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A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

**Characteristic changes of lignin in yellow poplar during
acid-catalyzed organosolv pretreatment**

산촉매 유기용매 전처리에 의한 백합나무 리그닌
특성 변화

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DEPARTMENT OF FOREST SCIENCES
GRADUATE SCHOOL
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Abstract

Characteristic changes of lignin in yellow poplar during acid-catalyzed organosolv pretreatment

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In the present study, the conversion of milled wood lignin (MWL) as a representative lignin after acid-catalyzed organosolv pretreatment was explained by the following three steps: 1) degradation, 2) re-distribution (degradation and condensation), and 3) condensation. In the degradation stage (0.5% H₂SO₄ at 120-180°C, 1% and 2% H₂SO₄ at 120-140°C), a large amount of lignin (56.44%~63.16%) was isolated, and mainly composed of syringyl unit lignin and degraded hemicellulose. In the re-distribution stage, degradation and condensation reactions occurred simultaneously (1% H₂SO₄ at 140-200°C, 2% H₂SO₄ at 140-180°C). During this stage, WIS (water insoluble solids) recovery rate, weight-average molecular weight (M_w), and ethanol organosolv lignin (EOL) yield were maintained; however, the amount of nitrobenzene oxidation (NBO) products declined due to degradation, and S/V (syringyl to vanillyl) ratio re-rose due to the condensation of S unit lignin to solid fraction. The condensation stage (2% H₂SO₄ at 180-200°C) occurred under severe conditions, and the condensation reaction was prominent at this stage along with associated phenomena.

As indicated by these results, characteristic changes of lignin in yellow poplar by acid-catalyzed organosolv pretreatment were very close to those of MWL. Most of the lignin was degraded from the solid fraction before reaching 150°C (from 26.57% to 5.35%); following that, the lignin ceased degradation from the solid fraction and underwent the condensation reaction. By precipitation of liquid hydrolysates, a maximum 12.87% of EOL was recovered with a slight amount of hemicellulosic sugars. After analyzing the lignin recovery from the solid residue and EOL, 44.41% of initial lignin content was not identified in the middle of the pretreatment condition, and at severe condition, initial lignin contents were exceeded, probably due to formation of pseudo-lignin. Unidentified compounds in supernatant obtained after EOL production were then determined and one third of initial lignin content of lignin-derived phenolic compounds was dissolved in supernatant mainly composed of lignin oligomers (from 595 Da to 921 Da).

Characterization of EOL during acid-catalyzed organosolv pretreatment corresponded to lignin behavior of MWL and yellow poplar. EOL was mainly formed by cleavage of lignin-hemicellulose structure in the secondary wall, thus having high M_w and S/V ratio. As the severity of pretreatment conditions increased, M_w , polydispersity, and NBO products of EOL were significantly decreased due to condensation. EOL produced at 140°C with 0.5% acid concentration was similar to MWL in functional groups, atomic ratios, and C9 molecular weight. Further physicochemical analysis, such as thermogravimetric analysis, FT-IR analysis, and ^{13}C -NMR analysis, supported the results above.

Addition of palladium reduced re-distribution of lignin fragment to solid fraction, and it resulted in slight enhancement of enzymatic hydrolysis (from 92.27% to 95.71% at 160°C), yield of EOL (from 13.36% to 16.30% at 160°C), and a minor prevention of sugar loss in liquid hydrolysates. As a result of supercritical ethanol treatment of EOL, yield and molecular weight

of oils varied from 23.79% to 26.67% and from 357 Da to 376 Da, respectively. Production yield of monomeric lignin compounds ranged from 2.33% to 4.04%; this was influenced by the degree of condensation of EOL. Thus optimization of both EOL production and the depolymerization process should be required.

In conclusion, the investigation on the characteristic changes of lignin and characterization of EOL in acid-catalyzed organosolv pretreatment might indicate feasible pretreatment conditions to maximize the economic value of the biomass feedstock by utilizing the EOL. This investigation could also help develop the potential of organosolv pretreatment for application in biorefinery processes.

Keywords : characteristic changes of lignin, acid-catalyzed organosolv pretreatment, ethanol organosolv lignin, yellow poplar, re-distribution of lignin

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List of Abbreviations

AFEX	ammonia fiber explosion
LHW	liquid hot water
ILs	ionic liquids
MWL	milled wood lignin
EOL	ethanol organosolv lignin
FT-IR	Fourier transform infrared spectrophotometer
NMR	nuclear magnetic resonance
GPC	gel permeation chromatograph
TGA	thermo-gravimetric analysis
DTG	differential thermal analysis
HPLC	high performance liquid chromatograph
RID	refractive index detector
GC-MS	gas chromatograph-mass spectrometer
FID	flame ionization detector
TEM	transmission electron microscopy
EDX	energy dispersive X-ray analysis
M_w	weight-average molecular weight
M_n	number-average molecular weight
PDI	polydispersity index
WIS	water-insoluble solid residue
Phenolic-OH	phenolic hydroxyl group
NBO	nitrobenzene oxidation
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide
S type	syringyl type
V type	vanillyl type
5-HMF	5-hydroxymethylfurfural
OMe	methoxyl group
DBE	double bond equivalents

Chapter 1

Introduction

1. Background

Fossil fuel has become the main energy and chemical source for a variety of industrial and household purposes since the industrial revolution (Amidon et al., 2008). Currently, world energy consumption is increasing by 2% annually (Mason, 2007), and oil demand is expected to reach 116 million barrels per day by 2030, up from 85 million barrels per day in 2006 (Leblond, 2006).

