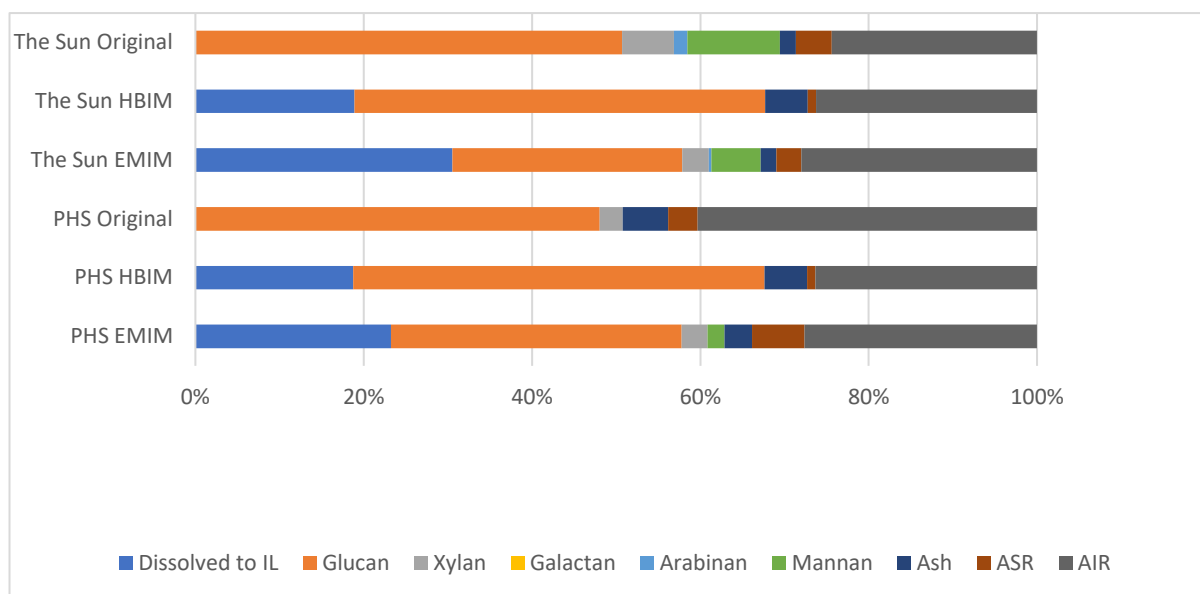


Figure 3 Compositional analysis of [HBIM][HSO₄] and [EMIM][OAc] pretreated samples of 'The Sun' and the PHS at 150 °C for 1 hour.

which is likely to inhibit saccharification. In addition, it is unknown how the fillers and ink in the newspaper could impact these pretreatment extractions. It is possible that the “lignin”/AIR that was original present on the newspaper also provided a surface that was conducive for the condensation or deposition of pseudolignin formed during the pretreatments. Pretreatment on the PHS decreases the “lignin”/AIR content in both cases suggesting a much more successful extraction (decreasing from 41.2% in the original to 29.2% in the [HBIM] and 23.8% in the [EMIM] samples). The significant decrease in the lignin content using EMIM is surprising and could be due to the heterogenous makeup of the “lignin” in the PHS, which have been extracted by the EMIM during the pretreatment process. In all the pretreatments the [HBIM][HSO₄] produced a pulp higher in overall glucan content (increasing from 47.6% in the original to 54.1% in *The Sun* and 49.1% in the PHS original to 54.3% in the pretreated) while [EMIM][OAc] extracted the glucans leaving a pulp lower in overall glucan content (29.5 % in *The Sun* and 29.7% in the PHS). Though [EMIM][OAc] can be useful as a benchmark comparison, in this case is unlikely to be feasible due to the cost of the ionic liquid and the energy required to recycling the ionic liquid compared to the relatively small quantity of glucans extracted. In addition, [EMIM][OAc] has issues with thermal stability at temperatures above 110 °C therefore focus was placed on pretreatments using [HBIM][HSO₄].¹⁰

The Sun and PHS pretreated with [HBIM] generated 10% wt (relative to starting mass) “lignin” recovery, while very little “lignin” was recovered from the [EMIM][OAc] samples. These samples were submitted to HSQC and revealed a very condensed lignin (see highlighted in dark blue in Figure 4), especially those found in the PHS. These areas also overlap with

regions expected for pseudolignin signals to appear. *p*-Hydroxyphenyl (H_{2,6}) are not found in *The Sun* “lignin” as these sub units are not generally found in softwoods. However, because PHS is generated from a mixture of organic waste and various lignocellulosic biomass sources the presence of H_{2,6} is not surprising, softwoods would be present in the PHS. Elemental analysis and heating values for the lignin were calculated to investigate the potential energy return should the lignin be used for combustion (elemental analysis values can be found in appendix 5A). The presence of nitrogen in the HSQC results suggest either the presence of unrecovered ionic liquid or the remnants of other biological materials or unusable compounds derived from the waste. If this is an indication of IL, it is possible that additional wash steps or reactor design would be needed to prevent the ionic liquid loss in the pretreatment process. A further possibility is the interaction of the ionic liquid with the fillers, plastics, and unknown materials in the samples. Either way the removal of nitrogen containing compounds would be necessary if the lignin is to be combusted for energy to prevent the release of nitrogen oxides.

A simple equation (reference chapter 2) to approximate higher heating value (HHV) from elemental analysis values presented by Jablonsky et al. was used to determine the HHV of the lignin produced from pretreatment of *The Sun* and PHS using [HBIM].¹¹ The approximate HHV values were calculated to be 25.78 MJ/kg for the PHS lignin and 25.84 MJ/kg for lignin precipitated from *The Sun*. These HHVs are lower than those of petrol, crude

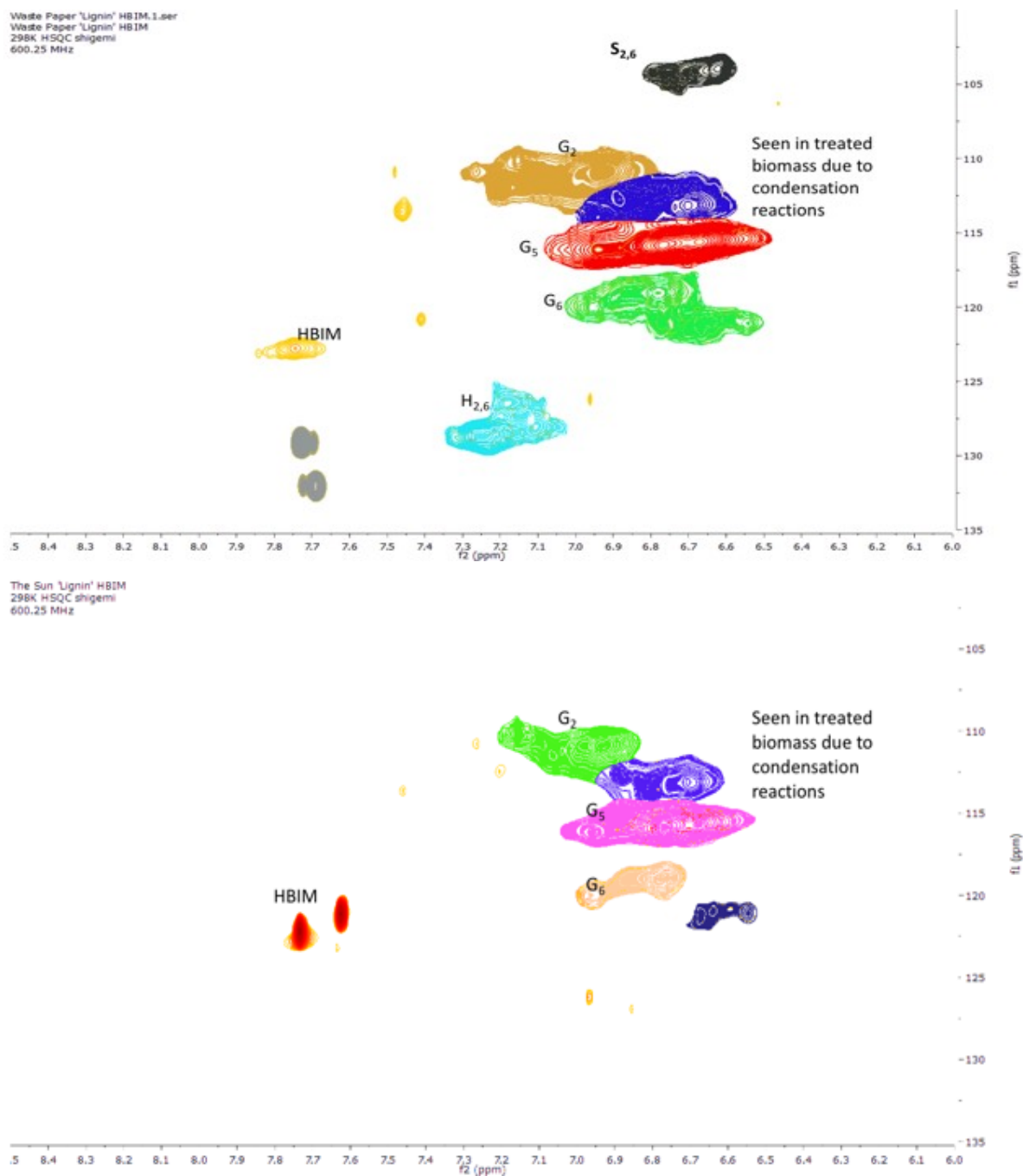


Figure 4 HSQC analysis of lignin recovered during pretreatment using [HBIM][HSO₄] at 150 °C for 1 hour of 'The Sun' (top) and PHS (bottom).

oil, and diesel (42-47 MJ/kg) but are in line with hard black coal and Sub-bituminous coal which range between 17-25 MJ/kg. Compared to conventional firewood, which has an HHV of 16 MJ/kg, these extracted "lignin" will provide greater energy by weight and could potentially be used as an energy source.

Saccharification of the solids were conducted for three days, which is shorter than the usual seven-day saccharification that we perform in our labs. The reduction in saccharification retention time is used to mimic the expected time limitations due to commercial feasibility. The saccharification results were no better than untreated values. It is hypothesized that the formation of pseudolignin and the potential impact of plastics and other fillers in the samples prevent enzymatic access to the glucans. Many plastics have a glass transition temperature ranging between 110-140 °C, pretreatment at 150 °C may have melted and coated the surface of the samples reducing saccharification.¹² The experiments were repeated at a lower temperature of 120 °C for 6 hours using only [HBIM][HSO₄]. Temperatures below 120 °C would not only result in much longer pretreatments but have been demonstrated to be less successful at lignin removal. Compositional analysis of the samples pretreated at 120 °C revealed a significant difference in the extraction of the sample compared with the pretreatment at 150 °C (see Figure 5). In the case of *The Sun* there is significant increase in glucan content (47.6 to 60.6%) and a modest decrease in the lignin content on the pulp from 22.9 to 18.4%. However, unlike the previous pretreatment at 150 °C much of the hemicellulose (xylans and mannans) remained intact on the pulp. The PHS results display modest lignin removal (41.2 to 37.9%) with a decrease in the glucan content (49.1 to 42.2%), possibly from the removal of amorphous cellulose regions.

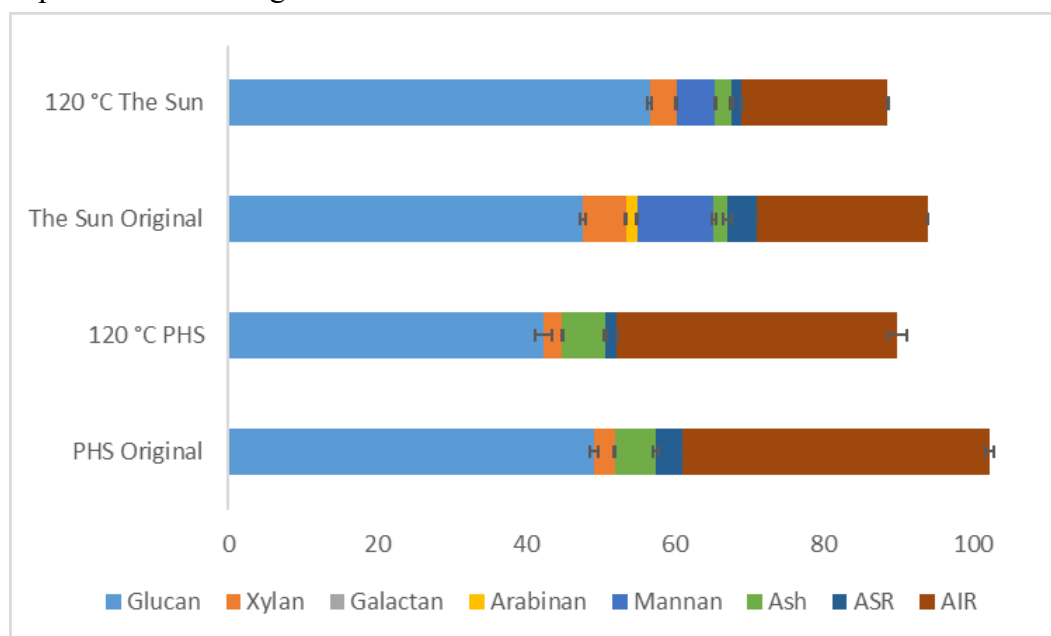


Figure 5 Compositional analysis of the 'The Sun' and PHS pretreated at 120 °C for 6 hours using [HBIM][HSO₄]

These less severe conditions did significantly improve saccharification for the PHS, but decreased saccharification yield for *The Sun* (see Figure 6). It was expected that the improvement would be modest as the extraction and pretreatment conditions are mild for lignin

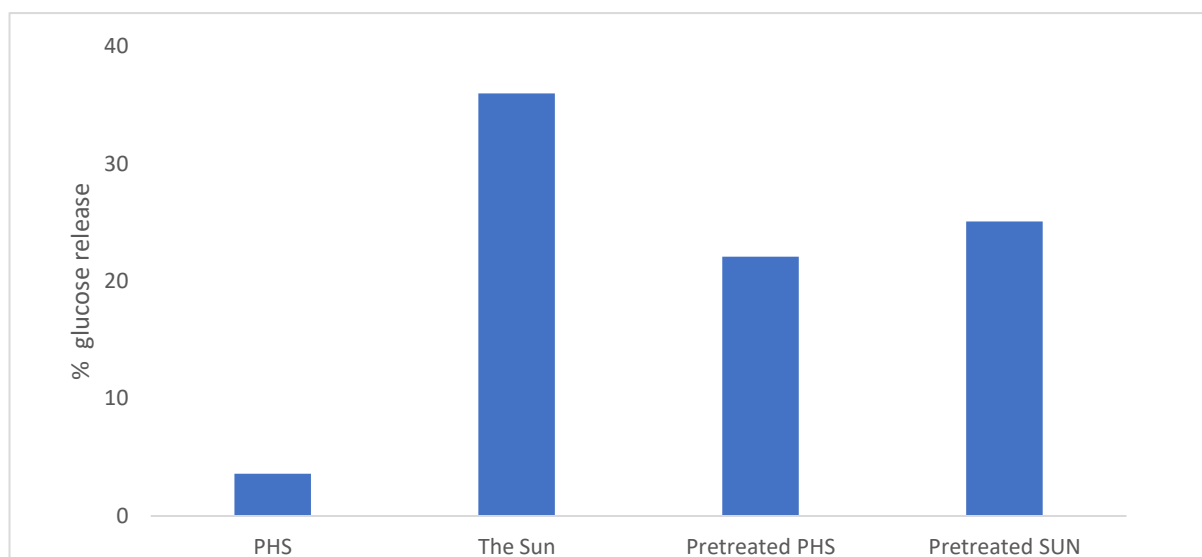


Figure 6 Three day saccharification yield of untreated PHS and 'The Sun' compared to pretreated PHS and 'The Sun' at 120 °C for 6 hours with [HBIM][HSO₄].

