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The Depolymerization and Utilization of Phenolic Compounds derived from Lignin

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The Depolymerization and Utilization of Phenolic Compounds derived from Lignin

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy
in Chemistry

by

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Dedicated to my family.

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PUBLICATIONS

1) Chongbo Cheng, Julianne Truong, Jacob A. Barrett,Dekui Shen, Mahdi M. Abu-Omar, and Peter C. Ford, Hydrogenolysis of Organosolv Lignin in Ethanol/Isopropanol Media without Added Transition-Metal Catalyst. *ACS Sustainable Chem. Eng.*, **2020**, 8, 2, 1023-1030.
2) Simin Li, Baoyuan Li, Julianne Truong, Zhongyang Luo, Mahdi M. Abu-Omar, and Peter C. Ford, One-pot hydrodeocygenation (HDO) of lignin monomers to C9 hydrocarbons co-catalysed by Ru/C and Nb_2O_5. *Green Chem.*, **2020**, 22, 7406-7416.

3) Rebecca Nishide, Julianne Truong, Mahdi M. Abu-Omar, Organosolv Fractionation of Walnut Shell Biomass to Isolate Lignocellulosic Components for Chemical Upgrading of Lignin to Aromatics. *ACS Omega*, **2021**,

PRESENTATIONS

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2) Truong, Julianne; Nishide, Rebecca; Liu, Baoyuan; Zhao, Shou; Abu-Omar, Mahdi, **"Fractionation of biomass and upgrading of lignin and cellulose streams"**, ACS National Meeting, San Diego, Monday, August 26, 2019.
3) Truong, Julianne; Cheng, Chong-bo; Ford, Peter; Abu-Omar, Mahdi, **"Efficient depolymerization of organosolv lignin in ethanol/iso-propanol media without catalyst"**, ACS National Meeting, San Diego, Monday, August 26, 2019.

ABSTACT

The Depolymerization and Utilization of Phenolic Compounds derived from Lignin

by

Julianne Truong

Biorenewable material has gained a greater focus in research to produce chemicals, fuels, plastics, and energy because of their abundance, diversity, and unique properties. Biorenewable materials include mainly terrestrial based plants that grow throughout the world. From the plant, there are constructed of three biopolymers, and in this study the biopolymer of interest is called lignin. Lignin is the second most abundant biopolymer and is known as the most abundant source of phenolic structures known to mankind. Current research focuses on utilizing lignin's aromatic monomers as replacements for phenolic monomers derived from nonrenewable resources. The key problem in accomplishing this idea is the recalcitrant structure of lignin. The functional properties of technical lignin must be characterized in more detail in order for it to become a renewable raw material for the chemical industries.

Herein, we have investigated the structure dependent properties of lignin from samples of fountain grass, walnut shell, and poplar. These samples were analyzed before and after successive organic solvent extractions. The lignin samples were fractionated according to their molar mass by these optimized organosolv extractions. Utilizing Ni/C for catalytic depolymerization achieved 50% aromatic products from fountain grass and walnut shell, respectively.

Previously in our laboratory, we utilized Ni/C for catalytic depolymerization of Poplar lignin and achieved reported yields of 69%. Stemming from this idea, we wanted to approach the depolymerization of lignin with the removal of metal catalysts. Herein, we describe lignin

hydrogenolysis/depolymerization of organosolv poplar lignin in ethanol/isopropanol solvent in the absence of added catalysts. Heating Organosolv Poplar Lignin (OPL) at 270 °C for 4 h in 50:50 (v:v) EtOH/i-PrOH in a closed pressure vessel gave an overall oil yield of 70 wt%, of which about 48% consisted of the monomers (E)-4-propenyl syringol and isoeugenol. Notably, these catalyst-free reactions in ethanol/ isopropanol media show monomer yields comparable to those reported for lignin depolymerization using precious metal catalysts and dihydrogen, which suggests unexpectedly favorable H-donor ability of this mixed alcohol medium.

With the advancements in discovering a mixture to utilize an alcohol mixture to depolymerize lignin, we wanted to finish by connecting the bridge gap between utilizing lignin aromatic monomers straight to materials such as thermosets and thermoplastics. We report novel methodology on the extraction, isolation, and depolymerization of different biomass towards monomeric compounds of interest in the goal of further utilizing them into material networks.

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Chapter 1. Introduction: Utilization of Lignin into valuable compounds

1.1 Abstract:

Lignin valorization is a candidate for production of value-added compounds, due to its high abundance, availability and eco-friendly properties. Lignin is the largest phenolic polymer that is found in the plant cell walls and is composed of monomeric subunits called monolignols.[1] Lignin accounts for over 20% of the cell wall in wood and interacts with two other biopolymers, cellulose and hemicellulose.[1] Due to its aromatic composition, research studies have been performed in order to utilize it towards a replacement form for petroleum. However, due to its complex and heterogeneous structure, lignin has obstacles in utilization, thus leading to its main use in combustion to produce low-grade heat. This chapter will show the background, difficulties, and options for lignin utilization.

1.2 Lignin: The Aromatic polymer from Lignocellulosic Biomass.

In society today, we are consumers of products that include energy, chemicals, plastics, materials, and transportation fuels, which primarily stem from fossil fuels. Since these products stem from fossil resources, it would be non-beneficial towards the contribution of CO_2 and climate change. Known as the most abundant, renewable resources on earth, lignocellulosic biomass offers possibilities as a feedstock for not only energy, but also the chemical industry due to its abundant availability and low costs. Lignocellulosic biomass mainly is formed by capturing CO_2 from the atmosphere and converting solar light into chemical energy.[11] The composition of the biomass cell wall is a heterogeneous natural nanocomposite of cellulose, hemicellulose, lignin (as the major components) and minor quantity compositions, such as resin, tannins, pigment, and pectin.[1] Shown in **Figure 1.1**, is the composition of the middle lamella which holds the lignin and hemicelluloses. Cellulose concentration increases from outer to inner layer with it being the highest in the secondary wall compared to the primary wall.

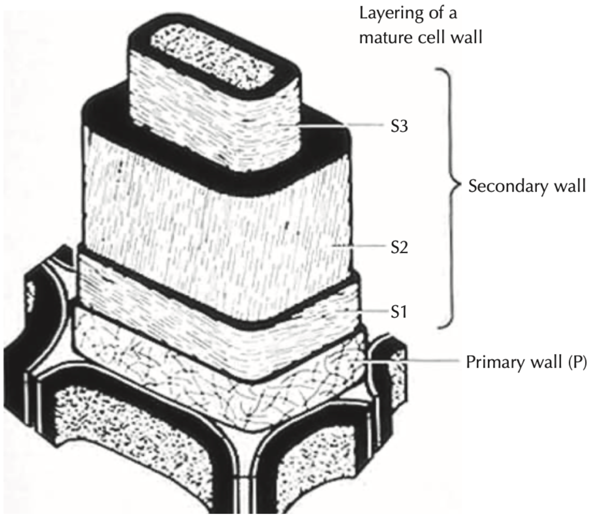


Figure 1.1- Simplified structure of cell from wood showing the composition of the middle lamella: Primary wall, outer, middle, inner layer of secondary wall. [95]

The main structural component in the biomass cell walls and most abundant natural polymer is cellulose. It is distributed in a wide range of biomass such as algae, plants, fungi, and bacteria; also, is produced annually world-wide: 75-100 billion tons.[2,3] Cellulose has a primary structure comprised of an unbranched (1,4)-linked β-D-glugan, in short glucose units, which comprise 30-50 wt% of the dry biomass.[4] Due to the parallel glucose linkages, it forms a partially crystalline microfibril through hydrogen bonding which gives the cell wall its strength. The discovery and isolation of cellulose was performed in 1938 by AnselmePayen who sparked a research branch exclusively on studying its physical and chemical properties.[5] The utilization of cellulose was solely in pulp and paper industry, but currently studies are now directed towards converting it into value-added products such as plastics and fuels.[6,7]

Having a backbone similar to cellulose, hemicellulose is composed of different units which include: arabinans, mannans, galactans, and xylans.[1] Depending on the species of plants, the composition of hemicellulose varies significantly. For example, in hardwoods xylan is the main composition (80-90%) whereas in softwoods, glucomannan is the main composition. Hemicellulose in plant cell walls mainly initiates the connectivity of cellulose fibrils together and adds flexibility to the structure. In the pulp and paper industry hemicellulose is produced as a byproduct.[2]

Lignin, derived from the latin word *lignum*, is a major polymeric component in biomass and known as the second most abundant biopolymer, after cellulose.[8-10] Lignin starts from the middle of the middle lamella (**Figure 1**), then in the primary wall and secondary wall. The structure of lignin is compiled of phenols that are randomly cross linked to mainly hemicellulose and cellulose. The main function of lignin is adding strength to the cell walls and acting as a defense mechanism against chemical degradation, pathogens, and herbivores. Lignin is a complex, cross-linked, hydrophobic and aromatic polymer that composes 15-50 wt. % of dry weight to a given biomass. The structure of lignin is highly branched with C-C bonds and ether linkages which are formed through oxidative radical polymerization. The oxidative polymerization, *via* enzymatic dehydrogenation, occurs with three precursor monolignols: p-hydroxycinnamyl (coumaryl) alcohol, 4-hydroxy-3-methoxycinnamyl (coniferyl) alcohol, and 3,5-dimethoxy-4-hydroxycinnamyl (sinapyl) alcohol. Phenoxyl radicals generated from the three monolignols form both C-O and C-C bonds, leading to a randomized heterogeneous structure and a three-dimensional network. The three monomers are typically found in grasses, but for hardwood only two are found which is coniferyl and sinapyl alcohol. For softwoods, lignin mainly is composed of coniferyl alcohol.[12-14] The three phenylpropane building blocks of lignin (**Figure 1.2**) correspond to *p*-hydroxyphenyl (1H), guaiacyl (1G), and syringyl (1S) which vary in grass, soft- and hard woods and differ in the number of methoxy groups. Softwood lignin is composed of mainly G-units with a small amount of H-unit lignin, while hardwood lignin consists of both G- and S-units. Herbaceous plant lignin contains all three monolignol units of G, S, and H units, and *p*-coumarate and ferulate, which are incorporated with normal G- and S-units. G-units and S-units with a C_α=O ($1G^1$, $1S^1$) also exist (**Figure 1.2**).

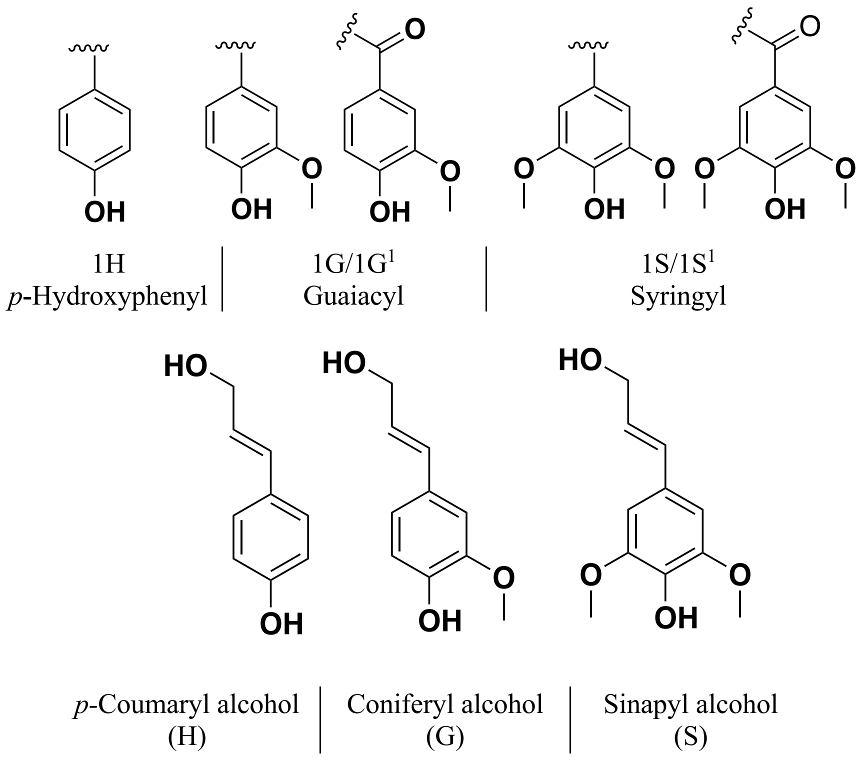


Figure 1.2 Lignin phenolic building blocks in lignocellulose: *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S).

To identify interunit linkages, lignin is generally degraded to low molecular weight products of monomers, dimers, and oligomers, then analyzed using common spectroscopic techniques such as: GC-MS, LC-MS, and NMR. From these techniques, the common interunit linkages found in lignin are β-O-4, 5-5, β-5, 4-O-5, β-1, and β-β (**Figure 1.3**). The abundance of each linkage depends on the biomass species, lignin type, and isolation method. The overall connectivity is randomly linked by given linkages: β-O-4 linkage is by far the dominant one in all species (45%-60%), followed by some other linkages such as β-5 linkage (10%-12%) and 5-5 linkage (10%-20%).

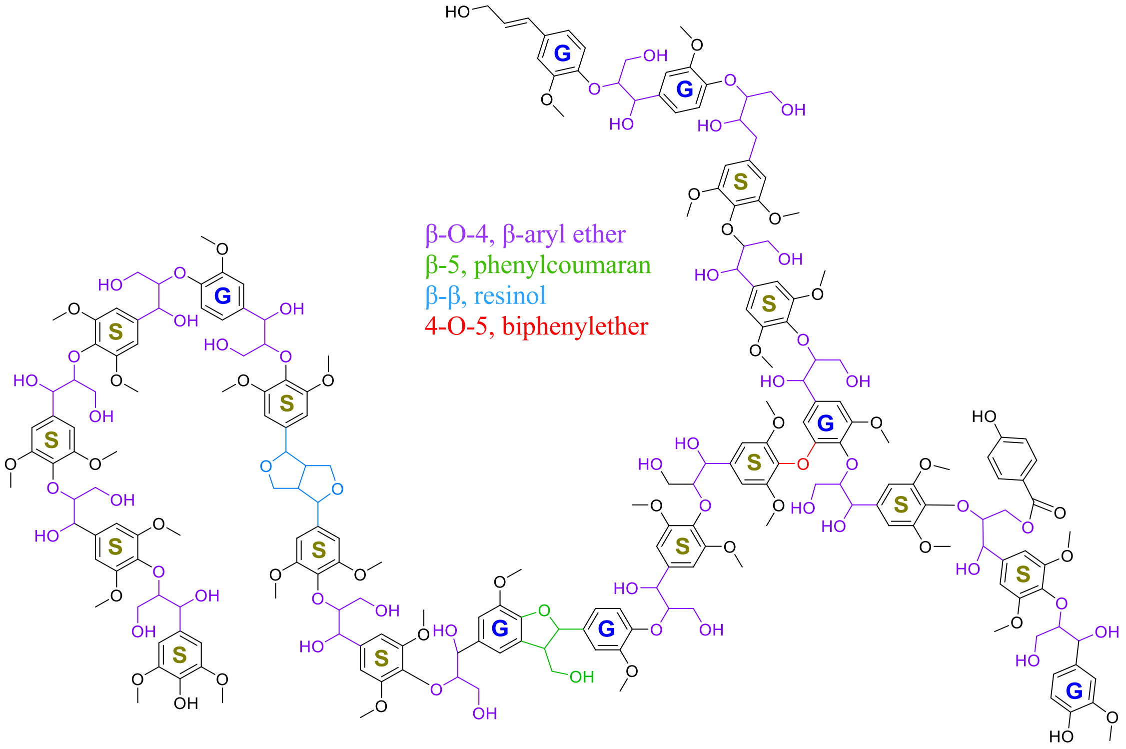


Figure 1.3- Representation of lignin from poplar, from NMR-based lignin analysis.

The arylglycerol- β-aryl ether (β-O-4) bond is the most common bond in native lignin and has a BDE of 226-290 kJ/mol relative to 524-532 kJ/mol for a typical C-C bond such as β-5.[41-44] Depending on the biomass source, the percentage of each linkage in lignin varies.

Known as phenylpropanes, they contain varying substitutions at the 3 and 5 positions.[13-15] Thanks to research performed by Ralph et al., they developed a process using reductive catalytic fractionation (RCF) which degrades the β-O-4 units using AcBr and Zinc.[95-98] This led to the use of HSQC-NMR as a quantitative method to measure the relative amounts of β-O-4 units found in the cell wall. Based on this research it confirmed β-O-4 as the predominant interunit linkage in lignin that is critical for depolymerization. Depending on the biomass, the type of linkage varies in quantity, but the most common that takes up two thirds are the aryl ether linkages. As of today, there is no conclusion to lignin's original structure because the chemical composition of lignin changes based on the biomass and the isolation method used.[21] Since the three inter-monomer linkages found in lignin (arylglycerol-β-aryl ether) are randomly connected, it lacks a repetitive structure for easy analysis.

Lignin previously mentioned holds together carbohydrates and how it does this is there are glycoside bonds, esters, and benzyl ethers that hold these two together. In pulping industry techniques, this lignin-carbohydrate complex makes isolation of lignin from carbohydrates difficult. Even though there are depolymerization methods focused on targeting β-O-4 linkages, the amount of this linkage varies depending on the S/G/H ratio. With more S, which has two methoxy groups in the 3rd and 5th position, it prevents the formation of β-5 and 5-5, which leads to an increase in the β-O-4 linkages in lignin.[10-16] Lignin represents about 30% of the non-fossil carbon on earth, and it accounts for 40% of the energy in biomass, due to its high carbon content. Natural abundance of lignin in the biosphere is around 300 billion tons (3.0×10^{14} kg), with an annual increase of 20 billion tons (2.0×10^{13} kg).[17]

1.3 Lignin Isolation Techniques.

Pulp consists of wood or other lignocellulosic materials that are broken down physically or chemically to generate fibers that can be dispersed in water and reformed into a web for paper. Pulping is the most common method where it uses the recovered wastepaper as a raw material. Paper mills are engaged in the manufacture of paper products from pulp. By-products of pulping processes and second-generation bio-ethanol production processes have been used on industrial scale to effectively utilize the lignin. There are four main industrial processes that are currently producing technical lignin; Kraft, Sulfite, Soda, and organosolv processes. Most isolated lignin observed from these extractions are brown amorphous powders. However, depending on the preparation method used and lignin studied, the color and shape of the lignin could change. Even though there are many methods for isolation, these main methods are recognized due to their recovery, maintenance, and utilization of lignin.

1.3.1 Kraft Lignin (Thiolignin, Sulfate Lignin).

Kraft pulping is done by dissolving the lignocellulose in hot alkaline sodium sulfide (Na_2S) solution. How the alkaline solution is prepared is by dissolving 16 grams of sodium sulfide per liter of 1 N sodium hydroxide (NaOH). In Wiley-milled form and alkaline solution at a 4:1 liquid to wood ratio, it is sealed in a stainless-steel bomb. For hardwoods, the temperature is ran at 155 °C and for soft woods, 170-180 °C.[18-23] This is run for 1-2 hours on a large scale. The steel bomb is cooled, and the resulting product is a black oil containing lignin, hemicellulose and degraded cellulose. Acetic acid is used to precipitate the lignin and then washed with distilled water and freeze-dried. What happens is the hydroxide and hydrosulfide anions aid in depolymerization of lignin into smaller water/alkali-soluble fragments. The Kraft lignin retrieved from this process mainly is applied as fuel for industrial boiler systems due to the process adding thiol groups to the lignin side chain. A limitation when extracting Kraft lignin with NaOH and Na_2S is the proposed structure of lignin (**Figure 1.4**) shows the lignin retrieved is of low molecular weight (1000-3000 g/mol).[23]

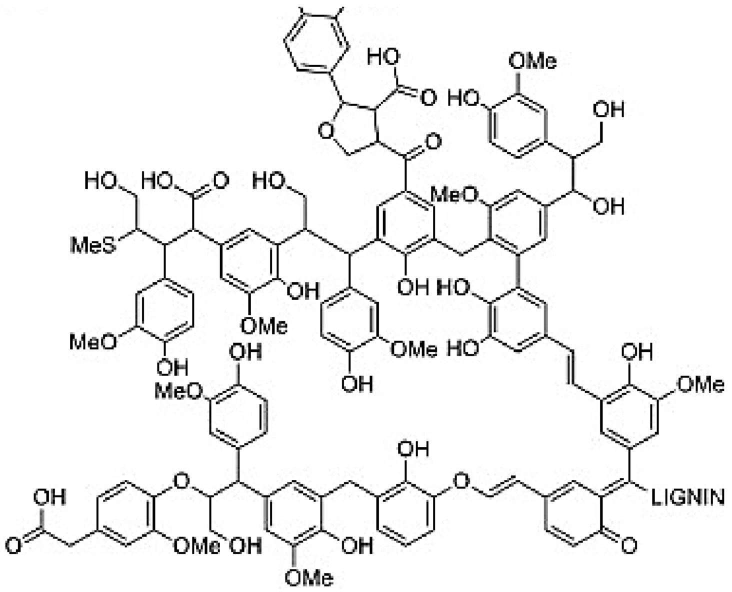


Figure 1.4- Proposed representative Kraft Lignin Structure[23]

1.3.2 The Sulfite Process.

The first known patent on work related to sulfite pulping was granted to Julius Roth in 1857 for treatment of wood with sulfurous acid.[99] Benjamin Tilghman is credited with the development of the sulfite pulping process and was granted a US patent in 1867.[99] Numerous difficulties (mainly from sulfuric acid impurities that led to loss of wood strength and darkening of the pulp) prevented commercialization of the sulfite process initially. This is the largest producer of lignins, using aqueous solution of sulfur dioxide with different pH. Shown in **Figure 1.5**, Sulfonate's are introduced to the α-position of the propyl sidechain in lignin's structure. The lignosulfonates are shown to be water-soluble which is different from the other techniques.

In Sulfite cooking, the full chemical pulping process uses mixtures of sulfurous acid and counter ions that include sodium, potassium, ammonium, calcium, and magnesium to solubilize lignin and form sulfonate functionalities and cleavage of lignin bonds. The temperature is between 130-160 °C for 4-14 hours. During this reaction, sulfonic groups are introduced into the partially hydrolyzed lignin converting it into a fully water-soluble lignosulfonate (**Figure 1.6**).

With these conditions, isolated lignosulfonate is usually very impure with a mixture of lignin and carbohydrates but does have high molecular weight. This makes the process not cost-manageable due to the need for more purification steps. Before the 1900s it was the most important pulping process but has decreased to less than 10% of pulp production due to the development of the Kraft pulping process. Lignosulfonates produced are usually produced on a scale of 1 Mt/year by many companies.[25,26]

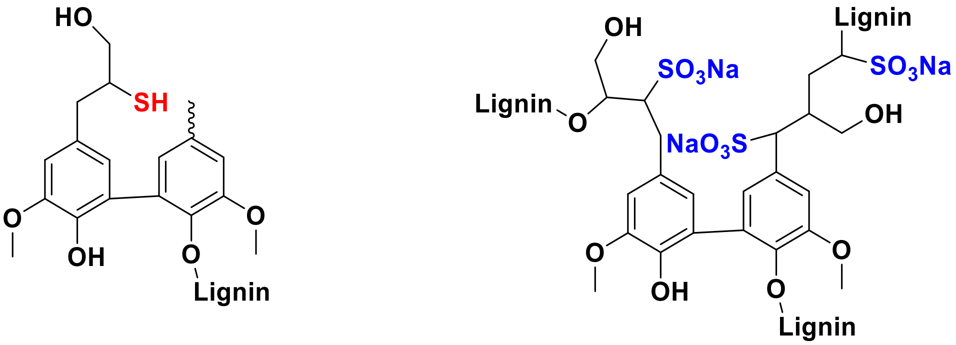


Figure 1.5- Kraft lignin (left) in comparison to Sulfite Process (right).[24]

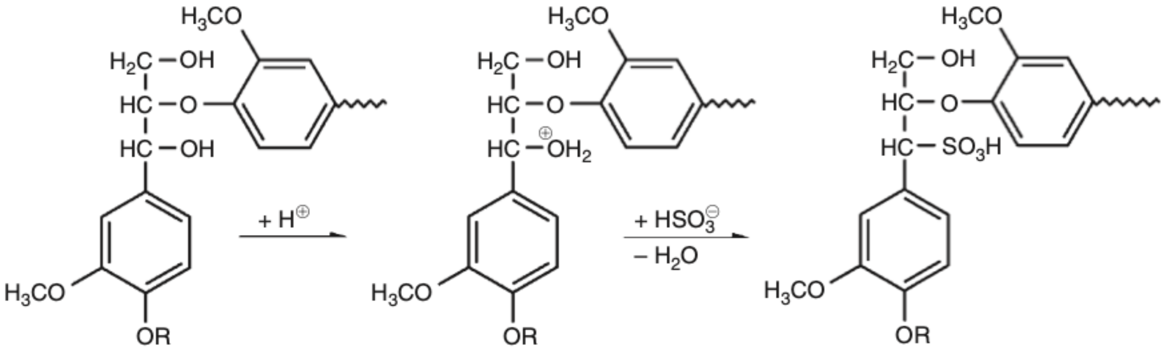


Figure 1.6- Basic Reaction during Sulfite Pulping.[100.101]

1.3.3 The Soda Process.

The Soda Process was invented in England in 1851 by Burgess and Watts using the base, sodium hydroxide as the main reactant.[99] Soda process is mainly used for non-wood biomass: sugar cane, bagasse, straw, etc.[27] This process uses sodium hydroxide which is ran at about 160-170 °C for 1.5 h. After the reaction mixture is cooled, a black solution is shown and then acidified to a pH of 5.5 with sulfuric acid to precipitate the lignin. Soda lignin is high in purity and sulfur-free which give researchers a better composition of native lignin. During the process, hydrolytic cleavage of the native lignin is observed giving smaller fragments that are soluble in the strong alkali mixture which is precipitated and recovered. The Soda lignin is a complex and heterogeneous mixture with a broad molecular weight distribution.[28] There are dilemmas using this method like no specific degradation to lignin. The use of high concentrated base degrades the carbohydrates which makes the mixture difficult to work with. There is still development with this process like the addition of oxygen which has been shown to be more selective to lignin and decreasing the degradation of the carbohydrates.[27,28]

1.3.4 The Organosolv Process.

The other processes listed are plagued with many environmental issues such as release of sulfur compounds due to it as air pollutants or the release of chlorine bleaching compounds as water pollutants. To overcome these issues, organosolv process takes meshed biomass material and uses a proportion of organic solvents to extract and solubilize lignin. Undergoing this process avoids the formation of sulfated byproducts and increases the production of high-quality cellulose and lignin. Typical solvents for the organosolv process involve the presence of mineral acid (acetic acid and formic acid) that acts as a catalyst, ethanol and an organic peroxide at a temperature range between 100-200°C.[29-32] Organosolv pretreatment dissolves hemicellulose and lignin, which leaves behind cellulose. To selectively remove lignin, water is added to insolubilize it from the hemicellulose. This method has become a selective protocol for producing monomers from lignin because of its purity from ash and sulfur. Another bonus is the consistent low molecular weight distribution, typically around 4,000 g/mol, when lignin is extracted.[35] Because of the high quality and purity given from organosolv, it makes it ideal for using it towards the production of high-value chemicals, such as phenolic derivatives.[24] This type of process is not commercial yet due to large excess of organic solvent and scalability but has shown promise. For example, companies like commercially available Lignol and CIMV are using this organosolv fractionation technology to optimize the upscaling of extracting lignin.[33,34]

1.4 Overview of Lignin Valorization.

There has been a lack of literature available with the utilization of lignin towards high-value chemicals.[35-40] Most papers have focused on the upgrade of cellulose with minimal review towards lignin. However, it has been noted that lignin is a prominent renewable source because of its phenolic properties. The USA has set a target of 20% substitution of conventional fuels by biomass-derived fuels by 2030.[61-63] Depolymerization of lignin into its monomeric species increases the solubility in solvents and decreases viscosity which brings it closer to prominent fuels, aromatics, or macromolecules. In the depolymerization of lignin, it is advantageous to break the ether linkages because of the lower bond enthalpy relative to C-C linkages. Breaking the ether linkages brings it a step closer to renewable energy.

Many lignin valorization techniques target fine chemical production; however, the processes often have low selectivity to specific compounds and requires difficult and expensive separation. Lignin depolymerization is usually divided into two categories: direct and indirect liquefaction. Direct liquefaction does not involve an intermediate gaseous product while indirect uses it. These categories have subclasses that fall underneath them: gasification, pyrolysis, catalytic hydrogenolysis, sub-/supercritical water and solvent depolymerization.

1.4.1 Gasification Studies: Lignin.

Gasification is a thermochemical process that converts a low value solid feed, to a gaseous form which in turn can be converted to a liquid product. It has been shown to be one of the most promising thermochemical processes because of the energy efficiency.[47-52] Gasification has been around since the early 1920s mainly for the production of transportation fuels. This form is a process that is also addressed as indirect liquefaction, meaning no addition of a solvent to turn the solid into a liquid state. A downfall of using indirect liquefaction is the large quantity of CO_2 produced due to this method utilizing air, oxygen, and steam to do partial oxidation on the feedstock at high temperatures, causing low selectivity in product.[39,45,46] Especially for biomass gasification, it involves a combination of primary and secondary reactions.[53] Primary reactions break the biomass into gas, hydrocarbons, tars and coke while secondary reactions take the hydrocarbons and crack/reform them into a lighter hydrocarbons with the production of more gas (CO_2, CO, H_2).

1.4.2 Pyrolysis Studies: Lignin.

Pyrolysis is an indirect liquefication process that has been around for quite some time, producing low molecular weight compounds and also seen in thermal processes such as hydrogenation.[39,54] Lignin thermally decomposes through pyrolysis without the aid of a catalyst or oxygen. Under pyrolysis, lignin degradation produces products such as solid char, liquid oil, and gases. From the products listed, it depends on the type of lignin used based on isolation methods. For example, hardwoods produce both guaiacyl and syringyl while softwoods produce only guaiacyl. The temperature range for thermal depolymerization ranges between 250˚C - 1000˚C, in the absence of oxygen.[45,55,56] The wide range in temperature is due to the lignin structure having a large quantity of functional groups, which each has their own thermal stability. Mentioned earlier, β-O-4 bond is the linkage that is dominant in the lignin structure and can be selectively cleaved at low temperatures. However, β-ether linkages have a cleavage temperature that depends on the cleavage mechanism due to the side-chain attached to the given structure.

Depending on the given reaction conditions, different compounds can be selectively chosen. When a pyrolysis reaction is heated at low temperature, it favors the formation of oxygen-containing compounds, while heating at higher temperatures give hydrocarbons and alkyl-phenol molecules.[57] Pyrolysis is a process that does produce selective material, but there are always side products that are formed. One product generated is char, which is chemically modified to form a stable structure, but has issues on being decomposed. Char also acts as an inhibitor towards the use of catalysts by sticking to it and causing deactivation.[57-59] Use of lignin rich feedstocks instead of whole biomass is a challenge in pyrolysis technologies as it results in lower oil yields and increased charring. Different variances in temperature towards different lignin gave varying yields in phenolics and has been studied for product selectivity with catalysts.

Due to the need of consistent heat transfer for biomass conversion, a way to keep it consistent is to have small particle sized feedstock. If there is non-uniformity in the temperature due to the size of the biomass particles, this results in the thermochemical processes yielding non-uniform gasses.