Excessive consumption of fossil fuel in urban areas has resulted in serious pollution especially related to greenhouse gas (GHG) emissions during the last few decades (Sarkar et al., 2011). Among GHGs, carbon dioxide concentration has increased in the atmosphere from 280 ppm to 390 ppm since the beginning of the industrial revolution (Firoozabadi & Cheng, 2010), despite the uptake of a large proportion of the emissions through various natural “sinks” involved in the carbon cycle. To limit the emission of GHG, most developed, and many developing, governments have devised and implemented strong policies and regulations. In addition, the rising price and finite nature of fossil fuels, accelerated by an increase of global energy consumption forces the search for new energy sources and collection techniques (Balat, 2010b).

Biomass is recognized as the only suitable primary energy resource that can replace fossil fuel (Hamelinck et al., 2005), and the term “Catch and release” is the key to biomass utilization (Figure 1-1). Carbon dioxide is captured from the atmosphere by plants, and the utilization and decomposition of bio-products releases carbon dioxide. In an optimally-balanced operation, carbon dioxide is just being recycled through the carbon cycle (Liu et al., 2006).

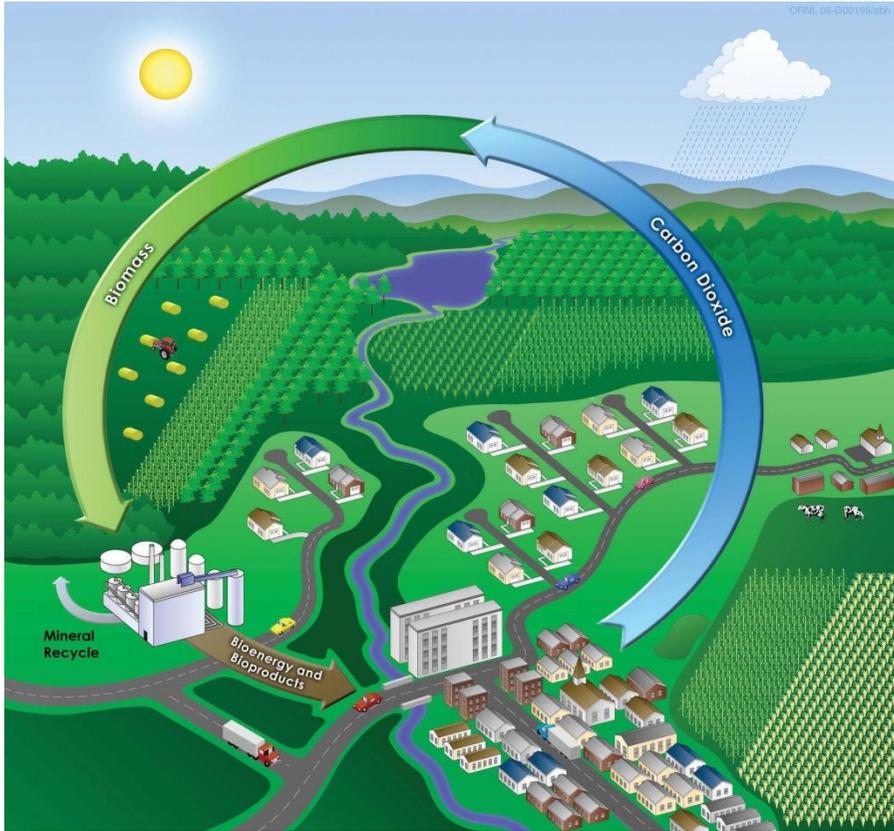


Figure 1-1. Reduced carbon dioxide emissions by bioenergy and bioproducts from biomass (DOE, 2006).

1.1. Bioethanol

Today, transportation fuel is responsible for 60% of world oil consumption (IEA, 2008). In addition, the transportation sector causes more than 70% of global carbon monoxide emissions and 19% of global carbon dioxide emissions (Goldemberg, 2008). Therefore the most significant challenge in many countries is to reduce the resulting this high oil dependency to improve energy supply security (Gnansounou, 2010).

For these reasons, the interest in biofuels from biomass has increased recently. Biofuels offer many advantages over fossil fuels: (1) biofuels are easily available from common biomass sources, (2) biofuels have a considerable potential to benefit the environment, (3) they can offer many benefits to economics and consumers, and (4) they contribute to sustainability (Demirbas, 2008; Puppen, 2001). Biofuels include bioethanol, biobutanol, biodiesel, biogas, bio-syngas, bio-oil, bio-char, and Fischer–Tropsch liquids are generally used for liquid fuels for the transportation sector (Balat, 2010a).

Bioethanol is the most widely commercialized liquid fuel for the transportation sector worldwide. Since the 1980s, interest in the use of bioethanol as an alternative transportation fuel has gradually increased. Global bioethanol production in 2006 was 39 billion liters and is expected to reach 100 billion liters in 2015 (Taherzadeh & Karimi, 2007). Brazil and the USA are the two largest bioethanol producers, together accounting for 62% of the world production (Kim & Dale, 2004).