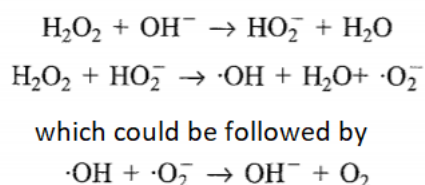
and hemicellulose removal. The PHS saccharification improved from 3.6% in untreated to 22.1%, suggesting that perhaps these lower temperatures has prevented some melting of plastics contained in the sample. However, this very low yield compared with that of traditional biomass suggest that the plastics and other components of the PHS have a largely inhibiting effect on saccharification. It is not clear if this inhibition is due to access prevention or some sort of enzyme neutralization. The saccharification of *The Sun* showed a decrease in glucose from 36% in the untreated sample to 25.1% after pretreatment. While it is unclear the reason for this decrease, these results may suggest that pretreatment of waste papers like *The Sun* may not be necessary and direct saccharification maybe a possibility and residual solids could be incinerated for energy recovery.

The strengths of the ionoSolv process in adaptability to multiple pretreatment feedstocks displayed some success in the pretreatment of both *The Sun* and PHS as a potential biofuel and energy source. Further studies will be needed not only to optimize the process for these types of samples, but to also explore how the plastics and fillers in these compounds may be interacting with the ionic liquid. It could be that the use of other types of ionic liquids would provide better extraction and saccharification yields. Future experiments should also explore the fractionation and extraction of plastics and fillers from these samples. These studies into the use of ionic liquids to extract plastics could potentially be applied in other fields of study such as removal of microplastics from aqueous environments.

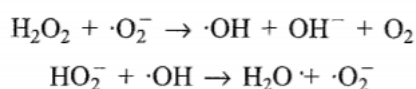
5.3.2 Bleaching of Miscanthus and Production of Nanocellulose

Traditionally, bleaching in the textile industry has been applied to cotton, wool, or linen as a method to whiten, temporarily brighten, and remove remaining hemicellulose from cellulosic fibers. Bleaching whitens and brightens by the destruction of chromophores, which are compounds (often aromatic or highly conjugated) that are responsible for color, for example why we see lignin as brown. Chromophores are also responsible for the yellowing of paper and textiles over time due to the gradual degradation of cellulosic material.¹³

Several methods are commonly used for bleaching, including: chlorine dioxide, oxygen, hydrogen peroxide, and ozone.¹⁴ Hydrogen peroxide, which is non-toxic, relatively inexpensive, and easily adaptable to the lab equipment available was used for the bleaching of our pulps. Though hydrogen peroxide bleaching does remove some amounts of lignin, it is generally more expensive and removes less lignin than traditional pulping or pretreatments processing designed specifically for lignin extraction.^{15,16} To use hydrogen peroxide for bleaching, alkali solutions between the pH of 10-12 are necessary to activate the peroxide and generate the perhydroxyl ion ($[\text{HO}_2]^-$) (see below equation for proposed perhydroxyl and radical formation pathways).



Another potential degradation pathway



From the formation of the perhydroxyl anion, it is hypothesized that in alkali media it is possible to generate radical species $\text{HO}\cdot$ or $\text{HO}_2\cdot$ which are powerful oxidants and likely responsible for reactions with chromophores, disrupting the aromaticity thereby removing color.¹⁷

The abundance of lignocellulosic biomass could make it a beneficial and more sustainable source to produce various materials. This initial study endeavors to understand the impact and potential for textile bleaching processes on the biomass pulps we typically produce for bioethanol production. No bleaching attempts had previously been performed on iSolv

pretreated pulps and initial trials were conducted to test the success of bleaching on *Miscanthus* pulps generated in previous experiments (see Figure 7 (left)). The initial bleaching experiment demonstrated the success for whitening and the potential removal of residual lignin. Subsequent trials were conducted to evaluate the effect of *Miscanthus* loading, ratio of alkali solution to hydrogen peroxide. Variations in alkali to peroxide ratios were tests (1:1, 1:2, 2:1 alkali to peroxide) in addition to varying the time of the pretreatment (1, 2, and 3 hours). These trials were conducted without stirring and found that a 5% loading of *Miscanthus* 1:1 ratio by

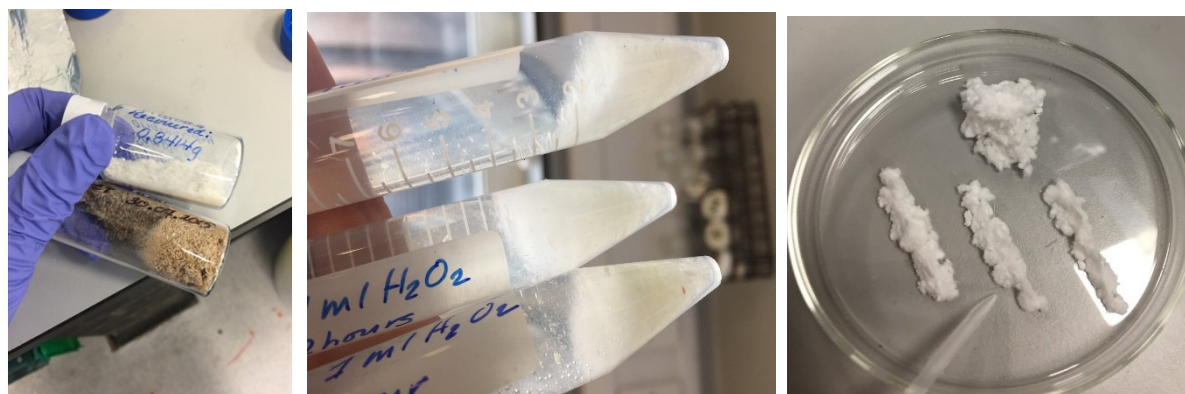


Figure 7 Bleached *Miscanthus* compared with *Miscanthus* pretreated for 1 hour at 150 °C with [TEA][HSO₄] (left photo). *Miscanthus* pulp bleached for 1 hour (bottom), 2 hours (middle), and 3 hours (top) with a 1:1 volume ratio (middle photo). Bleached *Miscanthus* produced using stirred reactor for 3 hours with 1:1 volume ration of hydrogen peroxide and 10% wt. NaOH solution (right photo).

volume of alkali solution to hydrogen peroxide for 3 hours produced the whitest bleached pulps (see Figure 7 (middle)). Thus demonstrating that loading and ratio of alkali solution to peroxide will affect the whiteness as well as consistency of the cellulose pulps generated. All other bleaching experiments were conducted under these conditions in the 50 ml reactor with constant 250 rpm stirring. Cellulose pulps produced from the reactor were consistent in visual color and texture (see Figure 7 (right)). Raw cotton fiber has a cellulose content between 88-95%, which generally increases to 95-99% after processes such as bleaching.¹⁸⁻²⁰ Compositional analysis conducted on the fibers of the bleached cellulose are lower than in Sigma cellulose (99.7 compared to 95.1%), however the bleached *Miscanthus* cellulose is within the purity range of cotton fibers used to produce textiles (see Figure 8 with full compositional graph at appendix 5B).

The cellulose pulp generated was submitted for morphological analysis via the Valmet FS5 fiber analyzer. International Organization for Standardization values for length and fiber width, coarseness, kink, and curl for the bleached fibers were recorded (see table 2). Fibers generated have an average fiber length of 0.38 mm with a weighted average of 0.47 mm and width of 15.8 μm. These are in line with other fibers produced from corn husks and jute after

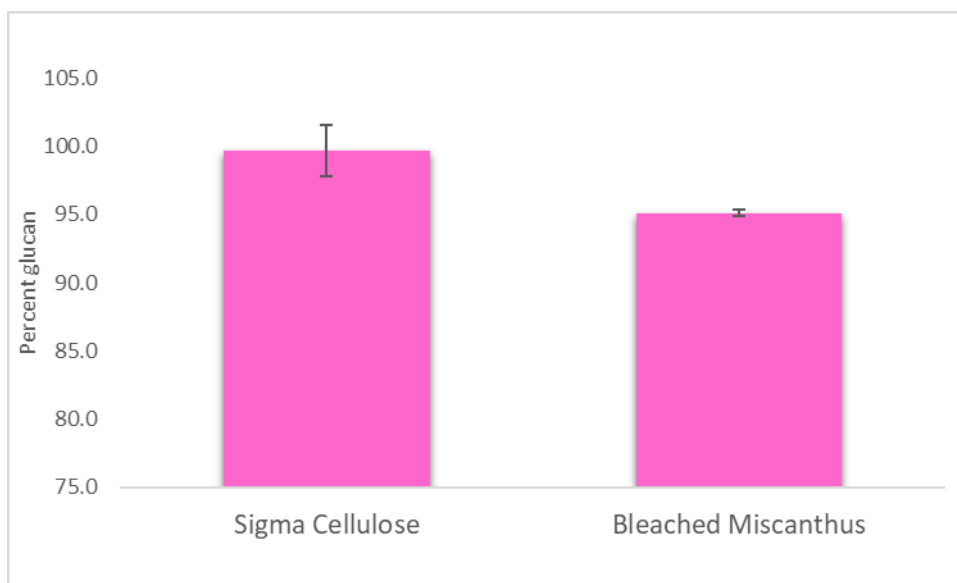


Figure 8 Percent glucan content of bleached *Miscanthus* produced from 1:1 ratio by volume alkali solution with hydrogen peroxide at 80 °C for three hours to 20 μ m Sigma cellulose.

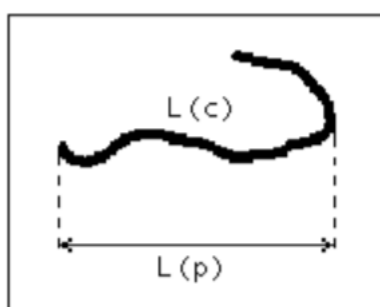
bleaching.²⁰ These fibers are much shorter and thinner than traditional cotton fibers produced for textile manufacture (which usually use fibers between 20 mm and 35 mm), but this difference could be modified depending on initial grinding applied to the *Miscanthus* prior to the pretreatment process.^{20–22} The lengths produced in this current process would be categorized as fines. These short dimension fibers have been found to strengthen mechanical properties of polymer composites and may be one possible application for these cellulose fibers.²³ In addition, fines impact the optical, surface properties, moisture control and retention in paper and other composites.²⁴

Table 2 The Valmet FS5 fiber analysis of bleached *Miscanthus* processed at 80 °C for 3 hours with a 1:1 volume ratio of alkali solution to 30% peroxide solution. International Standard (ISO) lengths and widths are provided

ISO Arithmetic average length [mm]	ISO Length weighted average	Fiber width [μ m]	Curl [%]	Opital Coarseness [mg/m]	Kink [1/1000]	Kink angle [deg]
0.38	0.47	15.8	13.1	0.08	439.6	39.1

Fiber curl is a result of mechanical and chemical force upon the pulp. It can impact tensile properties and can be seen and measured via the curvature of the fiber. High curl usually results in paper products with lower tensile strength but higher resistance to tearing.^{25,26} Curl is also an indication for moisture retention with higher curl materials shrinking more when dried.²⁷ Curl is calculated as:

$$curl = 100\% * \left(\frac{l_p}{l_c}\right)$$



Where l_p is the projection length of the fiber (from one end to the circle of the fiber) and l_c is the true length of the fiber. Mechanical processing and shearing forces often generate fibers with curls between 15-30% curl.^{25,28} This means that curl of the fibers could be controlled by how the pulp is processed. The pulps generated from the ionoSolv pretreatment and subsequent bleaching process produce a relatively low curl fiber at 13.1%. In addition, modifications to the cellulose via oxidation or sulfonation can potentially be used to reduce the curl of the fiber.²⁹

The coarseness of a fiber has direct impact on its resistance to tearing and optical properties. This property is important in the textile and paper industries and is determined by the weight of the fiber per unit length.²³ The coarseness is dependent on the thickness of cell walls, therefore the feedstock used to produce the cellulose pulps have a direct impact on the coarseness of the paper produced. High coarseness produces a stiffer and less flexible fiber making a stronger paper, such as those used to produce boxes and packing paper.³⁰ Low coarseness produces a flexible, dense sheet that has a smoother surface like those found in printing or notebook paper. The cellulose pulp produced after bleaching had a coarseness of 0.08 mg/m. This is relatively low in comparison to pulps produced after Kraft pretreatments of various softwood and hardwood feedstocks, which range from 0.12 mg/m for birch to 0.28 mg/m for southern pine (see appendix 5C). This means that the cellulose fibers produced from *Miscanthus* after pretreatment and bleaching would most likely not be suitable for heavy paper,

cardboard, or thick textile materials, but instead would be ideal for light paper or soft textile production.

Kinks, unlike curves, are abrupt angular changes in the curve of the fiber. Generally, kinks help to improve the wet strength of the fiber, though it decreases the tensile strength and tearing resistance. The kink index is calculated via the following equation:

$$KI = \frac{2N_{(20-50)} + 3N_{(50-90)} + 4N_{(90-180)}}{L_{total}}$$

where N represents the number of kinks found between those angles of 20-50, 50-90, and 90-180° and L is the total length of the fiber.^{23,28} In this case kink [1/1000] represents the sum of kink index per 1000 fibers. Kinks are measured via angle off a center axis, usually at 20° or greater and any angle greater than 60° is considered a major deformation.³¹ The *Miscanthus* fibers produced during this process have relatively low kink angles, suggesting that materials produced under these conditions would have a relatively low wet strength but high tensile strength.²⁶

This initial investigation into the potential of bleaching and production of cellulose fibers for materials production presented us with an opportunity to investigate not only the production of high purity cellulose microfibers, but also the production of nanocellulose particles during bleaching process. Though there is no defined limit for the size of nanocellulose, it generally refers to cellulose crystals produced between 10-1000 nm.³² It has also been demonstrated that different feedstocks will produce varying sizes of nanocellulose crystals and the method of pretreatment can also impact their production.³³ Nanocellulose has become a hot topic in the past few decades promoted as a sustainable biocompatible polymer whose properties have potential applications in a wide range of industries.^{34,35} Examples of these properties include but are not limited to: elasticity greater than that of Kevlar, high aspect ratio, and great tensile strength (similar to that of aluminum).³⁶ Research efforts also examine nanocellulose as a method for drug delivery, optical security productions, aero gels, strengthening of composite materials.