1.4.3 Catalytic Hydrogenolysis: Lignin Fractionation.

Direct processes like hydrogenolysis, also known as hydrogenation, can partake in two processes: pyrolysis or solvolysis with the addition of hydrogen. Hydrogen can be added to the reaction with a hydrogen donating solvent or as a gaseous state. Most studies that focus on hydrogenolysis report the use of a heterogeneous catalyst and hydrogen to create an environment for lignin to depolymerize.[64-67]

Another category that promotes hydrogen addition and oxygen removal is called hydrodeoxygenation (HDO). Hydrodeoxygenation (HDO) can upgrade bio-oils through various catalytic processes to produce deoxygenated fuels. In most studies, HDO involves treatment at high temperatures (250-500 °C) with high pressure H_2 (50-100 bar).[68-70] The high H_2 pressure required may limit the scalability of the reaction and is a safety hazard.[14]

Lower pressure and temperature are desired from an ease and cost-of-operation point of view. These obstacles encourage the research towards alternative approaches for phenolic upgrading under milder conditions.

1.4.4 Hydrothermal Liquefaction Process: Lignin Extraction.

Hydrothermal liquefaction, also known as sub- and supercritical fluid (SCF) technology was discovered in 1822 by Baron Charles Cagniard de la Tour while heating fluids in a sealed cannon.[71] From this discovery, it has been utilized in industry to obtain new products and has been promising to extract lignin from feedstock. Fluid is defined as sub-/supercritical fluid under sub-/supercritical conditions when the pressure and temperature is below or above the critical point for temperature or pressure. The subcritical region being the temperature range between normal boiling point and supercritical temperature. Supercritical is at the critical point where it causes the solvent to have low viscosity, high diffusion, and thermal stability.[72] SCF have been shown to be sustainable, environment friendly, and cost efficient. The main advantages is the easy separation and drying of the product by expansion of the solvent, allowing the gas to be collected and reused without purification steps. One solvent mainly used, water, facilitates the cleavage of ether linkages and cross linking to form small and large molecules from lignin. When the critical point is reached, it can readily dissolve and react with a range of different organic molecules. Though there are benefits there are disadvantages such as the requirement of harsh conditions and catalysts to make this process efficient.[73,74] Another disadvantage is the formation of char on high lignin content feedstocks lowers the yields of isolation making it not 100% free from using purification steps.

1.5 Lignin and Its Monomers.

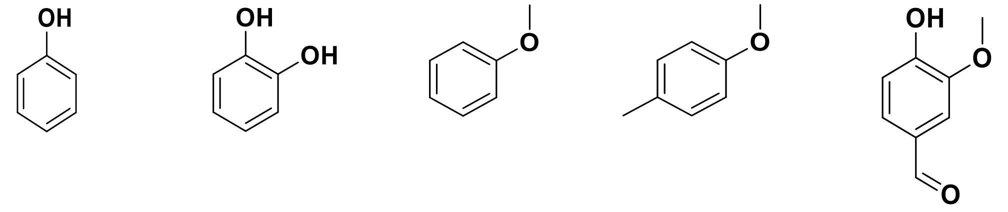
Lignin, as mentioned above, is a high molecular mass biopolymer (600-15000 g/mol) composed of three phenolic monomers connected by different chemical linkages, mainly ether bonds.[75] There is a vision for lignin application, but there are two major challenges in the utilization of lignin. First, lignin is difficult to decompose to higher valued products.[76] Second, even with successful decomposition, tar and char are often formed in the conversion processes reported.[77] Even with these challenges, lignin's chemical structure suggests it is a good source of valuable chemicals or intermediates which could be used towards monomers of polymers or liquid fuels. As of now, the production of a food additive, vanillin, has been commercialized.[78] To understand the structure of lignin, examination of model compounds which are used to represent lignin are used. It is useful to use lignin model compounds because it provides us added information on 1) designing the reaction system and 2) insight to the mechanism of lignin conversion.

1.5.1 Model Compound Studies for Lignin.

The structure of lignin has been studied, but the overall structure is still not confirmed due to the differences in the lignin samples from a given biomass. To study parallel with the lignin molecule itself, research geared towards the use of model compounds is employed. The study of model compounds simplifies the reaction due to the obstacle of lignin being a highly functionalized molecule. The model compounds focus on the linkages and functional groups that are found in

lignin. The different reactivity of the model compounds provides research insight into the different reactivity of the lignin polymer overall.

Figure 1.7- Lignin model compounds of monomers.



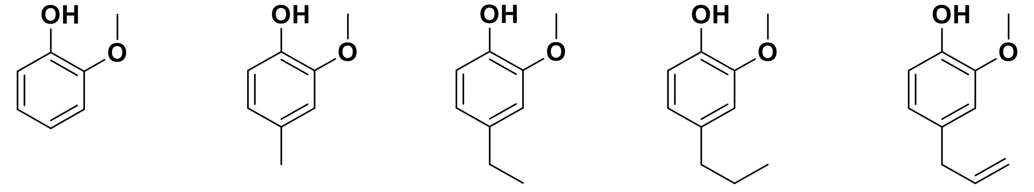
Phenol

Catechol

Anisole

4-methylanisole

Vanillin



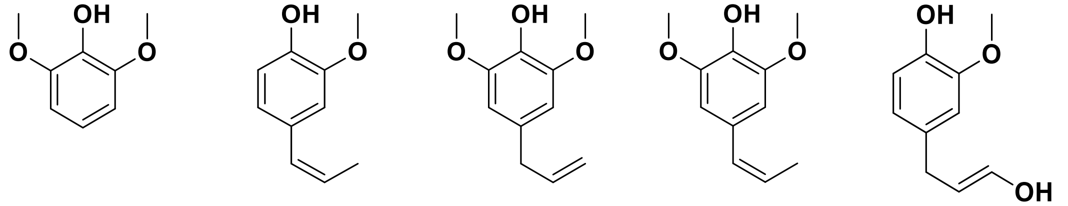
Guaiacol

4-methyl-guaiacol

4-ethyl-guaiacol

4-propyl-guaiacol

Eugenol



Syringol

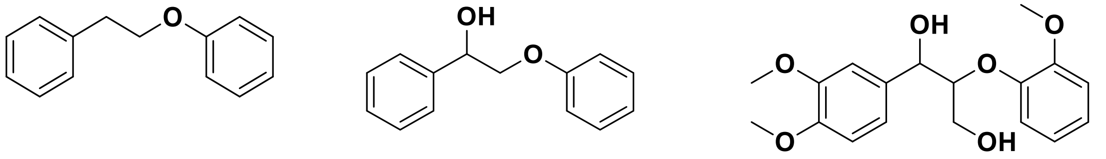
Isoeugenol

4-allyl-2,6-dimethoxyphenol

(Z)-2,6-dimethoxy-4-(prop-1-en-1-yl)phenol

Cinnamyl alcohol

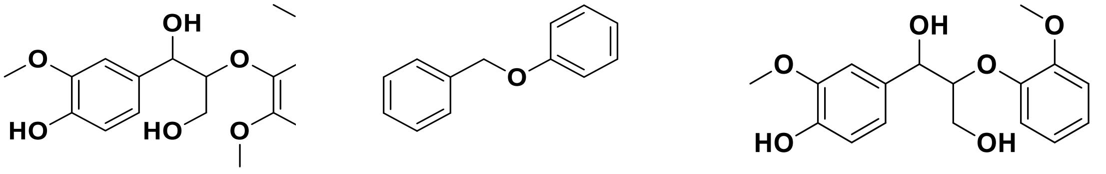
Figure 1.8- Lignin model compounds of dimers.



Phenethyl-phenyl-ether

2-phenoxy-1-phenethanol

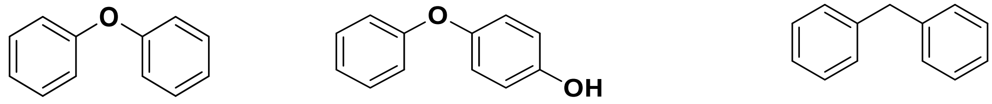
3,4-dimethoxyphenyl-gycerol-β-guaiacyl-ether



Veratryl-gycerol-β-guaiacyl-ether

Benzyl-phenyl-ether

Guaiacyl-glycerol-β-guaiacyl-ether



Diphenyl-ether

Phenoxy-phenol

Diphenyl-methane

There are two primary types of lignin model compounds: monomers and dimers. Monomers that are mainly used are shown in **Figure 1.7** which contain methoxyl, hydroxyl, and alkyl functional groups. Dimers that are used are shown in **Figure 1.8** which include: phenethyl-phenyl-ether, 2-phenoxy-1-phenethanol, guaiacyl-glycerol-β-guaiacyl-ether,veratryl-glycerol-β-guaiacyl-ether, dibenzodioxocin, benzyl-phenyl-ether, diphenyl-methane, diphenyl-ether, and phenoxy-phenol.[79-82] Selectivity in cleaving C-C and C-O bonds in lignin would be beneficial in upscaling in industry.

1.5.2 Lignin Conversion Studies.

Many research studies have focused on the depolymerization of lignin, but all differ based on the method used. One popular method uses hydrotreatment where it uses thermal conditions to break the lignin bonds into radical form which can later be stabilized by hydrogen from hydrogen-donor solvents. Another method called high-pressure direct liquefaction is used to produce bio-oil which contains different aromatic molecules such as phenolic derivatives. Depolymerization methods usually lead to the production of gasses, aromatic monomers, oligomers, and small organic compounds. Another method involves the use of supercritical fluids that converts organosolv lignin from both soft and hardwood into aromatic monomers.

The lignin conversion in liquid phase is utilized frequently due to its low vapor pressure and high polarity. Liquid-phase conversion uses aqueous phase reforming with a given catalyst, heat, and pressure to breakdown lignin into monomer units. However, using aqueous-phase reforming led to the lignin monomers undergoing recondensation. Due to this, solvent optimization was studied in order to prevent this formation.

From the years of lignin studies, research studies have impacted how the structure of lignin would look when depolymerized by certain reaction conditions. There needs to be more studies on different types of lignin from different biomass sources to compare how they differ depending on the region. To address this, lignin isolation is currently still under investigation and optimization to address further knowledge on this complex biopolymer.

1.6 Lignin towards polymer studies.

Lignin is a biopolymer that is composed of many aromatics linked together by ether bonds. Due to this, there have been studies for upgrading lignin towards material applications. However, because of lignin being recalcitrant, it makes the upgrading process by depolymerization difficult due to the high cost and high energy utilized. To avoid depolymerization process, there have been studies where modification of the biopolymer is made in order to avoid this issue. Lignin derivatives are used to functionalize a variety of polymers which are found in many different fields: films and coatings, adhesive resins, plastic, rigid foam formation, and fuels.[101-103]

1.6.1 Carbon Fibers.

The current market demand for carbon fibers has grown and the reasons why is for their unique and attractive mechanical properties. From government defense to general industrial markets, carbon fibers have been used for both light and strong materials. Carbon fibers are flexible and heat resistant with low thermal expansion and low density. Due to high costs of the material, around US $2.34 billion/tonnes, carbon fibers are produced at limited amount between 26,500-63,500 tons.[86] The main production of carbon fibers stems from fossil derived polyacrylonitrile (PAN). PAN is one of the major precursors used for carbon fibers and is the sole reason for making the material expensive. To make carbon fibers more economically friendly, different substitutes have emerged to suggest a more natural product.[83-85] From lignocellulose is lignin, which is becoming more valued because of how cheap it is to obtain the material and its high carbon content.

1.6.2 Phenol.

One platform chemical essential in polymer chemistry for the synthesis of different products like automotive and textiles are phenols.[87-91] Phenols carry important chemical groups for the synthesis of several thermostable and thermosetting polymers. Phenols are obtained from fossil oils which are toxic and difficult to obtain in nature. However, one promising natural aromatic biopolymer, lignin, can be utilized as a derivative for phenols. Once these phenolic derivatives are produced from lignin, they do not differ from fossil-derived phenol molecules. At the moment, there has been a challenge to separate lignin from a variety of aromatics into pure compounds. It is only reported to retrieve a total of 40% possible of pure phenol from lignin, theoretical being 60%. Taking into account the energy content of lignin, the energy amount for lignin costs $330 per ton, so to produce phenol from lignin would be almost triple the price today which is paralleled to today's world market on phenolic compounds.[93,94] Today research studies on lignin conversion to phenolic compounds is an on-going topic in the field. Phenols have also received an increased demand for converting them to Phenol-formaldehyde (PF) resins. PF resins are formed by a polycondensation of phenols with formaldehyde under basic or acidic conditions. Replacing PF from phenol with PF derived from lignin would decrease the cost and also reduce the toxicity.[92] 95% of phenol production involves the partial oxidation of isopropyl benzene by Hock process.[89] Now there is research dedicated to the synthesis of phenol by direct oxidation of benzene. As of now, there are no effective processes today that lead to the production of phenol from lignin at a high success rate.

1.6.3 Benzene, Toluene, and Xylene (BTX).

Benzene, toluene, and xylenes (BTX) are one of the most important aromatic compounds which can be hydrogenated to alkanes or utilized towards the synthesis of fiber, rubber, coatings, and pesticides. BTX is mainly produced as an important byproduct from oil refineries, generating over 102 million tonnes per annum. Shown in **Figure 1.9** are the current petrochemical products synthesized from benzene and phenol. BTX represents 60% of all aromatics on the market and 24% are in the global petrochemical market.[94] Shown in **Table 1.1** is the market value of BTX and their derivatives in 2020. To lower the market price for BTX, lignin is economically interesting, if successful, would lower the cost. The challenge is selectively targeting BTX from lignin which contains a mixture of aromatic structures, increasing the difficulty of purification/isolation.

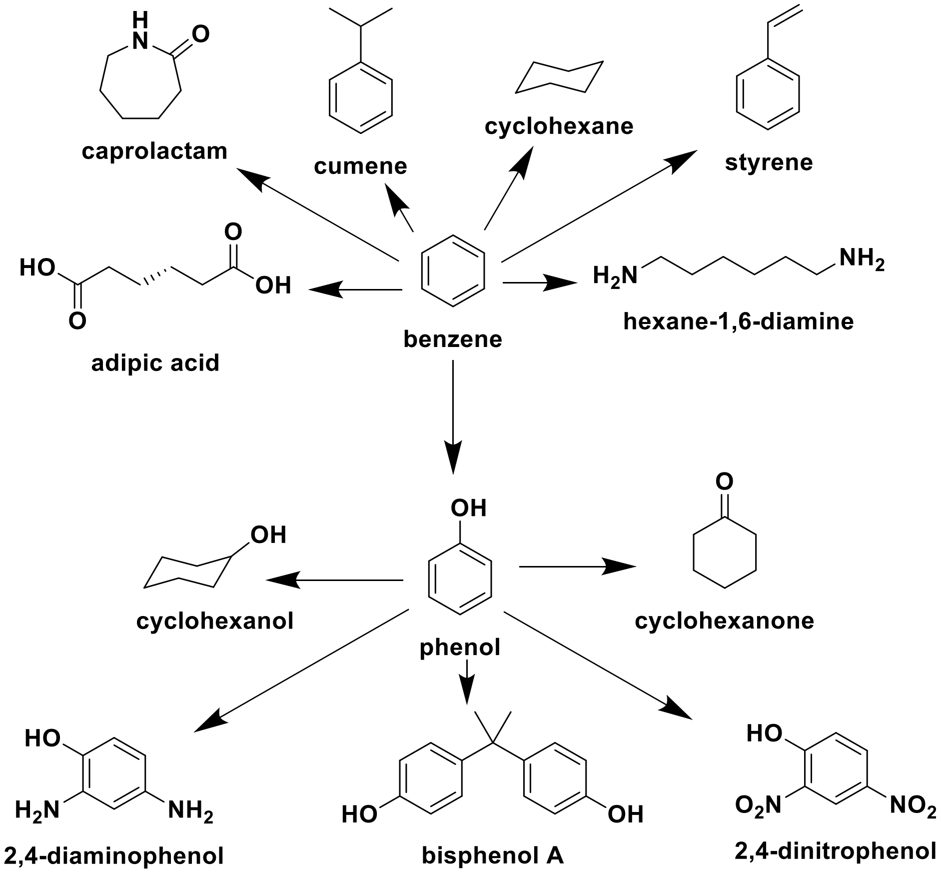


Figure 1.9- Current petrochemical products revolving around benzene and phenol.

Table 1.1- Prices of benzene, toluene, xylene, and their derivatives.

Chemical/Price	Derivative	Price (USD/MT)[-2*,3*]
Benzene	Cumene	1100-1500
(800-1200 USD/MT)[1*]	Styrene	700-1000
	Adipic Acid	1200-2000
	Caprolactam	900-2000
	Cyclohexanone	1000-2200
	Cyclohexane	1000-3000
Toluene	Benzoic acid	1000-1500
(700-1200 USD/MT)[1*]	Toluene diisocyanate	2000-2700
Xylene	Isophthalic acid	1000-2200
(800-1500 USD/MT)[1*]	Terephthalic acid	500-1100

[1*]www.platts.com [2*]www.echemi.com [3*]www.alibaba.com

1.7 Environmental benefits from the utilization of lignin.

Environmental awareness and political actions are important keys that influence the market for lignin-based products. Major sources of lignin are found in cooking liquors generated in wood pulping processes. The main liquor is called “black liquor” which is used as energy conversion at the pulp industries; but new technology is aimed towards using that black liquor towards value-added material. In current polymeric material, there is a search to find bio-based and safe alternatives to oil-based, toxic and expensive adhesive components. For example, phenol and formadehyde adhesives are found in plywood and laminates. Another material, resins, have a significant impact on the CO_2 footprint, linked to the use of plywood resins.[94] Replacement to lignin reduced the formaldehyde usage since there is more than six million tons of phenol-formaldehyde resins produced annually.[92,89] With new lignin manufacturing technology, it can be further integrated in pulp and paper industrial mills.

1.8 Conclusion.

Lignin and its derivative biopolymers have a prospective array of commercial applications and can be used in pulp industry, plastic, agriculture, and petrochemical industry. In order to utilize lignin to its full potential, extraction, derivatization, and functionalization gives an insight in the development of products for common use. The direct and indirect extraction processes stated earlier has given researcher a platform to develop and optimize new methods for retrieving natural lignin. With the need for well-designed extractions leads to the development of better biomaterials, biodegradable films, composites, and applications in industry. Research in developing lignin to biobased derivatives will benefit the future.

Organosolv fractionation is a promising process for effective separation of lignin. A wide range of organic solvents are used in this study, including acetone, methanol, ethanol, organic acids (i.e., acetic acid, formic acid), inorganic and combined solvents with and without acid or alkaline catalysts are used in biomass fractionation. These solvents providing selective solubilization of lignin from various lignocellulosic biomass including fountain grass, walnut shell, and genetically mutated poplar. Short-chain alcohols such as methanol and ethanol in the form of single or solvent mixture with water are the most commonly used organic solvents in this process which can be used in the presence or absence of catalytic promoters. The purpose of this thesis dissertation is to show the optimization of the isolation of high-purity lignin from three different promising lignocellulosic materials: fountain grass, walnut shell, and poplar using the clean fractionation-based organosolv process. The isolated lignin samples were then characterized for their physiochemical characteristics. Product profiles from depolymerization of lignin fractionated from these different biomass samples were then taken and analyzed. This study provides a platform for further study on the value-added utilization of lignin in integrated biorefineries. The utilization of organosolv fractionation was taken and a combination of novel methodology developed for the upgrading of lignin into selective monomers for biofuels and biomaterial was performed.

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Chapter 2. Isolation and Catalytic depolymerization of lignin from Fountain Grass using Ni/C.

2.1 Introduction.

Lignocellulosic biomass is an abundant, renewable, non-edible, and environmentally friendly staple crop which has been a main alternative resource to petroleum-based fuels and platform chemicals. Research has been focused on transforming biomass into useful chemicals in order to reduce global dependence on fossil fuel resources. In the past decade, there has been an increase in progress towards the development of methods for conversion of biomass to chemicals and fuels.

Biomass is composed of three biopolymers: cellulose (30-60%), hemicellulose (20-40%) and lignin (10-30%).[1-3] There has been a lack of literature available with the utilization of lignin towards high-value chemicals. Most papers have focused on the upgrade of cellulose and hemicellulose with minimal review towards lignin.[4-7] In lignocellulosic biomass, lignin is the main constituent that is composed of aromatics but is underrated and considered a byproduct in paper waste industry.[8,9] Research directed towards the upgrade of lignin not only removes it as a waste product, but aids in accessing the carbohydrate polymers easier due to lignin usually acting as an inhibitor towards them.

Lignin is a major polymeric component in biomass and known as the second most abundant biopolymer, after cellulose.[10-12] The structure of lignin is compiled of phenols that are randomly cross linked to mainly hemicellulose and cellulose. The main function of lignin is adding strength to the cell walls and acting as a defense mechanism against pathogens and herbivores.[1-3] Lignin is a complex, cross-linked, hydrophobic and aromatic polymer that composes 15-50 wt. % of dry weight to a given biomass. The structure of lignin is highly branched with C-C bonds and ether linkages which are formed through oxidative radical polymerization. The oxidative polymerization occurs with three precursor monolignols: p-hydroxycinnamyl (coumaryl) alcohol, 4-hydroxy-3-methoxycinnamyl (coniferyl) alcohol, and 3,5-dimethoxy-4-hydroxycinnamyl (sinapyl) alcohol.[11-20] The three monomers are all typically found in grasses and for hardwood only two are found which is Coniferyl and Sinapyl alcohol. For softwoods, lignin mainly is composed of Coniferyl alcohol.

The three monomeric building blocks are p-hydroxyphenyl (H), guaiacyl (G), and syringol (S) which differ in the number of methoxy groups. The common interunit linkages found in lignin are β-O-4, 5-5, β-5, 4-O-5, β-1, and β-β.[14-17] The overall connectivity is randomly linked by given linkages: β-O-4 linkage is by far the dominant one in all species (45%-60%), followed by some other linkages such as β-5 linkage (10%-12%) and 5-5 linkage (10%-20%). Depending on the biomass source, the percentage of each linkage in lignin varies. Known as phenylpropanes, they contain varying substitutions at the 3 and 5 positions. These phenolic building blocks give lignin a higher face-value as a precursor towards producing high-value phenolic derivatives. Due to this, lignin depolymerization studies remains a popular study in research.

Fountain grass (*Pennisetum alopecuroides*) is a popular ornamental grass biomass that has been used as a landscape perennial. Although most plant species may be beneficial or harmless in a new environment, fountain grass is harmful economically and ecologically. It has been shown to cause damage and extinction towards edible crops and other plant species due to its invasive nature. As this invasive species spreads, it causes the displacement or destruction of native species and may cause radical, and possibly permanent, changes in native ecosystems.[18-20] Fountain grass is wind-dispersed, perennial, and grows in dense strands and grows aggressively; it acts as a fuel source for fires as well.[19] Fountain grass has invaded California, Arizona, and Hawaii during the 20th century and is listed as one of the top 8 invaders in California by the California Exotic Pest Plant Council (CalEPPC).[20-30] The main areas of growth in California is on the roadsides and ruderal areas but could result in the harm of natural habitats. Due to this, it has been an issue to keep fountain grass contained. The main way to control this invasive species is by physical labor or using herbicides to deplete its population in some areas.[20-35] Refocusing the way fountain grass can be contained can be geared towards it being a biomass staple crop. In order to see its potential as a biomass feedstock, analysis in the amounts of the different biopolymers must be taken in account. Afterwards, the structure of lignin can be determined and then utilized to give more additional support for lignin research studies.

Lignin utilization still needs more work added to this study due to the biopolymer's complex and recalcitrant structure. These obstacles hinder the valorization of lignin into high value aromatic compounds. Thus, using fountain grass for the added information and development of efficient methods for lignin depolymerization is important for both scientific and economic perspectives. In this work, we report on organosolv extraction of fountain grass using different solvents for lignin isolation. The organosolv lignin isolated was characterized by two-dimensional Heteronuclear Single Quantum Coherence Nuclear Magnetic Resonance (HSQC-NMR) and Gel Permeation Chromatography (GPC). The organosolv lignin was then taken and subjected to catalytic depolymerization (CDL) over heterogeneous Ni/C catalyst. Herein, we see that pre-treatment is required in order to isolate lignin from FG using the Soxhlet extraction. With this added, the performance of CDL was increased. Four monomeric phenolic products were obtained from lignin isolated at a high yield of 57%. These monomeric products were later subjected to other research in upgrading them to material.

2.2 Lignin Isolation from Fountain grass using Organosolv-Acetic Acid and Formic Acid.

Using acetic acid/formic acid/ water (AA/FA/H_2O) organosolv mixture was performed on a 16-gram scale in a 500 mL round bottom flask. To the 500 mL round bottom flask, 150 mL of AA/FA/H_2O (50:30:20 ratio) was added. The contents in the round bottom flask was stirred for 1 hour at 60°C then increased to 110°C for 3 hours at reflux. The reaction was cooled to room temperature then the solid and liquid phases separated by filtration. The solid was washed with 0.5 N acetic acid 5 times then the filtrate was taken and concentrated for the extracted lignin. To the concentrated liquid, 200 mL of nano pure water was added in order to precipitate the lignin. Once precipitated, the lignin was collected by filtration and then analysed by Heteronuclear Single Quantum Coherence Nuclear Magnetic Spectroscopy (HSQC-NMR) and Gel Permeation Chromatography (GPC). In Figure 2.1 and Table 2.1 are the HSQC-NMR and GPC of the isolated organosolv lignin from AA/FA/H_2O.

2.3 Lignin Isolation from Fountain grass using Organosolv-MeOH.

Fountain Grass was taken and studied in methanol/water and acetic acid/formic acid/water mixture in order to obtain isolated lignin from both processes. In the methanol/water treatment, 2 grams of grounded raw biomass was added with 25 mL 0.045 N H_2SO_4, 25 mL MeOH, and 4 mL 37 wt % aqueous formaldehyde. The reaction was purged with H_2 gas 5 times and stirred at 700 rpm. 10 bar of H_2 gas was added to the Parr® vessel and the system was heated at 160 °C for 0.5 hour. Once cooled to room temperature, the contents of the Parr® reactor were filtered to separate the solid residue from the filtrate. The filtrate containing MeOH and dilute acid was concentrated to remove the MeOH. Once all MeOH was removed, the remaining aqueous layer was then filled with 150 mL of nano pure water to crash out the lignin. The extracted lignin is then collected by filtration and washed 3 times with nano pure water. The solid lignin was then dried overnight then verified by HSQC-NMR and GPC. In Figure 2.1 and Table 2.2 is the HSQC-NMR of the isolated organosolv lignin from MeOH.

Table 2.1. Assignments on the common carbon-proton correlations of organosolv lignin in HSQC-NMR spectra (a) side chain region and (b) aromatic region.

(a)

Carbon-proton correlations	**Assignments**[I]
71.0-72.0/4.9-5.1	A_α
83.0-84.0/4.3-4.5	A_β (H,G)[II]
86.0-87.0/4.1-4.3	A_β (S)[III]
60.0-61.0/3.4-3.7	A_γ
84.0-85.0/4.6-4.7	B_α
53.0-54.0/3.0-3.1	B_β
71.0-72.0/3.8; 71.0-72.0/4.2	B_γ
86.8-87.1/5.5-5.6	C_α
53.0-54.0/3.5-3.6	C_β
61.6-62.5/3.7-3.8	C_γ

[I] For the assignments on carbon-proton correlations for the side chain region, A is used to represent β-O-4 linkage, B is used to represent β-β linkage, C is used to represent β-5 linkage. A_α is for the α position of β-O-4 linkage, B_α for the α position of β-β linkage, C_α for the α position of β-5 linkage, etc.

[II] β position of β-O-4 linkage in H and G type lignin.

[III] β position of β-O-4 linkage in S type lignin.

(b).

Carbon-proton correlations	**Assignments**[I]
128.0-129.0/7.2-7.3	$H_{2,6}$
114.0-115.0/6.7-6.9	$H_{3,5}$
110.0-111.0/7.0-7.1	G_2
114.0-115.0/6.7-6.9	G_5
118.0-119.0/6.8-6.9	G_6
103.0-104.0/6.6-6.8	$S_{2,6}$
130.0-131.0/7.7-7.8	$H'_{2,6}$[II]
105.0-106.0/7.3-7.4	$S'_{2,6}$[III]

[I] For the assignments on carbon-proton correlations for the aromatic region, H is used to represent H type lignin, G is used to represent G type lignin, S is used to represent S type lignin. $H_{2,6}$ for the 2 and 6 positions of H lignin, G_2 for the 2 position of G lignin, $S_{2,6}$ for the 2 and 6 positions of S lignin, etc.

[II] Oxidized H unit (p-Hydroxybenzoic Acid).

[III] Oxidized S unit (Syringic Acid)

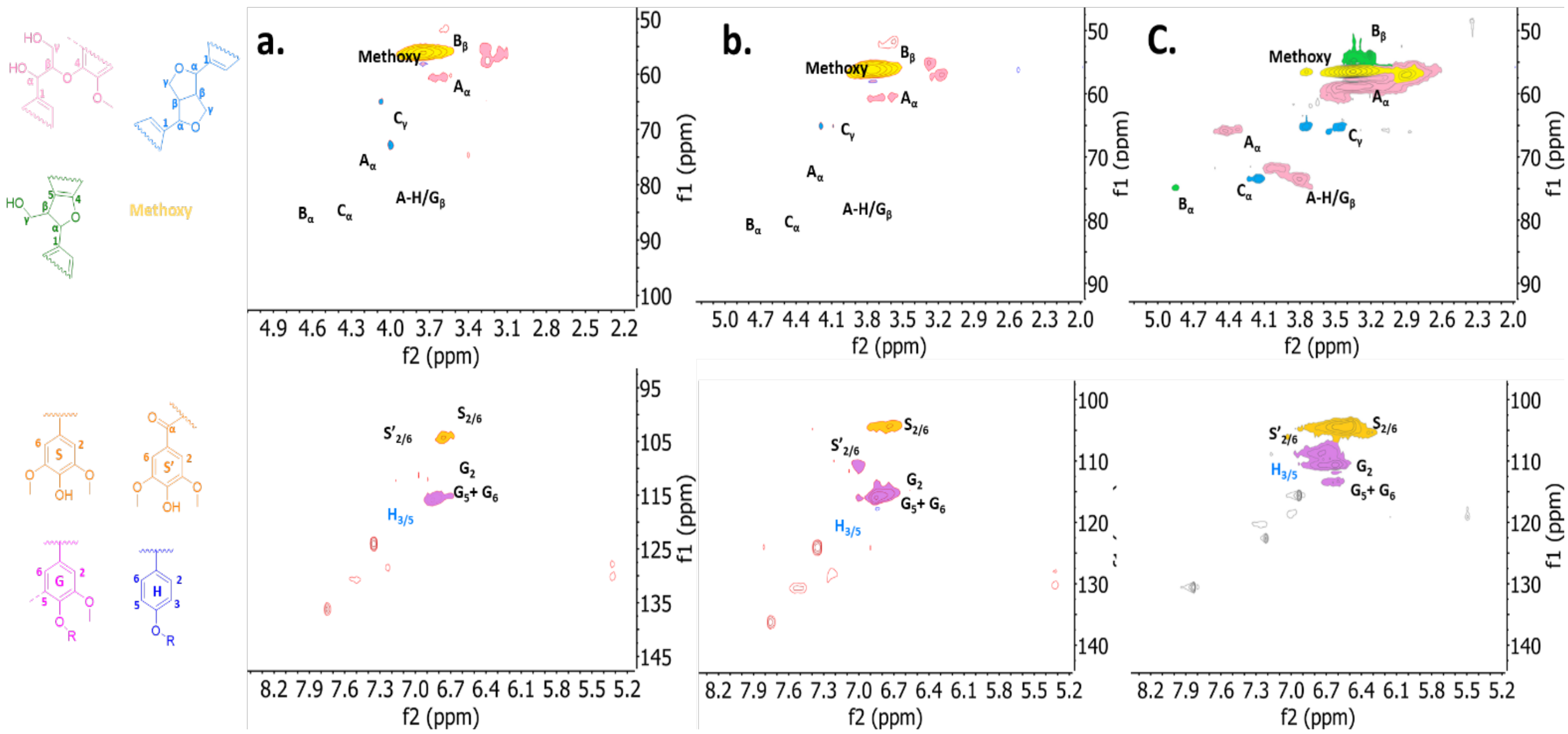


Figure 2.1- Two-dimensional HSQC-NMR spectra of aliphatic region and the aromatic region (δ_C/δ_H 100-145/5.2-8.2) using two different treatment media for organosolv fountain grass lignin: AA/FA/H_2O (a), MeOH (b), and (c) Control spectra of MeOH Poplar Organosolv Extraction

Table 2.2- GPC data for AA/FA/H_2O and MeOH.