Biomass feedstocks for bioethanol production are divided into three major groups: sucrose-based biomass (sugar cane, sugar beet, sweet sorghum, and fruits), starch-based biomass (corn, wheat, potatoes, cassava, sweet potatoes and barley), and lignocellulosic biomass (wood, straw, and grasses) (Balat & Balat, 2009). Brazil utilizes sugarcane for bioethanol production

while the United States and Europe mainly use starch-based biomass (corn, wheat and barley). Sugarcane production yield in Brazil is about 82.4 tons/ha (Prabhakar & Elders, 2009), and the yield of bioethanol has been around 6650 l/ha in recent years (Trostle, 2010). In the United States, bioethanol production derived from corn is predominant, and the production is concentrated in Midwestern states with abundant corn supplies (Asher, 2006).

Thus current bioethanol production as a transportation fuel is almost entirely dependent on sugarcane and corn which are food crops (Smith, 2008). This may be undesirable due to the crops' value as food and feed. In addition, sugar- or starch-based bioethanol has little potential for technical improvements, and low environmental effect, with 20%-40% of carbon dioxide reduction when it replaces gasoline (Fulton et al., 2004; Gray et al., 2006; Ingram & Doran, 1995).

On the other hand, lignocellulosic biomass is a promising feedstock to produce bioethanol in the mid- and long-term due to its low cost, large yield, and high availability (Gnansounou et al., 2005). In particular, bioethanol obtained from lignocellulosic biomass is favorable from a GHG perspective, with around an 85% net reduction in GHGs compared to gasoline (Farrell et al., 2006). Thus, the US Department of Energy provided more than one billion dollars toward lignocellulosic bioethanol projects in 2007 to achieve cost-competitive bioethanol (1.33 dollar per gallon) by 2012 (Slade et al., 2009). During the past few years, lignocellulosic bioethanol has been extensively studied, and sufficient literature is available.

However, despite significant advantages over conventional bioethanol, several factors limit the development of lignocellulosic bioethanol and technology using enzymatic hydrolysis:

- (1) To overcome the recalcitrance characteristics of lignocellulosic biomass, a feasible pretreatment process should be developed (Wyman, 2007).

- (2) To improve the yield and rate of the enzymatic hydrolysis, the hydrolysis process should be optimized and cellulase activity should be enhanced (Han & Chen, 2007; Lu et al., 2007).
- (3) To improve ethanol yield, pentose in the hydrolysate should be effectively converted into ethanol (Hou et al., 2007).

1.2. Pretreatment

The basic process steps in producing bioethanol from lignocellulosic biomass are: pretreatment, hydrolysis, fermentation and product separation/distillation (Figure 1-2) (Balat, 2010b). The recalcitrance of lignocellulosic biomass is one of the major barriers to the economical production of bioethanol (Wyman, 2007). Pretreatment, the technical approach to overcome recalcitrance, has been recognized as one of the most important and expensive processing steps in lignocellulosic bioethanol production (U.S.DOE, 2010); thus there is huge scope for lowering the cost of pretreatment process through extensive R&D approaches.

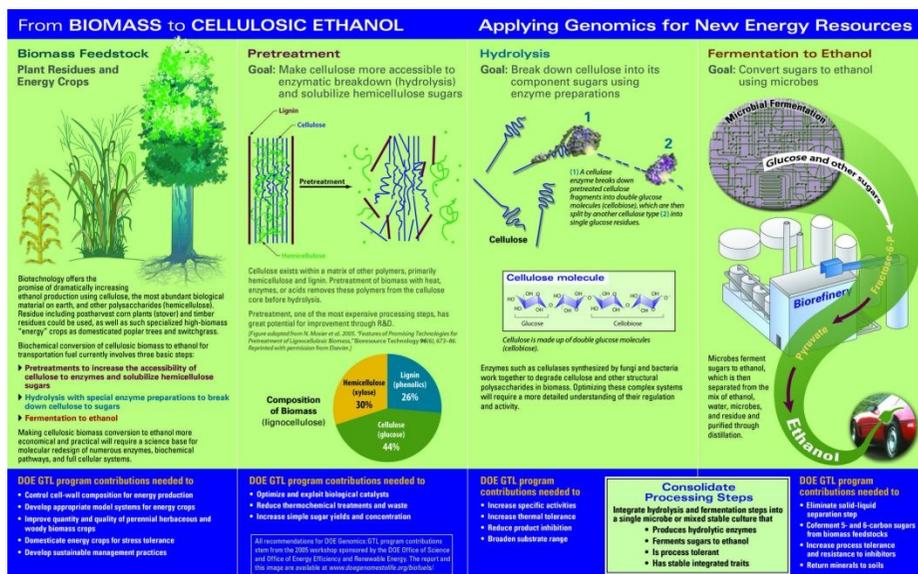


Figure 1-2. From Biomass to Cellulosic Ethanol (DOE, 2007).

Researchers have summarized the prerequisites for an ideal lignocellulose pretreatment; it should: (1) produce of reactive cellulosic fiber for enzymatic hydrolysis, (2) avoid decomposition of hemicelluloses and cellulose, (3) minimize formation of possible inhibitors for hydrolytic enzymes and fermenting microorganisms, (4) reduce the energy demand and cost, (5) reduce the cost of size reduction for feedstocks, and (6) produce less waste residues (Taherzadeh & Karimi, 2008).

Various pretreatment methods for lignocellulosic biomass have been widely studied. Physical (milling and grinding), physico-chemical (steam explosion/autohydrolysis, hydrothermolysis, and wet oxidation), chemical (alkali, dilute acid, oxidizing agents, and organic solvents), and biological processes have been widely studied for pretreatment of lignocellulosic materials. All the pretreatments have been conducted to increase the efficiency of enzymatic hydrolysis of the lignocellulosic biomass. Advantages and disadvantages of various pretreatment processes for lignocellulosic materials are summarized in Table 1-1 (Kumar et al., 2009).