Dynamic light scattering (DLS) measurements were taken of the supernatant produced during bleaching after one wash with deionized water to confirm the production and presence of nanocellulose. Another sample was generated via neutralization of that water sample with hydrochloric acid to see if pH and the presence of salts would impact the zeta potential of the suspended cellulose in the aqueous solution. Five DLS samples was measured in triplicate for

a total of 15 runs for each sample (see Figure 9 left). After pretreatment (at 150 °C for 1 hour with [TEA][HSO₄]) and bleaching (3-hour bleaching at a 1:1 volume ratio of hydrogen peroxide with a 10% by weight solution of sodium hydroxide) *Miscanthus* produces nanocellulose particles around the size of 250 nm. This is consistent with other nanocellulose particles created by grasses like bagasse (240 nm produced from ball milling), cotton linters (210-480 nm from sulphuric acid hydrolysis), and corn cobs (approximately 215nm from sulphuric acid hydrolysis).^{37,38}

The stability of nanocellulose particles in aqueous solutions is was measured via the Malvern Zeta potential analyzer (see Figure 9). The ability of the nanocellulose to stay dispersed and stable, in this cause, in aqueous media (though other solvents medias are possible) can aid in the success of the nanocellulose particle distribution through composite materials. These particles could be modified to affect their colloidal properties using various surface modification such as oxidation, polymer grafting, esterification etc.³⁵ Flocculation is also impacted by the solvent, with higher dielectric constant solvents proving more favorable to dispersion than hydrophobic ones.³²

Generally, a high zeta potential above -25mV is preferred and the ability to alter and control the gelation of the nanocellulose particles is necessary to produce nanocellulose materials that have consistent properties throughout.³⁹ In the nanocellulose particles generated during the bleaching process, there was little difference in the zeta potential between the samples that were neutralized and the samples that were just washed with deionized water (-28.7 vs -27.4 respectively). Zeta potential can be affected by the salt concentration in a solution and there may be a risk that the increase in salts via the addition of hydrochloric acid would affect the potential.⁴⁰

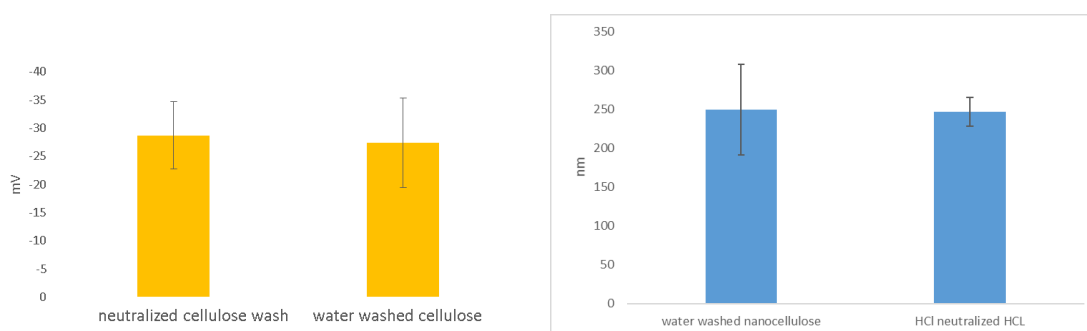


Figure 9 Dynamic light scatter of nanocellulose produced during bleaching of *Miscanthus* (left) and zeta potential of cellulose particles suspended in aqueous solution (right).

However, this was not the case in these trials. The deionized water wash still contained enough charges from the alkali solution from the bleaching process which could have affected and possibly suppressed the zeta potential. A higher zeta potential could be achieved if samples were first diluted and the salts removed from the water. It is unlikely that sulphate anions from pretreatment has bound to the surface of the cellulose or that oxidation from bleaching had occurred. Surface potential would be much higher in those cases (absolute results between 53-66 mV have been observed).⁴¹ The absolute value of the potential in our results is above what is considered a stable colloid solution (25 mV). In addition, these results suggest that there has been little modification to the surface of the cellulose by the pretreatment and bleaching process leaving flexibility for further modification for application specific needs.⁴²

Unfortunately, the yield of nanocellulose production is low, between 1-3% by weight of the dry biomass. Therefore, this process could be used as a potential value add or additional product stream but could not be the only process for the biorefinery of any lignocellulosic biomass used. However, the bleaching of cellulose using hydrogen peroxide is easy, straight forward and much less toxic compared with conventional chlorine-based bleaching. This provides the ionoSolv pretreatment an opportunity to produce cellulose fibers and nanocellulose for various applications from one process. Larger size particles with length of close to 1 μm and high aspect ratios could be used to produce aqueous aerogels.⁴³ Aerogels have a wide variety of applications ranging from thermal insulation, particle detectors, and cryogenics just to name a few.⁴⁴ These cellulose based aerogels would be highly useful in applications that require biocompatibility and low toxicity. Smaller particles produced from this process (such as those around 250 nm) could be used for composite reinforcement. Nanocellulose with a density of 2.6 g cm^{-3} can have Young's modulus (a measure of the stiffness or elasticity of a solid material; how much tensile or compressive stress the material can withstand) similar to that of Kevlar (60–125 GPa, with an approximate density of 1.45 g cm^{-3}).⁴³

5.4 Conclusions and Future Studies

The production of micro and nanocellulose opens an additional value stream for the ionoSolv biorefining process. In addition, the initial exploration has demonstrated the potential for bleaching as an additional process that can be applied to ionoSolv pretreated lignocellulosic feedstocks. Future research in the bleaching of pulps produced from the ionoSolv process

should include the use of metal catalysts as well as stabilizers to improve stability of the bleaching process and consistency of pulp produced. Adjustments to solvent loading, biomass loading, temperature, and bleaching time should be investigated with focus placed on pulp properties including crystallinity, molecular weight, dispersity, and tensile/compressions strength. Whiteness and brightness tests should be conducted and direct applications for the microcrystalline cellulose produced could be explored. Wash steps to collect different size fractions of the micro fibrillated cellulose and nanocellulose should also be considered. Collection and modification of nanocellulose produced would need to be investigated. Initial experiments into consistent high yield production would be necessary. Beyond these studies, the ability to produce nanocellulose would open the door to a great deal of research for nanocellulose modifications and applications. Characterization of the nanocellulose, chirality, modification for stabilization of the colloid suspension, dispersion, and hydrophilicity/hydrophobicity, should be explored.

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6 Chapter 6 Thesis conclusions

6.1 Key Findings

This PhD research project investigated the impact of the ionoSolv process on cellulose quality and cellulose-derived materials from lignocellulosic biomass, specifically *Miscanthus*. An easily synthesized low cost ionic liquid was used to demonstrate the effects of the pretreatment, as well as the potential materials that could be produced as value add streams during the biorefining process.

Chapter 3 focused on the characterization of the *Miscanthus* pulps produced under varied time conditions. Variations on pretreatment conditions past the optimum temperatures or times can lead to the formation of pseudolignin on the pulps which leads to lower pulp purity and saccharification yields. The formation of pseudolignin at 120 °C begins shortly after 8 hours of pretreatment. Up to 8 hours of pretreatment there is a continual decrease in pulp yield (extraction of the biomass), decrease in particle size, and removal of lignin (removal of aromatics as demonstrated from the FT-IR). However, after 24 hours of pretreatment pulp recovery yields begin to increase and pulp particle size increases demonstrating the deposition and formation of pseudolignin on the surface of the pulps. Pretreatment conditions will also impact the cellulose pulp molecular weights. This suggests that the ionoSolv process could be utilized to produce nanocellulose and microcellulose particles after pretreatment for potential applications in industry or materials production. These experiments suggest that this pretreatment is effective at producing a cellulose rich pulp by disrupting the linkages between lignin, hemicellulose and cellulose, and leaves the cellulose I crystalline structures intact. These pulps can undergo further treatments and modifications allowing for a diverse range of products.

Chapter 4 examined the production of pseudolignin during the pretreatment process, with a focus on glucose, xylose, HMF and furfural. Xylose was most reactive in generating humic compounds when pretreated with fresh ionic liquid while furfural was most reactive in generating pseudolignin when recycled ionic liquid was used. These experiments demonstrated that these sugars and degradation products react quite differently when using fresh or recycled ionic liquid; the solutes in the ionic liquid have a huge impact on the pretreatment results. In any economically feasible production system, recycled ionic liquid would be used. In every test case, the use of recycled ionic liquid outperformed its fresh ionic

liquid counterpart. Recycled ionic liquid provided better extraction, which lead to pulps with higher cellulose purity and better saccharification yields. Recycled ionic liquid seem to buffer the formation and deposition of pseudolignin onto the surface of the pulps by improving pseudolignin solubility in the ionic liquid. In addition, the use of naturally occurring additives (such as HMF) seem to aid in the extraction of the biomass pulp and solubility of pseudolignin. These results are promising for the economics of the use of ionic liquids on a larger scale for pretreatment and biorefining.

For the use of ionic liquids to be feasible for biorefining, the process needs to be able to provide multiple value streams. In chapter 5 the ionoSolv process was extended to waste pretreatment and cellulose materials production. Though there was limited success with pretreatment of PHS (post hydrolysis solids), it was shown that bleaching and the production of high purity cellulose and nanocellulose is possible with the ionoSolv process. The bleached cellulose pulp produced using a 1:1 ratio of alkali solution to 30% wt. hydrogen peroxide solution was the visually whitest pulp. These fibers produced from the pretreatment of *Miscanthus* ground between 180-860 μm would be classified as fines (though size could be adjusted if larger particles were pretreated and bleached). These fines had relatively low curl and a low kink index (suggesting moderate tensile strength) and low coarseness which is suitable for soft paper materials. In addition, the combined pretreatment and bleaching process produced nanocellulose around 250 nm in size with a zeta potential of -25 mV, suggesting that an unmodified stable colloidal dispersion is achieved. Considering the projected future demand for these cellulose fibers and nanocellulose, these products may aid in the economic feasibility for the use of ionic liquids on a large scale.

6.2 Perspectives and Future works

The research completed in this PhD project has improved the understanding of the ionoSolv pretreatment process, its impact on the lignocellulosic biomass, the quality of the pulps produced and potential for high value materials production. This work also demonstrated the complexities inherent in this pretreatment process and further research is needed to answer some key questions prior to commercialization.

It is clear that the solutes in the ionic liquid have a great impact on the results of the pretreatment. Further work focused directly on the impact of the solutes, solute concentration, and the potential for using selective membranes, zeolites or filters to remove these solutes would need to be conducted. Additional pretreatments looking at hemicellulose pre-extracted

biomass and the recycled ionic liquid recovered from these pretreatments would provide insights into the effect of hemicellulose sugars on pseudolignin formation, pulp purity, and saccharification. Studies could be conducted using predictive modelling to determine results using these recycled ionic liquids in batch vs continuous flow reactors to determine the optimum scenarios. These findings would need to consider reactor design, heat profile, solute concentrations, the addition of additives, as well as techno-economic variables prior to empirical experimentation.

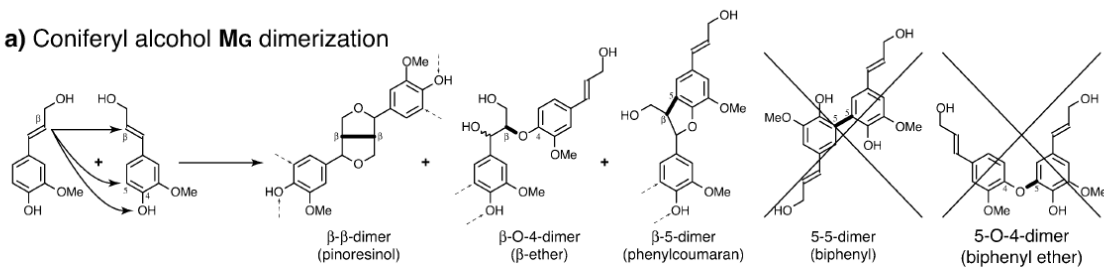
The PhD project provided an initial proof of concept that pure cellulose fibers and nanocellulose production are possible. Effects of parameter adjustments as well as optimization of this process are still needed. Solvent and biomass loading, bleaching times, and further characterization of the cellulose produced are still necessary (such as crystallinity, molecular weight). Additional mechanical tests on tensile and compression strength of the fibers produced and their optical properties would need to be examined. In addition, a more efficient method for size separation would be needed. Further research would need to be conducted into producing cellulose materials and modification of the cellulose produced.

Most importantly toxicological research is needed. No matter how efficient the pretreatment or the value of the products produced, if the ionic liquid and the recycled ionic liquid with the solutes are toxic and environmentally persistent, this process will never be realized. Life cycle analysis and arguably end of life analysis for this process will be extremely important if this process is ever to be commercialized. LD50 (lethal dose) and LC50 (lethal concentrations) should be conducted in addition to closed bottle tests to determine degradation of the major ionic liquids used in the ionoSolv pretreatment process. In catalysis, as solvents, and in extractions, ionic liquids show great potential to improve and change almost every aspect of industry. However, further research is needed to optimize and commercialize the use of ionic liquids for pretreatment of lignocellulosic biomass as well as the production of materials and platform chemicals.