Lignin Sample	M_n (g/mol)	M_w (g/mol)	PDI
AA/FA/H_2O	751-891	1906-2064	2.7-4.31
MeOH	853-1032	1511-3345	3.96-4.12
MeOH-Poplar	1300-1600	3000-6000	1.30-5.3

The comparison of HSQC-NMR for AA/FA/H_2O (a) and MeOH (b) to the control spectra (c) showed that there was lignin isolated, but not great quality due to the missing linkages found between 3.5-4.4 ppm in the aliphatic region and 6.4-7.3 ppm for the aromatic region. This states that the lignin extraction for both methods was not delivering intact lignin. Using gel permeation chromatography (GPC), weight average (M_w) and number average (M_n) were given for isolated FGL samples. Comparing the FGL samples analyzed, AA/FA/H_2O resulted with the smaller molecular weight and MeOH was the highest. However, AA/FA/H_2O and MeOH are not near the range of the control lignin sample, MeOH-Poplar which is validated in **Table 2.2** where the GPC M_n and M_w are lower than the expected isolated lignin (**Table 2.1**). To analyze the content of the types of linkages that were intact in the organosolv lignin, the characteristics of the lignin were calculated in **Table 2.3**.

Table 2.3- Characteristics of the organosolv fountain grass lignin.[a]

Type	S	G	H	β-O-4	β-β	β-5
FGL-AA/FA/H_2O	43	23	b	43	6	<2
FGL-MeOH	45	25	b	46	6-7	<2
MeOH-Poplar	60	41	b	79	18	<3

[a]S: syringyl units, G: guaiacyl units, H: p-hydroxyphenyl units. [b]Too small to ascertain. [c]$\%X = X/(S_{2/6} + S'_{2/6} + G_2 + G_5 + G_6)$, $X = S_{2/6} + S'_{2/6}$, $G_2 + G_5 + G_6$. [d]$\%Y = Y/(A_\alpha + B_\alpha + C_\alpha)$, $Y = A_\alpha, B_\alpha, C_\alpha$

Aromatic region between 130-100/7.3-6.1 ppm and a side chain region between 90-50/5-2 ppm is shown in Figure 2.1. The side chain region shows the assignment of several peaks to the methoxy group and interunit linkages of β-O-4, β−β, and β-5. These linkages can be calculated by the integration of the contour signals. Based on integration of β-aryl ether (Aα), phenylcoumaran (Bα), and resinol (Cα) contour signals, the linkage percentages of these three linkages in FG lignin for AA/FA/H_2O and MeOH are 43, 46% β-O-4, 6, 7% β−β, and a small amount of β-5 (see Table 1), respectively. The aromatic region of the HSQC spectra also show the distribution of H:G:S (see Figure 1 caption). Similarly, the integration of (S2/6 + S′2/6)/(G2 + G5 + G6) contour signals shows that FG lignin contains half the amount of syringyl units as well as guaiacyl units in comparison to the control, MeOH-Poplar(see Table 1). Due to this discrepancy, further experiments were run to find the cause for the decrease in organosolv lignin extraction.

2.4 Soxhlet extraction performed on Fountain grass.

On the outside stems of the fountain grass are different waxes and oils that coat the biomass which could potentially inhibit the process in lignin isolation. To test this hypothesis, Soxhlet extraction was performed on the fountain grass to remove these oils and waxes. How the extraction works is it transfers partially soluble compounds of the biomass to the liquid phased using the Soxhlet extractor. Using the NREL procedure, 10 grams of fountain grass was loaded to the glassware in a filter paper thimble which was placed into the extractor and using first water to remove water-soluble compounds then using ethanol to extract organic soluble compounds. The compounds collected in both water and ethanol then are taken and weighed in order to determine how much was loss from a 10-gram scale. In fountain grass, 0.5 grams of water-soluble oils were obtained, and 0.45 grams of organic soluble oils were obtained. Shown in **Figure 2.2** is the comparison of organosolv-MeOH weighed after runs with and without Soxhlet extraction.

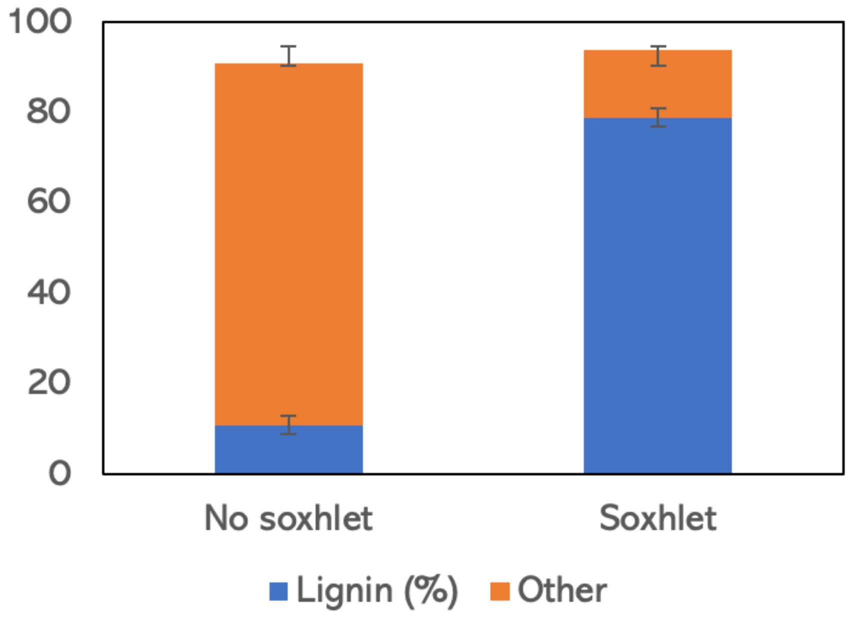


Figure 2.2- Fountain grass lignin isolated with and without Soxhlet extraction.

In **Figure 2.2** what is noted is that the mass balance is both around the same for with and without Soxhlet ranging from 82-95%. However, the extraction of lignin is significantly higher when using Soxhlet, giving isolation yields averaging 79%. Whereas for no soxhlet, there was an average of 12% lignin isolated. This states that when leaving the oils on the fountain grass, this acts as an inhibitor for the organosolv system, preventing a clean separation of lignin from the other carbohydrates.

2.5 HSQC-NMR Analysis of Soxhlet extraction performed on Fountain grass.

Fountain Grass was taken and studied in based on the addition of a pre-extraction method called Soxhlet extraction to remove any contaminants such as oils and waxes from our: methanol/water and acetic acid/formic acid/water mixture in order to increase the amount of isolated lignin from both processes and lower the recondensation occurring. The solid lignin obtained from AA/FA/H_2O and MeOH Organosolv lignin extraction was dried overnight then verified by HSQC-NMR and GPC. In **Figure 2.3 and Table 2.4** is the

HSQC-NMR of the isolated organosolv lignin from MeOH and the calculations on the content of linkages for both extractions.

The comparison of HSQC-NMR for AA/FA/H_2O (left) and MeOH (right) in Figure 2.3 to Figure 2.1 shows a significant visual improvement to the contouring of the linkages found between 3.5-4.4 ppm in the aliphatic region and 6.4-7.3 ppm for the aromatic region. This states that the lignin extraction was improved by the addition of the soxhlet extraction step for both methods and delivered better quality/intact lignin.

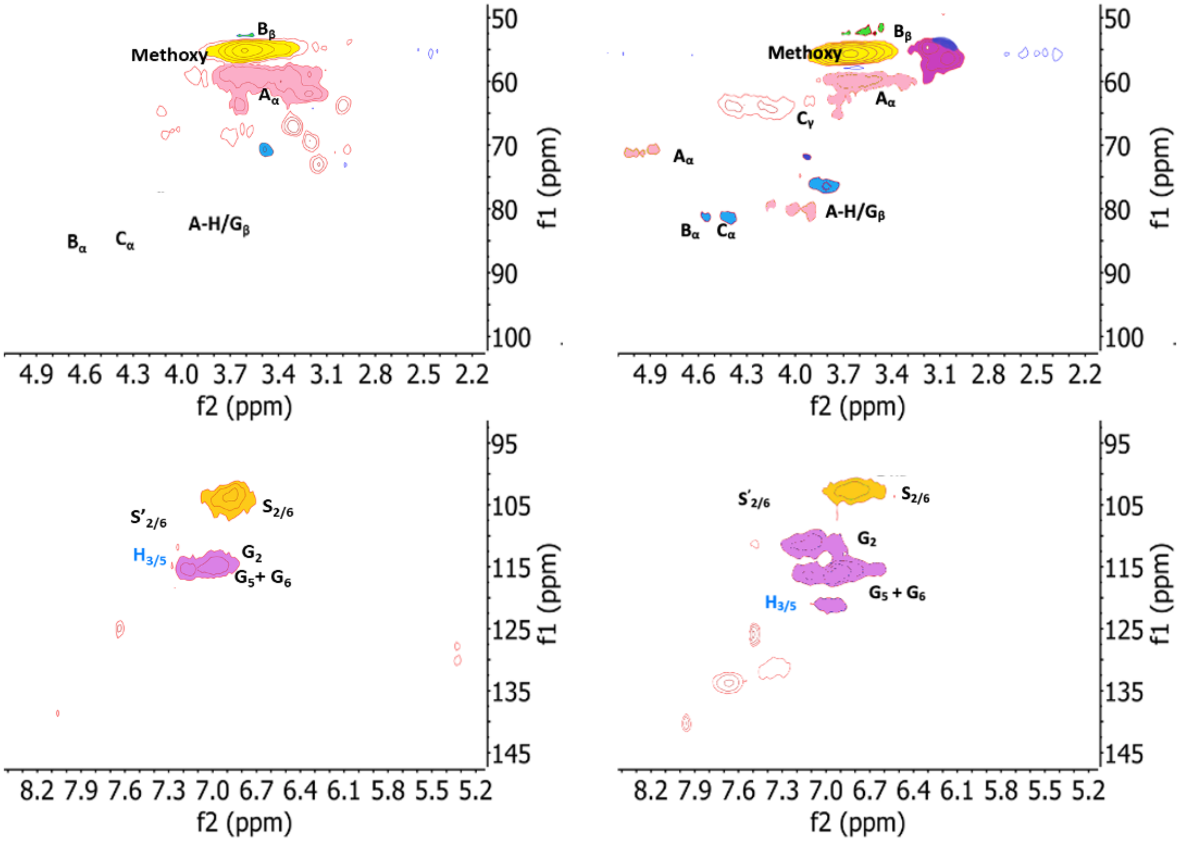


Figure 2.3- Two-dimensional HSQC-NMR spectra of aliphatic region and the aromatic region (δ_C/δ_H 100-145/5.2-8.2) using two different treatment media for soxhlet-organosolv fountain grass lignin: AA/FA/H_2O (left) and MeOH (right).

Table 2.4- Characteristics of the organosolv fountain grass lignin.[a]

Type	S	G	H	β-O-4	β-β	β-5
FGL-AA/FA/H_2O	43	23	b	43	6	<2
FGL-MeOH	45	25	b	46	6-7	<2
MeOH-Poplar	60	41	b	79	18	<3

[a]S: syringyl units, G: guaiacyl units, H: p-hydroxyphenyl units. [b]Too small to ascertain. [c]$\%X = X/(S_{2/6} + S'_{2/6} + G_2 + G_5 + G_6)$, $X = S_{2/6} + S'_{2/6}$, $G_2 + G_5 + G_6$. [d]$\%Y = Y/(A_\alpha + B_\alpha + C_\alpha)$, $Y = A_\alpha, B_\alpha, C_\alpha$

2.6 Organosolv lignin analysis by gel permeation chromatography (GPC).

Using gel permeation chromatography (GPC), weight average (M_w) and number average (M_n) were determined for isolated FGL samples. Comparing the FGL samples analyzed, Soxhlet FG resulted with the smaller molecular weight and Without Soxhlet FG was the highest. In the study of fountain grass with Soxhlet, studies suggest that the use of acetone in our AA/FA/H_2O, stabilizes the carbocation intermediate that is formed during the AA/FA/H_2O Organosolv reaction to produce relatively small molecular weight monomers due to the acetone being a polar aprotic solvent. When we look at **Table 2.5**, it shows that the PDI is narrower in comparison to the FG without Soxhlet, indicating the need for pre-extraction before the performance of the organsolv process. The same is noted for the methanol soxhlet organosolv extraction where the same trend is shown where we have an improvement in the lignin isolated with a polydispersity index (PDI) being a narrow range of the molecular weight distribution. The given molar mass of the lignin monomers is about 200 g mol^{-1}, thus the range of the observed M_w for both AA/FA/H_2O and MeOH of with/without Soxhlet indicates that the isolated organosolv lignin chains are 10-12-mers.

Table 2.5. Weight average molecular weight (M_w), number average molecular weight (M_n), and polydispersity index (PDI) of isolated lignin from Acetic Acid/Formic Acid (AA/FA), Acetone, and MeOH treatments.

Fountain grass Substrate	Organosolv Lignin Extraction[a]	Isolated Lignin (%Yield)[b]	M_w (kg mol^{-1})	M_n (kg mol^{-1})	Polydispersity Index (PDI)
Soxhlet	AA/FA	**40-52**	**2.6**	**1.8**	**1.41**
Without Soxhlet	AA/FA	**9-16**	**3.5**	**2.3**	**2.17**
Soxhlet	Methanol	**60-79**	**2.1**	**1.5**	**1.61**
Without Soxhlet	Methanol	**10-14**	**3.2**	**2.5**	**2.48**

[a] Organosolv lignin extraction medium: AA/FA = acetic acid/formic acid and H_2SO_4/formaldehyde , Methanol = MeOH/water and H_2SO_4/formaldehyde.

[b] Lignin isolated yields are calculated based on the theoretical amount of lignin in raw biomass. Isolated lignin (%) = $\frac{Isolated\ Lignin\ (g)}{Theoretical\ Lignin\ (g)} \times 100$

2.7 Catalytic Depolymerization of fountain grass organosolv lignin.

After optimizing the performance of organsolv lignin extraction with the aid of Soxhlet extraction, the FG lignin was taken and subjected to catalytic depolymerization. Catalytic depolymerization fo lignin (CDL) was performed with the addition of Ni/C as the catalyst. Lignin was depolymerized and deoyxygenated using 10-wt.% Ni/C catalyst as reported by Luo et. al.[36,37] In a 75 mL Parr® vessel, 50-100 mg WSL or raw WS was combined with 5-10 mg 10 wt% Ni/C catalyst and 20 mL MeOH. After purging with H_2 five times, the reaction was run under 35 bar H_2 at 225 °C for 12 h while stirring at 700 rpm. Once the reaction was completed and cooled, the contents were filtered to remove solid catalyst, which was rinsed with three 3 mL portions of MeOH. Wash and filtrate were combined and concentrated by rotary evaporation until an oil was produced. The oil was further dried under vacuum in a tared vial untill the mass stabilized. The final product was analyzed by HPLC with a Zorbax Eclipse XDB-C18 column (250 × 74.6 mm) set at 30°C. The aromatic biophenol products were compared and quantified versus calibration curves of standards. All experiments were replicated at least three times. Yields are reported as wt. % of starting acid soluble lignin in dry FG material.

From the CDL of isolated organosolv lignin, there were 4 major phenolic products with alkenyl side chains that are shown in **Table 2.5 and Figure 2.4**. Results show a high selectivity of Ni/C catalyst towards the depolymerization of FG organosolv lignin with mass balance of the four major products and byproducts which was reported to achieve 90-93% mass recovery (**Table 2.6**).

Figure 2.4. Aromatic products (HPLC-UV) after catalytic depolymerization of (a) organosolv lignin- AA/FA/H_2O and (b) oganosolv lignin-MeOH.

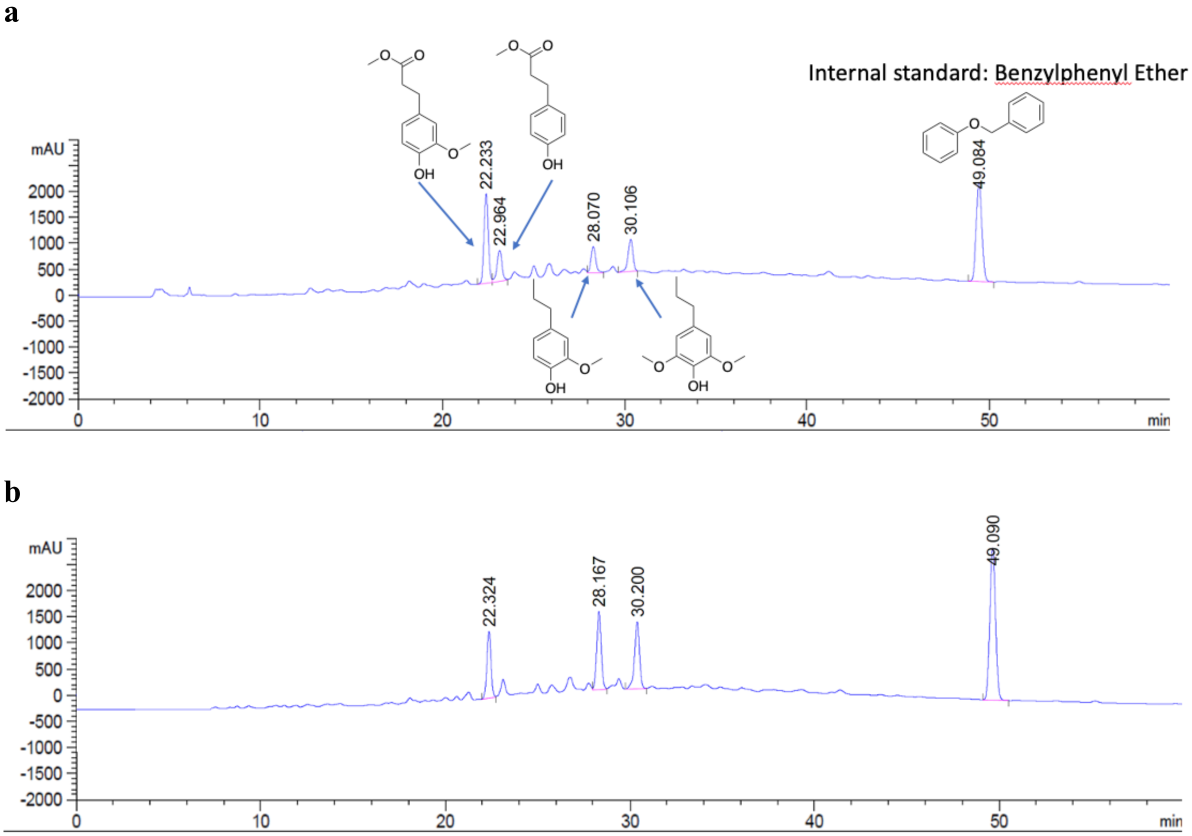
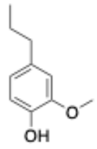
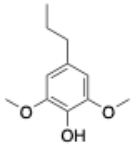
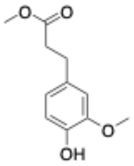
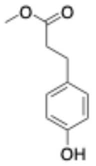


Table 2.5. Catalytic depolymerization of organosolv lignin from different treatment with 10 wt% Ni/C catalyst.[a]

Organosolv Treatment Medium[b]	Poplar Variant	% Yield of Dihydroeugenol 	% Yield of 2,6 dimethoxy-4-propylphenol 	% Yield of methyl 3-(4-hydroxy-3-methoxyphenyl)propanoate 	% yield of methyl 3-(4-hydroxyphenyl)propanoate 	% Total Yield[c]
Methanol	Soxhlet FG	9	21	19	8	57
AA/FA/H_2O		5	8	10	3	26

[a] Organosolv lingin (50 mg) with 10wt% Ni/C catalyst in 20 mL of MeOH, at 225 °C and 35 bar H_2 for 12 h.

[b] Organosolv lignin samples were named as the solvent used for extraction followed by the specific poplar species.

[c] Yields are calculated based on initial organosolv lignin substrate.

Table 2.6. Mass balance calculation after catalytic depolymerization of organosolv lignin.[a]

Substrates[b]	Toluene soluble aromatics%	Water precipitated oligomer & unconverted organosolv lignin%	Total%[c]
AA/FA/H_2O - FG	26	65	91
Methanol-FG	57	36	93

[a] Organosolv lignin (50 mg) with 10wt% Ni/C catalyst in 20 mL of MeOH, at 225 °C and 35 bar H_2 for 12 h.

[b] Organosolv lignin samples were named as the solvent used for extraction followed by the specific poplar species.

[c] Total mass balance is calculated based on initial organosolv lignin substrate.

As can be seen from **Table 2.5.** and **2.6**, products yield for organosolv lignin made from the methanol treatment is significantly higher than from the AA/FA/H_2O treatment. This is attributed to the degree of lignin re-condensation during the organosolv process. When methanol is used it acts as a protecting group on the C_α position minimizing C-C bond formation. The use of acid during organosolv extraction leads to irreversible condensation of extracted lignin. In specific, the protonated hydroxyl group on C_α becomes a good leaving group, resulting in the formation of carbocation. This carbocation can attack another electron rich aromatic ring in lignin to form stable carbon-carbon bonds. The resulting structure is highly recalcitrant towards further upgrading because carbon-carbon bonds are quite inert towards hydrogenolysis catalysts.[38,39] Methanol prevents the formation of carbon-carbon bonds by reacting with the carbocation to form C-OMe linkages. MeO- Cα is observed in 2D-HSQC NMR spectra (**Figure 2.3**). Organosolv lignin from AA/FA/H_2O did not perform as well as a protectant in comparison to MeOH.

Based on our study, catalytic depolymerization of organosolv lignin into monomeric phenolic products is feasible if lignin re-condensation is prevented or minimized with a pre-extraction by Soxhlet extraction. In case of organosolv lignin depolymerization, four products were formed. Catalyst and product separation is straightforward. As all the monomers together with unconverted organosolv lignin are soluble, simple filtration can efficiently separate the liquid phase from solid catalyst. Finally, residual unconverted lignin can be precipitated subsequently and separated from the four phenolic products.

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Chapter 3. Organosolv Fractionation of Walnut Shell Biomass to Isolate Lignocellulosic Components for Chemical Upgrading of Lignin.

3.1 Abstract.

Renewable carbon sources are a rapidly growing field of research because of the finite supply of fossil carbon. The lignocellulosic biomass walnut shell (WS) is an attractive renewable feedstock because it has high lignin content (38-44 wt. %) and is an agricultural waste stream. Lignin, a major component of lignocellulosic biomass that is currently a waste stream in pulping processes, has unique potential for chemical upgrading because its subunits are aromatic. In the interest of improving the sustainability and reducing the environmental impact of biomass processing, valorization of agricultural waste streams is important. Herein, three lab-scale, batch organosolv procedures are explored in the interest of optimal isolation of protected WSL (walnut shell lignin). One system uses acetic acid, one MeOH, and the final EtOH as the primary solvent. The optimal condition for protected WSL isolation, which resulted in 64% yield, was methanol and dilute sulfuric acid with formaldehyde to act as a protecting group at 170 °C. Select samples were upgraded by hydrogenolysis over a nickel catalyst. Protected lignin recovered from the optimal condition showed 77% by weight conversion to monomeric phenols, demonstrating that the protected WSL can selectively afford high value products. One key finding from this study was that MeOH is a superior solvent for isolating WSL versus EtOH because the latter exhibited lignin recondensation. The second was that the Ni/C catalyzed reductive catalytic fractionation (RCF) directly of WS biomass was not selective relative to RCF of isolated WSL; conversion of raw WS to monomers produced significantly more side products.

3.2 Introduction.

Industrial scale chemical manufacturing is currently dependent on carbon in the form of coal, natural gas, and liquid oil as feedstock.[1] Increasing consumption of single use plastics and other products made from these non-rewable feedstocks is a positive climate forcer. To mitigate global warming and to move towards a more sustainable industrial system for chemical and material production, an alternative carbon feedstock is necessary. Non-food lignocellulosic biomass is a promising alternative; it is renewable and readily available in existing agricultural waste streams.[2] The primary components of typical lignocellulosic biomass are acid soluble (ASL) and insoluble lignin (AIL) (combined 10-25 wt. %), cellulose (40-60 wt. %), and hemicellulose (20-40 wt. %).[3] Non-organic or other unquantified material in biomass is categorized as ash, which is subcategorized as acid soluble (ASA) and insoluble (AIA).

In recent years, many studies have focused on the chemical valorization of lignin, a structural component of biomass. Lignin is an amorphous, three-dimensional polymer composed primarily of three aromatic monomers (Figure 1a), which vary by species in ratio and linkage frequency. Notably, β-O-4 bonds make up 40-60% of all linkages in lignin, and β-5 bonds 6-12% (Figure 1b).[4] As an abundandant, naturally occuring aromatic polymer, lignin has unique potential to serve as a feedstock for bulk aromatic chemicals if effective and economic depolymerization methods can be realized.[5,6]

Currently the presence of lignin in industry is primarily as a byproduct of pulping processes such as Kraft. The black liquor produced by Kraft pulping, which is comprised of highly degraded lignin, is commonly burned for heat, while intact cellulose fibers are used to produce valuable products such as paper.[7] Delignification pretreatment is vital to cellulose fed processes because entanglement between the structural lignin network and linear cellulose makes lignocellulosic biomass recalcitrant.[8-11] Hence, lignin removal and valorization has become a hot reseach topic, with notable industrial progress such as the product "liquid wood," registered as ARBOFORM by TECNARO.[12]

There is a variety in composition between all the available lignocellulosic species, so certian species will be better suited for production of certian chemical compounds. Woody biomass is between 14-25% lignin depending on if it’s soft versus hard wood, and many of these biomass feedstocks have been well-studied.[13,14] Grass-type biomass ranges from 15-20% lignin and has ferulate/diferulate linkages in their cell walls. Under hydrodeoxygenation (HDO) conditions, their isolated monomers have methyl ferulate tails.[15-17] Nut shells, which contain 26-45% lignin, fall into niether of the preceding categories.

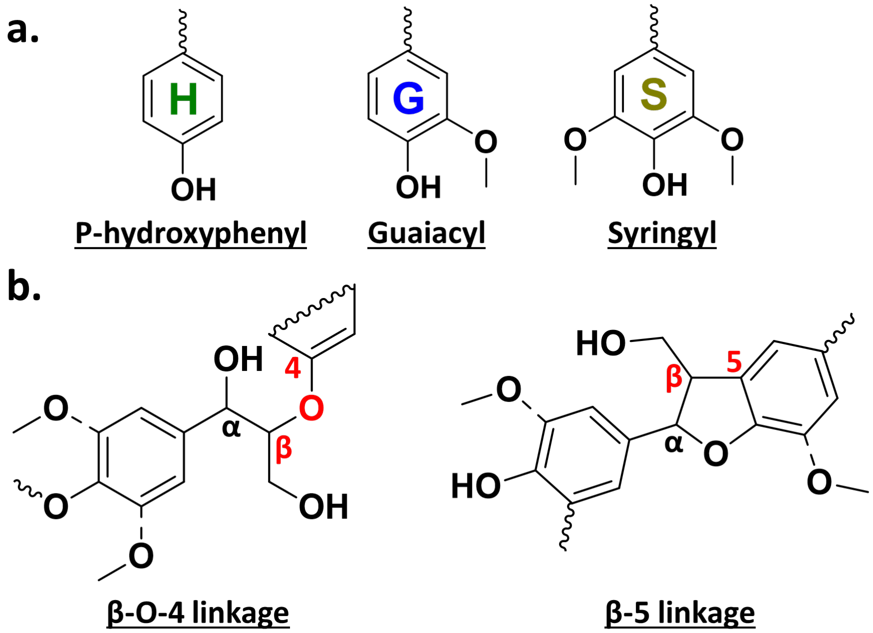


Figure 3.1: Lignin (a) monomers and (b) predominant linkages.