Table 1-1. Advantages and disadvantages of various pretreatment processes for lignocellulosic materials (Kumar et al., 2009)

Pretreatment process	Advantages	Limitations and disadvantages
Steam explosion	Causes hemicellulose degradation and lignin transformation; cost-effective	Destruction of a portion of the xylan fraction; incomplete disruption of the lignin-carbohydrate matrix; generation of compounds inhibitory to microorganisms
AFEX	Increases accessible surface area, removes lignin and hemicellulose to an extent; does not produce inhibitors for down-stream processes	Not efficient for biomass with high lignin content
CO ₂ explosion	Increases accessible surface area; cost-effective; does not cause formation of inhibitory compounds	Does not modify lignin or hemicelluloses
Ozonolysis	Reduces lignin content; does not produce toxic residues	Large amount of ozone required; expensive
Acid hydrolysis	Hydrolyzes hemicellulose to xylose and other sugars; alters lignin structure	High cost; equipment corrosion; formation of toxic substances
Alkaline hydrolysis	Removes hemicelluloses and lignin; increases accessible surface area	Long residence times required; irrecoverable salts formed and incorporated into biomass
Organosolv	Hydrolyzes lignin and hemicelluloses	Solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost
Biological	Simple equipment degrades lignin and hemicelluloses; low energy requirements	Very low rate of hydrolysis

1.3. Biorefinery

Lignocellulosic bioethanol is a promising renewable transportation fuel; however, several limitations persist, such as pretreatment technologies to overcome lignocellulosic biomass recalcitrance. In addition, there is a basic and critical problem in the single bioethanol process. Table 1-2 shows the chemical composition of lignocellulosic biomass materials.

Table 1-2. Composition of various types of lignocellulosic biomass materials (% dry weight) (Balat, 2010b)

Material	Cellulose	Hemicellulose	Lignin	Ash	Extractives
Algae	20-40	20-50	-	-	-
Cotton	80-95	5-20	-	-	-
Grasses	25-40	25-50	10-30	-	-
Hardwoods	45±2	30±5	20±4	0.6±0.2	5±3
Hardwoods barks	22-40	20-38	30-55	0.8±0.2	6±2
Softwoods	42±2	27±2	28±3	0.5±0.1	3±2
Softwood barks	18-38	15-33	30-60	0.8±0.2	4±2
Cornstalks	39-47	26-31	3-5	12-16	1-3
Wheat straw	37-41	27-32	13-15	11-14	7±2
Newspapers	40-55	25-40	18-30	-	-
Chemical pulps	60-80	20-30	2-10	-	-

Bioethanol is mainly produced by fermentation of the glucose from cellulose. However, hardwoods, softwoods, cornstalks, and wheat straw only have about 40%-50% cellulose composition. As mentioned above, to improve ethanol yield, hemicellulose in the hydrolysate should be efficiently converted into ethanol, but technical barriers to achieve this remain. Above all, considerable amounts of lignin are not utilized or simply combusted to produce heat or electricity.

To be the bioethanol production from lignocellulosic more feasible, the biorefinery concept continues to gain momentum, with emerging research trends in the production of bio-based products. A biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power, materials, and chemicals from biomass. The biorefinery concept is analogous to today's petroleum refinery, which produces multiple fuels and products from petroleum (Kaparaju et al., 2009). A biorefinery has the advantages of producing diverse products, maximizing the value of the biomass feedstock. For instance a biorefinery could produce one or several low-volume, high-value chemical products as well as a low-value, but high-volume liquid transportation fuel such as biodiesel or bioethanol. The high-value chemicals and materials, which can be produced by lignin derived compounds, increase profitability and the high-volume fuel production helps meet energy needs (FitzPatrick et al., 2010).

The biorefinery can maximize the economic value of the biomass feedstock while reducing the waste streams produced (especially streams of lignin production) (Thomsen, 2005), and converting them into by-product streams rather than waste streams. This concept is illustrated in Figure 1-3. A biorefinery approach involves multi-step processes. The first step is conventionally referred to as pretreatment, preparing biomass for further processing. Following pretreatment, the biomass is subject to a combination of biological and/or thermo-chemical treatments. The products from

pretreatment could be further converted to biofuels (bioethanol or biobutanol) or chemical building blocks for further processing uses (Werpy & Petersen, 2004).