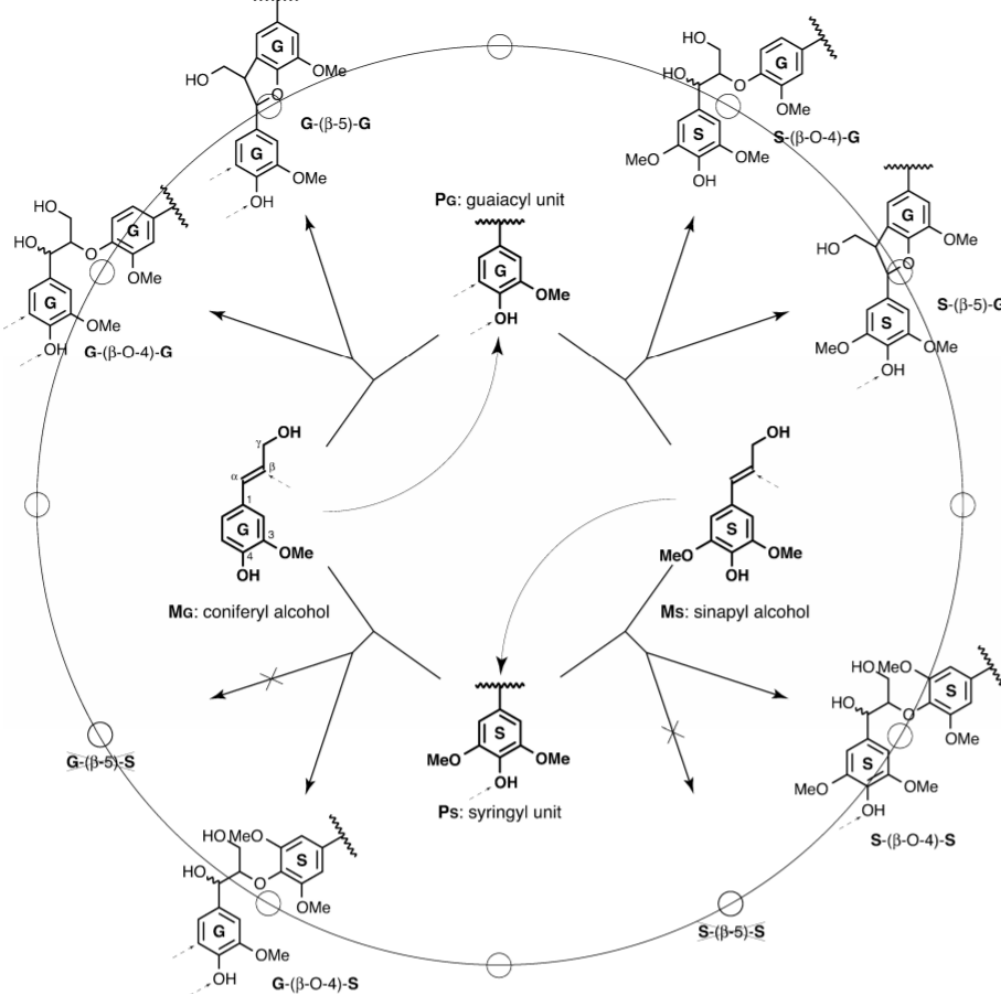
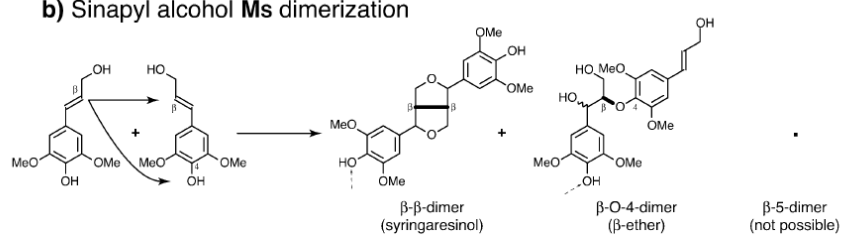
7 Appendix

Appendix 1A: Formation of dimers from monolignols differ from lignification cross-coupling¹

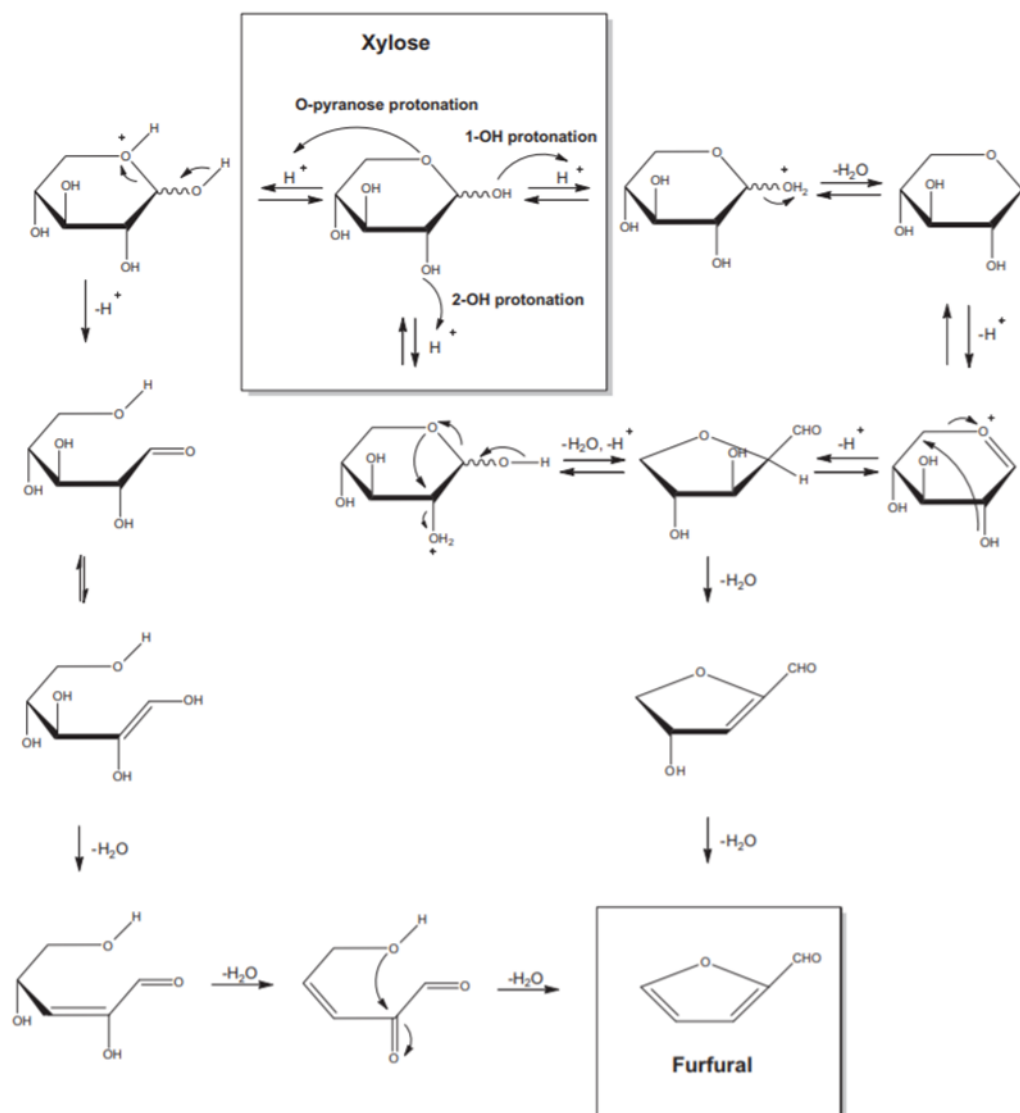
a) Coniferyl alcohol **Mg** dimerization



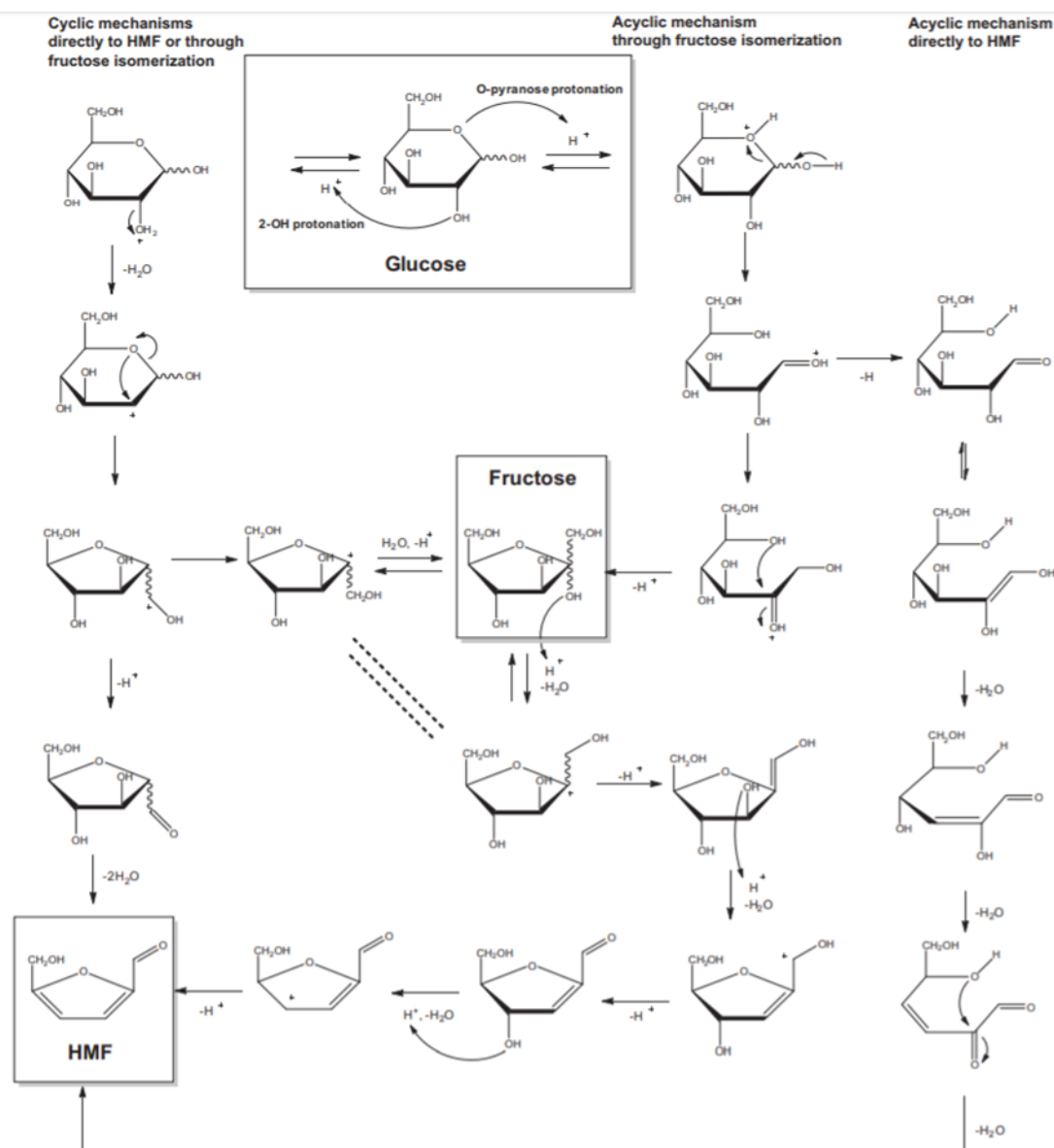
b) Sinapyl alcohol **Ms** dimerization



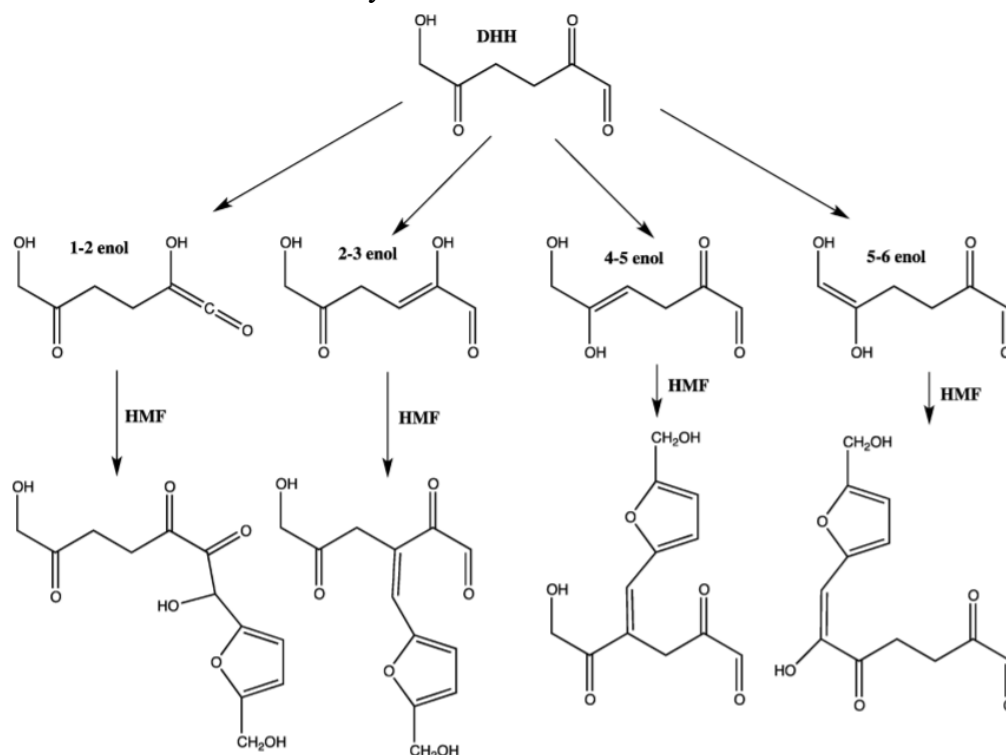
Appendix 1B: Potential pathways for xylose transformation to furfural cyclic and acyclic.²



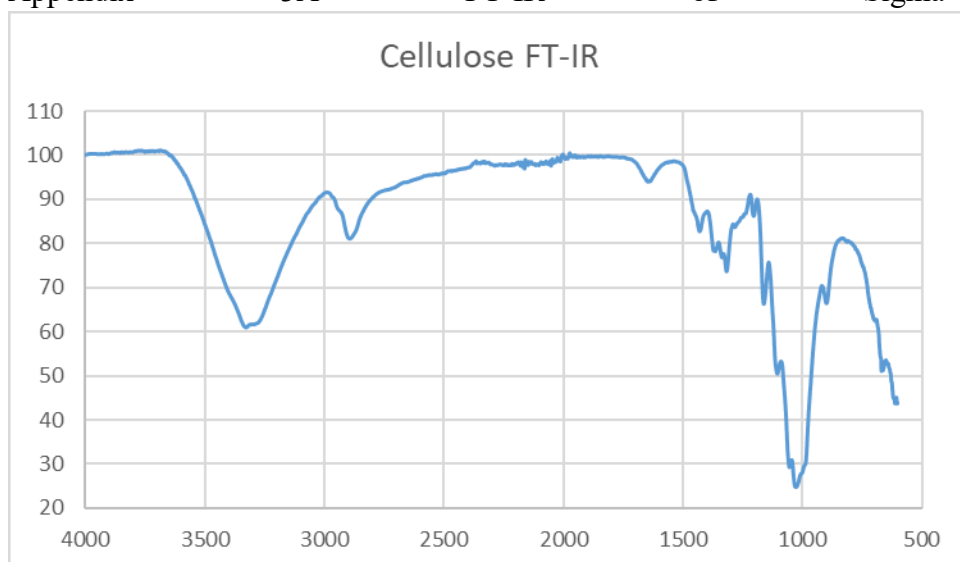
Appendix 1C: Potential pathways for glucose transformation to HMF cyclic and acyclic.²