While they have been studied to some degree, nut shell lignins are less characterized.[18,19] Due to large variation in lignin content, ash content, and lignin character, lignocellulosic feedstocks must be studied by species to determine if they are amenable to pretreatment and upgrading. Methods that work for one biomass may not necessarily work for another due to the complexity of the feedstock.[20]

Industrial scale agriculture uses large amounts of land, fertilizer, pesticides, and water. Implications of these demands are habitat loss, disruption in the water cycle, environmental contamination and injustices, and distruptions in local ecologicial cycles.[21] Therefore, it is sensible to use agricultural waste that is already being generated rather than fueling new agricultral growth. In California alone, walnuts are the sixth most valuable crop, valued at $1.3 billion in 2019.[22] The only other nuts in the top fifty highest value crops are pistachio in fourth place, and almonds in first. Thus, any of these nut-shell waste streams is a potentially untapped resource for non-food lignocellulosic material. In this study, WS was investigated because it has both the highest lignin content by mass and the highest crop yield by land area (Figure 2).[22,23]

Notable past work using WS biomass with a variety of different organosolv systems, lignin valorization methods, and characterization approaches have been reported, although no comprehensive studies were found.[24-30] Other examples of low environmental impact and abundant biomasses used in similar studies are plant trimmings such as banana leaf, other nut shells, or invasive species.[18,19]

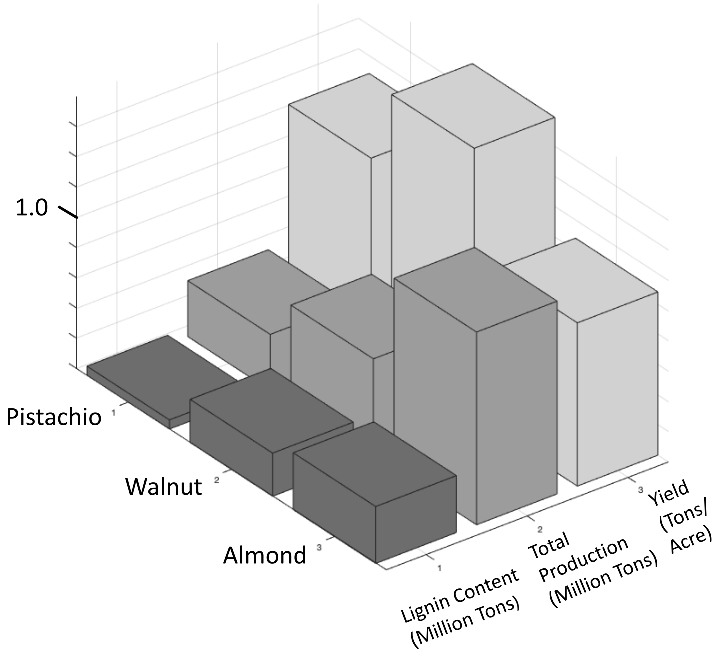


Figure 2. Lignin content, crop production, and crop yield comparing pistachio (P), walnut (WS) and almond (A).[21,46]

A process called reductive catalytic fractionation (RCF) of lignocellulose is an emerging biorefinery method that combines the fractionation of biomass with lignin depolymerization, referred to as "lignin first" strategy.[31-35] Currently, the literature for RCF of WS has not been reported. Rather, the main focus of the utilization of walnut shell waste has been through pyrolysis. Naderi et al. reported the use of water and high temperature (300 °C) to convert walnut shell into hydrochar.[36] However, these were performed on 10-30 mg scales of WS and the converted WS overrall is turned into carbon-densified hydrochar, which needs further characetrization. Kar et al. reported pyrolysis on WS which yielded 30% which required harsh conditions in order to convert the WS to hydrochar. [37]

Organosolv pretreatment was chosen as the delignification method of focus after direct RCF of WS was found to give a large mixture of untractable products. Organosolv lignin isolation can be accomplished under relatively mild conditions using alcoholic medium, which circumvents the need for toxic volatile organic solvents (VOC's). Organosolv lignin extraction for valorization and pretreatment purposes has been widely reported as an effective, green, and non-destructive delignification method.[4,11,28-46] The non-destructive nature of organosolv delignification as a pre-treatment is especially attractive because cellulose valorization processes often require harsher conditions which lead to lignin recondensation. To reduce waste, it is important that lignin fractions are isolated and stabilized while leaving cellulose intact, so that utilization of the whole biomass is possible.[47]

In this study, three organosolv methods for WSL removal are investigated. The first (**System A**), a formic acid (FA)/acetic acid (AA)/water system under atmopsheric pressure, was found to be the least effective; with lignin yields ranging from 23-27%. The second system, refluxing 5-7 wt.% hydrochloric acid (HCl) in ethanol (EtOH) or methanol (MeOH) at ambient pressure (**System B);** was found to give high product recovery, up to 70% yield of isolated lignin, but with severe loss in lignin quality due to recondensation. The third system (**System C**), organosolv in aqueous sulfuric acid (H_2SO_4), MeOH, and formaldehyde under 15-20 bar hydrogen (H_2) in a Parr reactor; gave 60-64 % yield of stablized lignin. This lignin showed high selectivity and yield for monomeric products when subjected to our chosen method of Ni/C catalyzed hydrogenolysis. In contrast, the same catalytic hydrogenolysis reaction applied directly to raw WS gave a large mix of products, which cannot be easily upgraded.

Our three systems were selected due to the acccesibility of the solvents and reagents as commodity chemicals. EtOH is the major product of biorefining. AA is a commodity chemical that is less hazardous than halide based acids such as HCl. Finally, formaldehyde was added to the organosolv process because of its recognized protecting group reactivity; it helps prevent carbocation based recondensation of lignin as has been described by Luterbacher.[47]

Organosolv lignin products were analyzed by heteronuclear single quantum coherence nuclear magnetic resonance (HSQC-NMR) and gel-permeation chromatography (GPC). WSL which was deemed intact by these analysis methods was subjected to hydrogenolysis over a nickel catalyst.

3.2 **Experimental Section.**

3.2.2 Materials.

Untreated, ground WS was purchased from Agra Grit in 35/50 particle size. 10 wt. % nickel catalyst on activated carbon support (Ni/C) was synthesized following previously reported procedures.[47,48] Formic acid (88 wt.% in water) and methanol were purchased from Fischer. Sulfuric acid (95-98 wt.%), hydrochloric acid (38 wt.%), and acetic acid (glacial, 99 wt.%) were purchased from Sigma-Aldrich. Ethanol (200 proof) was purchased from Gold Shield. Commercial chemicals were used as received. Water was filtered to nano-purity at 18-Ω resistance by a Millipore "Q-Guard 2" machine. Reactions which took place under pressure were done in a Parr® 5000 multi reactor instrument.

3.2.3 WS Composition Analysis.

Composition of WS biomass was analyzed following the NREL procedure.[49] A scale of 300 mg of raw WS biomass was combined with 3 mL 72 wt.% H_2SO_4 in a dry pressure tube and stirred with a dry glass rod. The tubes, still equipped with stir rods, were placed on a shaker rack immersed in a 35°C water. Starting at 0 minutes, the contents of each pressure tube were stirred every 10 minutes for one hour while continuously shaking.

At the end of the hour, 84 mL of nanopure water was added to each pressure tube. Pressure tubes with the mixtures were then capped, agitated by inversion, and autoclaved on liquids setting; 121 °C for 1 h. Tubes were allowed to cool completely to room temperture before removal of the cap. They were inverted before aliquots were removed to ensure homogeneity.

To measure AIL, solids post-hydrolysis were removed by gravity filtration and allowed to dry for 4-6 hours in a 105°C oven, then weighed to obtain oven dry weight. In a tared crucible, the dry solid underwent pyrolysis in a furnace on ramp setting with the program; room temperature to 105 °C, hold at 105°C for 12 minutes, ramp to 250°C at 10°C/min., hold at 250°C for 30 minutes, ramp to 575°C at 20°C /min., hold at 575°C for 180 minutes, temperature drop to 105°C, then hold until a full 24 h from initial ramp is complete. Contents of the crucible after pyrolysis were AIA, the mass of which was subtracted from the oven dry weight taken earlier, to give the mass of AIL. The filtrate from the pressure tubes was diluted a factor of 10 with water. UV-VIS measurement of the resulting solution was used to calculate a range of ASL content assuming Beer's Law was obeyed. The known extinction coefficients published in the referenced NREL procedure were used to give upper and lower limits for ASL content.[38] Agilent Technologies 1260 Infinity high performance liquid chromotgraphy (HPLC) equipped with UV and refractive index detection was used to quantify xylose, glucose, and fructose against a 10 mM tert-butanol internal standard and the corresponding calibration curves. Composition analysis was replicated five times, and final values were determined by averaging results weighted by starting biomass.

3.2.4 Biomass Fractionation.

Biomass fractionation methods were optimized starting from published procedures.[23,50] Organosolv methods differ in solvent systems, but generally follow the pathway in Figure 3. Three solvent systems were chosen for this study; formic acid/acetic acid/water (FA/AA/H_2O) [**System A**], EtOH or MeOH and concentrated HCl [**System B**], and H_2SO_4 (aq.)/MeOH/formaldehyde [**System C**]. For **System A**; 20/30/50 v/v/v of FA/AA/H_2O solution was combined with biomass in a 10 mL/1g ratio in a round bottom flask. The flask was then heated to 60°C for 1 h, then 110-135°C for 3 h, with constant stirring at 500 rpm. For **System B**: 5-10 % volume concentrated HCl in EtOH or MeOH was combined with biomass in a 10mL/1g ratio in a round bottom flask, then heated to reflux at 80-120°C for 6 h while stirring at 500 rpm. For **System C**: in a 75 mL stainless steel Parr® vessel, 2 g biomass was combined with 20 mL MeOH, 20 mL 0.045 N H_2SO_4, and 4 mL formaldehyde. The reactor was sealed and purged five times with nitrogen (N_2) and then heated for 30 minutes under 15-20 bar N_2 while stirring at 700 rpm.

Workup for each system was comparable. The organosolv solution was allowed to cool, and then the biomass pulp was removed by filtration and rinsed with EtOH or MeOH depending on starting solvent, or 0.05 M AA in the case of **System A**. The washes and filtrate were combined and concentrated to approximately 10% original volume by rotary evaporation. For **System B**, the resulting concentrate was added dropwise to 150 mL water while stirring at 200 rpm. Once the dropwise addition was complete, another 100 mL of water was added. For **Systems A and C**, water was added to the concentrate to bring the volume up to twice the original. For all three systems, after water was introduced, the solution was placed in a refrigerator overnight and then filtered. Water was used to rinse the recovered precipitated WSL. The WSL was air dried overnight and then weighed. The organosolv lignin (WSL) was analyzed by GPC and HSQC-NMR. Let it be noted that lignin recovered from **Systems A and C** are orange-brown in color, while lignins recovered from **System B** are pale pink-purple. Each organsolv experiment was replicated at least three times. Yields are reported as wt.% of theoretical acid soluble lignin (ASL) in starting dry WS material.

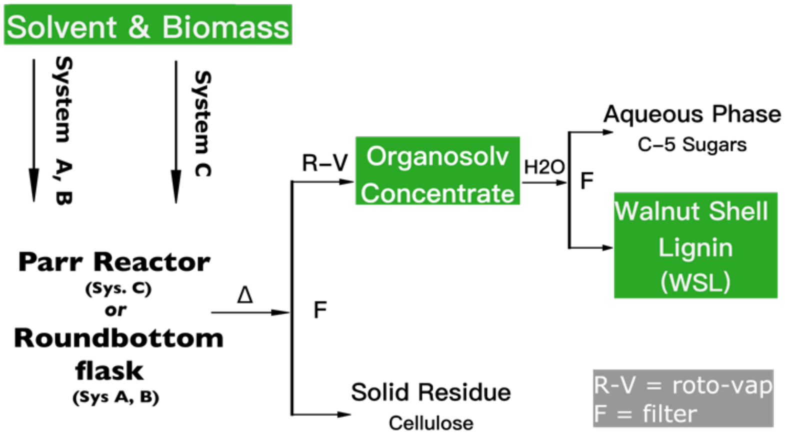


Figure 3. Organosolv process diagram.

3.2.5 Lignin Re-precipitation.

Isolated organosolv lignin from **Systems B** or **C** was heated in the alcholic solvent it was originally extracted in (either EtOH or MeOH) at a 1g/100mL ratio at 70°C until either complete solvation was achieved, or 24 h had elapsed. The liquor was then decanted and concentrated by rotary evaporation. The concentrate was added dropwise to 150 mL of water, while stirring. Precipitate was filtered out, dried overnight, weighed, and characterized by GPC and HSQC-NMR. All experiments were replicated at least three times.

3.2.6 Reductive Catalytic Upgrading of Lignin.

Lignin was depolymerized and deoyxygenated using 10-wt.% Ni/C catalyst as reported by Luo et. al.[51,52] In a 75 mL Parr® vessel, 50-100 mg WSL or raw WS was combined with 5-10 mg 10 wt% Ni/C catalyst and 20 mL MeOH. After purging with H_2 five times, the reaction was run under 35 bar H_2 at 225 °C for 12 h while stirring at 700 rpm. Once the reaction was completed and cooled, the contents were filtered to remove solid catalyst, which was rinsed with three 3 mL portions of MeOH. Wash and filtrate were combined and concentrated by rotary evaporation until an oil was produced. The oil was further dried under vacuum in a tared vial untill the mass stabilized. The final product was analyzed by HPLC with a Zorbax Eclipse XDB-C18 column (250 × 74.6 mm) set at 30°C. The aromatic biophenol products were compared and quantified versus calibration curves of standards. All experiments were replicated at least three times. Yields are reported as wt. % of starting acid soluble lignin in dry WS material.

Table 1. Walnut Shell (WS) Biomass Composition

Component	wt.%
Acid Soluble Lignin (ASL)	37 ± 4
Acid Insoluble Lignin (AIL)	7
Cellulose	12
Hemicellulose	24
Acid Insoluble Ash (AIA)	13
Acid Soluble Ash (ASA)[a]	7 ± 4

[a] To bring mass balance to 100%.

3.3 Results and Discussion.

3.3.1 Composition.

Untreated WS was found to be 8% moisture by mass. The composition of dry WS mass was determined by this study as follows; 12 wt.% cellulose, 24 wt.% hemicellulose, 37 wt.% ASL, 7 wt. % AIL, and 20 wt. % inorganics.[53] Our findings are in accordance with reported values.[54] The uncertainty in lignin content is due to uncertainty in the extinction coefficient for ASL. The upper and lower limits for ASL composition are determined by the upper and lower molar absorptivity coefficients defined by the NREL method which was followed.[53] Inorganic or un-characterized content in the biomass is classified as ash, which is further categorized as AIA or ASA. Full composition is presented in **Table 1.** Reported yields are based on dry lignocellulosic biomass, meaning the 8 wt.% moisture is excluded. For lignin yields, because the utilized organosolv methods are all acidic, only ASL is considered accessible, and the maximum theoretical lignin recovery is 37 wt.% of dry WSL mass. Yields represent the weight percent recovered out of the total theoretical acid soluble lignin.

3.3.2 Reductive catalytic fractionation (RCF) of walnut shell biomass over Ni/C.

To assess the viability of "lignin first" strategy with walnut shell biomass, 10 wt% Ni/C catalyst was used in both MeOH and EtOH at 225 °C. These conditions have been shown to achieve 40-50% yield of monomeric phenols based on lignin content in a diverse array of woody biomass.[42,47,50,52] In the case of WS biomass, 53% of lignin oil was produced in EtOH. However, analysis showed a large number of products (**Figure 4**). The same reaction in MeOH led to 100% conversion of lignin based on WS mass dissolved into MeOH. However, unlike what was observed for woody biomass, an intractable number of

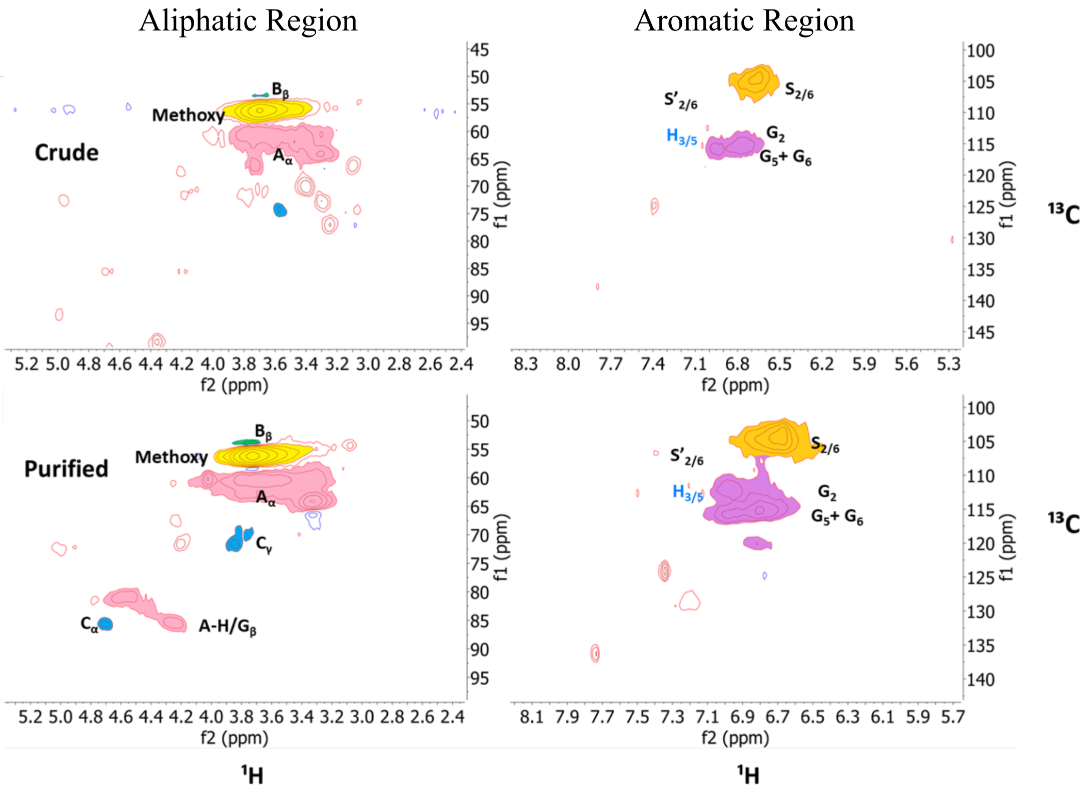
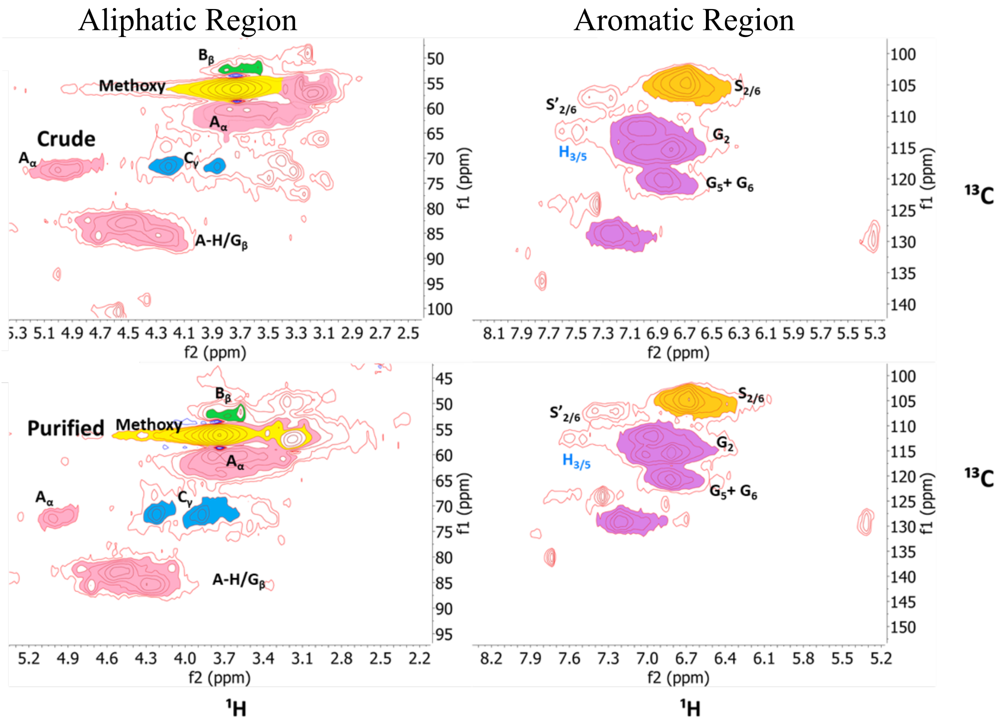


Figure 4. Above: Color coded HSQC-NMR for **System B** with 93/7 EtOH/HCl solvent at 120 ˚C, before and after purification by recrystallization. **Below:** Color coded HSQC-NMR for **System B** with 95/5 MeOH/HCl solvent at 85˚C, before and after purification by recrystallization.



products were afforded (**Figure 4**). These results showed that WS biomass is not suitable for direct RCF. Lignin extraction, stabilization, isolation and subsequent upgrading is necessary to access meaningful aromatic/phenolic products from walnut shell lignin. We describe next three organosolv lignin extraction systems, their characterization and hydrogenolysis catalysis over Ni/C catalyst.

3.3.3 Solvent, pre-treatment, and the effect of pressure.

In the organosolv lignin isolation process, acid induces carbocation formation in the benzylic positions. The carbocation reacts with other lignin molecules in a condensation reaction, or with the solvent.[55] As reported by Luterbacher, formaldehyde facilitates production of uncondensed lignin by converting the α- and γ- hydroxyl groups to a stable 1,3-dioxane/acetal motifs to block the formation of the benzylic cations.[46]

It has been previously reported that the organosolv extraction is dependent on the solvent to biomass ratio, with higher ratios giving higher yields.[56] To test if the solubility of lignin in the organosolv solution is a limiting factor for the extraction of WSL at 1:10 WS/solvent g/mL for **System A**, an analogous experiment was run with a 1:20 WS/solvent g/mL ratio. Results shown in **Table 2** indicate that the solution was not being saturated with lignin at the 1:10 ratio, since yields with the 1:20 ratio were comparable. Solvent to biomass ratio experiments were examined with **Systems B** and **C**. For **System B**, it was found that doubling the solvent to biomass ratio did result in higher yields, but the effect was modest.[56]

To check if extractives hinder the solvolysis of WSL, **System A** organosolv was run with WS that had been pre-treated by Soxhlet extraction in EtOH and water.[55] Only 2% of WS mass was extracted after 24 h of EtOH followed by 24 h of water pretreatment. Furthermore, there was no notable difference in isolated lignin yield or quality from WS that underwent Soxhlet extraction pretreatment versus WS that did not. The small amounts of extractives and negligible effect on the organosolv results led to the conclusion that Soxhlet treatment is unnecessary for WSL extraction, and all subsequent experiments were conducted without Soxhlet extraction of WS biomass. An organosolv experiment in **System A** was also conducted under an inert N_2 atmosphere, as opposed to air, to check the extraction process for air sensitivity. Results indicated no air sensitivity; thus, further experiments were conducted without protection from air. However, **System C** is conducted under pressure in a Parr® reactor and inert atmosphere N_2 was used. Complete results from the aforementioned screening experiments are summarized in Table 2. The higher yields obtained by **Systems B** and **C** indicate that WSL is more suited for alcohol solvated extractions. Therefore, **B** and **C** were pursued for further optimization.

Table 2. Effect of solvent and Soxhlet extractives on lignin extraction **Systems A, B, & C**.

Entry	System	Soxhlet[a]	Biomass/ Solvent (g/mL)	Isolated Lignin (%Yield)[b]
1	A	N	1/10	30
2	A	Y	1/10	27
3	A	N	1/20	30
4	A	Y	1/20	23
5[c]	A	N	1/10	27
6	B[d]	N	1/10	34
7	B[d]	N	1/20	43
8	B[e]	N	1/10	35
9	B[e]	N	1/20	48
10	C	N	1/10	67
11	C	N	1/20	65

[a] WS pretreated with Soxhlet extraction in ethanol and water for 24 h each

[b] Based on theoretical acid soluble lignin of 37 wt.% of dry WS biomass

[c] Reaction under N_2 atmosphere

[d] EtOH

[e] MeOH

Table 3. Optimization of Organosolv Systems for WSL Isolation

Entry	Temperature (°C)	Time (h)	Pressure[a] (bar)	Solvent System (System Symbol)	Solvent ratio (by volume)	Biomass Recovered (wt.%)	Lignin Yield (wt.%)[d]
1	85	6	1	HCl/EtOH (B)[c]	5/95	83 ±6	46±8
2	120	6	1	HCl/EtOH (B)[c]	7/93	71	55±16
3	85	6	1	HCl/MeOH (B)[c]	5/95	70	35 ±2
4	150	0.5	15-18	H_2SO_4/MeOH/HCOH (C)[b]	5/5/1	56 ±2	26 ±4
5	160	0.5	15-18	H_2SO_4/MeOH/HCOH (C)[b]	5/5/1	50	52 ±8
6	170	0.5	15-18	H_2SO_4/MeOH/HCOH (C)[b]	5/5/1	32 ±8	62 ±2
7[e]	180	0.5	15-18	H_2SO_4/MeOH/HCOH (C)[b]	5/5/1	41 ±2	88 ±7
8[e]	190	0.5	15-18	H_2SO_4/MeOH/HCOH (C)[b]	5/5/1	26 ±2	37 ±2

[a] Nitrogen pressure

[b] 0.045N aqueous sulfuric acid

[c] concentrated HCl

[d] Theoretical acid soluble lignin assumed to be 37 wt.% of dry lignocellulosic WS

[e] Product significantly degraded

3.3.4 Optimization of WSL isolation.

Solvent acidity, reaction time, and temperature were explored as reaction parameters. Optimization results are given in Table 3. Generally, higher acid concentration and temperature improved lignin yield, a trend which has been previously reported for various other organosolv processes.[57] It was observed that WSL yields in **System C** afforded a smaller range over multiple experiments than did **System B**. It was also found that temperatures above 180 °C or reaction times longer than 0.5 h for **System C** resulted in charred material (dark color and coarser) rather than the orange, fine powder collected from WSL isolation at lower temperatures. **Table 4,** shows previous reports of walnut shell lignin extraction for comparison to our work. Our optimized conditions offer higher yields and shorter reaction times for WSL isolation.

Table 4. Comparison of reported literature results on the extraction of lignin from walnut shell.

Ref.	Reaction Conditions	Lignin %
WS[25]	0.2 M HCl, EtOH or ButOH/H2O, reflux 6 h	6-32
WS[270]	0-1.0M KOH or Na$_2$CO$_3$, 200-300°C, 1 h	N/A
WS[28]	80:20 n-prOH/H$_2$O, 0.18 M H$_2$SO$_4$, 120 °C, 5 h	55-57%

WSL Characterization.

GPC in dimethylformamide (DMF) was used to characterize the molecular weight and dispersity of isolated WSL (**Figure 5).** WSL from **Systems A, B** with MeOH, and **C** gave by GPC a single distribution with average M_n in the range of 5-7 kg mol^{-1} and a polymer dispersity index (PDI) in the range of 4-7; typical of organosolv lignin (**Figure 5, Table 5**).[44,58] However, crude lignin isolated from System B in EtOH organosolv afforded a bimodal GPC distrubution. One peak is in the expected range, with a M_n value of 4-5 kg mol^{-1}, while the second larger peak has an M_n over 100 kg mol^{-1}. It should be noted that samples which afforded this bimidal distrubution took up to a day to dissolve in DMF, while those which displayed a single distrubution dissolved readily in less than ten minutes.

To investigate the difference in GPC results between the MeOH and EtOH **System B** organosolv lignins, a sample from the EtOH process was reprecipitated. Only 30-40% of the original WSL by weight was recovered after reprecipitation. As seen in **Figure 5**, GPC on the purified product gave a single peak distrubution of lower molecular weight (M_n = 5-7 kg mol^{-1}), which closely resembled those of **Systems A, B** with MeOH, and **C**. The solubility of the reprecripitated lignin product was also improved; it dissolved readily in DMF in under ten minutes. Analysis of the reprecipitated products of all other samples did not return different GPC results, and no change in solubility was observed.

In summary, EtOH derived WSL had poor solubility, gave a bimodal distribution by GPC, and low repreciptation yield. MeOH, a stronger nucleophile, yielded WSL samples which did not show these characteristics under the same conditions. Organosolv lignin is typically of molecular weight between 2-5 kg/mol, and high molecular weight fractions (>> 5 kg/mol) are often recondensed material.[6,60] Therefore, we attribute these characteristics to inadequate protection of the carbocation intermediates by EtOH, which resulted in recondensation.

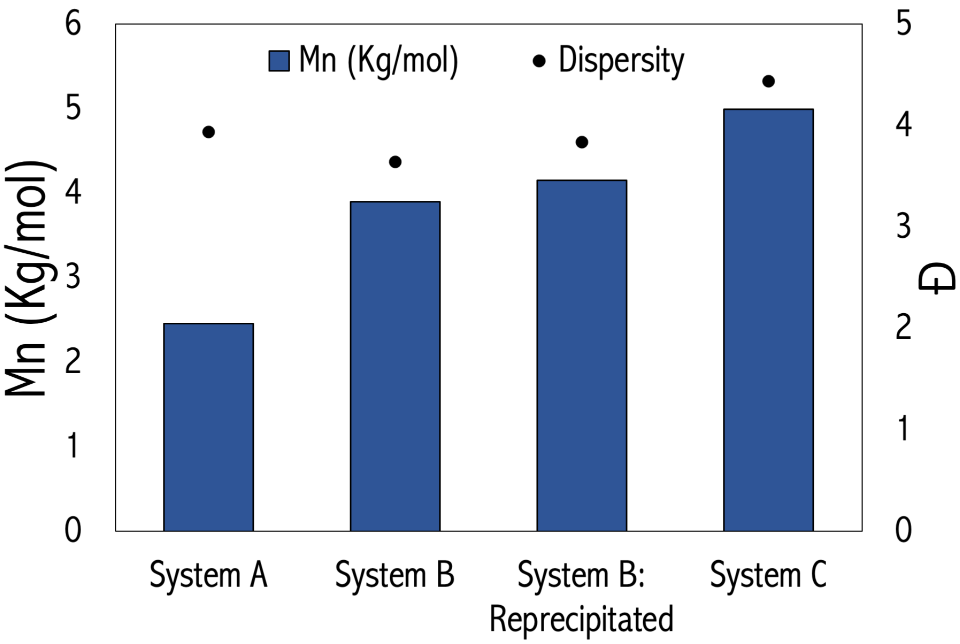


Figure 5. WSL Characterization by GPC in DMF is presented above with x-axis referring to lignin samples from different extraction methods, y-axis (left) is the number average molecular weight (M_n) and y-axis (right) is dispersity (Đ). System B data corresponds to the low molecular weight fraction, and System C for reactions run at 170 °C.

HSQC-NMR is a well-established tool for lignin analysis, as the chemical shifts for peaks corresponding to the monomers and monomeric linkages found in lignin are well characterized.[59] β-O-4 and β-β linkages and aromatic monomer peaks are assigned in the HSQC spectra, displayed in **Figure 6a and 6b,** for **System C** crude WSL products at 160 °C and 170 °C, respectively. The detection of intact linkages and lignin monomers indicates that **System C** organosolv at 160-170 °C yields native like lignin which has been properly protected and does not have a high frequency of recalcitrant carbon-carbon bonds. Although the WSL spectra, and therefore native character and quality, is very similar for WSL from both 160 and 170 °C extractions, the yield is about 15% higher at 170 °C than at 160 °C (see Table 3). Therefore higher heat must aid the solvation process. Following this trend, **System C** at 180 °C yielded the highest organosolv product recovery at a theoretical lignin yield of 81-95%. However, HSQC-NMR of the isolated solid, which was darker in color and coarser in texture, revealed that the native lignin structure was not preserved. The spectra, seen in **Figure 6c,** display monomer and linkage peaks which are far less prominent than those in **Figures 6a and 6b**. It follows that, while the higher heat aided delignfication, it must have also accelerated degradation/recondensation reactions.

Table 5. WSL Characterization by GPC in DMF

Entry	System and Experimental Parameters	M_n (g mol^{-1})	PDI
1	**A**, 20/30/50 FA/AA/H_2O, 110°C	2455	3.94
2	crude **B**, 95/5 EtOH/HCl, 85°C	3902, 118405	3.64, 2.49
3 [a]	**B**, 95/5 EtOH/HCl, 85°C	4153	3.84
4	crude **B**, 93/7 EtOH/HCl, 120°C	4718, 370098	6.10, 3.68
5 [a]	**B**, 93/7 EtOH/HCl, 85°C	3959	3.46
6	crude **B**, 95/5 MeOH/HCl, 85°C	4127	3.42
7 [a]	**B**, 95/5 MeOH/HCl, 85°C	2957	2.99
8	**C**, 160°C	5634	5.24
9	**C**, 170°C	4994	4.44
10 [a]	**C**, 180°C	5101	6.35

[a] Sample was recoverd after reprecipitation in alcohol solvent used in original organosolv isolation

Higher heat also led to larger mass loss, which means that more cellulose and hemicellulose is extracted at higher temperatures. These findings align with those reported by Jiang et al, where it is stated that harsh temperature and acidic conditions for organosolv ethanol pre-treatment of various lignocellulosic biomass samples often results in high extraction by mass, but signficant recondensation of lignin.[54] Longer exposure to heat, 160 °C for 1 h, also produced a lignin product with non-native HSQC-NMR peaks and a more charred physical appearance. It was concluded that 170 °C for ½ h is optimal for yield and quality for **System C** organosolv process.

For quantitative analysis, the β-O-4, β-β, and β-5 linkages for (a) 160 °C, (b) 170 °C, and (c) 180 °C were calculated by integrating the contour signals (Table 5). Based on the integration of the β-aryl ether (A_α), phenylcoumaran (B_α), and resinol (C_α) contour signals, the following percentages were found (Table 5): For β-O-4, 66%, 77%, and 58%, were found for (a) 160°C, (b) 170°C, and (c) 180°C, respectively. For β-β, 19%, 21%, and 11%, were found for (a) 160°C, (b) 170°C, and (c) 180°C, respectively.