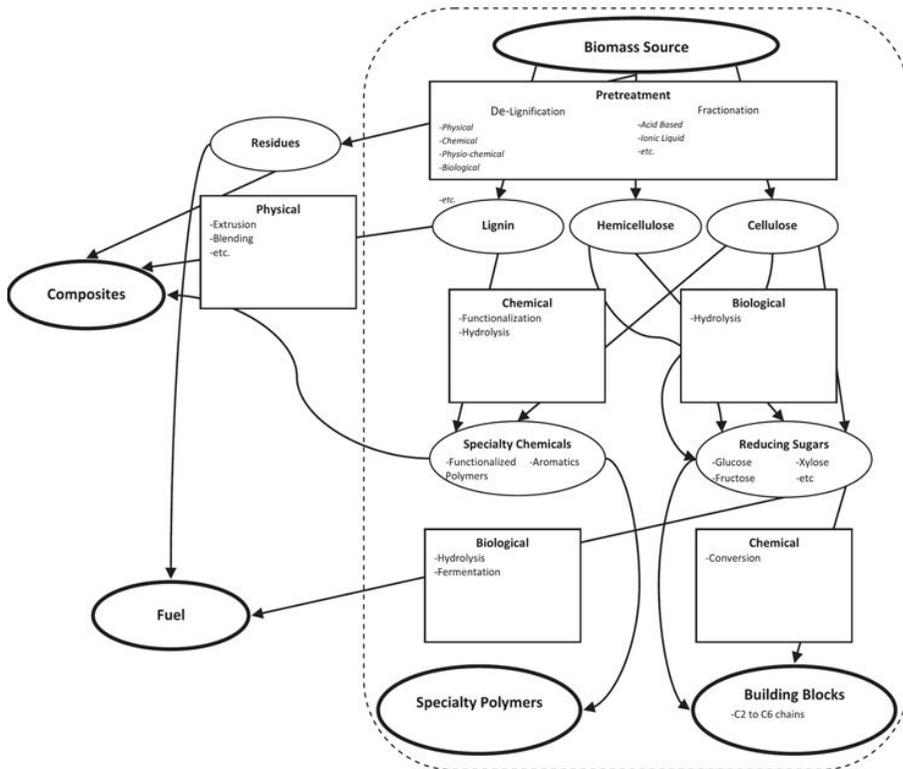


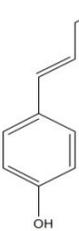
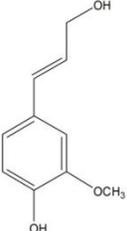
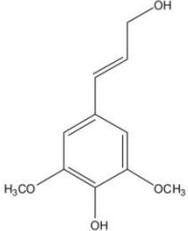
Figure 1-3. Conceptual biorefinery schematic (FitzPatrick et al., 2010).

1.4. Lignin

Lignin is the key factor related to the lignocellulosic biomass recalcitrance in both bioethanol and biorefinery processes. Thus, most pretreatment processes try to remove or redistribute lignin for enhancing the cellulose hydrolysis yield. Although lignin is one of the most abundant organic polymers (Gosselink et al., 2004) and natural aromatic polymer (Lora & Glasser, 2002), it is not considered a high-value product because of its heterogeneity and lack of a defined primary structure.

Lignin is polymer of phenylpropane units and a cross-linked racemic macromolecule with molecular masses in excess of 10,000 Da. There are three monolignol precursors: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Table 1-3).

Table 1-3. Lignin contents and chemical structures of precursors of lignocellulosic biomass (Azadi et al., 2013)

Lignin (%)		Phenylpropane unit (%)		
Structure				
		p-coumaryl alcohol	coniferyl alcohol	sinapyl alcohol
Softwood	27-33	-	90-95	5-10
Hardwood	18-25	-	50	50
Grasses	17-24	5	75	25

These precursors are polymerized by ether bond linkage (α -O-4, β -O-4, 4-O-5) and carbon-carbon bond linkage (β -5, 5-5, β -1, β - β) (Figure 1-4). Among the lignin linkages, ether linkages more frequent linkages compared to carbon-carbon linkages, and hardwood lignin contains approximately one and a half times more β -O-4-linkages compared to that of softwood. There are various functional groups in the lignin structure including methoxyl, phenolic hydroxyl, aliphatic hydroxyl, benzyl alcohol, noncyclic benzyl ether, and carbonyl groups. These functional groups give reactivity to lignin in various chemical reactions (Azadi et al., 2013).

Using lignin, it is possible to obtain low molecular weight aromatic compounds used in a variety of different industries as solvents or chemical reagents- for example, most commonly used aromatics chemicals, such as BTX (benzene, toluene, and xylene) and alkylated phenols. Those are easy to incorporate into existing industrial processes and products, and they seem to offer major technical and economic advantages. However production of aromatic compounds is deeply influenced by physico-chemical characteristics of lignin substrate. Therefore, prior to depolymerization of lignin, it is very important to evaluate the characteristics of lignin products of biorefinery process.

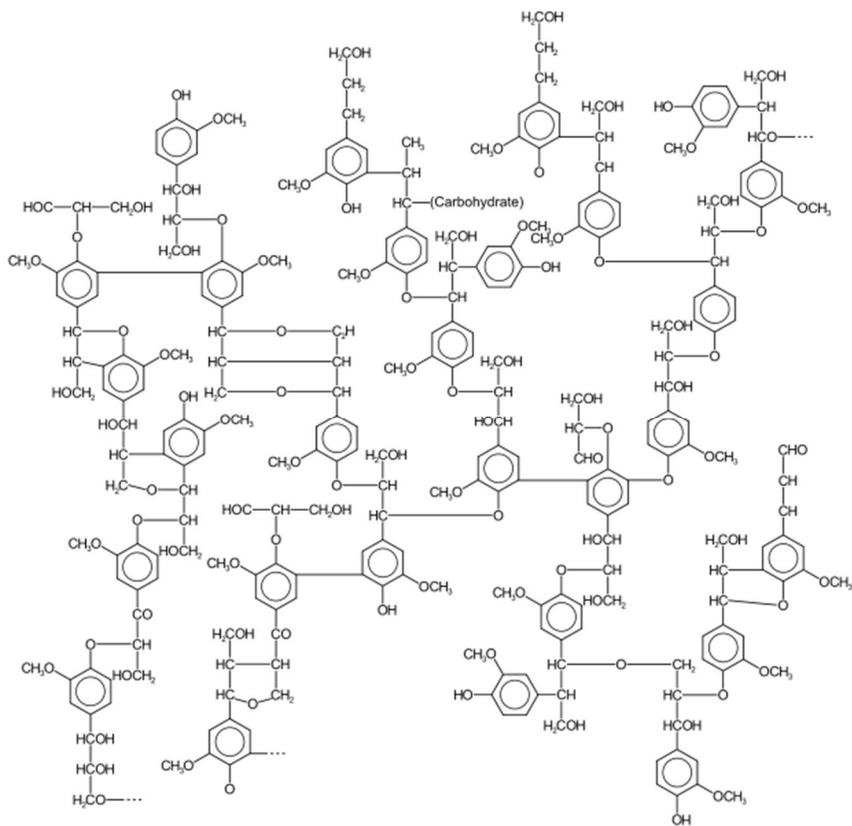


Figure 1-4. An example of a possible lignin structure (Glazer & Nikaido, 2007).