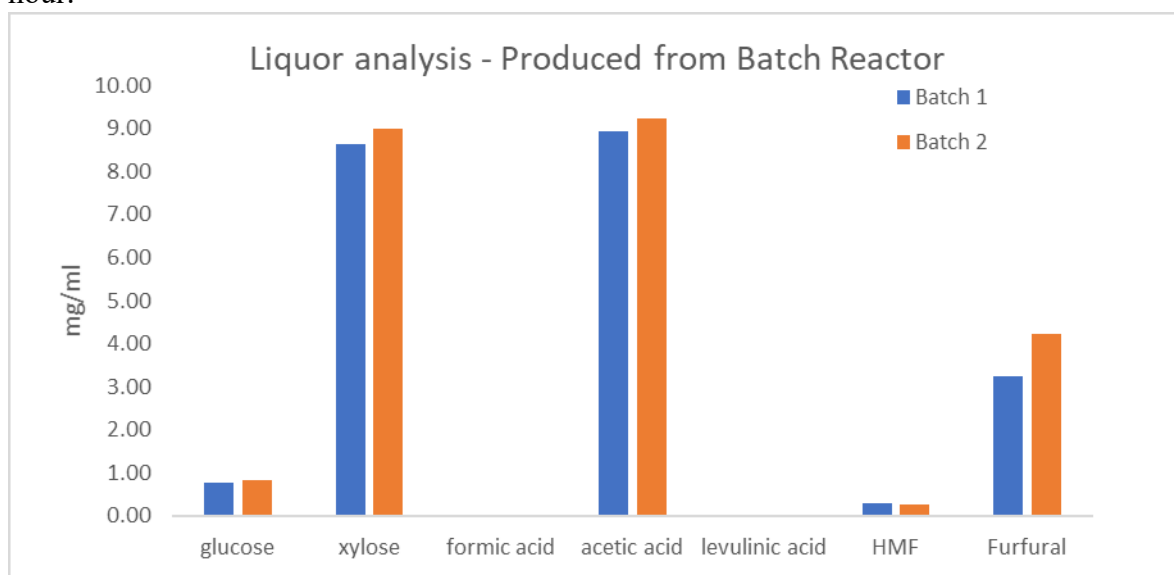
Appendix 1D: Various ways in which DHH can combine with HMF³



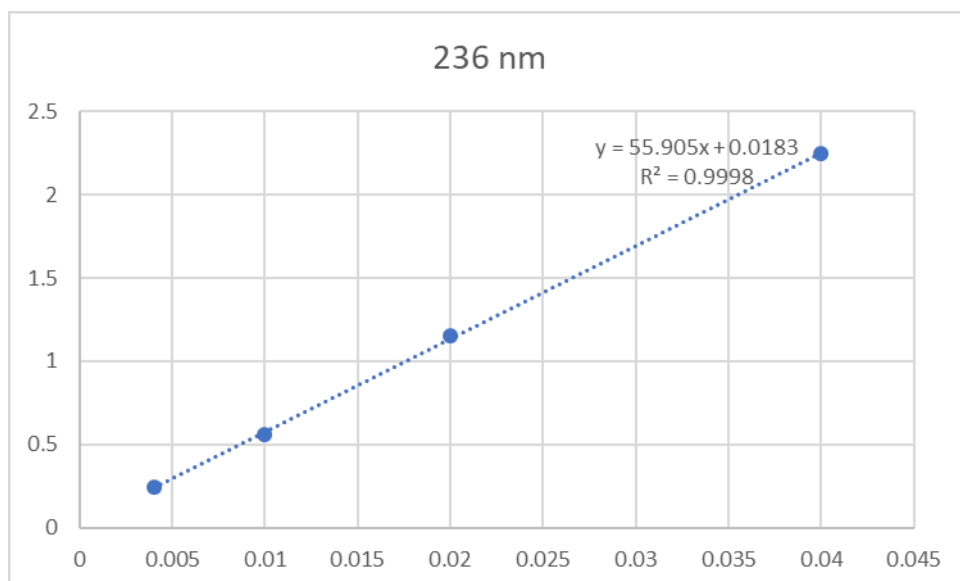
Appendix 3A FT-IR of Sigma Cellulose

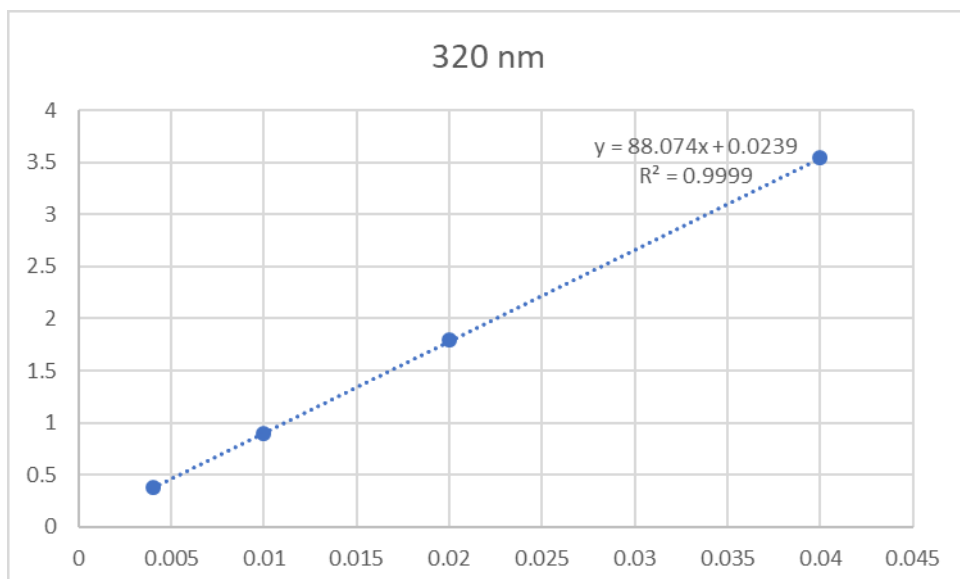
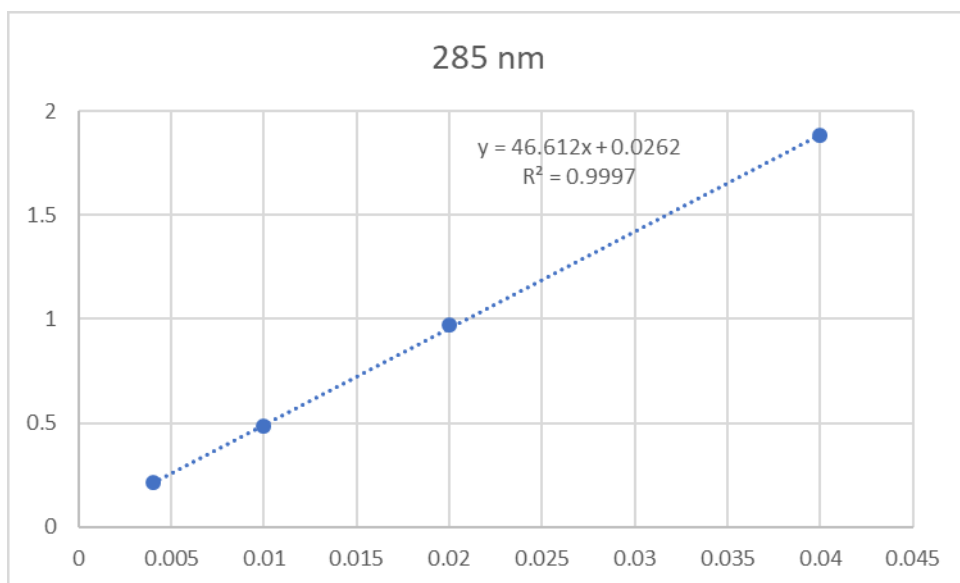


Appendix 4A Liquor analysis from two separate reactor runs with Miscanthus at 150 °C for 1 hour.

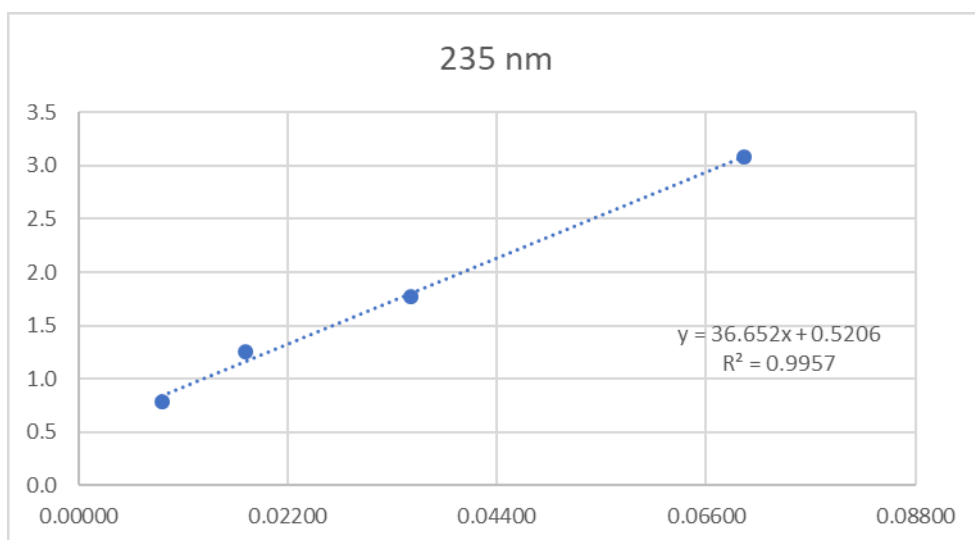


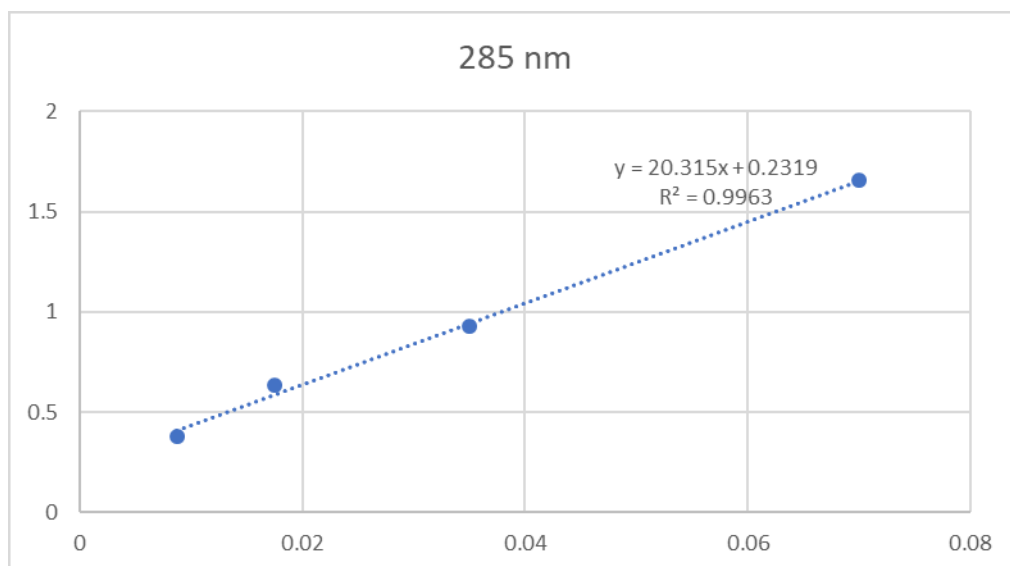
Appendix 4B Linear regression of ferulic acid standards