The distribution of H:G:S was also determined based on the aromatic region of the spectra (see Table 5). Lignin isolated at 170 °C showed higher connectivity for β-O-4 in comparison to 160°C and 180°C. The quantitative analysis shown in **Table 6** confirms that at 180°C, there is significant decrease in intact β-O-4 and β-β linkages. Thus, **System C** at 170°C is considered optimal for both yield and quality.

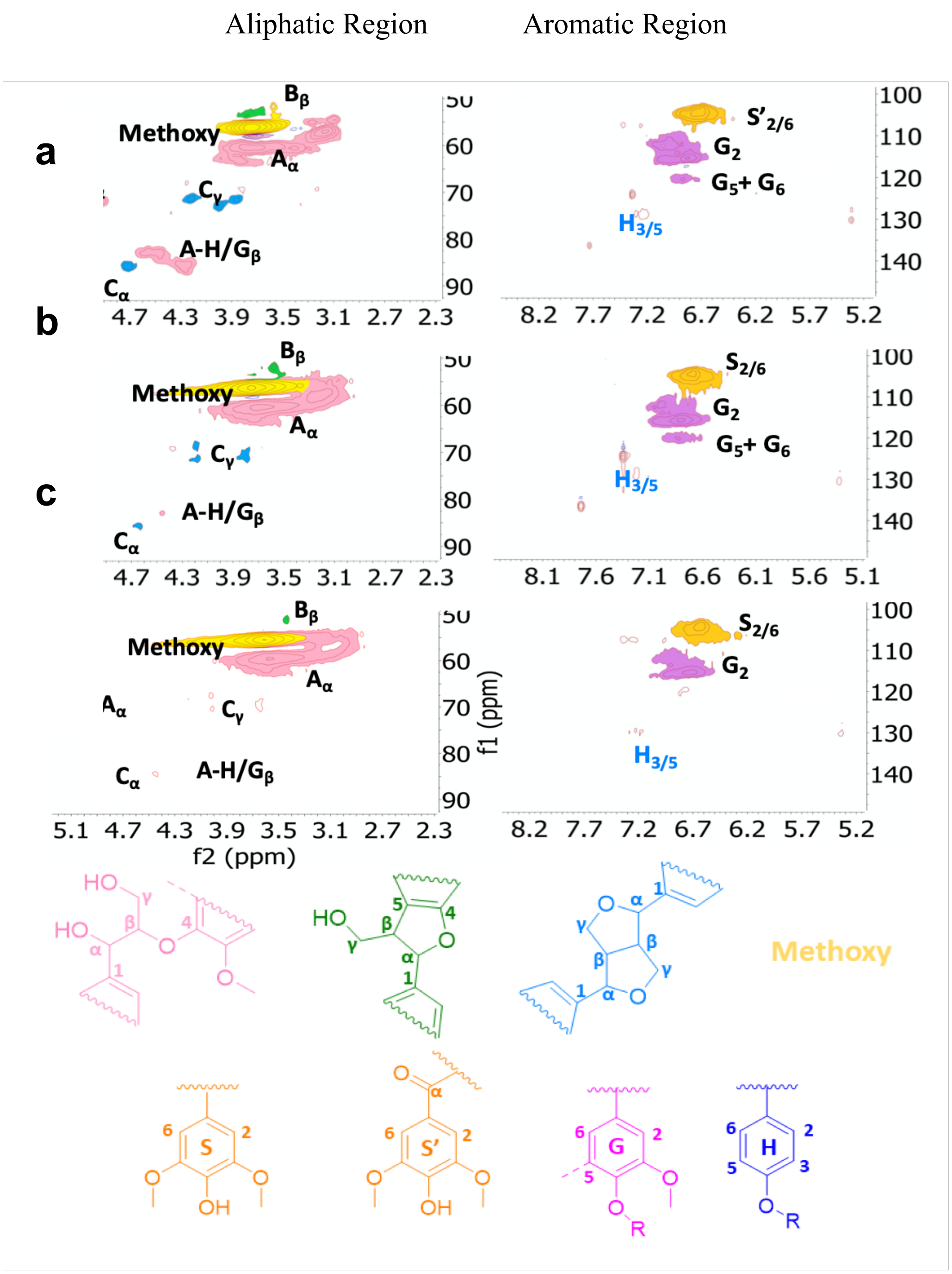


Figure 6. HSQC-NMR spectra for **System C** organosolv products at (a) 160°C, (b) 170°C, and (c) 180°C. Horizontal axes correspond to ^{1}H chemical shift, vertical to ^{13}C.

Table 6. Characteristics of the Organosolv Walnut Shell Lignin based on System C[a]

System	S	G	H	β-O-4	β-β	β-5
160 °C	58	42	b	66	19	<2
170 °C	57	43	b	79	21	<2
180 °C	68	32	b	58	11	<3

[a] S: syringyl units, G: guaiacyl units, H: p-hydroxyphenyl units.

[b] Too small to ascertain. c %X = X/($S_{2/6}$ + $S_{'2/6}$ + G_2 + G_5 + G_6), X = $S_{2/6}$ + $S_{'2/6}$, G_2 + G_5 + G_6. d %Y = Y/(A_α + B_α + C_α), Y = A_α, B_α, C_α

Crude WSL from **System B** in EtOH, which gave a bimodal GPC distribution, took nearly 24 h to dissolve in the 5:1 deuterated dimethylsulfoxide- d_6:pyridine-d_5 solution for NMR analysis. Other samples dissolved in less than ten minutes. Upon dissolution of the recalcitrant **System B** with EtOH derived lignin, the NMR solution became viscous and gel like. The sample returned an HSQC-NMR spectra which contained unidentified peaks and was not reminiscent of native lignin spectra. Examination of the HSQC-NMR spectra in **Figure 4**, specifically the β-O-4 region, reveals that there is modification of the lignin due to significant incorporation of the alcoholic solvent, which is consistent with previous reports.[26,59] To investigate in parallel to the GPC experiments, the spectra of the reprecipitated product from **System B** in EtOH WSL was taken. The resulting spectra has peaks as expected for native organosolv lignin, and similar to those corresponding to **System C** at 160°C and 170°C. Notably, defined β-O-4 peaks, which were not observed in the crude product spectra, were visible in the purified product spectra. Additionally, S, G, β-β, and β-5 peaks appear in higher volume in the spectra for the purified lignin samples than in those for the crude product (see **Figure 4 and Table 6**). Presumably, the native characteristic peaks were overwhelmed by modified-lignin peaks, observed in high volume between 3-3.5 ppm in the ^{1}H spectrum and 65-75 ppm in the ^{13}C spectrum for the crude sample HSQC-NMR (**Figure 4**).

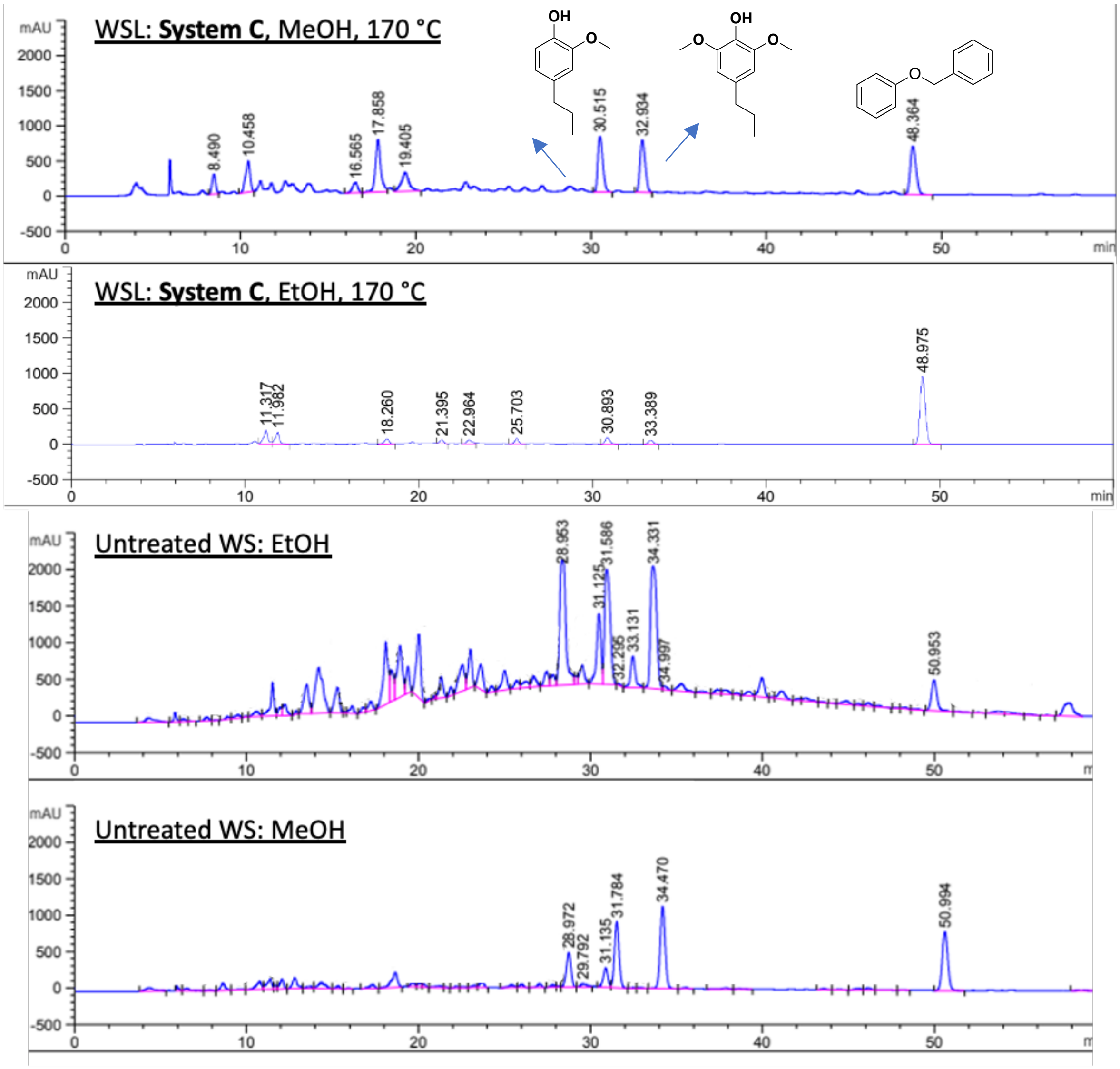


Figure 7. HPLC of bio-oil obtained after RCF of optimized **System C**, MeOH and EtOH, 170 °C derived WSL, Raw WS in EtOH, and Raw WS in MeOH.

Table 7. Depolymerization Products ^{a}of WSL with 10 wt.% Ni/C in MeOH, 225°C,12 h

Entry	Feedstock Sample	Monomer Products[b] (wt.%)	Phenolic monomers % Yield[c]
			Total
1	WSL: **System C**, MeOH, 170 °C	46	29
2	WSL: **System C**, EtOH, 170 °C	15	2
3	WSL:**System B**, 85 °C, 95/5 EtOH/HCl	23	5

[a] Conversion and monomer yield percents based on isolated mass of monomer oil and of indicated monomeric products, respectively, relative to total theoretical acid soluble lignin

[b] Mass of monomeric oil represented as percent from theoretical lignin.

[c] Mass of S and G monomers obtained represented as percent from theoretical lignin.

Table 8. Depolymerization Products ^{a}of WS and WSL with 10 wt.% Ni/C in MeOH, 225°C, ,12 h

Entry	Feedstock Sample	Crude oil Yield[b] (wt.%)	Primary Product Monomer % Yields[c]				
			i-DMPP	Isoeugenol	DMPP	DHE	Total
1	WSL: **System C**, MeOH, 170 °C	46	4	3	10	12	29
2	WSL: **System C**, EtOH, 170 °C	15	1	0.8	N/A	N/A	1.8
3	WSL:**System B**, 85 °C, 95/5 EtOH/HCl	23	2	3	0	0	4
4	Untreated WS: EtOH	53	9	4	0	0	13
5	Untreated WS: MeOH	100	14	11	0	0	25

[a] Conversion and monomer yield percents based on isolated mass of monomer oil and of indicated monomeric products, respectively, relative to total theoretical acid soluble lignin

[b] Mass of monomeric oil represented as theoretical acid soluble lignin

[c] Mass of monomers obtained represented as percent of starting dry ligoncellulosic

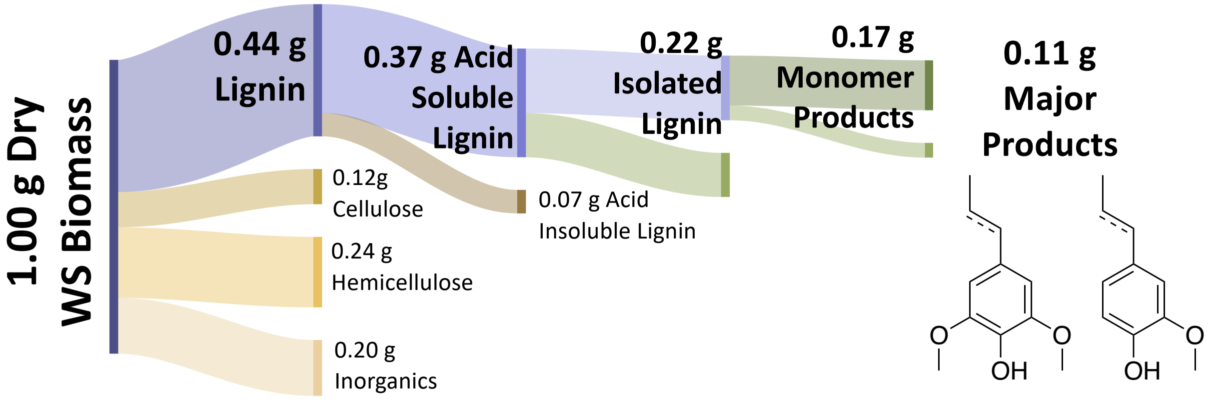


Figure 7. Sankey diagram of lignocellulosic WS mass to illustrate mass flow throughout entire reported process. Major products are also depicted.

3.4 Reductive Catalytic Upgrading of Lignin.

Isolated lignin must be selectively depolymerized to yield a narrow range of monomeric products for complete biomass valorization. Nickel is an attractive catalyst for this process due to its earth abundance and subsequently low cost, tendency to catalyze hydrogenolysis reactions, and selectivity for carbon-oxygen bonds and allyl alcohols.[53-56] Because of these properties, nickel catalysts have been thoroughly studied and utilized in the field of biomass valorization, with notable advances in RCF, which offers an industrially viable biomass valorization process.[62-65]

Selected WSL samples were subjected to hydrogenolysis over Ni/C catalyst to evaluate the potential of isolated WSL for obtaining monomeric phenol products. These experiments also demonstrate the benefit of isolating lignin before upgrading reactions, on top of the benefit which lignin removal provides to cellulose based processes. Samples from **Systems B** and **C** were selected such that MeOH/H_2SO_4 and EtOH/HCl pretreatments were both represented. Only the two samples which showed highest β-O-4 freqency from NMR analysis were used for the comparative catalytic upgrading study, because the hydrogenolysis mechanism targets this linkage specfically.[66]

Catalysis with 10 wt.% Ni/C resulted in conversion of System C at 170 °C with MeOH lignin to a an oil product, comprised of two major phenolic products (**Figure 7, Figure 8, and Table 7 Entry 1).**[50,51] For Systems B and C with EtOH the yield of lignin oil as well as selectivity for phenolic monomers was significantly lower (**Figure 7, Table 7, and Table 8**). The success of organosolv lignin from MeOH/H_2SO_4 (**System C**) is attributed to sucessful protection of the α-carbon in the β-O-4 linkage reducing C-C bond formation (recondensation).

3.5 Conclusion.

In this study, WS was used to study 3 different organosolv processes. EtOH and HCl under ambient pressure proved to effectivly fractionate lignin from WS, but failed to adequately protect the extracted lignin. Recalcitrant, recondensed lignin made up over half the mass of lignin isolated by this method. This fraction, which had a large molecular weight (4000-37000 kg mol^{-1}), was insoluble in EtOH, indicating that EtOH is unfit for isolation of protected and upgradable organosolv WSL.

Our study suggests that an aqueous H_2SO_4, MeOH, and formaldehyde solvent system is optimal for WS. The optimized method resulted in WSL recovered in 60-64% yields, based on theoretical lignin content in WS, with a typical molecular weight for organosolv lignin (5 kg mol^{-1}). HSQC-NMR confirmed that native structure was preserved. Subsequent batch catalytic hydrogenolysis using earth abundant Ni/C catalyst confirmed that 80% of the WSL lignin isolated under optimized conditions can be converted to monomeric phenolic products.

3.6 Acknowledgements.

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3.7 Publication.

This chapter has been accepted in ACS omega where I worked on the analysis of walnut shell lignin and depolymerization using a heterogeneous catalyst: Ni/C which has been previously studied in our laboratory.

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Chapter 4. Hydrogenolysis of Organosolv Lignin in Ethanol/Isopropanol Media without Added Transition-Metal Catalyst.

4.1 Abstract.

Lignin is the largest renewable source of aromatic chemical building blocks on the planet and has great potential to produce value-added chemicals. Herein, we describe lignin hydrogenolysis/depolymerization of organosolv poplar lignin (OPL) in ethanol/isopropanol solvent in the absence of added catalysts. Different EtOH/i-PrOH ratios as well as various reaction conditions were evaluated. OPL depolymerization was more effective in the mixed media than in ethanol or isopropanol alone. Heating OPL at 270 °C for 4 h in 50:50 (v:v) EtOH/i-PrOH in a closed pressure vessel gave an overall oil yield of 70 wt %, of which about 48% consisted of the monomers (E)-4-propenyl syringol and isoeugenol. Notably, these catalyst-free reactions in ethanol/ isopropanol media show monomer yields comparable to those reported for lignin depolymerization using precious metal catalysts and dihydrogen, which suggests unexpectedly favorable H-donor ability of this mixed alcohol medium.

4.2 Introduction.

With the increasing consumption of fossil-carbon resources and the impact of the resulting CO_2 release on the global climate, there has been a growing interest in utilizing biomass as a sustainable feedstock for producing fuels and chemicals.[1-3] Lignocellulose, the main non-food component of biomass, which consists of cellulose, hemicellulose and lignin, represents the best candidate to provide sustainable chemicals and fuels. [4-6] The chemical conversion of cellulose and hemicellulose to higher value products has seen substantial progress;[7] however, lignins, which are major byproducts of biorefineries and of the paper pulping industry, are poorly utilized.[8,9] Lignin, the second most plentiful biopolymer after cellulose, has the potential to serve as a renewable feedstock in the production of aromatic chemicals.[10-12] The challenge in such utilization lies in lignin's amorphous structure and propensity to cross link into a recalcitrant refractory material under hydrolytic conditions. These properties, make efficient and economic chemical conversion of lignin into useful aromatic products problematic.[13-16]

Thermochemical methods proposed for converting lignin into fuels and chemicals include pyrolysis[17-19], acid and base-catalyzed depolymerization,[20] oxidation,[21,22] and hydrogenolysis[23-27] hydrogenolysis being an especially effective strategy. For example, Xiao et al.[28] reported the hydrogenolysis of poplar lignin over a heterogeneous MoO_x/CNT (carbon nanotube) catalyst at 260 ºC under 3 MPa H_2 giving a 28 wt% overall yield of monomeric phenols. In other examples, Zhai et al.[29] achieved 23 wt% yield of propylguaiacol and propylsyringol upon hydrogenolysis of organosolv birch lignin over a Ni-Fe/activated carbon (AC) catalyst at 225 °C under 2 MPa H_2. For these and most other examples of lignin hydrogenolysis, metal catalysts are required for lignin depolymerization. However, catalyzed hydrogenolysis in some cases also leads to undesired hydrogenation of aromatic rings of the phenolic products. [27,30]

Another approach is catalytic hydrogen transfer hydrogenolysis (HTH) from reductive organic compounds such as alcohols, formic acid and formates.[31-33] Alcohols that readily dissolve lignin and are potential hydrogen donors, such as methanol,[27,34] ethanol[35] and iso-propanol,[36] have been the most widely used solvents in this regard. Toledano et al.[37] reported HTH of lignin over a (Ni/Al)SBA-15 catalyst in various hydrogen donor solvents (tetralin, isopropanol, glycerol, and formic acid). No char was generated, and the highest bio-oil yield was obtained in formic acid solvent. Song et al.[38] investigated HTH of birch lignin over a Ni/AC catalyst in different solvents (methanol, iso-propanol, 1,4-dioxane, glycerol, cyclohexane). Methanol gave the highest monomer yield among these solvents. Ethanol with formic acid proved to be a more reactive hydrogen source than added H_2 for lignin hydrogenolysis.[39] In addition, ethanol could be produced by biomass fermentation,[40] while iso-propanol, it can be manufactured by bioprocessing lignocellulose with a bacterial consortium.[41] By using such alcohols as hydrogen donors, it is possible to achieve the fully sustainable transformation process of lignin.

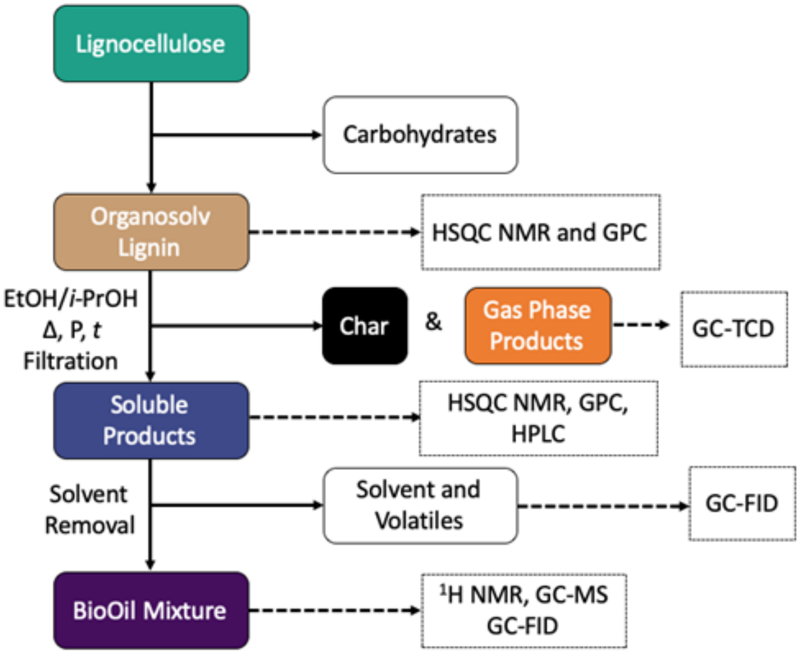
Mixed solvents may have synergistic capability by suppressing the formation of solid residues and promoting the liquefaction conversion of lignocellulose.[42] Kloekhorst at al.[43]investigated the depolymerization of Alcell lignin over a Ru/C catalyst in three mixed solvents (iso-propanol/formic acid, ethanol/formic acid, methanol/formic acid). The highest yield of bio-oil was obtained in iso-propanol/formic acid solvent. Formic acid (FA) acted as a protecting group to inhibit the repolymerization reaction. Wu et al.[44] reported the depolymerization of industrial lignin in a mixture solvent of ethanol/1,4-dioxane/formic acid. Their results showed that depolymerization of lignin carried out in the mixed media was more effective than the reaction in ethanol alone. In this case FA also acted as an acid catalyst. Each of these systems used a transition metal catalyst.

Inspired by the possibility that mixed solvents may exhibit synergistic capabilities in hydrogen transfer hydrogenolysis, we describe herein an investigation of lignin disassembly by catalyst-free ethanol/iso-propanol mixtures in a closed pressure reactor at elevated temperature. Notably, this mixed solvent system is surprisingly effective at the hydrogenolysis/depolymerization of organosolv poplar lignin (OPL) to generate phenolic lignin monomers. Moreover, the catalyst-free EtOH/*i*-PrOH system shows phenolic monomer yields that rival several reported methods using transition metal catalysts and added H_2.

4.3 Experimental.

Two methods were used to extract lignin from Poplar wood. Biomass substrates were milled to 40 mesh by a Mini Wiley Mill (Thomas Scientific, Swedesboro, NJ) before use. Organosolv poplar lignin (OPL) was isolated from poplar shavings obtained from ACE Hardware lumberyard, Santa Barbara. 2-Phenoxy-1-phenylethanol was synthesized and characterized according to previously reported methods.[45] Ethanol (200 proof), isopropanol (HPLC grade), methanol (ACS Reagent Grade), glacial acetic acid, formic acid (88wt%), sulfuric acid (98 wt%), formaldehyde (37 wt%), and dichloromethane were purchased from Fischer Chemical and used as received.Scheme 1 provides a graphical roadmap of the isolation and characterization of the organosolv lignins and of the procedures used to analyze the products from the reaction with the EtOH/*i*-PrOH mixed solvent.

Scheme I. Lignin processing sequence and analysis used.



4.4 Isolation and Depolymerization of Lignin from Poplar

4.4.1 Lignin Extraction and Isolation.

Poplar wood substrates were treated in methanol/water mixtures to separate lignin.[46] A 2 g sample of 40 mesh raw biomass was added to a mixture containing 0.045 N H_2SO_4 (25 mL), MeOH (25 mL), and 37 wt % aqueous formaldehyde (4 mL) in a Parr pressure vessel. The reaction was purged with N_2 gas five times and stirred at 700 rpm. The N_2 gas pressure was increased to 10 bar, and the contents were allowed to react at 160 °C for 0.5 h. After cooling to room temperature, the contents were filtered to separate the solid residue, mainly carbohydrates, from the filtrate. The filtrate was concentrated by rotary evaporation to remove the MeOH. Nanopure water (150 mL) was added to the remaining acidic aqueous solution to precipitate the lignin. The extracted lignin was then collected by filtration and washed three times with Nanopure water. The solid lignin was dried overnight and then characterized by heteronuclear Single Quantum Coherence NMR

(HSQC) and Gel Permeation Chromatography (GPC). This lignin is referred to in this study as MPL. Elemental analysis of MPL: C, 60.8%; H 6.6%; N, 0.34%.

In the other method ,[47] poplar wood shavings were treated with 1:1 ethanol:toluene, filtered, and dried overnight. In a 10 L round bottom flask, 4.5 L of methanol, 600 g of treated poplar wood shavings and 12 mL of concentrated HCl were added. The suspension was heated to 110 °C and stirred for 12 days. The deep brown solution was separated from residual solids, mainly carbohydrates, by filtration and the volume reduced to half in vacuo. One liter of ice was added to precipitate the lignin as a beige solid. The solid was collected by filtration and washed with cold water until the pH of the filtrate was 7. The resulting organosolv poplar lignin was then dried in vacuo overnight, yielding 21.6 g of material. This lignin is referred to in this study as OPL. Elemental analysis of OPL: C, 59.8%; H 6.6%.

4.4.2 Lignin depolymerization in mini-autoclave reactors.

Stainless-steel mini-reactors consisting of a ¾ inch Swagelok® union with ¾ inch Swagelok plugs were used for small scale reactions (<5 mL solvent).[48] The internal volume of these reactors is 10 ml. A typical run contained 30-150 mg lignin, 5 mL 50:50 (v:v) ethanol:isopropanol mixture, and 20 µL of n-decane as an internal standard. The reactors were sealed and placed in an aluminum heating block in a preheated oven set at a specified temperature. The contents of the reactor after cooling to ambient temperature were filtered using a 10 mL syringe fitted with a 0.22 µm Acrodisc nylon membrane filter. Both types of lignin were tested under these conditions. Since the results were comparable, OPL was used as the main focus in this paper.

4.4.3 OPL depolymerization in a Parr® reactor.

The depolymerization of lignin was also performed in a stainless-steel 75 mL 6-series pressure vessel (Parr Instrument Company). The lignin was dried at 60 °C overnight before use. For a typical experiment, 180 mg of lignin and 30 mL volume of the 50:50 (v:v) ethanol:isopropanol mixture were loaded into the vessel, and the sealed reactor was purged with N_2 five times. The reaction system was then pressurized with 10 bar N_2 at room temperature and heated to 270 °C at a stirring speed of 700 rpm. The desired reaction temperature was typically established within 30 min. After reaching 270 °C for 4 h, the reactor was removed from the heating mantle and cooled to room temperature using a water bath. All experiments were reproduced in triplicates and in different high-pressure reactors to ascertain that these observations are not artifacts of a specific reactor.

After cooling the reactor, the gas phase products were collected and analyzed by gas chromatography using thermal conductivity detection (GC-TCD). The liquid sample was filtered, and the solid residue was further washed with 5 mL of ethanol twice. The solid residue was dried in a desiccator under vacuum prior to determining its mass by weighing. All liquid fractions were combined and diluted to a total of 50 mL. From the 50 mL sample, 100 μL of the sample was added to 500 μL of MeOH and 400 μL benzyl phenol (Internal Standard) to give a total volume of 1 mL. This sample was then analyzed by High Performance Liquid Chromatography (HPLC). Afterward, the ethanol and isopropanol in the 50 mL sample were removed by rotary evaporation in order to measure the actual weight of the oil product. This oil was analyzed by ^{1}H NMR and GPC in triplicate.

4.5 Instrumental Analysis Methodology

4.5.1 GC analysis.

Gas chromatography with mass spectrometric detection (GC-MS) was used to identify volatile liquid products using a Shimadzu model 2010 QP2010 GC-MS, equipped with a 60 x 0.25 mm x 0.25 µm Agilent DB-5 Column (0.25 µm (5%-phenyl)-methylpolysiloxane lining). The injector temperature was set at 250 °C. Samples were diluted in methanol. The temperature was held at 40 °C for 10 min. and increased at 10 °C/min to 270 °C. The MS detector was kept at 285 °C with a solvent cutoff of 2.25 min. to avoid oversaturation by the solvents.

Gas chromatography with flame ionization detection (GC-FID) was used to quantify the components of the liquid products using the previously described effective carbon number (ECN) methodology.[49] An Agilent 6890N (G1530N) gas chromatograph equipped a 30 m × 0.25 mm Agilent DB-5 Column (0.25 µm (5%- phenyl)-methylpolysiloxane lining) and with flame ionization detector was used. The inlet temperature was 200 °C with a split ratio of 80:1 and helium carrier gas at 27 mL/min flow rate. One µL of the filtered liquid mixture was injected. The column temperature was held at 70 °C for 2 min, then ramped to 250 °C at 12 °C/min where it was held for 2 min. The FID was set at 250 °C with a 30 mL/min H_2 flow rate and 350 mL/min air flow rate.

GC-TCD was used to identify gas phase products. The same Agilent 6890N (G1530N) gas chromatograph was also equipped with a thermal conductivity detector and 30 m × 0.53 mm Fused Silica Carboxen 1010 capillary column. Samples were collected using a gas tight sampling bag and injected into the inlet at 230 °C using a gas-tight Hamilton syringe. The carrier gas, helium, was set at a constant flow rate of 1 mL/min. The column temperature was held at 35 °C for 14 min., then ramped to 245°C at 10 °C/min where it was held for 60 min. The detector was set at 250 °C with a 7 mL/min He flow rate and 8 mL/min air flow rate.