2. Objectives

As mentioned above, to develop an economically feasible biorefinery process, it is very important to maximize the value of the biomass by reducing the production of waste streams. However, a large amount of lignin is not utilized as high-value products because characteristic conversion changes of lignin depending on pretreatment conditions have not been adequately studied. Characteristics of lignin undergo wholly or partly physico-chemical modification depending on the pretreatment conditions. However those change differently because of other major components, such as cellulose and hemicellulose.

Accordingly, in this study, to understand the characteristic changes of the lignin, milled wood lignin (MWL), taken as a representative lignin and lignin in yellow poplar (*Liriodendron tulipifera*) will be subjected to acid-catalyzed organosolv pretreatment under various conditions (temperature and catalyst concentration) to analyze the effect of pretreatment condition on the lignin structure. In addition, characterization of ethanol organosolv lignin (EOL) will be evaluated. In particular, the changes of degree of condensation and S/V (syringyl to vanillyl) ratio of EOL depending on the pretreatment conditions will be analyzed. Moreover, lignin recovery analysis will be conducted to support the results above. After understanding the characteristic changes of the lignin, pretreatment processes will be modified and adjusted to produce more favorable lignin. Depolymerization of EOL can be accomplished through the supercritical ethanol treatment.

To support understanding of the above mentioned characteristics, a variety of structural and analytical methods (FT-IR, ¹³C-NMR, GPC, TGA, HPLC, GC/MS, and Pyrolysis GC/MS) will be applied.

The objectives of this study are:

- (1) To analyze characteristic changes of MWL and lignin in yellow poplar by acid-catalyzed organosolv pretreatment.
- (2) To characterize EOL depending on acid-catalyzed organosolv pretreatment conditions.
- (3) To establish the acid-catalyzed organosolv pretreatment process for the production of favorable lignin and the application of the biorefinery process.

3. Literature review

3.1. Characteristics of lignin in lignocellulosic biomass

Lignin serves as a binding agent to hold the adjacent cells together while the lignin within the cell walls gives rigidity through chemical bonding with hemicellulose and cellulose microfibrils (Figure 1-5). Additionally, lignin offers several unique characteristics such as resistance to decay and biological attacks and water impermeability. Thus lignin content and distribution in cell walls limits enzyme accessibility.

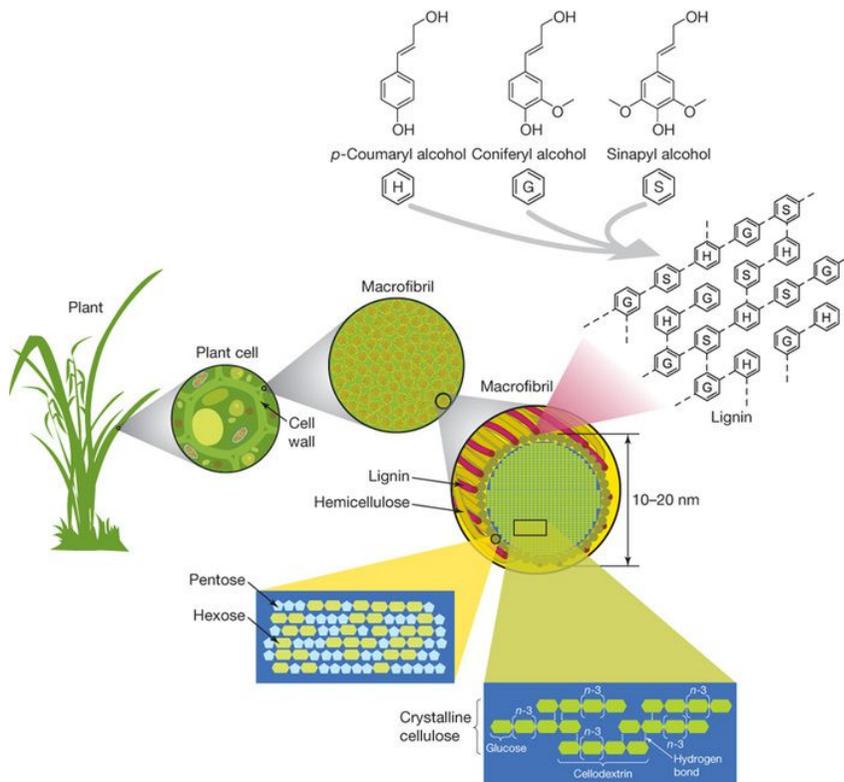


Figure 1-5. Structure of lignocellulosic biomass (Rubin, 2008).