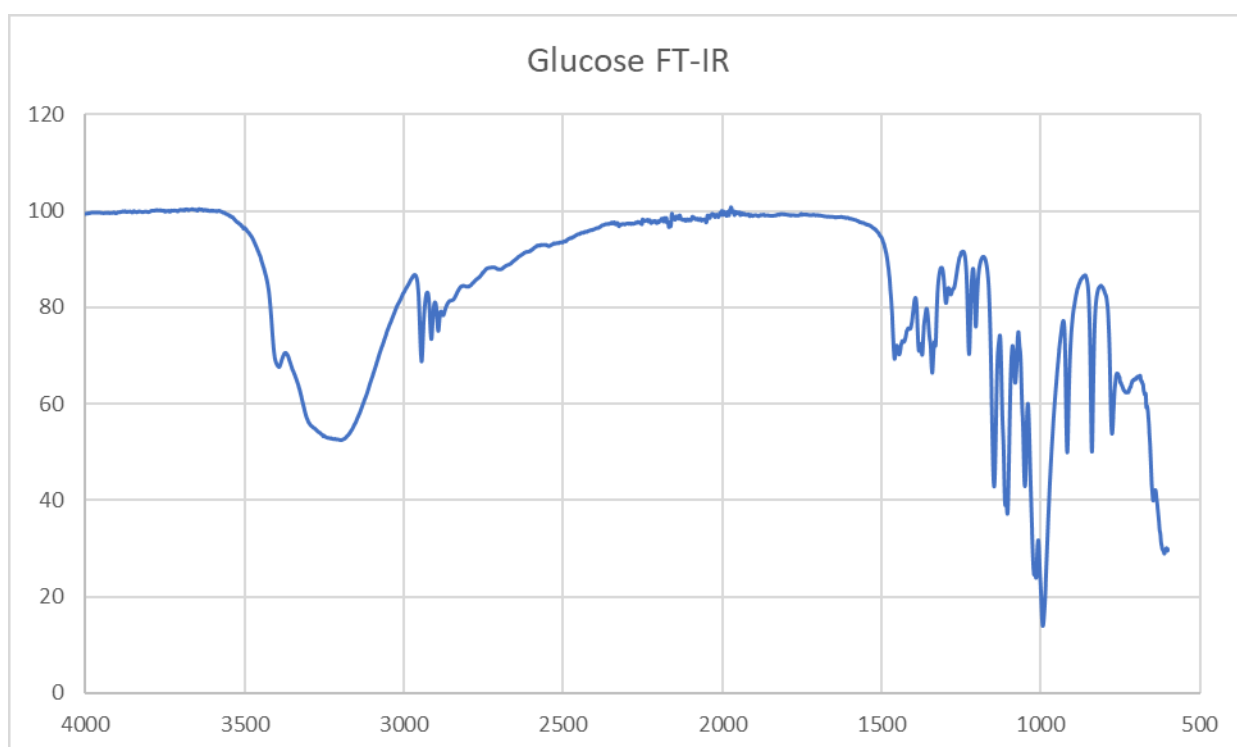


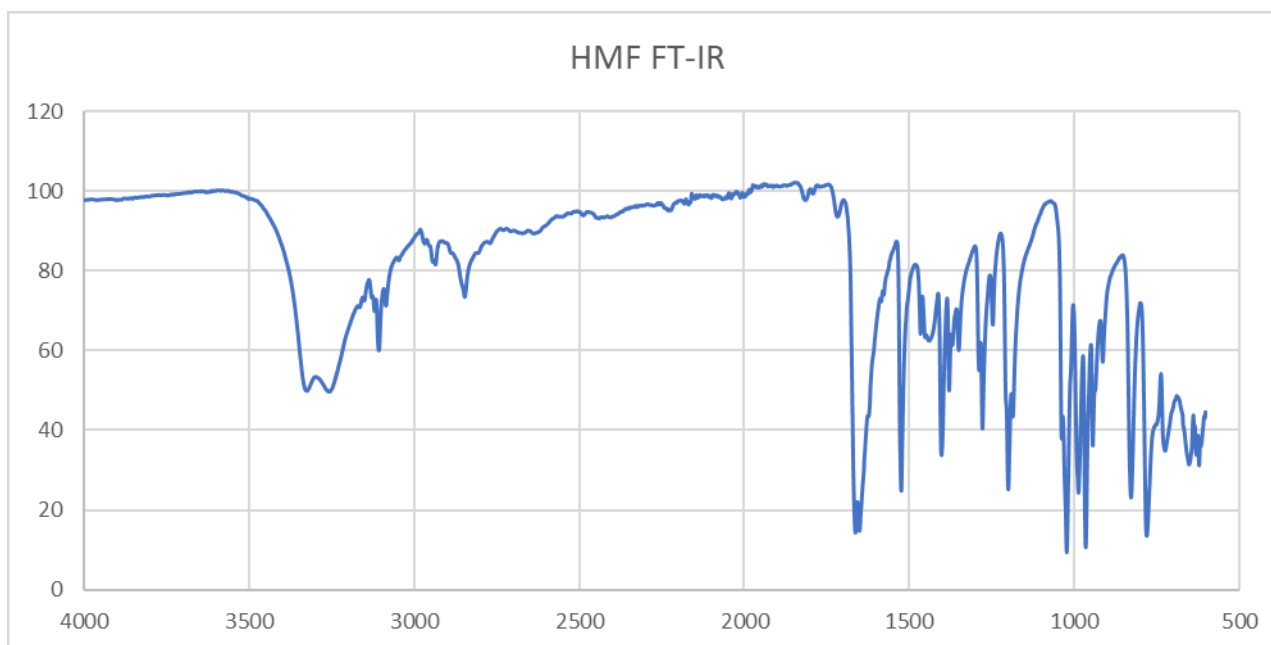
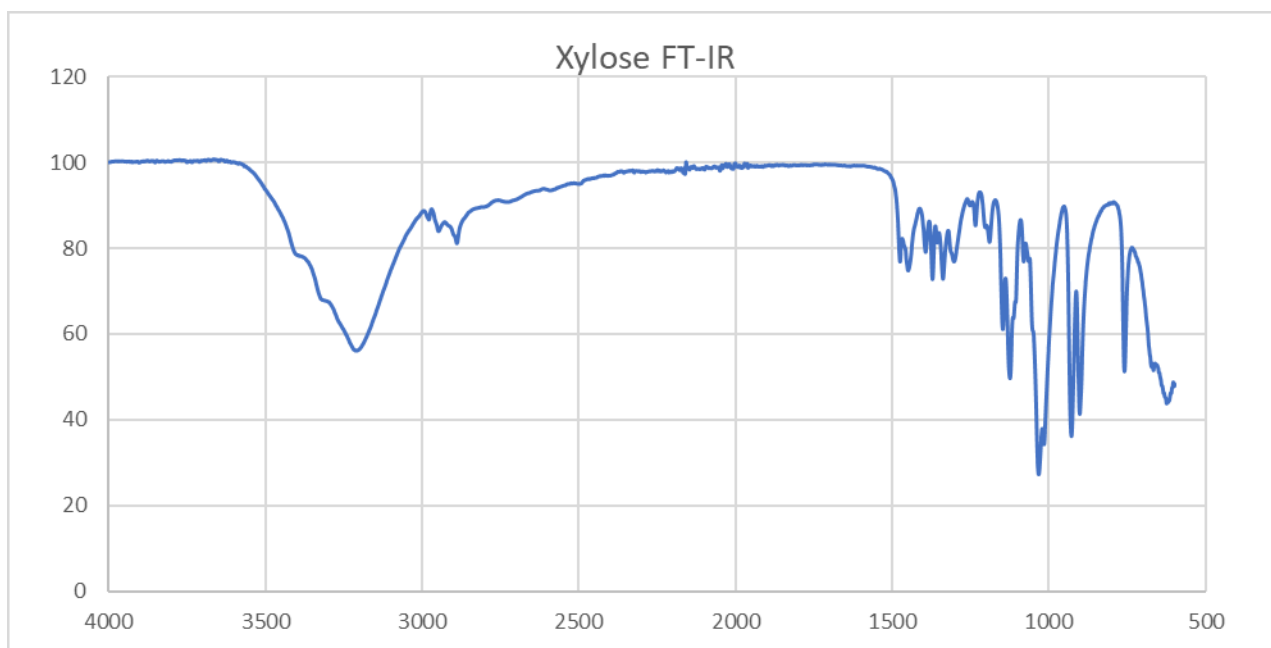
Appendix 4C Linear regression of Kraft lignin standards



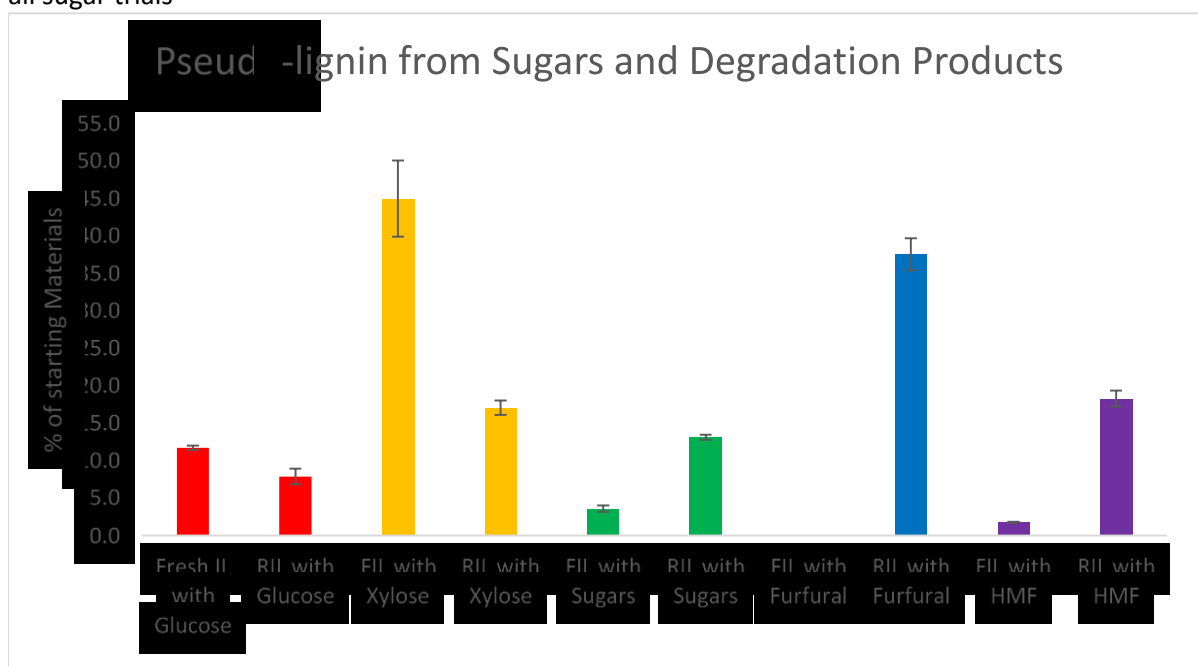


Appendix 4D FT-IR of Glucose, Xylose, HMF, and Furfural



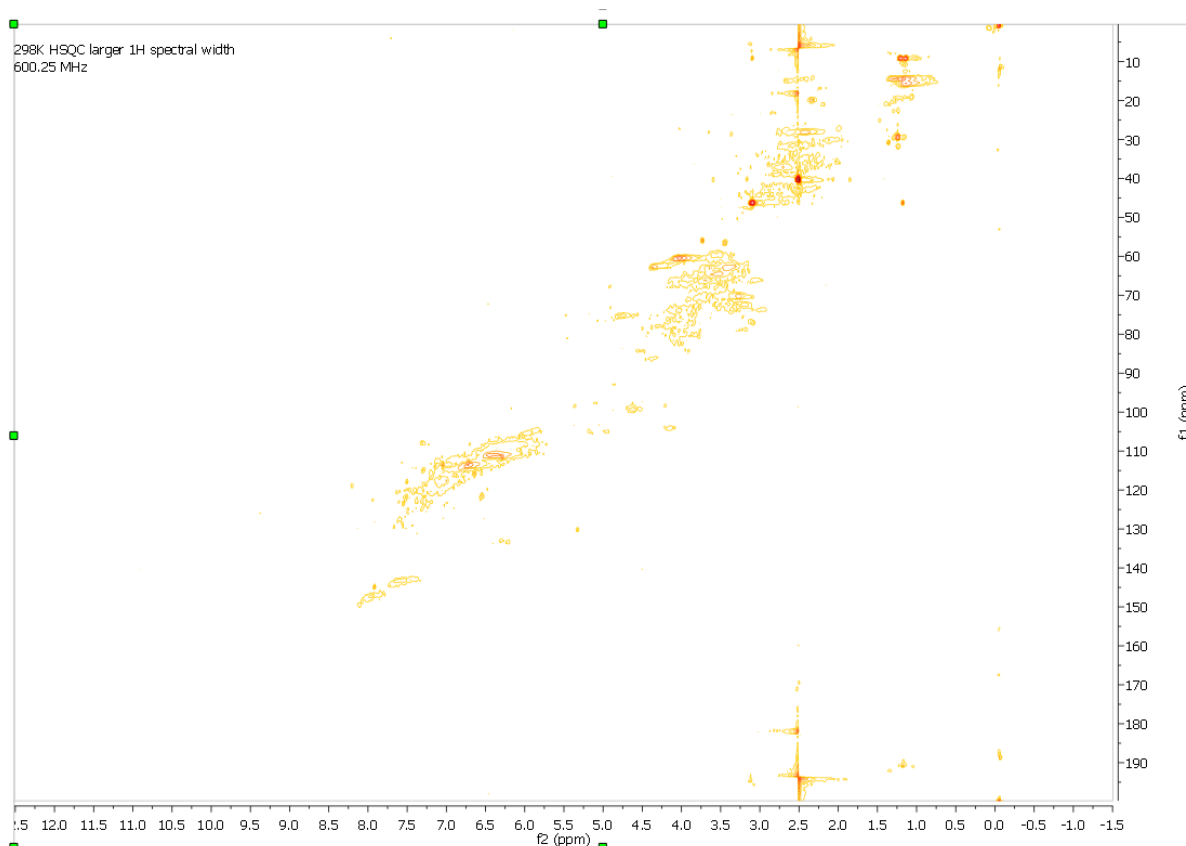


Supplementary 4E Comparison of humin and pseudolignin formation as a % of starting materials for all sugar trials