4.5.2 HPLC analysis.

The liquid phase from lignin depolymerization was filtered using a 0.22 µm Acrodisc nylon membrane syringe filter and analyzed with an Agilent 1260 Infinity Quaternary High-Performance Liquid Chromatography system, using a Zorbax Eclipse XDB-C18 column (250 x 74.6 mm) set at 30 ˚C. The HPLC instrument was equipped with a G1315D Diode Array Detector (DAD). The mobile phase was a mixture of H_2O (A) and acetonitrile (B) at a flow rate of 0.5 mL/min and a nonlinear gradient from 80% A and 20% B and to 5% A and 95 % B at 55.0 min. A fixed amount (400 µL) of a 10 mM internal standard solution (benzyl phenyl ether) was added into each sample for quantification purposes. Standard curves for all the aromatic products were made by comparison of the products to the internal standard. All results were analyzed and quantified according to standard curves.

4.5.3 GPC analysis.

Gel permeation chromatography was carried out on a Waters (Millford, MA) chromatograph equipped with a Waters Alliance HPLC pump (2695 Separation Module) and two Tosoh TSKgel Super HM-M columns with N,N-dimethyl formamide (DMF) A Waters 2414 differential refractometer was used as the detector. Number average molecular weights (M_n) and weight average molecular weights (M_n) were calculated relative to linear polystyrene standards.

4.5.4 NMR analysis.

^{1}H-NMR spectra were recorded on a Varian Unity Inova 400 MHz. Eight spectra were collected with a 5s relaxation delay. A Bruker AVANCE500 spectrometer was used for HSQC which has a narrow AS600 magnet that is charged to 500 MHz. The spectrometer operates at 500.13 and 125.77 MHz for ^{1}H and ^{13}C nuclei, respectively. For 2D-HSQC, we used an echo-antiecho experiment called HSQCETGP. Spectra of isolated lignin (40 mg) was acquired in 700 μL 5:1 v:v DMSO-d_6:pyridine-d_5 solvent.

4.6 Results.

4.6.1 Lignin Substrates.

The choice of lignin feedstock greatly influences the outcome of conversion studies. It is understood that lignin substrates with abundant ether linkages, resembling native lignin in plants, are more amenable to depolymerization and upgrading than lignin substrates containing C-C bond cross-linkages that are formed during extraction processes. Organosolv Poplar Lignin (OPL) was selected for this study and compared to Methanol-extracted Poplar Lignin (MPL), which has been shown to retain significant native ether linkages.

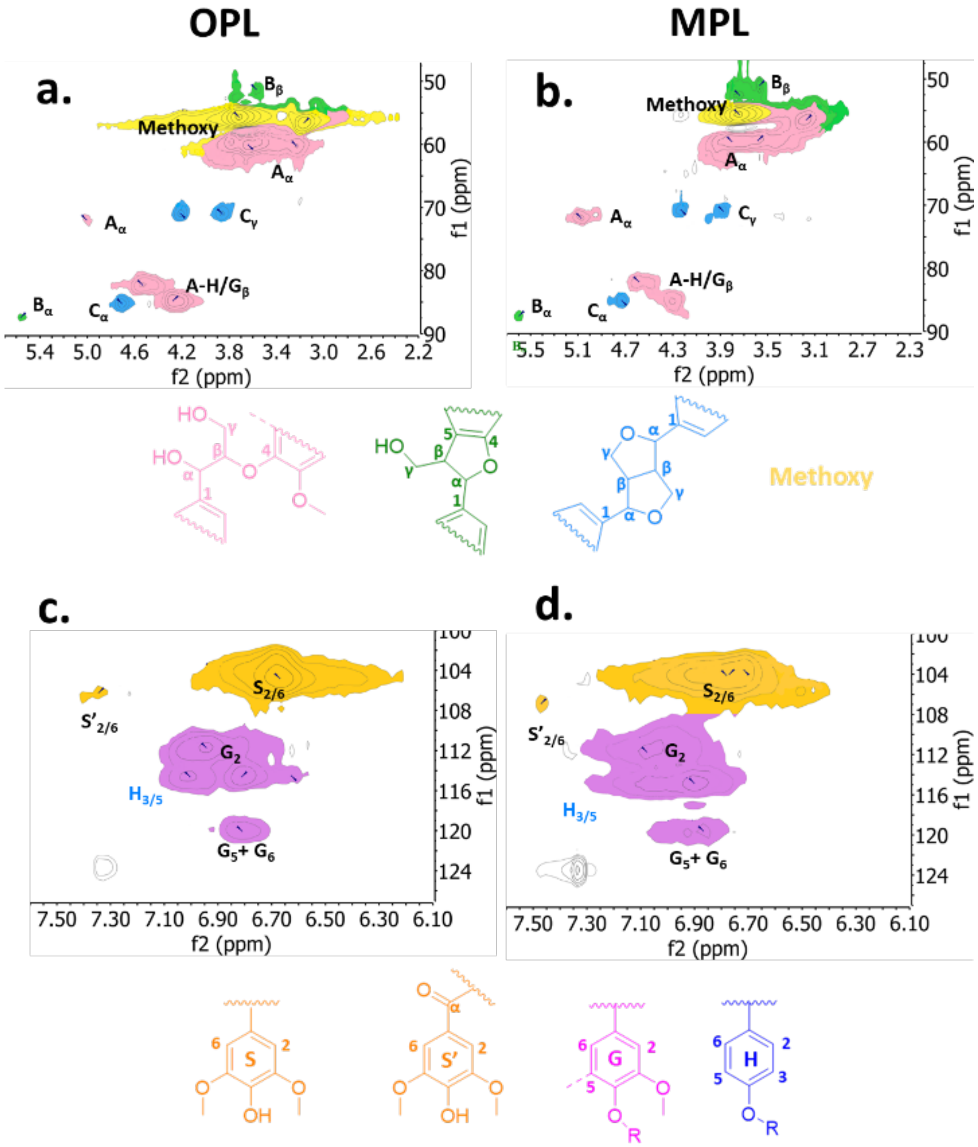


Figure 1. Shown above is a 2D ^{1}H-^{13}C HSQC NMR spectra ran in DMSO-d_6:pyridine-d_5 solvent showing characteristics of the organosolv poplar lignin (OPL) in comparison to methanol organosolv poplar lignin (MPL). The interunit linkages are color coded in order to depict which linkages are shown in the Chemical shift assignments are presented as '^{1}H/^{13}C ppm. β-O-4 linkage (pink), β-5 linkage (green), β-β (light blue) in the aliphatic region. S and S' (orange), G (purple), and H (indigo) in the aromatic region. The top spectrum is depicting the aliphatic region (a. and b.: δ_C/δ_H of 50–90/2–5.5 ppm). The spectra, a. and b., show similarities where the β-O-4 is consistently shown in the 3.1-4.2/55-65 ppm region for threo and erythro diasteromers. The β-5 linkage is present in the 3.0-4.0/50-55 ppm region. β-β appears also in the 3.8-4.6/70-75 ppm region. The bottom spectrum is depicting the aromatic region (c. and d.: δ_C/δ_H of 100–130/6.1–7.5 ppm). S region is shown from 6.2-7/100-110 ppm. G region is shown from 6.5-7.3/110-120 ppm. Assignments in linkages were determined based on literature.[50]

The HSQC-NMR spectra of OPL (Figure 1) are divided into two regions. Chemical shift assignments are presented as '$^{13}C/^{1}H$ ppm'. An aromatic region between 126 – 100 / 7.5 – 6.1 ppm and a side chain region between 90 – 50 / 5.6 – 2.2 ppm are evident in Figure 1. In the side chain region, several peaks can be assigned to the methoxy group and interunit linkages of β-O-4, β-β, and β-5 motifs. The proportions of β-O-4, β-β, and β-5 in OPL can be calculated by integrating the contour signals and compared to MPL (Table 1).[50] Based on integration of β-aryl ether (A_{α}, phenylcoumaran (B_{α} and resinol (C_{α} contour signals, the linkage percentages of these three linkages in OPL are 82% β-O-4, 16% β-β, and a small amount of β-5 (see Table 1). The analogous analysis of MPL gave nearly the same result (Table 1). The aromatic region of the HSQC spectra also show the distribution of H:G:S (see Figure 1 caption). Similarly, the integration of $(S_{2/6}+S'_{2/6})/(G_2+G_5+G_6)$ contour signals shows that OPL and MPL contain substantial amounts of syringyl units- as well as guaiacyl units (see Table 1). These results agree with a previous report by Luo et al.[26] where the native lignin connectivity was not altered significantly by the extraction and isolation methods.

GPC analysis gives a M_n 1939 g mol^{-1} and a dispersity of 2.84 for OPL which is typical for organosolv lignin. Semi-quantitative evaluation of inter-unit bonds in lignins can be expressed as the numbers of specific inter-unit bonds per 100 lignin monomers or C9 units. In order to perform a semi-quantitative evaluation of the inter-unit bonds in OPL and MPL, it was necessary to use as an internal standard with a cluster of signals that are representative of all C9 units present in the sample. According to the research by Sette et al.,[50] the overall number of C9 units present in hardwood can be quantified by the sum of half the syringyl signal plus the G signal (0.5(S+S')+G). Thus, the total linkages (β-O-4, β-5, β-β) in every 100 basic lignin units can be calculated by the signal of $(A_\alpha + B_\alpha + C_\alpha)/[0.5(S + S') + G]$. Accordingly, OPL contains 71 linkages (58 β-O-4, 11 β-β, and 2 β-5) in every 100 units compared to 75 linkages in MPL.

Table 1. Characteristics of the organosolv poplar lignin. [S: syringyl units, G: guaiacyl units, H: *p*-hydroxyphenyl units]

Type	S	G	H	β-O-4	β-β	β-5
OPL	62	38	a.	82	16	~2
MPL	59	41	a.	79	18	~3

a. Too small to ascertain

b. c %X = $X/(S_{2/6} + S_{'2/6} + G_2 + G_5 + G_6)$, $X = S_{2/6} + S_{'2/6}$, $G_2 + G_5 + G_6$. d %Y = $Y/(A_\alpha + B_\alpha + C_\alpha)$, $Y = A_\alpha, B_\alpha, C_\alpha$

4.6.2 Reactions in Different Solvent Mixtures.

All the reactions described in this section were carried out without any added metal-based catalyst. A sample of OPL (180 mg) was heated at 270 °C in a 75 mL Parr reactor with pure ethanol (30 mL) for 4 h and then the solution was filtered to recover the char formed (20-30 mg). The remaining solution was concentrated by rotary evaporation to give a soluble oil (115-120 mg, ~64-67 WT% based on the original OPL). GC-MS and GC-FID analysis demonstrated that this soluble oil contains the monomers. 4-propenyl-syringol ((2,5-dimethoxy-4-propenylphenol or i-DMPP) and isoeugenol (2-methoxy-4-propenylphenol), but the total yield of these was small (12 mg, ~10 wt% of the soluble oil).

When the analogous reaction was conducted in pure iso-propanol as the solvent, the yield of the soluble oil was slightly higher at ~ 70% (126 mg) but more significantly, the soluble oil contained a much higher amount (~30 wt%) of the monomers, isoeugenol and i-DMPP (Figure 2). This result led us to explore the effect of using different ethanol/iso-propanol mixtures as the solvent. Notably, the yield of oil from the reaction in these solvent mixtures under supercritical solvent conditions (270 °C) was consistent (65-75% in each case), but the fractions of these oils that are identifiable monomeric products varied with the solvent composition. The 50:50 (v:v) EtOH/*i*-PrOH ratio gave optimal selectivity toward monomer yield. Thus, there appears to be a distinct synergy of such mixtures in the depolymerization pathway.

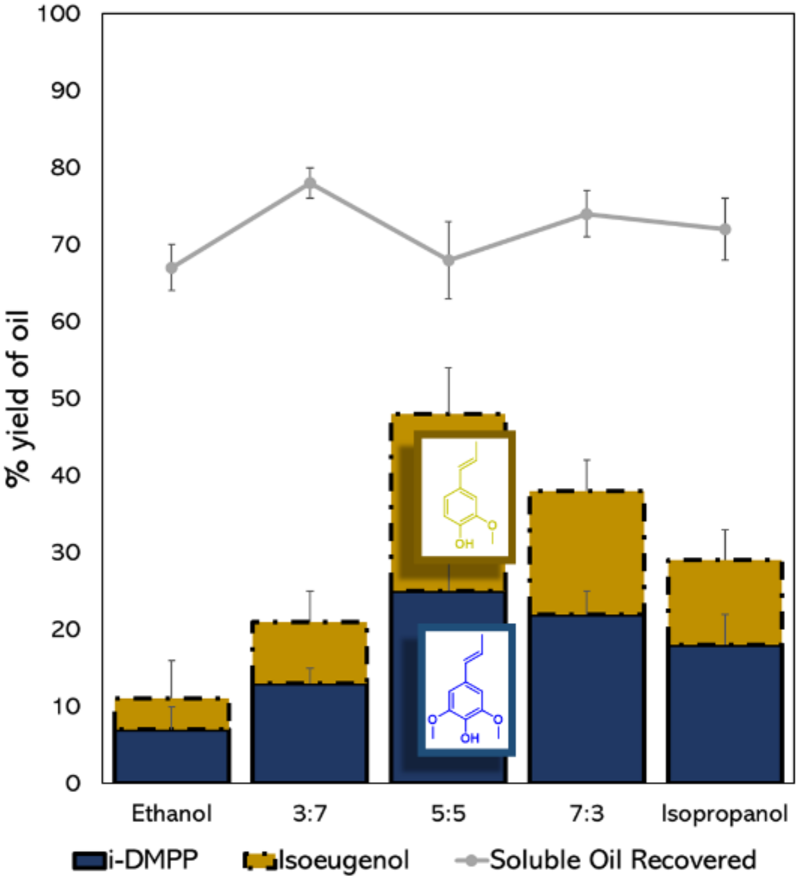


Figure 2. Specific product percent yield of oil as a function of ethanol/isopropanol determined by using quantitative HPLC analysis. Conditions: 10 bar N_2, 270 °C, 4 h reaction time, stirred at 700 rpm in a Parr reactor.

HPLC analysis of the recovered oil shows that the principal monomeric products are i-DMPP and isoeugenol. The remaining material in the oil is apparently a mixture of dimers, trimers and other cross-coupled products that we did not identify. Figure 3 displays the aromatic region HSQC spectra for the OPL solution before and after reaction in 50:50 (v:v) EtOH/*i*-PrOH. A key feature is that the reaction products display the same S:G ratio as seen in OPL, thus the process does not disrupt the aromatic methoxy groups. However, in the aliphatic region, approximately 14% of the inter-lignol linkages remain, the apparent composition being 4% β-O-4, 9% β-β, and 2% β-5 linkages This result indicates that the supercritical solvent conditions readily cleave the predominant β-O-4 linkages (apparently by hydrogen transfer hydrogenolysis) accompanied by deoxygenation of aliphatic sites. However, β-β and β-5 C-C bond cross-links remain intact under these conditions.

4.6.3 GPC Analysis.

This was conducted in order to confirm that OPL was depolymerized. Table 2 summarizes the number-average (M_n), weight-average (M_w) molecular weight, and polydispersity (M_w/M_n) of the residual lignin (relative to polystyrene standards) obtained after lignin depolymerization by the EtOH/*i*-PrOH solvent system. Compared to untreated OPL (entry #1), entries 2-4 show that the molecular weight and dispersity have decreased; therefore, depolymerization has occurred. The recovered lignin when re-dissolved for GPC analysis dissolved into the solution with no signs of repolymerization/condensation.

Table 2. GPC analyses. Entry #1 is OPL before the reaction is performed. Entries #2 and #3 are for the soluble oils isolated after reaction. Entry #4 is OPL with a Ni/C catalyst under H_2. Conditions: 50:50 EtOH:*i*-PrOH (30 mL), 4 h reaction at 270 °C in a 75 mL Parr reactor stirred at 700 rpm.

#	Sample	M_n/g mol^{-1}	M_w/g mol^{-1}	PDI
1^a	OPL	1939	5515	2.84
2^b	OPL + 10 bar N_2	523	1056	2.02
3^b	OPL + 10 bar H_2	583	1075	1.84
4^b	OPL+ 10 bar H_2 Ni/C	591	1153	1.95

^{a} before the reaction. ^{b} from soluble oil collected after reaction.

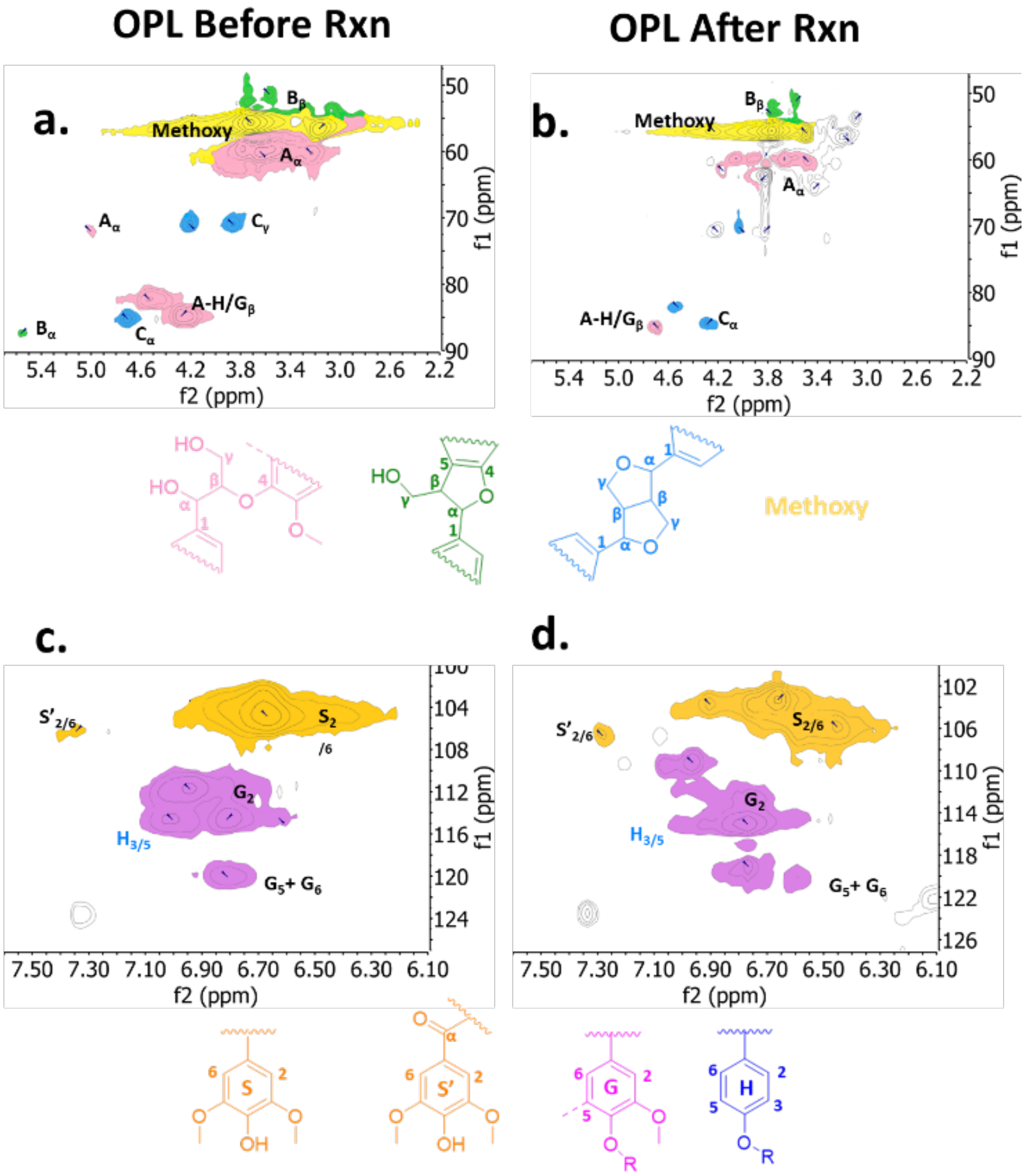


Figure 3. 2D ^{1}H-^{13}C HSQC NMR spectra showing characteristics of OPL before reaction vs. OPL after reaction using 50:50 EtOH/*i*-PrOH. Conditions: 180 mg OPL, 50:50 EtOH/*i*-PrOH (30 mL), 270 °C, 10 bar N_2, 4 h stirring at 700 rpm in Parr vessel. The top spectrum is the aliphatic side chain region (a. and b.: δ_C/δ_H of 50–90/2–5.5 ppm). The spectra, a. show the β-O-4 in the 3.1-4.2/55-65 ppm region, but a decrease in spectrum b. suggesting cleavage of the linkage. The β-5 linkage is present in the 3.0-4.0/50-55 ppm region for spectrum a. but decreased in b. β-β appears also in a. 3.8-4.6/70-75 ppm region, but not as prevalent in b. The bottom spectrum is depicting the aromatic region (c. and d.: δ_C/δ_H of 100–130/6.1–7.5 ppm). S region is shown from 6.2-7/100-110 ppm. G region is shown from 6.5-7.3/110-120 ppm. Assignments in linkages were determined based on literature.[50]

4.6.4 Temperature effects.

The reactivity of OPL in 50:50 EtOH:*i*-PrOH was investigated at 215 °C, 235 °C, 255 °C, and 270 °C (Swagelok). Notably, the supercritical temperature of iso-propanol is 235.6 °C (5.4 MPa) while that for EtOH is 241 °C (6.3 MPa), and we assume that the mixtures enclosed in the reactors are supercritical above 240 °C. Not surprisingly, the reaction proved to be quite sensitive to temperature, a significant drop in monomer yields being observed at temperatures below 255 °C (Figure 4). At 215°C and 235 °C, the yields of i-DMPP and isoeugenol were negligible, while the HPLC analysis indicated the presence of a large number of side products (unidentified). Temperatures at or above 255 °C result in more complete hydrogenolysis of OPL. However, again it was found that the yields of recovered oil (ca. 70%) and char (ca. 30%) remain roughly the same.

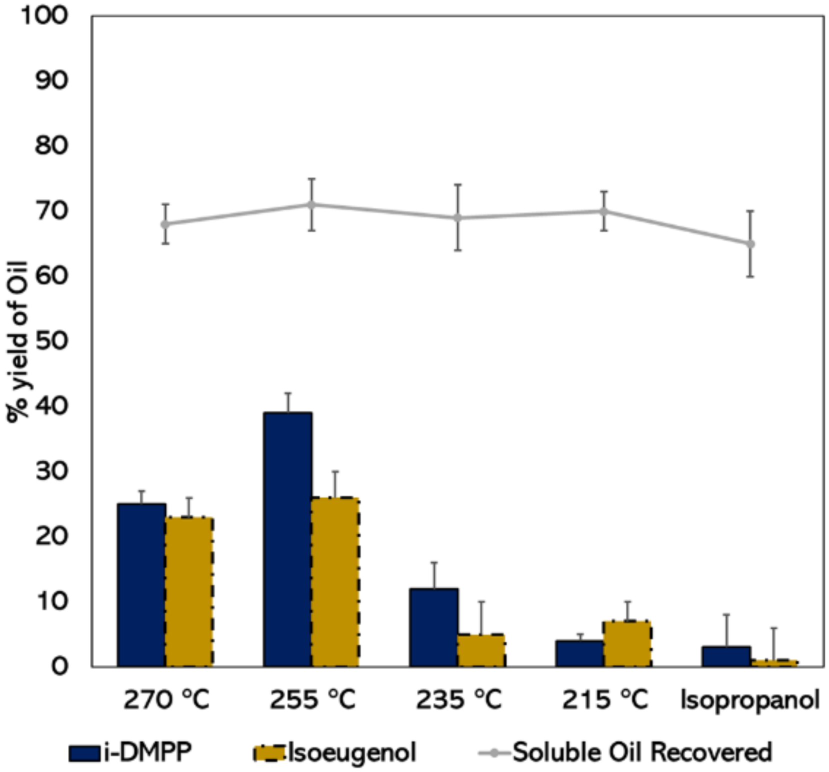


Figure 4. Temperature effects on yields of monomeric products from conversion of OPL in 50:50 (v:v) EtOH/*i*-PrOH as determined by HPLC analysis . All reactions were for 4 h

in a Parr reactor stirred at 700 rpm and pressurized to 10 bar (N_2) with no added metal-based catalyst. The data of the 5th column was derived from an analogous run at 235 °C in iso-propanol only.

4.6.5 Pressure effect.

Table 3 summarizes results for reactions carried out in the Parr reactor while pressurizing the system with an added gas (N_2 or H_2) at 1-35 bar (starting pressure at room temperature). The yields of recovered oil and char do not show major differences. However, the yield of monomers i-DMPP and isoeugenol did improve at higher pressure, with an increase for H_2 versus N_2 at 35 bar.

Table 3. The effects of added gasses and pressure in 50:50 (v:v) EtOH/*i*-PrOH solvent system. Conditions: 180 mg OPL, 270 °C, 10 bar N_2, 4 h, in Parr reactor stirred at 700 rpm.

Pressure (Bar)	Char (mg)	Soluble Oil (mg)	Monomers from Oil (mg)	Wt% Yield of monomers[a]
1 **(N_2)**	62	98	40	22
10 **(N_2)**	57	120	59	33
35 **(N_2)**	71	103	52	29
10 **(H_2)**	62	120	65	36
35 **(H_2)**	45	130	83	46

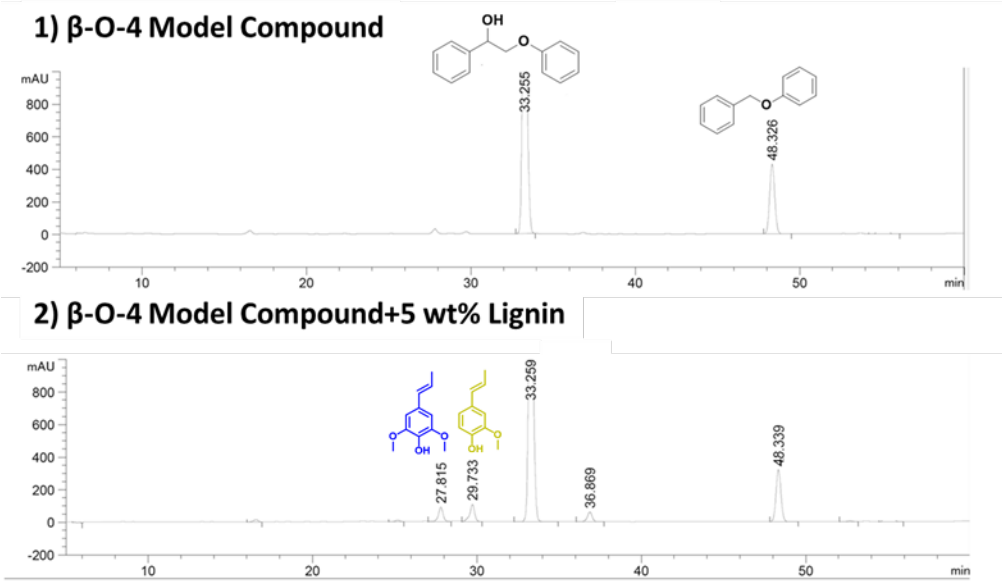
[a] = (monomers from soluble oil/180 mg) x 100

4.6.6 Mass Balance.

For a 180 mg OPL reaction in 50:50 EtOH:*i*-PrOH solvent under 10 bar N_2 gas, 275 °C, and 4 h, complete mass balance was taken for this reaction. Filtered char was weighed and dried to give 57 mg of solid. The recovered oil was 120 mg; thus the sum of the recovered solid and soluble oil corresponds to 98% of the original weight of OPL. Analysis of the soluble oil indicated the presence of 32 mg and 27 mg of i-DMPP and isoeugenol, respectively, corresponding to a 33 wt% yield of identifiable monomers yield of 33% from the starting 180 mg OPL. Similar mass balance and monomer yields were obtained with other applied pressures of N_2 or H_2 (Table 3)

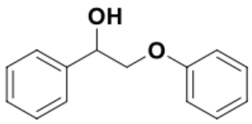
4.6.7 Reactivity studies with model compounds.

In order to gain greater insight into the depolymerization pathways, two β-O-4 model compounds 2-phenoxy-1-phenylethanol (MC1) and 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (MC2) were subjected to the same conditions as shown above for OPL substrate. Remarkably, MC1 proved to be refractory when heated at 270 °C for 4 h in 50:50 (v:v) EtOH/*i*-PrOH as analyzed by HPLC (Figure 5). When a small amount of OPL (5 wt% relative to the MC1 substrate) was added to the reaction mixture, some products were observed, but these may have largely originated with the OPL. Within experimental uncertainty none of the MC1 was consumed.

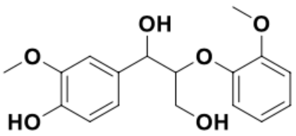

1) β-O-4 Model Compound

2) β-O-4 Model Compound+5 wt% Lignin

Figure 5. HPLC chromatograms of the soluble oil products of the following reactions: (1) MC1 (50 mg) alone (2) MC1 (50 mg) OPL (2.5 mg). In each case the solvent mixture was 5:5 EtOH:*i*-PrOH (30 mL), the temperature was 270 °C, and the reaction time was 4 h. Each reaction were run in a 75 mL stainless steel Parr reactor stirred at 700 rpm under a N_2 atmosphere (10 bar at ambient T). The peak at 48 min is benzylphenylether (10 mM) was added after the reaction as an internal standard.



MC1



MC2

In contrast, the substrate MC2 is more reactive under the standard conditions in 50:50 EtOH/*i*-PrOH (270 ^{o}C), although after 4 h, conversion was only 48%. (Figure 6 and Table 4). Neither isoeugenol nor guaiacol was formed in significant quantities, although some other unidentified species are seen in the HPLC chromatogram. However, when the same reaction was carried out in the presence of small amount of added OPL (5 wt%, relative to the substrate), the conversion increased to 100% under otherwise identical conditions (Figure 6). Furthermore, the product distribution as analyzed by HPLC was significantly different with substantial generation of both guaiacol (~62% of theoretical) and isoeugenol (~ 40% of theoretical), which are not formed in the absence of OPL. Thus, OPL appears to contain a component that clearly affects substrate reactivity and product distribution.