The composition and distribution of lignin might also be as important as the concentration of lignin. The distribution of lignin is varied by species and location and it plays specific rolls in cell wall (Figure 1-6). It is reported that some pretreatments show different effects such as melting and lignin re-distribution (steam explosion and organosolv) or disruption of lignin-carbohydrates linkages (AFEX) (Laureano-Perez et al., 2005). Some softwoods are more recalcitrant than hardwoods, and this might be related to the type of lignin. Softwoods are mainly composed of guaiacyl lignin, while hardwoods have a mix of guaiacyl and syringyl lignin. It has also been suggested that guaiacyl lignin has extra recalcitrance characteristics by more effectively restricting fiber swelling and enzyme accessibility than syringyl lignin does (Ramos et al., 1992).

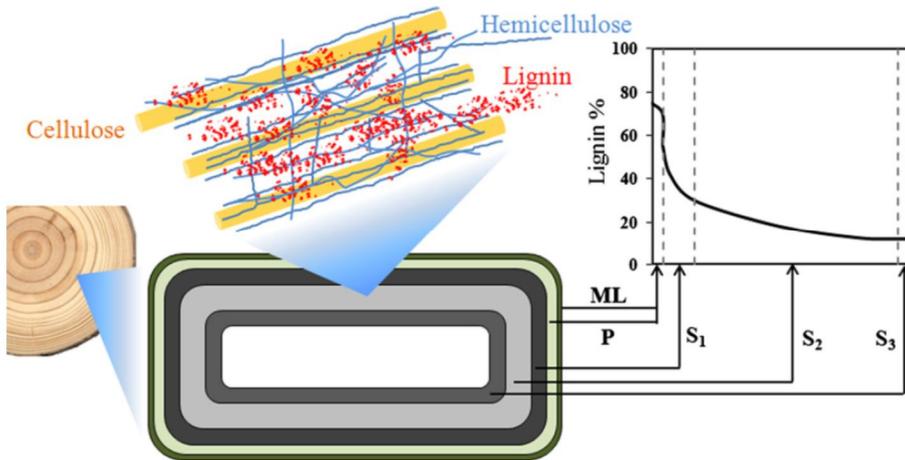


Figure 1-6. Lignin distribution within the middle lamella (ML), primary wall (P), and secondary wall (S1, S2, S3) (Azadi et al., 2013).

It was well known that hardwood lignin is composed in equal parts of guaiacyl unit lignin and syringyl unit lignin. However distributions of each component differ significantly. In white birch wood, most syringyl unit lignin is in the secondary wall of fiber and ray parenchyma, which also contains almost all the cellulose and hemicellulose (Table 1-4). On the other hand, guaiacyl unit lignin mostly exists in the vessel and middle lamella, which demands high rigidity.

Table 1-4. S/G ratio of white birch by TEM-EDX analysis (Saka & Goring, 1988)

White Birch	G	S
Fiber, S2 layer	12	88
Vessel, S2 layer	88	12
Ray parenchyma, S layer	49	51
Middle lamella (fiber/fiber)	91	9
Middle lamella (fiber/vessel)	80	20
Middle lamella (fiber/ray)	100	0
Middle lamella (ray/ray)	88	12

3.2. Characteristic changes of lignin by leading pretreatments

The main components of lignocellulosic biomass, such as cellulose, hemicellulose, and lignin, have been polymerized by different intra- and interpolymer linkages; thus, the altered characteristics differ among to pretreatment methods (Table 1-5).

Most of the pretreatment methods have focused on overcoming biomass recalcitrance for effective enzymatic hydrolysis. A number of studies have sought to improve glucose conversion yield, xylose recovery, and lignin removal in the pretreatment process (Negro et al., 2003; Pan et al., 2005b; Saha & Cotta, 2006; Saha et al., 2005a; Sun & Cheng, 2002a). In particular, lignin removal increases enzymatic hydrolysis by reducing non-productive adsorption sites for enzymes and increasing cellulose accessibility (Kim & Holtzaple, 2006).

However, research has not been focused on the mechanism and kinetics of lignin behavior depending on the various pretreatment conditions recently. For scale-up and commercial implementation of pretreatment technologies, it is essential to understand the characteristic changes of lignin in lignocellulosic biomass depending on pretreatment conditions. Thus, in the present research, feasible pretreatment conditions for producing compatible lignin, adequate for the depolymerization process for end-use lignin-derived compounds production, are proposed.

Table 1-5. Overview of linkages between the monomer units of lignocellulosic biomass (Harmsen et al., 2010)

Bonds within different components (intrapolymer linkages)	
Ether bond	Lignin, hemicellulose, cellulose
Carbon to carbon	Lignin
Hydrogen bond	Cellulose
Ester bond	Hemicellulose
Bonds connecting different components (interpolymer linkages)	
Ether bond	Cellulose-Lignin
Ester bond	Hemicellulose-lignin
Hydrogen bond	Cellulose-hemicellulose
	Cellulose-Lignin
	Hemicellulose-Lignin

3.2.1. Physical pretreatments

In lignocellulosic biomass pretreatment, physical pretreatments mainly consist of the size reduction process. Mechanical size reduction is a chemical-free intensive operation, and contributes to decreasing particles size and cellulose crystallinity and increasing accessible surface area. It has been suggested that particles sizes must be reduced to 0.5 mm-2 mm in order to enhance heat and mass transfer and to reach a proper level of sugar conversion (Barakat et al., 2013). However, mechanical size reduction steps are not cost-effective because of too high energy demands of dry grinding operations; therefore, innovative grinding and milling processes or combinations of mechanical size reduction with other pretreatments are required.