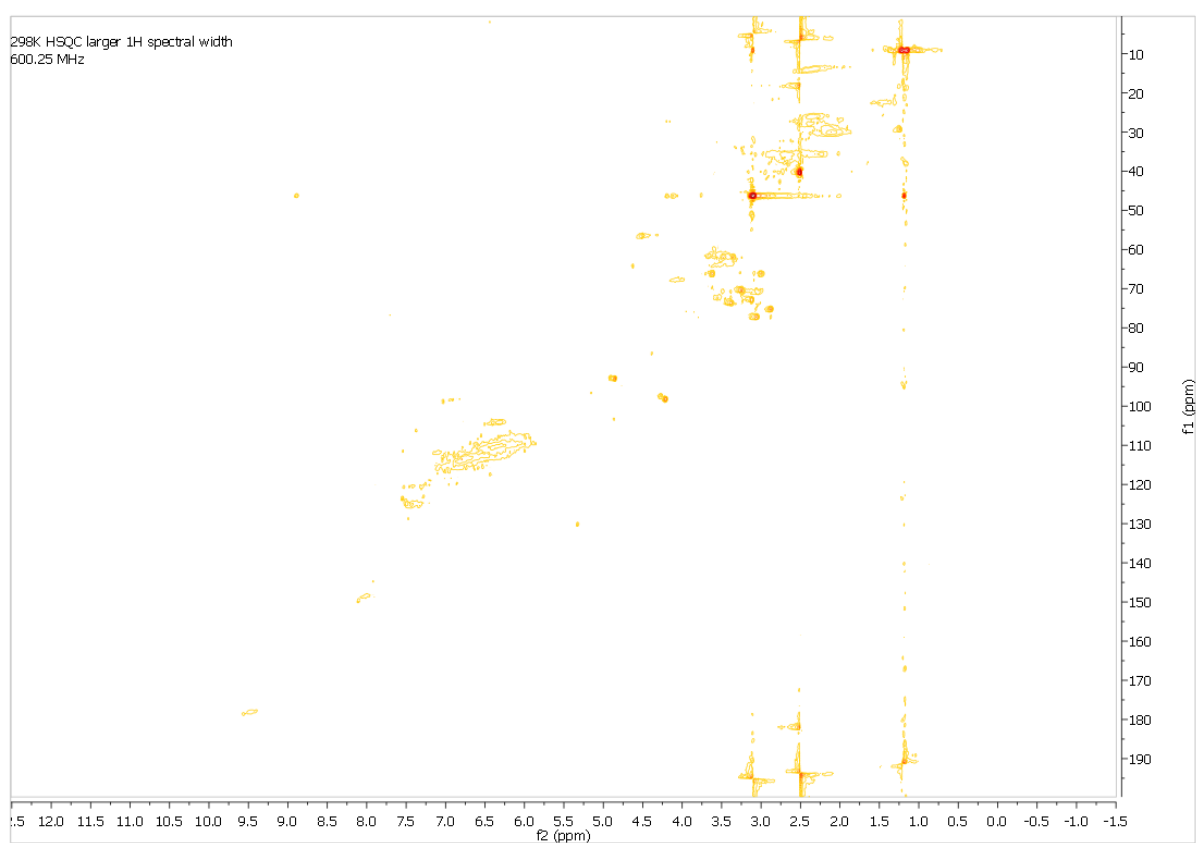


Appendix 4F

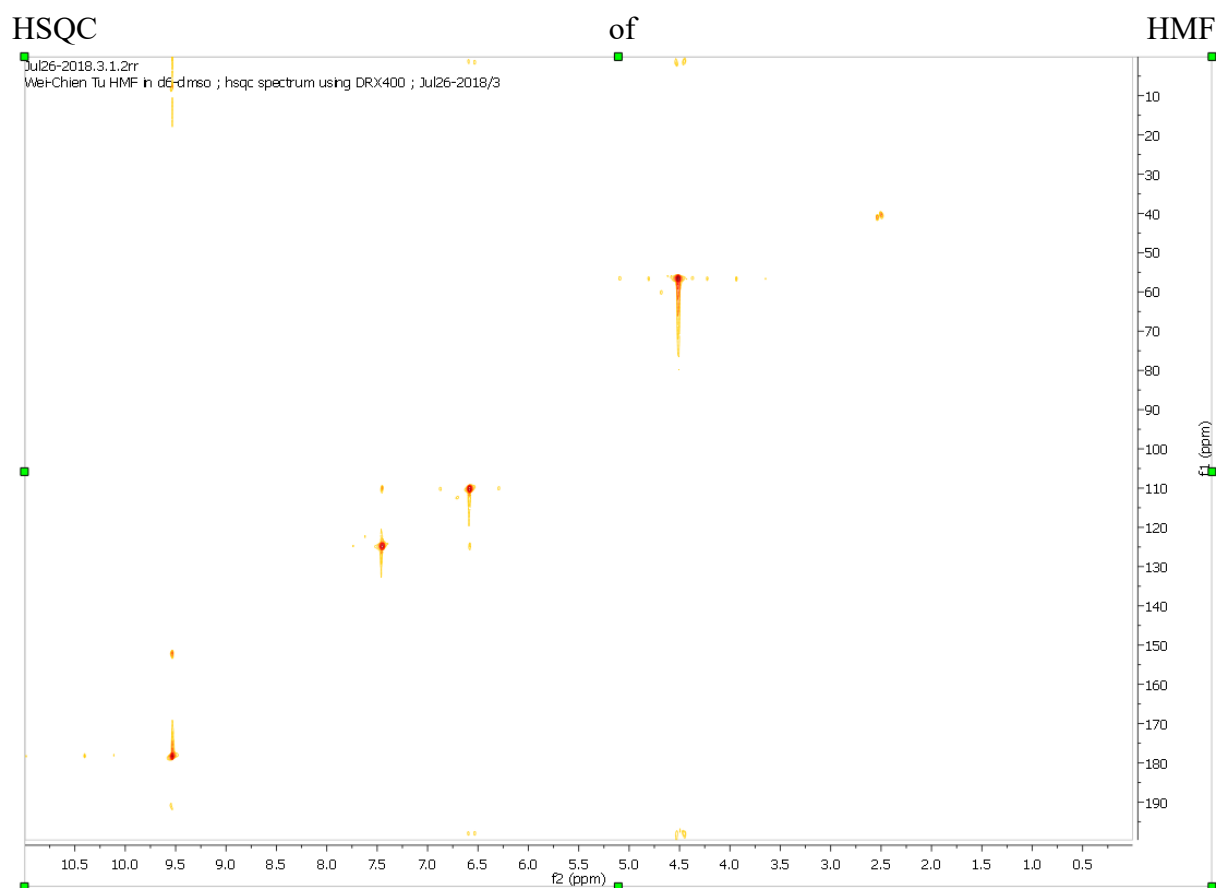
Solids from Glucose pretreatment with FIL



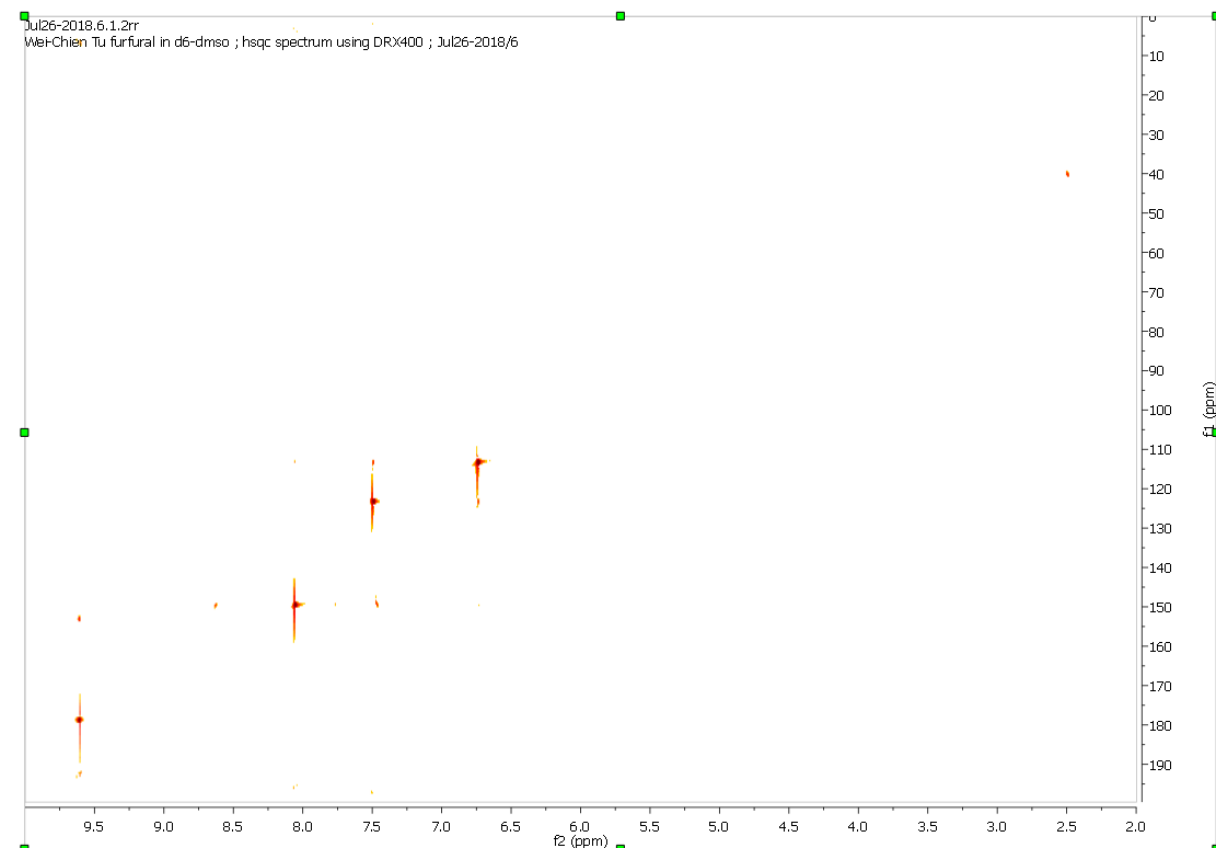
Solids from Xylose pretreatment with FIL



Appendix 4G



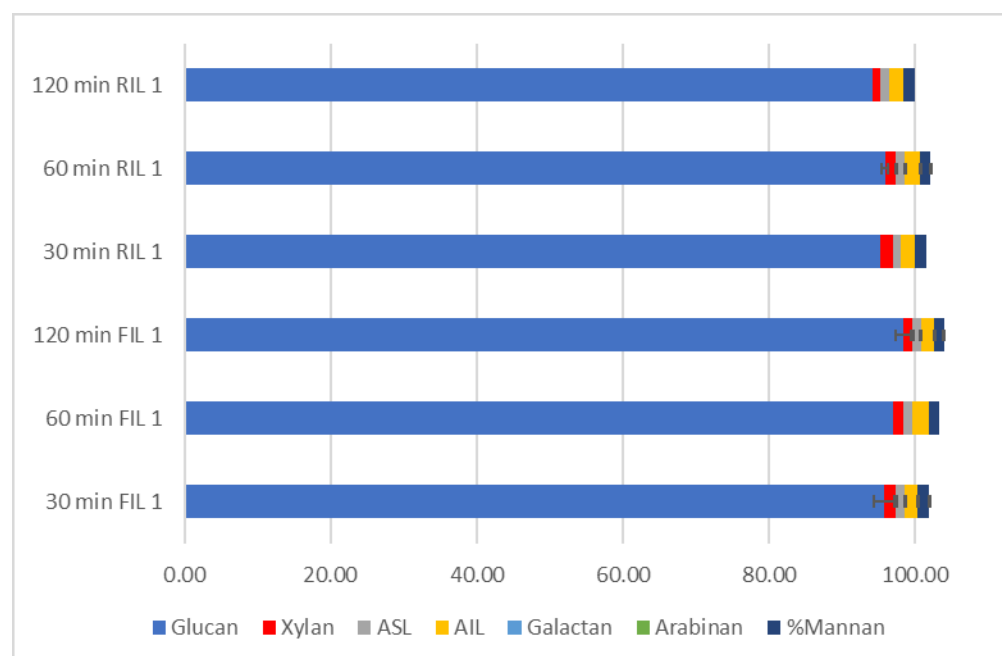
HSQC of furfural



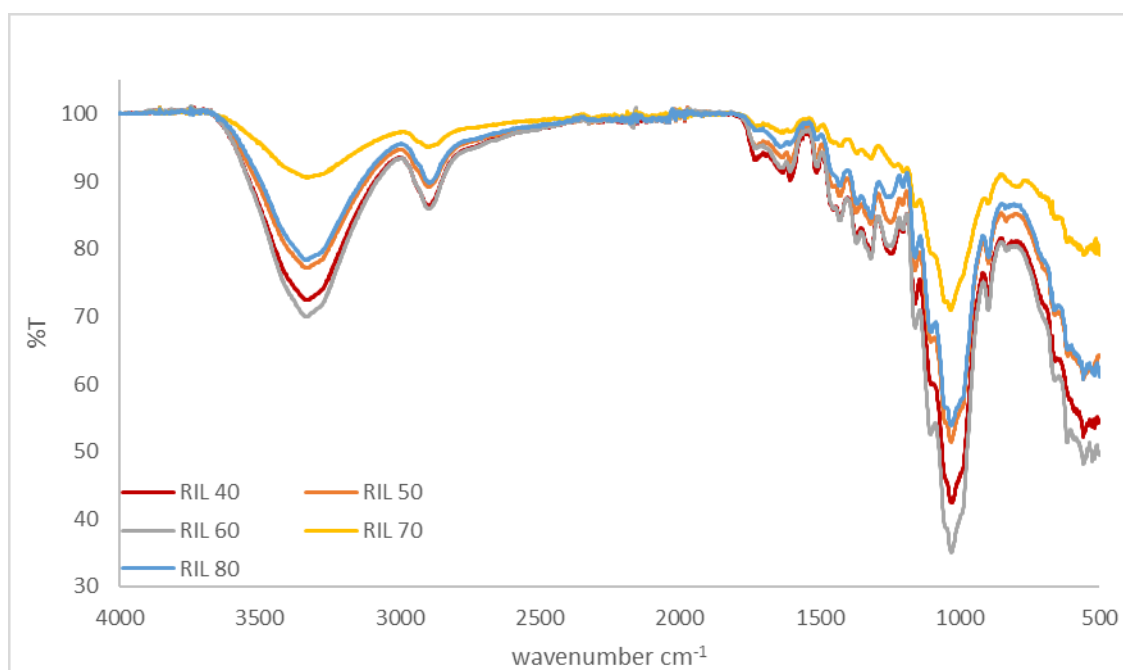
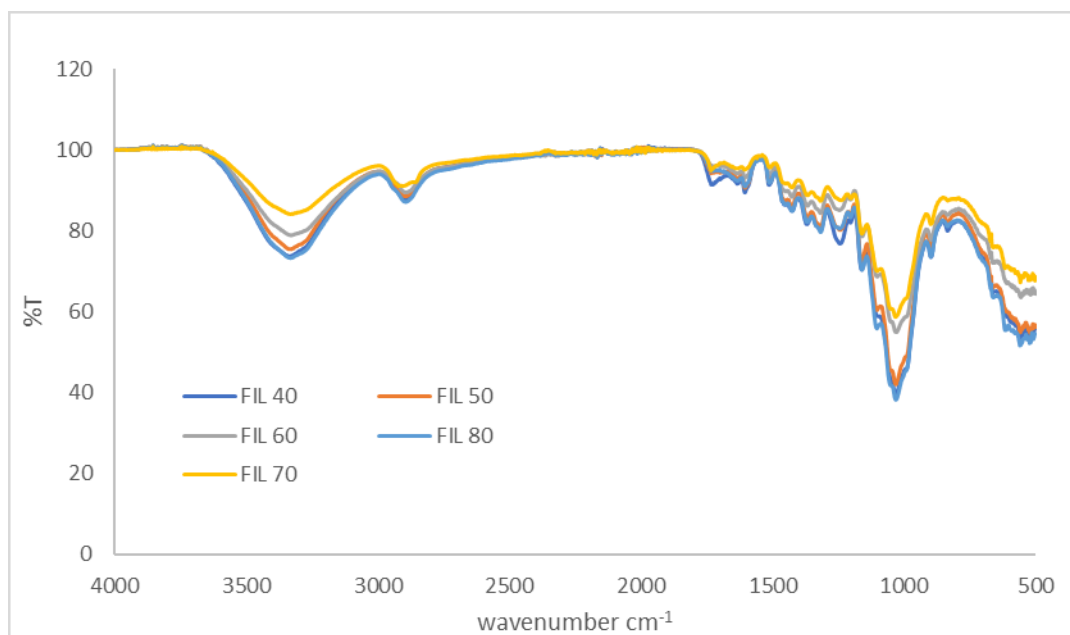
Appendix 4H: HSQC for pretreated HMF