The question remains regarding what OPL component may be responsible for this activity. A previous study from this laboratory[51] found that OPL prepared by an HCl extraction method as described here contains significant chloride impurities. Thus, the organosolv extraction method may lead to HCl impurities that could be acting as a catalyst for the depolymerization of lignin. In this context, Figure 6 illustrates the HPLC results for different 4 h reactions of MC2 at 270 ^{o}C in 50:50 EtOH/*i*-PrOH to which additives relevant to potential OPL impurities have been added. The quantitative data are summarized in Table 4. Notably, these data show that addition of HCl or NaCl (1000 ppm in the solution) leads to complete consumption of MC2 coupled with substantial yields of guaiacol (83%

and 55%) and isoeugenol (59% and 41%, respectively). The analogous reaction with added KOH also led to complete consumption of the substrate but the only

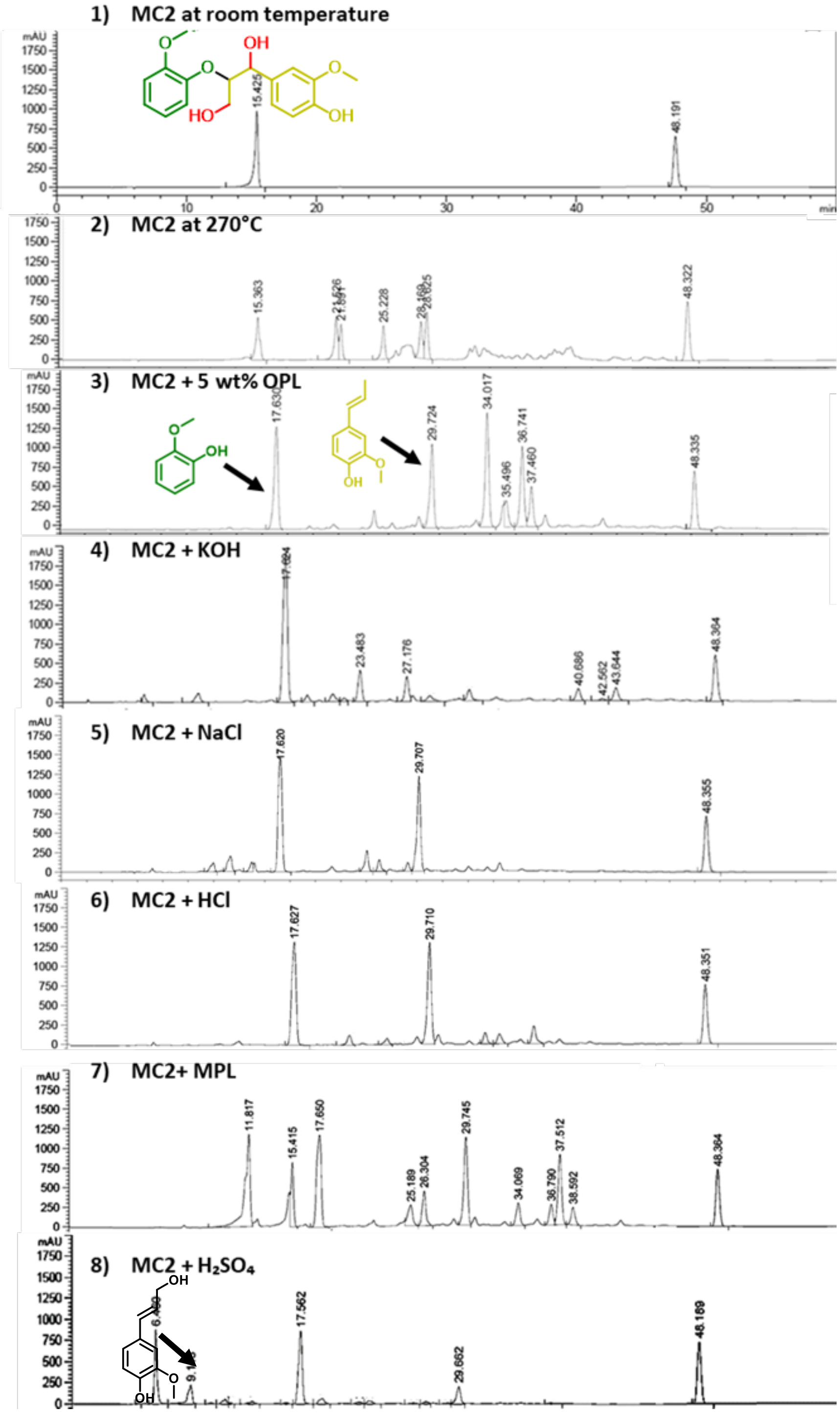


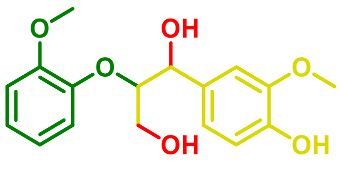
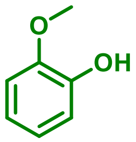
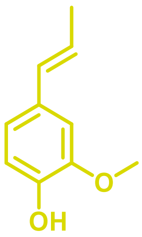
Figure 6. HPLC chromatograms of products from reaction of MC2 in the presence of the following additives: (1) none. (2) 5 wt% OPL. (3) 1000 ppm KOH. (4) 1000 ppm HCl. (5) 1000 ppm NaCl. (6) 5 wt% MPL. (7) 1000 ppm MPL. (8) 1000 ppm H_2SO_4. In each case,

the solvent was 50:50 EtOH:*i*-PrOH (30 mL), T = 270 °C, and the reaction time was 4 h in a 75 mL Parr reactor stirred at 700 rpm under N_2 (10 bar). The peak at RT 48 min is benzyl phenyl ether added after the reaction as an internal standard.

major identified product was guaiacol (~90%), which can be formed by simple hydrolysis of the β-O-4 bond. There was very little or no isoeugenol formation.

When the analogous of MC2 was carried out with added MPL (5 wt% to the substrate), ~90% of the substrate was consumed with guaiacol (~55%) and isoeugenol (40%) being among the products. Sulfuric acid is used in the extraction of MPL, so the 4 h reaction of MC2 at 270 °C in 50:50 EtOH/*i*-PrOH containing H_2SO_4 (1000 ppm to the solution) was also examined. Notably, while nearly complete consumption of MC2 was observed, the HPLC product chromatogram proved to be different from the other systems studied (Figure 6), although both guaiacol (~88%) and isoeugenol (19%) were among the products.

Table 4. Quantitative summary of Figure S2 for the formation of i-DMPP and isoeugenol from MC2 the reaction of MC2 in the presence of different additives.

	 **Starting: 50mg; 0.156 mmol**		
	320.34 g/mol	**124.14 g/mol**	**164.20 g/mol**
Additive[a]			
(2) None	0.081 mmol	0 mmol	0 mmol
(3) 5 wt% OPL	0 mmol	0.096 mmol	0.062 mmol
(4) KOH	0 mm ol	0.14 mmol	0 mmol
(5) NaCl	0 mmol	0.086 mmol	0.064 mmol
(6) HCl	0 mmol	0.13 mmol	0.092 mmol
(7) 5wt% MPL	0.016 mmol	0.085 mmol	0.064 mmol
(8) H_2SO_4	0 mmol	0.137 mmol	0.030 mmol

[a] Amount **of** HCl, KOH, NaCl or H_2SO_4 added is 1000 ppm relative to the solvent.

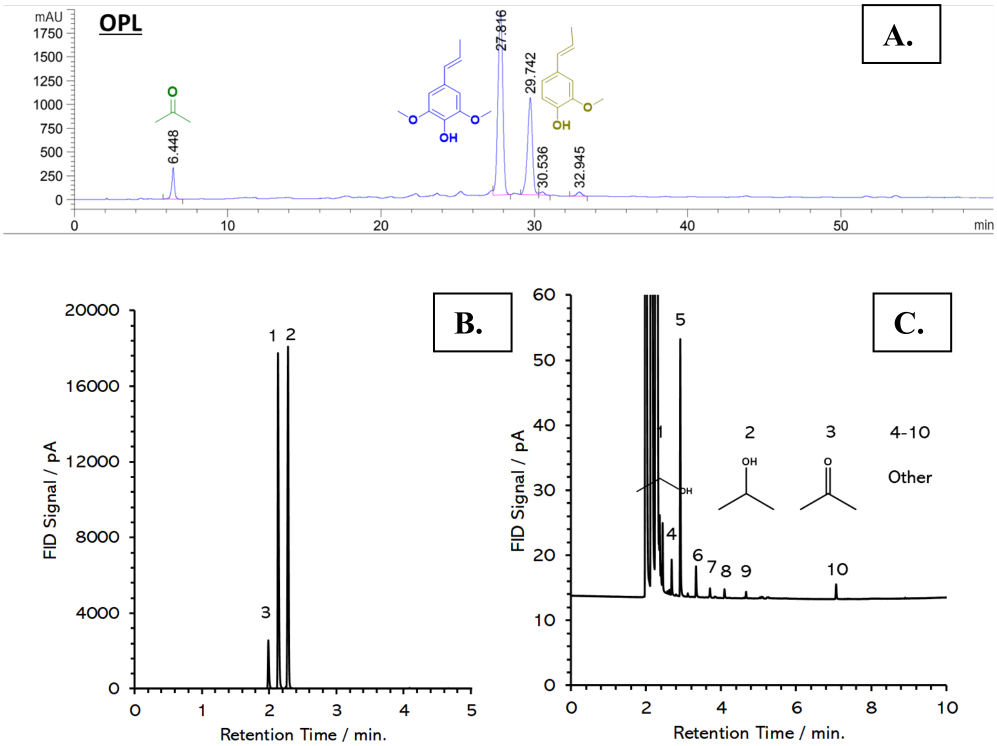


Figure 7. HPLC and GC-FID chromatograms of the solvent collected after the reaction of 180 mg OPL in 50:50 EtOH:*i*-PrOH (30 mL), 10 bar of N_2, 270 °C, in a stainless steel Parr reactor stirred at 700 rpm. **A**: HPLC: Major peaks: acetone (6 min), i-DMPP (27-28 min), isoeugenol (29-30 min). **B**: GC-FID: Major peaks: ethanol (1), iso-propanol (2) and acetone (3). **C:** Same chromatogram as **B** expanded to show minor products labeled 4-10.

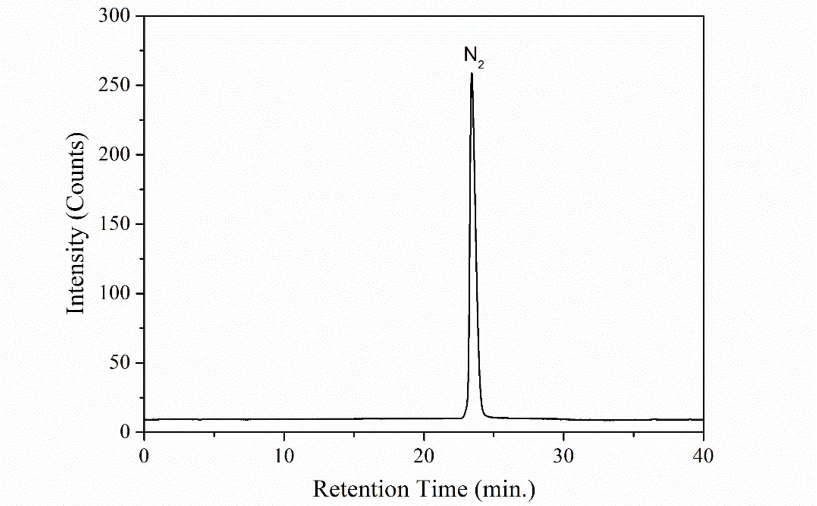


Figure 8. The GC-TCD analysis of the gas collected from the experiment described in Figure 7. The only gas evident was N_2.

4.6.8 GC analysis of solvent based products.

The principal monomeric products, especially isoeugenol, are qualitatively the result of the hydrogenolysis of phenolic ether linkages in OPL. Given the absence of added H_2 the likely source of the necessary hydrogen equivalents would be the solvent itself-most likely *i*-PrOH. In this context, we analyzed both the solvent and gas phase collected after the reaction for 180 mg OPL in 50:50 EtOH/*i*-PrOH (30 mL), 10 bar of N_2, 270 °C, in a 75 mL stainless steel Parr reactor stirred at 700 rpm. GC-FID analysis of the solvent showed, in addition to EtOH and *i*-PrOH, a strong peak at a retention time (2 min) corresponding to acetone (Figure 7). The amount of acetone is ~5 mmol in the after-reaction solvent calculated by ECN method.[51] Expanding the vertical axis of this chromatogram also demonstrated the presence of at least 7 very minor species in quantities very much smaller (<1%) than that for acetone. GC-TCD analysis of the corresponding gas phase (Figure 8) showed only N_2 and that no H_2 was evident. In this context, it appears that direct hydrogen transfer from *i*-PrOH is the principal source of the reducing equivalents leading to the HTH of OPL ether linkages under these conditions. Given that the reaction does occur in neat EtOH, albeit with lower efficiency, it is likely that ethanol is a lesser source of reducing equivalents in the mixed solvent system.

4.7 Discussion.

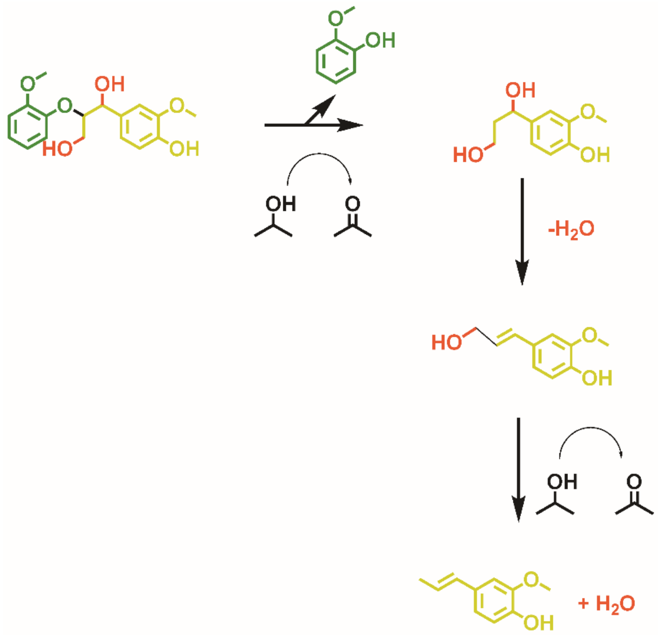
Reported is the thermochemical reaction of organosolv poplar lignin in catalyst-free ethanol/iso-propanol solvent mixtures. The depolymerization of OPL in EtOH/*i*-PrOH gave solid char and liquid oil fractions with yields of the phenolic monomers within the soluble oil product dependent on the solvent ratios (Figure 2). Although the yields of soluble oil were comparable for different EtOH/*i*-PrOH ratios, yields of the monomers i-

DMPP and isoeugenol proved to be optimal in the 50:50 solvent mixture. With ethanol or iso-propanol alone, the selectivity toward these monomers decreased, although pure *i*-PrOH did perform better than pure ethanol. Thus, it appears that the mixed EtOH/*i*-PrOH medium shows synergistic effects for the hydrogen transfer hydrogenolysis of OPL.

To our knowledge, there are no previous reports of using EtOH/*i*-PrOH mixtures alone for lignin depolymerization and HTH, although there have been reports for using supercritical ethanol or iso-propanol as sources of hydrogen equivalents for metal catalyzed HTH.[52-59] It has also been shown potassium isopropoxide aids in transfer hydrogenation from iso-propanol to coal in the formation of acetone and the equivalent of H_2 transfer to substrate.[60] In our work, the observation of acetone in the liquid phase after reaction with OPL (Figure 7) clearly indicates that *i*-PrOH is the major source of hydrogen reducing equivalents needed for HTH of OPL. Hydrogen donor solvents such as *i*-PrOH have also been used to suppress reactions of radical intermediates produced in the high temperature pyrolysis of lignins.[61]

The catalytic effect of OPL was much more evident with MC2. There are clear differences in the two model compounds where MC2 contains electron-donating methoxy substituents on the aryl rings, and thus would be a better model of lignin. For MC2, there was a low conversion in 50:50 EtOH/*i*-PrOH, but when 5 wt% OPL was added, there was markedly increased selectivity towards the generation of guaiacol and isoeugenol. One known OPL impurity is chloride, given the procedure used in the organosolv extraction process. Sulfur is another possible impurity in organosolv lignins. Addition of NaCl, HCl, or H_2SO_4 to the reaction medium changed the product distribution to favor hydrogenolysis. These results imply that such impurities in the OPL assist in hydrogenolysis cleavage of the β-O-4 bonds of both MC2 and OPL into guaiacol as well as polyols that are subsequently hydrodeoxygenated to isoeugenol.

Although guaiacol can be formed from MC2 by simple hydrolysis or hydrogenolysis of the β-O-4 linkage, the formation of isoeugenol requires a much more complex series of steps. Scheme II illustrates a possible reaction sequence including hydrogenolysis, dehydration and hydrogenation. Regardless the exact sequence, which remains to be determined, the formation of this product requires two H_2 equivalents, the primary source presumably being *i*-PrOH give the observed acetone formation. Li and coworkers concluded that the disassembly of a model substrate very similar to MC2 using a Re/activated carbon catalyst and *i*-PrOH as the hydrogen source proceeded initially by β-O-4 hydrogenolysis followed by additional reductions as shown in this Scheme. However, how the cleavage of the β-O-4 linkage in MC2 (and presumably in lignin) is activated by OPL or the impurities included remains an open question.



Scheme II. Proposed sequence of reactions for MC2 conversion to guaiacol and isoeugenol in 50:50 EtOH:*i*-PrOH at 270 °C.

4.8 Conclusion.

In summary, our findings show a new process for lignin depolymerization and hydrogenolysis in which EtOH/*i*-PrOH solvent mixture provides the reducing equivalents through a hydrogen transfer pathway. The identity of the active hydrogen transfer species is not known, but acid or ions introduced along with the lignin substrate act as a catalyst in this unique depolymerization/hydrogenolysis system. The reaction does not require the addition of a transition-metal catalyst and affords monomer phenols in yields (~33% based on the mass of starting lignin substrate) that are competitive with those obtained with most transition metal catalysts.

4.9 Publication.

This chapter was accepted in ACS Sustainable Chemistry & Engineering where I worked on the analysis of the synergistic performance of EtOH/i-prOH with the depolymerization on organosolv lignin.

4.10 References.

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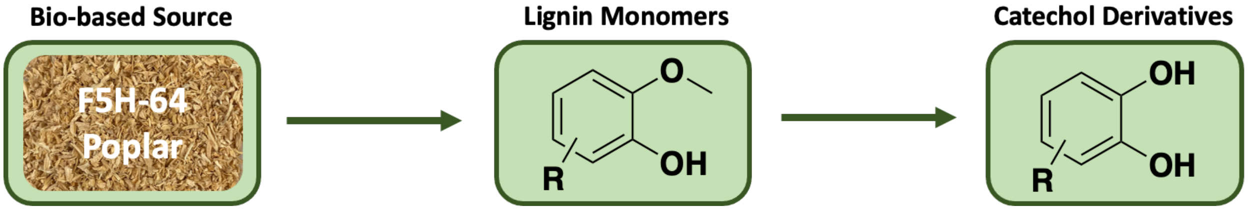
Chapter 5. Selective depolymerization and modification of genetically modified Poplar into catechol derivative: DMPPO.



5.1 Introduction.

Currently we live in a world that is surrounded by plastics ranging from vehicles to the packaging of clothing, there is endless possibilities to different commodities produced from plastic.[1-4] These plastics largely stem from petroleum, and the increased concern with the green-house emissions and climate change provide a strong incentive for the search for clean and renewable alternatives.[5-12] This is a challenge due to the high performance and dependance of current fossil-derived plastics which has set a high bar for biobased plastics. Research has shown the progress in the replacement of petrol-derived monomers by using biobased analogs which provide the same characteristics but are derived from renewable sources.[13-18] Even though biobased-derived building blocks have similar properties as petro-derived monomers, there is still a variance that makes it not the most direct replacement. Some disadvantages that are currently faced in research in both the industry and academia is the optimization of the price and availability of the monomers from the renewable resource.[21-33]

Lignocellulosic biomass is the most abundant and renewable source of organic carbon known which presents the best alternative for sustainable bio-based materials.[34-39] Research in the past decade has been focused to convert biomass components into 1. Biofuel and 2. Value-added products. With the dilemma of it becoming economically feasible, the success of the future towards these bio-based materials is dependent of lignin valorization. Lignin, which is the second most abundant aromatic polymer can be obtained from woody biomass, agricultural residues, and energy crops. The world annually produces around 100 million metric tons of lignin which is worth $732.7 million USD, cheaper in comparison to fossil fuels.[33] With lignin being a cheap and abundant alternative, the research spent on lignin valorization has focused on production of valuable products such as polymers, resins, and adhesives.[40-46]

Organosolv fractionation is a promising process for effective separation of lignin. A wide range of organic solvents are used in this study, including acetone, methanol, ethanol, organic acids (i.e., acetic acid, formic acid), inorganic and combined solvents with and without acid or alkaline catalysts are used in biomass fractionation.[47-53] Short-chain alcohols such as methanol and ethanol in the form of single or solvent mixture with water are the most commonly used organic solvents in this process which can be used in the presence or absence of catalytic promoters.[55-57] Product profiles from depolymerization of lignin fractionated from these different biomass samples were then taken and analyzed. This study provides a platform for further study on the value-added utilization of lignin in integrated biorefineries. The utilization of organosolv fractionation was taken and a combination of novel methodology developed for the upgrading of lignin into selective monomers for biofuels and biomaterial.

Due to the complexity, lignin depolymerization and upgrade of monomers for the target compound production is important. 2-methoxy-4-propylphenol (DHE) and 2,6-dimethoxy-4-propylphenol (DMPP) is a promising model compound of phenolic fractions of lignocellulosic bio-oil derived by catalytic depolymerization of lignin (CDL). The importance of these molecules is that it consists of several key functional groups, including phenyl, hydroxyl, methoxy, and allyl groups, which are part of all of the three main constituents of lignin. The bio-oil produced by CDL cannot be directly used as a transportation fuel as it contains a large number of methoxy-functional groups and has to be upgraded. Catalytic methods are used to reduce oxygen content and improve hydrogen/carbon ratios.

Catalytic hydrogenation, hydrodeoxygenation (HDO), and isomerization are the most promising routes for the upgrade of lignin-derived bio-oils to biofuel and fine chemicals. A platform chemical of interest for us is catechols. Catechols represent an important and versatile building block for a variety of materials such as pesticides, perfumes, and pharmaceuticals. In this regard, 2-methoxy-4-propylphenol and 2,6-dimethoxy-4-propylphenol is chosen as a lignin monomeric model compound having olefin, methoxy, aromatic and hydroxy functionality and is a potential molecule to understand the catalytic behavior and production of these catechol derivatives.

The demethoxylation and aromatic hydrogenation of lignin model compounds, DHE and DMPP under moderate conditions is a challenging task and is the subject of investigation. In our previous work, we identified the main function Nb_2O_5 partook in was catalyzing the intramolecular cyclization and hydrogenation of lignin oil. Here, we report the use of niobium oxide (Nb_2O_5) using only an aqueous medium as the major solvent for the demethoxylation of the model compounds. Niobium oxide was shown to successfully demethoxylated both model compounds under new optimized conditions and starting with raw biomass, we were successful in the conversion of the lignin monomers into polymer building blocks.

What is interesting about our work is the conversion of DMPP to 5-propylpyrogallol (DMPPO), a polymeric building block that is not as well known for its properties. We have selectively targeted the production of DMPPO from genetically modified poplar in order to study its chemical structure and mechanical properties. In this study, we demonstrate a synthesis chain from genetically modified poplar wood (high-S poplar) to produce DMPPO selectively and the DMPPO can be upgraded to tri-epoxides which shed the light on making a real lignin-based sustainable polymer.

5.2 Experimental.

5.2.1 Reagents and feedstocks.

All commercial chemicals were purchased and used as received. Biomass substrates were milled to 40 mesh by a Mini Wiley Mill (Thomas Scientific, Swedesboro, NJ) before use. 2-Methoxy-4-propylphenol (≥99%), 2,6-dimethoxy-4-propylphenol (99%), Pd/C (5 wt%), $ZnCl_2$, and niobium(V) oxide (325 mesh, 99.9%) were purchased from Sigma-Aldrich. Methanol (ACS reagent grade), ethanol (200 Proof), and ethyl acetate (ACS reagent grade) were purchased from Fisher Chemical. Cetyltrimethylammonium bromide (CTAB, 98%) and n-dodecane (99%) were purchased from Alfa Aesar. Hydrochloric acid (GR ACS) was purchased from EMD Millipore Corporation. Niobium(V) chloride (≥99%) was purchased from Strem Chemicals. Hydrogen gas (5.0 grade) and nitrogen (99.998%) were purchased from Praxair. Water used for reaction and sample preparation was obtained from an A10 Milli-Q water purification system by Millipore.

5.2.2 Catalyst preparation: Nb_2O_5.

Nb_2O_5 was synthesized using a hydro- thermal method according to a modified literature pro- cedure.35 Typically, a 20 mmol portion of the precursor $NbCl_5$ was dissolved in 20 mL ethanol with rigorous stirring for 10 min, and then the solution was added to a water solution of CTAB (1 g in 15 mL distilled water) dropwise. The mixed solution was then stirred for 0.5 h, followed by adding 20 mL of aqueous HCl (pH 1) that was previously prepared by dissolving a specific amount of hydrochloric acid in water and stirring for another 1.5 h. The resulting solution was then placed into a Teflon-lined autoclave and aged at 160 °C for 24 h. Subsequently, the solid was separated and washed with dis- tilled water and dried at 60 °C overnight. After this, the sample was ground and packed for calcination in air. A Thermolyne F6020 1200 C Muffle furnace was used to calcine the niobia sample. Ramping rate of the furnace was pre-set to 1 °C min^{-1}. After 6 h of calcination at 450 °C, the active Nb_2O_5 catalyst was collected at room temperature.

5.2.3 Catalyst preparation: Pd-Zn/C.

Pd-Zn/C was prepared with 10 wt% Pd/C (5 wt%) with the addition of $ZnCl_2$ in 25 mL MeOH, and a glass stir bar was added into a stainless-steel Parr reactor and sealed then purged with H_2 gas and pressurized with 10 bar of H_2. The reaction was heated at a temperature of 150°C for 8 h at 700 rpm. The reaction was then left to cool to rt and then the catalyst was filtered and rinsed 3 times with 25 mL MeOH. The catalyst was tested in intact biomass and lignin reactions to test reactivity and selectivity.

5.2.4 Catalyst Characterization Techniques.

NH_3-temperature programmed desorption (TPD).

To evaluate the acid sites on Nb_2O_5, NH_3-TPD was performed on a Micromeritics AutoChem 2920 instrument. A 200 mg sample of Nb_2O_5 was placed into a U-shaped quartz tube. This material was first pretreated by heating under flowing helium (25 cm^3 min^{-1}) at 300 °C for 0.5 h. A mixture of NH_3 in He (1 : 9 v/v) was then passed through the tube at a flow rate of 15 $cm^3\ min^{-1}$ at 25 °C for 1 h. After this, the sample was flushed with He (25 $cm^3\ min^{-1}$) at 100 °C for another hour. TPD measurements were carried out over the temperature range 100–500 °C at a ramp rate of 10 °C min^{-1} and the ammonia concentration in the effluent was monitored using a filament thermal conductivity detector (TCD). The amount of desorbed ammonia was determined based on the integrated peak area.

Gel Permeation Chromatography (GPC).

Gel permeation chromatography was carried out on a Waters (Millford, MA) chromatograph equipped with a Waters Alliance HPLC pump (2695 Separation Module) and two Tosoh TSKgel Super HM-M columns with N,N-dimethylformamide (DMF). A Waters 2414 differential refractometer was used as the detector. Number-average molecular weights (M_n) and weight-average molecular weights (M_n) were calculated relative to linear polystyrene standards.

Organosolv Lignin Preparation from F5H-64 Poplar.

Abu-Omar Method.[52]

Lignin Extraction and Isolation. Poplar wood substrates were treated in methanol/water mixtures to separate lignin. A 2 g sample of 40 mesh raw biomass was added to a mixture containing 0.045 N H_2SO_4 (25 mL), MeOH (25 mL), and 37 wt % aqueous formaldehyde (4 mL) in a Parr pressure vessel. The reaction was purged with N_2 gas five times and stirred at 700 rpm. The N_2 gas pressure was increased to 10 bar, and the contents were allowed to react at 160 °C for 0.5 h. After cooling to room temperature, the contents were filtered to separate the solid residue, mainly carbohydrates, from the filtrate.

The filtrate was concentrated by rotary evaporation to remove the MeOH. Nanopure water (150 mL) was added to the remaining acidic aqueous solution to precipitate the lignin. The extracted lignin was then collected by filtration and washed three times with Nanopure water. The solid lignin was dried overnight and then characterized by heteronuclear single quantum coherence NMR (HSQC) and gel permeation chromatography (GPC). This lignin is referred to in this study as MPL. Elemental analysis of MPL: C, 60.8%; H, 6.6%; N, 0.34%.

Ford Method.[53]

Poplar wood shavings were treated with 1:1 ethanol:toluene, filtered, and dried overnight. In a 10 L round-bottom flask, 4.5 L of methanol, 600 g of treated poplar wood shavings, and 12 mL of concentrated HCl were added. The suspension was heated to 110 °C and stirred for 12 days. The deep brown solution was separated from residual solids, mainly carbohydrates, by filtration, and the volume reduced to half in vacuo. A 1 L volume of ice was added to precipitate the lignin as a beige solid. The solid was collected by filtration and washed with cold water until the pH of the filtrate was 7. The resulting organosolv poplar lignin was then dried in vacuo overnight, yielding 21.6 g of material. This lignin is referred to in this study as OPL. Elemental analysis of OPL: C, 59.8%; H, 6.6%.

Deuss Method.[58]

10 g scale with alcoholic solvent (EtOH) (80 mL), 20 mL of H_2O, and 2 mL of a 37% (12 M) aqueous HCl/H_2SO_4 solution were added and the setup was equipped with a reflux condenser. The mixture was heated and stirred at 80 °C (oil bath temperature). After

the desired extraction time, the mixture was cooled to room temperature, the extract was collected by filtration and concentrated by rotary evaporation. The obtained solid was redissolved in a minimal amount of acetone and the lignin was subsequently precipitated by addition to 300 mL of water. The product was collected by filtration, washed with water, and air-dried overnight to obtain organosolv lignin as a powder, which was analyzed by NMR ($DMSO_6$) and GPC [DMF] The mass balance of a 5 h ethanosolv extraction is calculated. The lignin was analyzed with HSQC-NMR, GPC, and HPLC after depolymerization.

Barakay Method.[59]

10 g scale (EtOH 65%, H_2O 35%) with 8 mmol L−1 catalyst in a 500 mL autoclave (Autoclave France®). A benchmark experiment was carried out by using 4.4 mmol L−1 H_2SO_4 in the same conditions. The mixture was stirred during 2 h at 160 °C. Upon completion of the reaction, the reactor was cooled down to room temperature. Pulp and black liquor were separated from the reaction mixture using a nylon filter. The pulp was washed three times with 300 mL of an aqueous ethanol solution at 60 °C and the washes combined with the black liquor. Three volumes of water were added to the resulting liquor in order to precipitate the lignin. The solution obtained was filtered and washed with 3 times EtOH in 25 mL portions. The precipitate was washed with water and dried at room temperature for three days. Water was added to the filtrate to precipitate the lignin then taken analyzed with HSQC-NMR, GPC, and HPLC after depolymerization.

Hosur Method.[60]

1:8 ratio biomass A mixture of 85% organic acid (ratio of formic acid/acetic acid mixture was 70:30 by volume) was added to the biomass in the flask at a fiber to liquor ratio of 1:8 and allowed to boil on a hot plate for 2h. After 2h, flask and its content were allowed to cool to ambient temperature. Fibers were filtered in a Buchner funnel and washed with 80% formic acid followed by hot distilled water. The filtrate contained lignin that was precipitated with water and filtered and washed. The lignin was analyzed with HSQC-NMR, GPC, and HPLC after depolymerization.

5.2.5 Catalytic reaction and product analysis.

Reactions in a Parr reactor. Batch reactions were carried out in a stainless steel 75 mL 6-series pressure reactor (Parr Instrument Company, 5000 series). The reactor vessel was equipped with a magnetic stirring system. For a typical reaction, 0.2 g Nb_2O_5 were physically mixed in the vessel with 12 mL distilled water as the solvent. To this were added the substrate (0.2 mL). The reactor was then sealed and purged with N_2 three times. Then, the reactor was filled with N_2 (10 bar). The reactor was heated to 230 °C and held at that temperature for a defined time (20 h). The stirring rate was kept at 700 rpm during the whole reaction period. Subsequently, the reactor was cooled to room temperature. The products in the liquid phase were extracted using ethyl acetate and the gas phase products were collected in a sealed gasbag for further analysis.