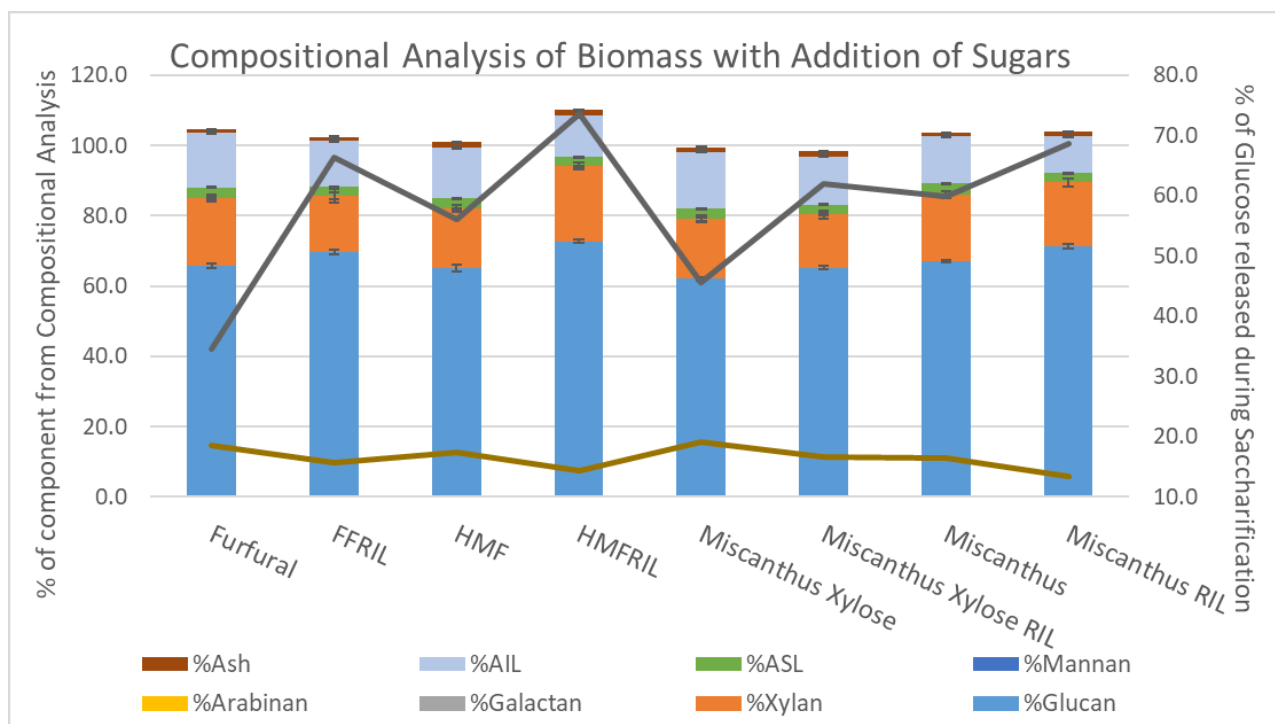
Appendix 4I: Compositional analysis of sigma cellulose pretreated with FIL and RIL



Appendix 4J FT-IR for FIL(top) and RIL (bottom) time course pulps

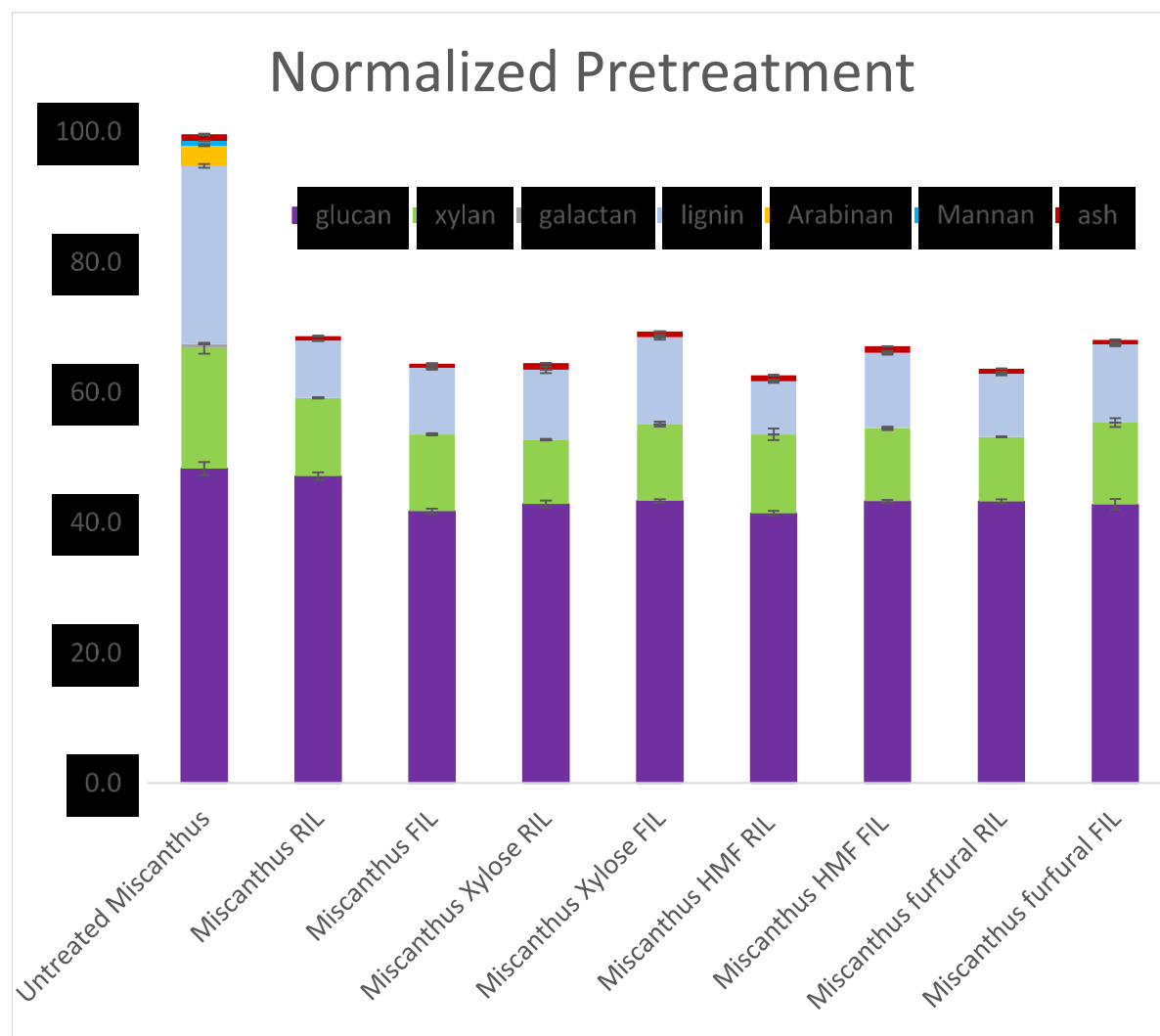


Appendix 4K Compositional analysis, lignin recovery, and saccharification of *Miscanthus* with additives:

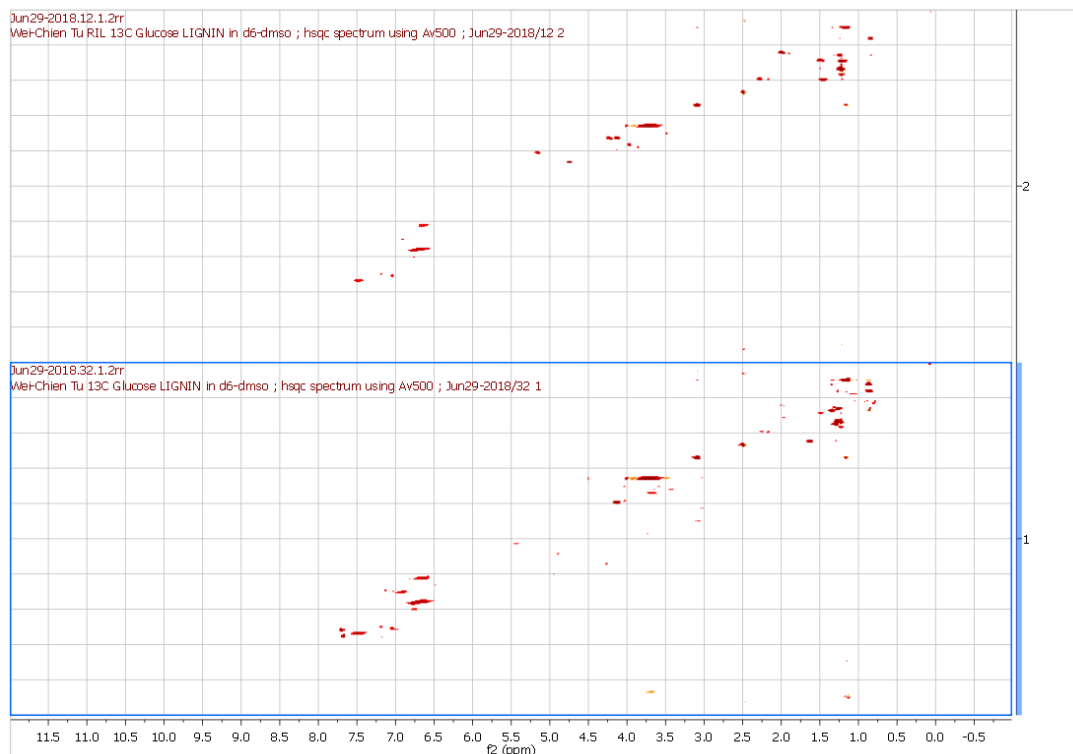


Appendix

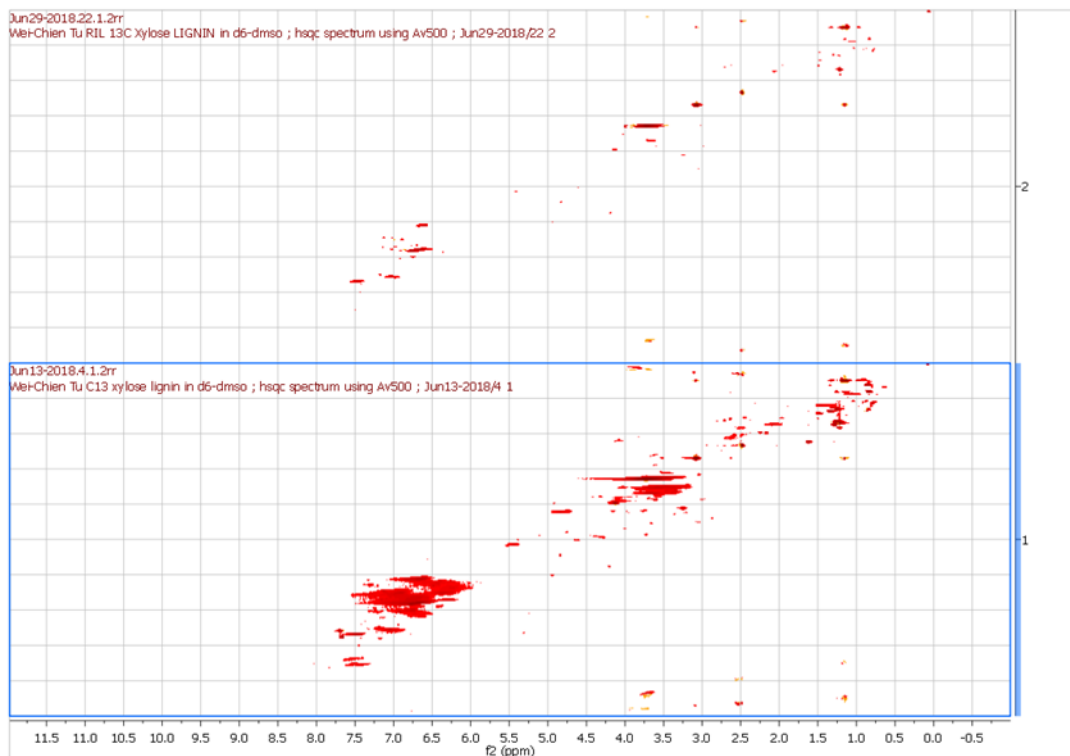
4L Normalized compositional analysis for Miscanthus with additives compared with untreated Miscanthus:



Appendix 4M HSQC comparison of ¹³C labelled glucose Miscanthus lignin pretreated with recycled ionic liquid (top) and fresh ionic liquid (bottom)



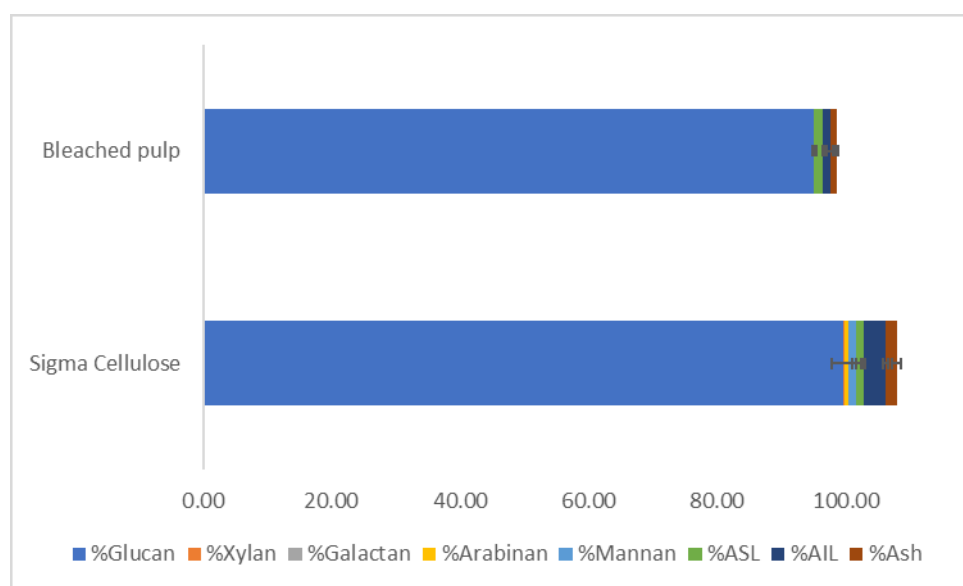
Appendix 4N HSQC comparison of ¹³C labelled xylose Miscanthus lignin pretreated with recycled ionic liquid (top) and fresh ionic liquid (bottom)



Appendix 5A Elemental analysis of lignin produced from The Sun and PHS using [HBIM][HSO₄]

Sample	%C	%H	%N
HBIM PHS1			
average	63.40	6.56	2.43
SD	0.03	0.08	0.08
HBIM The Sun1			
average	63.55	6.68	2.30
SD	0.06	0.05	0.07

Appendix 5B Compositional analysis of Sigma Cellulose and Bleached Miscanthus pulp



Appendix 5C coarseness of pulps produced from

Feedstock	Coarseness [mg/m]
Spruce	0.14
W. Hemlock	0.19
Douglas Fir	0.26
Southern Pine	0.28
Oak	0.14
Gum	0.27
Birch	0.12
Aspen	0.13

1. Ralph, J. *et al.* Lignins: Natural polymers from oxidative coupling of 4-hydroxyphenylpropanoids. *Phytochem. Rev.* **3**, 29–60 (2004).
2. Rasmussen, H., Sørensen, H. R. & Meyer, A. S. Formation of degradation compounds from lignocellulosic biomass in the biorefinery: sugar reaction mechanisms. *Carbohydr. Res.* **385**, 45–57 (2014).
3. Patil, S. K. R., Heltzel, J. & Lund, C. R. F. Comparison of Structural Features of Humins Formed Catalytically from Glucose, Fructose, and 5-Hydroxymethylfurfuraldehyde. *Energy & Fuels* **26**, 5281–5293 (2012).