5.2.6 Synthesis of tri-epoxides from DMPPO.

Tri-epoxide DMPPO was prepared by reaction of DMPPO (1 g) and epichlorohydrin (15 g). Tetrabutylammonium bromide (155 mg) was used as a phase-transfer catalyst. The mixture was heated at 60 °C for 3 h and followed by a dropwise addition of 50% w/w

NaOH solution. The reaction was kept for another 3 h, and the mixture was washed with acetone, filtered to remove salt, and concentrated with a rotary evaporator. The triepoxidized DMPPO was analyzed by ^{1}H NMR and mass balance was calculated by measuring the weights before and after reaction.

5.2.7 Analytical Techniques.

HPLC Analysis.

The liquid phase from lignin depolymerization was filtered using a 0.22 μm Acrodisc nylon membrane syringe filter and analyzed with an Agilent 1260 Infinity Quaternary High-Performance Liquid Chromatography system, using a Zorbax Eclipse XDB-C18 column (250 × 74.6 mm) set at 30 °C. The HPLC instrument was equipped with a G1315D diode array detector (DAD). The mobile phase was a mixture of H_2O (A) and acetonitrile (B) at a flow rate of 0.5 mL/min and a nonlinear gradient from 80% A and 20% B to 5% A and 95% B at 55.0 min. A fixed amount (400 μL) of a 10 mM internal standard solution (benzyl phenyl ether) was added into each sample for quantification purposes. Standard curves for all the aromatic products were made by comparison of the products to the internal standard. All results were analyzed and quantified according to standard curves.

Gas Chromatography analysis (GC-FID).

An Agilent 6890N gas chromatograph equipped with a flame ionization detector (FID) was used to quantify the reaction mixtures. A J&W DB-5 GC column (30 m × 0.250 mm I.D. × 0.25 μm film thickness) was selectively used for separation. The liquid product sample was first passed through a 0.2 micron PTFE syringe filter to remove solid particles, and then diluted to 25 mL in a volumetric flask. A 10 mM n-dodecane solution was pre-made as the internal standard for GC quantification. The sample solution was mixed with internal standard (1:1 v/v) in a 2 mL Agilent GC vial. The sample was injected using an autosampler. The inlet temperature was kept at 280 °C, while the detector temperature was 310 °C. The initial temperature of the oven was 40 °C and held for 7 min. Then the oven was heated to 250 °C at a ramp rate of 10 °C min^{-1} and kept at the final temperature for 5 min. The split mode was used with a split ratio of 10:1. Helium was used as a carrier gas at a flow rate of 14 mL min^{-1}. The instrument was calibrated using the known samples of the products. The analytes were then identified according to their retention time. The quantification of each analyte was acquired from a calibration curve which represented the relationship between concentration versus the ratio of peak area over internal standard.

NMR Analysis.

^{1}H NMR was obtained using a Varian Unity Inova 600 MHz spectrometer. The analyte was extracted using 700 μL DMSO and packed in a glass NMR tube for analysis. A Bruker AVANCE500 spectrometer was used for HSQC, which has an AS600 magnet that is charged to 500 MHz. The spectrometer operates at 500.13 and 125.77 MHz for ^{1}H and ^{13}C nuclei, respectively. For 2D-HSQC, we used an echo anti-echo experiment called HSQCETGP. Spectra of isolated lignin (40 mg) were acquired in 700 μL of 5:1 v:v DMSO-d_6 :pyridine-d_5 solvent.

5.3 Results and Discussion

The biomass sample that was used was High-S modified poplar named: F5H-64. This biomass contains: 20% lignin, 44% cellulose, and 21% hemicellulose. What is different about this biomass is that the G-unit is suppressed leading to high-S production after utilizing catalytic depolymerization techniques.

5.3.1 Lignin Substrates.

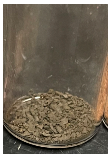
Even though poplar has been pulped with organic solvents before, there must be a careful study of the organosolv pulping of this material in order to produce the highest-quality lignin with high yields. With that, six different organosolv pulping methods were performed on F5H-64 poplar which is shown in **Table 1.** What we see is the isolation of lignin varies depending on the method that was utilized. The lignin color also visually is different in each entry giving a prediction that all of them vary in what monomers they would depolymerize to. To compliment **Table 1**, HSQC-NMR, GPC, and CDL was performed to analyze the extracted lignin samples.

5.3.2 HSQC-NMR Quantification.

Looking at **Figure 1**, the HSQC-NMR of the 6 different methods is divided into two regions: Aliphatic and Aromatic. Chemical shift assignments are presented as $^{13}C/^{1}H$ ppm. An aromatic region between 126–100/7.5–6.1 ppm and a side chain region between 90–50/5.6–2.2 ppm are evident in Figure 1. Looking at A-D and F, the side chain region shows several peaks that can be assigned for methoxy group and interunit linkages of: β-O-4, β–β, and β-5 motifs. However, for Spectra E, there are no linkages present and this shows that the interunit linkages were not maintained and degraded in this method. Looking at Spectra E, the conditions were for entry 5 where high heat was applied in comparison to the other entries.

The proportions of β-O-4, β–β, and β-5 in OPL were calculated by integrating the contour signals and comparing each method **(Table 2)**. Based on integration of β-aryl ether (A_α), phenylcoumaran (B_α), and resinol (C_α) contour signals, the linkage percentages of these three linkages vary in a range of: 29-85% β-O-4, 15-72% β–β, and a small amount of β-5. The aromatic region of the HSQC spectra also show the distribution of H:G:S **(Figure 1)**. When looking at the linkage percentages, the one that stood out with the best linkage preservation was B and F. All of these samples were taken and ran under CDL except **Table 1, Entry 5**. Similarly, the integration of $(S_{2/6} + S'_{2/6})/(G_2 + G_5 + G_6)$ contour signals shows that Spectra A-D and F contain substantial amounts of syringyl units as well as some guaiacyl units **(Table 2)**. Spectra E, Figure 1 does not show any aromatics indicating that the overall quality is not useable. After looking at the analysis for the methods from **Table 1**, the lignin samples were taken and CDL using Pd-Zn/C was performed.

Table 1. Results obtained through tests of pulping F5H-64 poplar.

Entry	Method	Lignin	Lignin (g)	Solid (g)
1	2 g of treated poplar wood shavings, 0.045 N H_2SO_4 (25 mL), MeOH (25 mL), and 37 wt % aqueous formaldehyde (4 mL) in a Parr pressure vessel, 0.5 h.		0.120	1.53
2	100 g of treated poplar wood shavings, and 12 mL of concentrated HCl were added. The suspension was heated to 110 °C and stirred for 12 days		7.12	88.34
3	10 g of treated poplar wood shavings, 40 mL H_2O, 4 mL 37 wt% H_2SO_4, stir at 80 °C for 5 h.		0.284	9.70
4	10 g of treated poplar wood shavings, FA/AA 70:30, 1:8 ratio biomass to solvent, 2 h to boil.		0.828	8.42
5	2 g of treated poplar wood shavings, EtOH 65% H_2O 35% 4.4-8 mmol H_2SO_4 for 2 h at 160° C 16 bar N_2		0.129	1.20
6	10 g of treated poplar wood shavings, 40 mL H_2O, 4 mL 37 wt% HCl, stir at 80 °C for 5 h.		0.436	7.72

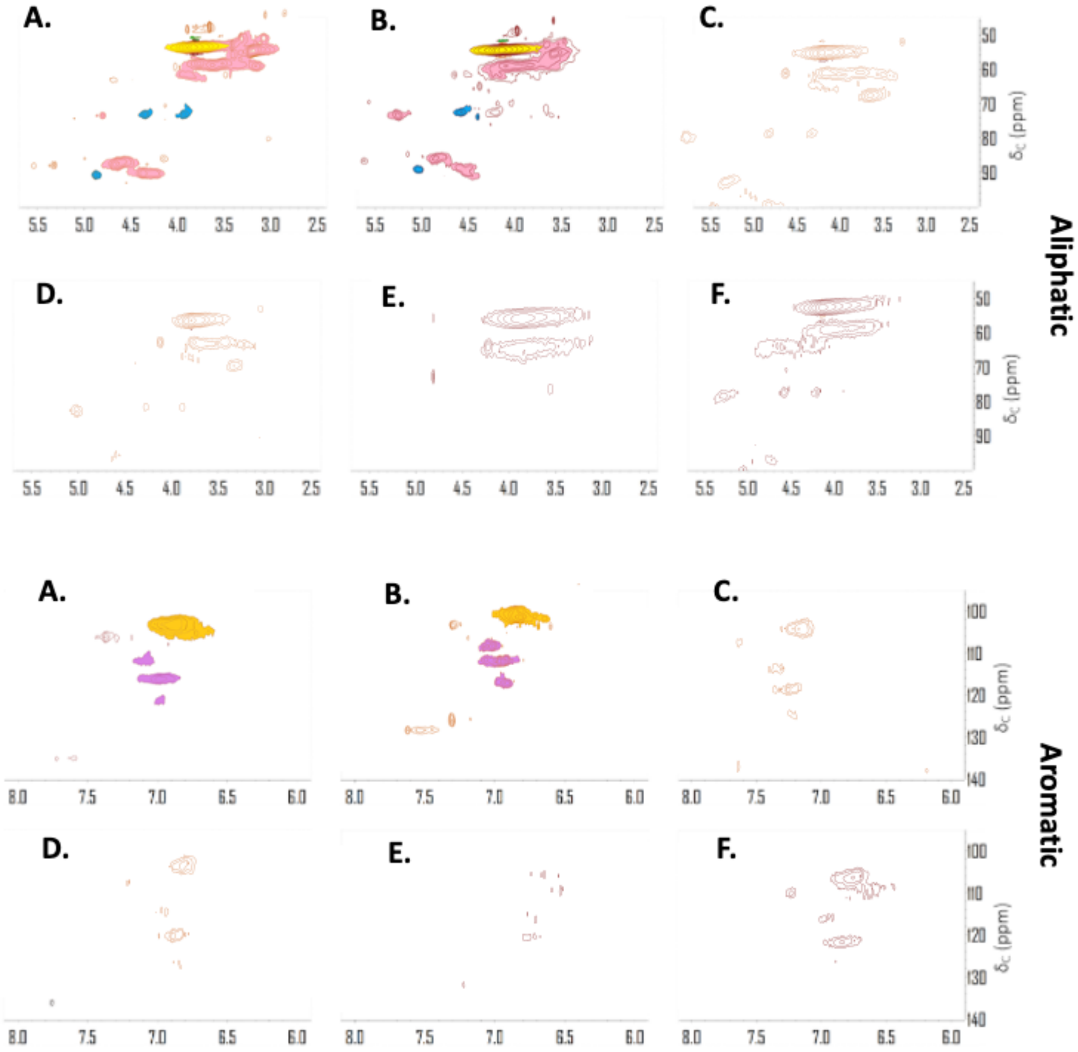


Figure 1. Shown above is a 2D ^{1}H-^{13}C HSQC NMR spectra ran in DMSO-d_6:pyridine-d_5 solvent showing characteristics of six different methods: OPL (A), MPL (B), H_2O/H_2SO_4 (C), FA/AA (D), EtOH (E), and H2O/HCl (F). The interunit linkages are color coded in order to depict which linkages are shown in the Chemical shift assignments are presented as '^{1}H/^{13}C ppm. β-O-4 linkage (pink), β-5 linkage (green), β-β (light blue) in the aliphatic region. S and S' (orange), G (purple), and H (indigo) in the aromatic region. The top spectrum is depicting the aliphatic region (a. and b.: δ_C/δ_H of 50–90/2–5.5 ppm). The spectra, a. and b., show similarities where the β-O-4 is consistently shown in the 3.1-4.2/55-65 ppm region for threo and erythro diasteromers. The β-5 linkage is present in the 3.0-4.0/50-55 ppm region. β-β appears also in the 3.8-4.6/70-75 ppm region. The bottom spectrum is depicting the aromatic region (c. and d.: δ_C/δ_H of 100–130/6.1–7.5 ppm). S region is shown from 6.2-7/100-110 ppm. G region is shown from 6.5-7.3/110-120 ppm. Assignments in linkages were determined based on literature.

Table 2. Characteristics of different Organosolv F5H-64 Lignin[a]

Sample	S	G	H	β-O-4	β-β	β-5
F5H-64-MPL	78	22	b	69	25	6
F5H-64-OPL	62	38	b	82	16	-
40 mL H_2O, 4 mL 37 wt% H_2SO_4, stir at 80 °C for 5 h	67	33	b	85	15	<1
FA/AA 70:30, 1:8 ratio biomass to solvent, 2 h to boil.	79	21	b	78	22	<1
EtOH 65% H_2O 35% 4.4-8 mmol H_2SO_4 for 2 h at 160° C 16 bar N_2	N/A	N/A	b	N/A	N/A	N/A
40 mL H_2O, 4 mL 37 wt% HCl, stir at 80 °C for 5 h.	72	28	b	80	20	-

[a] S: syringyl units, G: guaiacyl units, H: p-hydroxyphenyl units.

[b] Too small to ascertain.

[c] $\%X = X/(S_{2/6} + S'_{2/6} + G_2 + G_5 + G_6)$, $X = S_{2/6} + S'_{2/6}$, $G_2 + G_5 + G_6$.

[d] $\%Y = Y/(A_\alpha + B_\alpha + C_\alpha)$, $Y = A_\alpha, B_\alpha, C_\alpha$

5.3.3 GPC Analysis.

GPC in dimethylformamide (DMF) was conducted in order to characterize and confirm the molecular weight and dispersity of isolated lignin from the six different methods: OPL, MPL, H_2O/H_2SO_4, FA/AA, EtOH, and H2O/HCl. Shown in **Table 3** is the summary of the number-average (M_n), weight-average (M_w) molecular weight, and polydispersity (M_w/M_n) of the residual lignin (relative to polystyrene standards) obtained after lignin depolymerization different solvent systems. From **Table 3**, the best lignin points to entry 1 and 2, OPL and MPL, respectfully. The other lignin samples range in their expected M_n which could state the isolated polymer is smaller upon the isolation treatment.

Table 3. Lignin Characterization by GPC in DMF presents the number-average (M_n), weight-average (M_w) molecular weight, and polydispersity (M_w/M_n) of OPL, MPL, H_2O/H_2SO_4, FA/AA, EtOH, and H2O/HCl.

Entry	Sample	M_n (g mol^{-1})	Mw	PDI
1	OPL	1131	3866	3.42
2	MPL	1403	2113	3.04
3	H_2O/H_2SO_4	802	1631	2.34
4	FA/AA	525	2538	4.45
5	EtOH	610	2687	4.40
6	H_2O/HCl	391	1180	3.02

5.3.4 Pd-Zn/C Depolymerization with different lignin samples from F5H-64.

Using raw biomass as a control to compare the six different methods utilized, F5H-64 monomers should be a 2.5:1 ratio of DMPP:DHE. For catalytic depolymerization of lignin (CDL), Pd-Zn/C was synthesized and added to MeOH as the organic solvent under 35 bar H_2 and ran at 225 °C for 12 h. After, HPLC analysis of the different materials was run and calculated from amounts of DMPP and DHE produced. In raw biomass, a 1-gram scale produces 72 mg DMPP to 7.1 mg of DHE (**Figure 2**). In Figure 2B, an HPLC spectrum is shown to depict where each monomer elutes on the reverse-phase column ran with an I.S. of benzyl phenyl ether. **Table 4** depicts the monomer percent breakdown of i-DMPP, isoeugenol, DMPP, and DHE from the six different organosolv lignin extractions which shows that entry 1 gives the best % isolation of DMPP (21%) whereas entries 2-6 fall below this. Note that entry 5 does not have any analysis due to the run not having any specific monomers of interest for this study. The reason for comparing between raw biomass to organosolv lignin is that we can perform large scale lignin when isolated whereas for a 1 g scale of F5H-64 poplar only has a max yield of 200 mg of monomeric material. With separation and isolation of the organosolv lignin, we can scale up the reaction in order to demethylate DMPP to DMPPO.

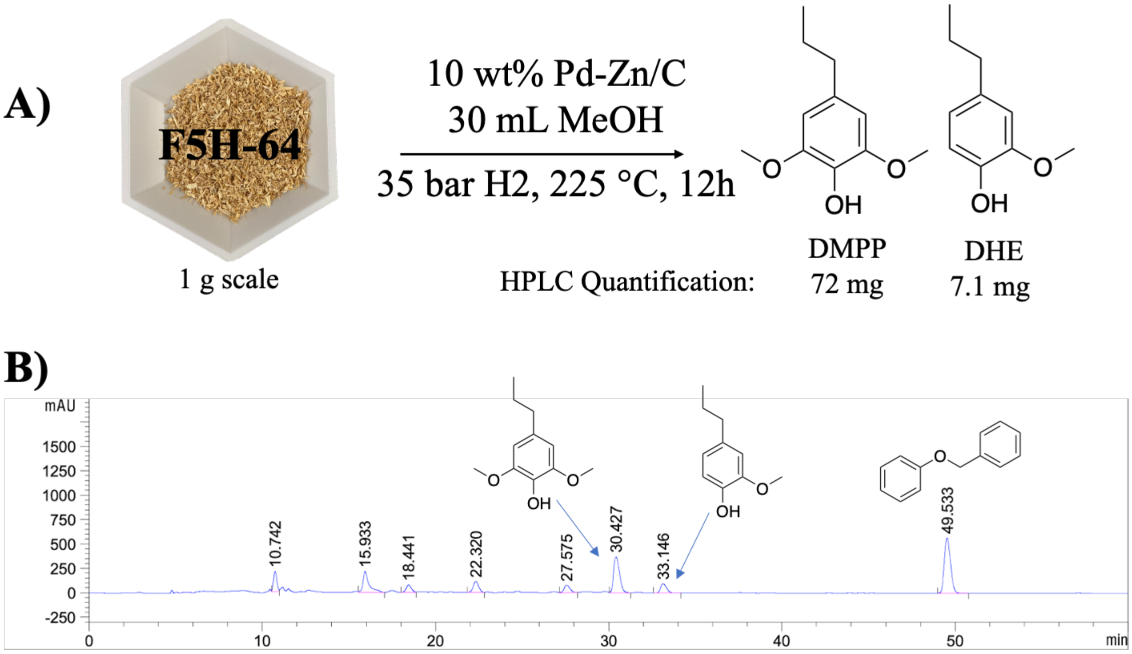


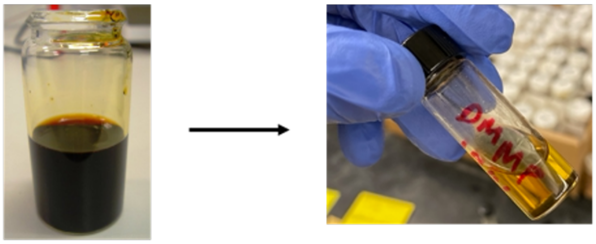
Figure 2. A) F5H-64 (High S) Poplar was used and depolymerized using 10 wt% Pd-Zn/C, 30 mL MeOH, 35 bar H_2, 225 °C, 12h. B) HPLC of the Pd-Zn/C depolymerization to give DMPP and DHE using I.S. Benzyl Phenyl Ether.

Table 4. HPLC analysis of lignin under Pd-Zn/C depolymerization. % yield of each monomer is shown for Isoeugenol, i-DMPP, DMPP, and DHE.

Entry	Sample	Isoeugenol	i-DMPP	DMPP	DHE
1	OPL	-	-	21	7
2	MPL	-	-	17	3
3	H_2O/H_2SO_4	-	-	14	3
4	FA/AA	1	7	3	0.3
5	EtOH	-	-	-	-
6	H_2O/HCl	-	8	9	1

5.3.5 Isolation of DMPP from depolymerized lignin.

After scaling collecting enough crude oil from F5H-54 poplar, it was subjected to flash column chromatography using a gradient of hexanes and ethyl acetate: x2 100 mL 95% Hex:EtOAc, x2 100 mL 90% Hex:EtOAc, x2 100 mL 85% Hex:EtOAc, x 2 100 mL 80% Hex:EtOAc. The fractionated products were checked by TLC and the combined DMPP fractions were combined. After this, the final product was ran for 1H-NMR and shown in **Figure 3**, the structure was confirmed to be DMPP. From a 1.32 g scale 523 g of DMPP was isolated which was then taken and utilized for the production of DMPPO.



F5H-64 crude oil in MeOH:
1.32 gram

After Column Chromatography:
523 mg of isolated DMPP

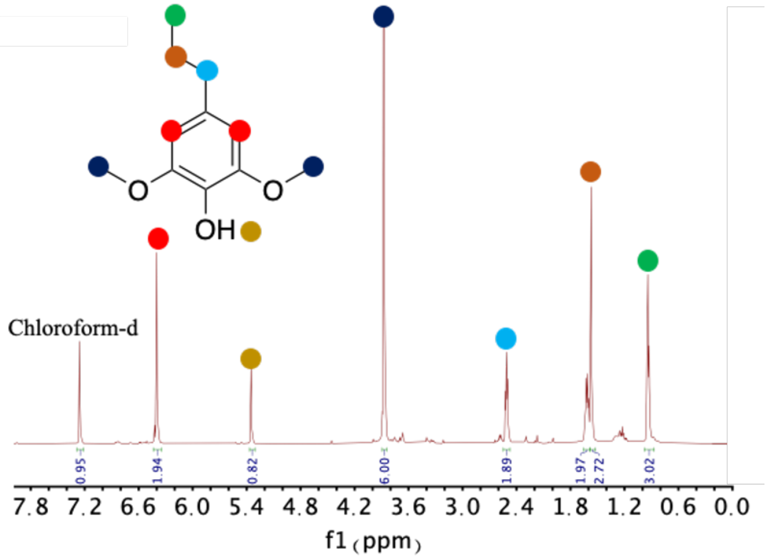
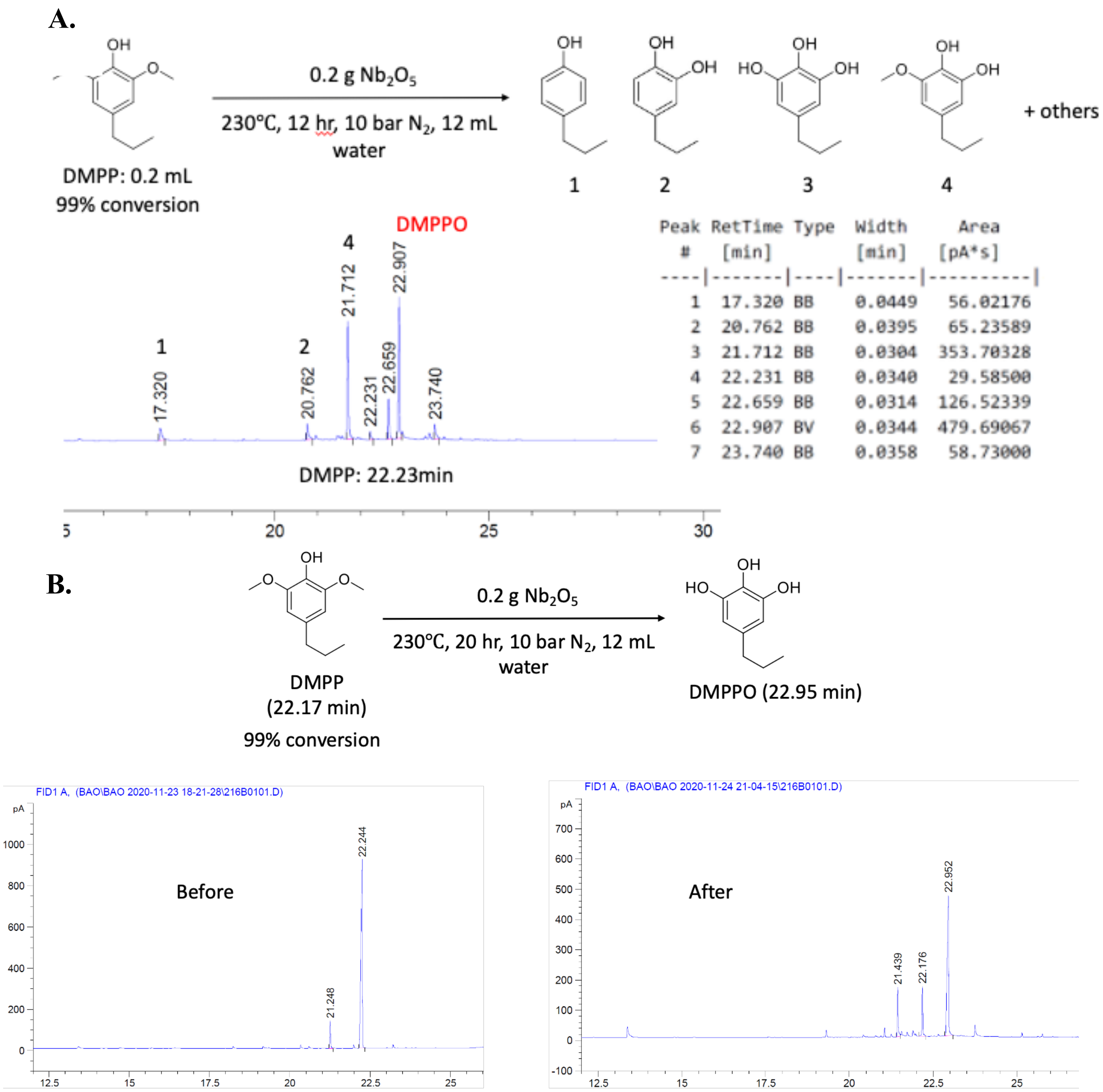


Figure 3. 1H-NMR of isolated DMPP from crude oil gathered from depolymerization using Pd-Zn/C.

5.3.6 Demethylation of DMPP to DMPPO utilizing Nb_2O_5.

With DMPP purified by column chromatography and verified by ^{1}H-NMR, the DMPP was taken demethylated using niobium pentoxide catalysis where according to our past publication, we show the hydroprocessing of dihydroeugenol (DHE) in an aqueous medium involving this catalyst. Nb_2O_5 is an air stable, water insoluble white solid that exhibits both strong Lewis and Brønsted acid sites that was proved to be an effective catalyst for the demethylation of DHE. With this previous study we optimized the conditions to suit the selective conversion of DMPP to DMPPO. Shown in **Figure 4a**, with 0.5 g of DMPP at 0.2 g Nb_2O_5, 230 °C, 10 bar N_2, 12 mL water ran for 12 h, we see that the DMPP has a 99% conversion but no selectivity for our desired product shown in the GC-FID, DMPPO (3). To increase the selectivity, longer reaction times were optimized from 12 to 20. and because of this, there is a dramatic change to 99% selectivity for DMPPO **(Figure 4b).**



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Peak RetTime Type  Width      Area
  #   [min]        [min]    [pA*s]
----|-------|----|-------|----------|
  1  17.320 BB    0.0449    56.02176
  2  20.762 BB    0.0395    65.23589
  3  21.712 BB    0.0304   353.70328
  4  22.231 BB    0.0340    29.58500
  5  22.659 BB    0.0314   126.52339
  6  22.907 BV    0.0344   479.69067
  7  23.740 BB    0.0358    58.73000
```

Figure 4. (A) Shows the conversion of DMPP to products 1, 2, 3, 4 identified by GC-FID. (B) Optimized time for DMPP to DMPPO conversion verified by GC-FID (GC-FID ran by Baoyuan Liu).

Further verification was performed by ^{1}H NMR to identify the product by Nb_2O_5 demethylation. Shown in **Figure 5**, the product was identified as DMPP's catechol derivative: DMPPO with small amounts of ethyl acetate. The ^{1}H-NMR (500 MHz, $CDCl_3$) was determined with the chemical shifts labeled in the spectra. The peak at δ 0.61, 1.46, and 2.27 corresponds to the propyl tail group of DMPPO. After the o-demethylation using

Nb_2O_5 in aqueous medium, the $-OCH_3$ peak is not present which confirms complete demethylation. This is important to note that the demethylation from DMPP originates from raw biomass which is the first time this has been reported. With these findings future work is aimed toward the functionalization of DMPPO into triepoxide motifs in order to synthesize different polymer networks derived straight from biomass.

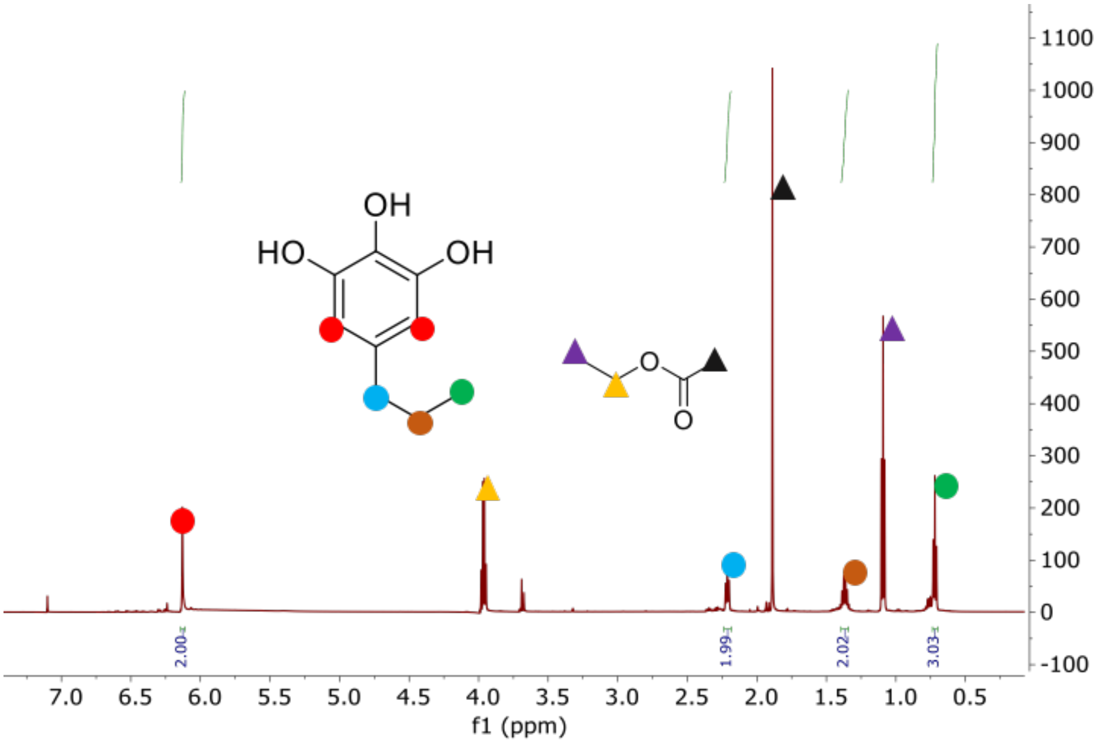

Figure 5. ^{1}H-NMR spectra of isolated DMPPO from Nb_2O_5 demethylation of DMPP.

5.4 Conclusion.

This study depicts a successful extraction, depolymerization, and upgrading of lignin and further research will be geared towards the production of triepoxides. The comprehensive synthesis and characterization of monomeric lignin-based epoxides have been demonstrated. The cleavage of DMPP methoxy was made possible with niobium oxide making the method a more sustainable route for the demethylation of these lignin monomers. The whole synthesis process, from demethylation to epoxidation was represented in ^{1}H NMR spectra. What is most important to note is the step-by-step route to triepoxides starting with actual biomass lignin. This is a route that is important to connect the bridge gap between biomass to materials and avoiding using commercially available monomers. The next steps towards this project would be to test the mechanical properties of materials straight from biomass.

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