In the size reduction process, lignin contents are not significantly affected by the extent of milling. On the other hand, it has been asserted that severe chemical modification of the lignin occurs. For example, increases in carbonyl content and phenolic hydroxyl content (Bjorkman, 1957; Chang et al., 1975), as well as decreases in molecular weight (Chang et al., 1975), and cleavage of aryl ether linkages (Gellerstedt & Northey, 1989) have been reported as a result of the MWL isolation procedure. Researchers further showed that MWL was not representative of the all lignin in wood, but primarily originates in the secondary wall of the cell due to inherent differences in the chemistry of lignin in the middle lamella and the secondary wall (Whiting & Goring, 1981).

3.2.2. Physico-chemical pretreatments

Steam explosion is a thermo-mechano-chemical process that is the most widely employed physico-chemical pretreatment for lignocellulosic biomass. Usual conditions of steam explosion involve steam at 180-220°C (corresponding to a pressure of 1-2.3 MPa) and 2-10 min of retention time. This allows the breakdown of lignocellulosic biomass constituent by steam heating, as well as hydrolysis of glycosidic bonds by the organic acid formed during the process and by shearing forces due to the expansion of moisture (Jacquet et al., 2011). In combination with the partial hemicellulose hydrolysis and solubilization, the lignin is re-distributed and, to some extent, removed from the material (Wang et al., 2009). Typically, lignin-carbohydrate complexes are not disrupted completely, meaning degree of lignin removal is not considerable. However, it has been reported that after steam explosion treatment, phenolic hydroxyl group contents were significantly increased (from 23 to 47 per 100 aromatic units) while β -O-4 ether linkage contents were decreased (from 64 to 36 per 100 aromatic units) (Martin-Sampedro et al., 2011). Furthermore, with severe steam explosion conditions, the average molecular weight and a more heterogeneous lignin structure increased (Li et al., 2007). Such observations were probably results of the lignin condensation reactions.

A typical ammonia fiber expansion (AFEX) process is carried out with 1-2 kg ammonia/kg dry biomass at 90°C for 30 min. AFEX has been reported to decrease cellulose crystallinity and disrupt lignin-carbohydrates linkages (Laureano-Perez et al., 2005). This leads to cellulose decrystallization, hemicellulose solubilization, and lignin depolymerization (Holtzapfel et al., 1991), although only a small amount of the solid material is solubilized. The major advantage of AFEX pretreatment is that no additional water wash is required. However, recent work has shown that part of phenolic fragments of

lignin and other components may remain on the surface of pretreated solid thus washing with water might be necessary (Chundawat et al., 2007b). Above all, AFEX pretreatment is not effective on woody biomass and other high-lignin feedstocks (Wyman et al., 2005).

Liquid hot water (LHW) is another hydrothermal treatment that does not require rapid decompression and does not employ any catalyst or chemicals. In LHW pretreatment, lignin is partially depolymerized and solubilized as well; however complete delignification is not possible using hot water alone, because of the re-condensation of soluble components originating from lignin (Alvira et al., 2010a).

3.2.3. Chemical pretreatments

Alkali pretreatment, based on the effects of swelling caused by the addition of dilute bases, increases the internal surface of cellulose and decreases the degree of polymerization and crystallinity, which provokes lignin structure disruption (Taherzadeh & Karimi, 2008). Sodium hydroxide has been reported to increase hardwood digestibility from 14% to 55% by reducing lignin content from 24-55% to 20% (Kumar et al., 2009). Relatively mild process conditions of the alkali pretreatment prevent condensation of lignin, resulting in high lignin solubility. However this was effective only for biomass with relatively low-lignin content, of 10-18% (Bjerre et al., 1996).

Dilute acid pretreatments typically use sulfuric acid as a catalyst at low acid concentrations (0.05-5%), and temperatures ranging from 160-220°C (Farrell et al., 2006; Kim et al., 2001). Dilute acid pretreatment has the advantage of solubilizing hemicellulose and converting solubilized hemicellulose into fermentable sugars. Nevertheless, depending on the process temperature, some sugar degraded compounds such as furfural and 5-HMF and lignin degraded aromatic compounds were formed, affecting the microorganism metabolism in the fermentation step (Saha et al., 2005b). It was also demonstrated that lignin droplets were produced on the pure cellulosic fiber during pretreatment under acidic condition and above 130°C by re-distribution (Selig et al., 2007). By acid pretreatment, β -O-4 ether linkages decreased while contents of condensed aromatic C were increased which is caused by re-polymerization of fragments of β -O-4 (Samuel et al., 2010).

Ozone is a powerful oxidant that shows high delignification efficiency (Sun & Cheng, 2002b). The lignin polymer was converted into smaller degraded products such as carboxylic acids, because of the high reactivity of oxidizing chemicals with the aromatic ring (Harmsen et al., 2010). This lignin

removal significantly increases the yield in the enzymatic hydrolysis that follows; however an important drawback to consider is the large amounts of ozone needed, which can make the process economically unviable.

Ionic liquids (ILs) pretreatment can simultaneously dissolve carbohydrates and lignin because ILs form hydrogen bonds between the non-hydrated chloride ions of the IL and the sugar hydroxyl protons in a 1:1 stoichiometry. As a result, the intricate network of non-covalent interactions among biomass polymers of cellulose, hemicellulose, and lignin is effectively disrupted while minimizing the formation of degradation products (Alvira et al., 2010a). Kim et al. reported that the amounts of functional groups of ionic liquid lignin were similar to that of MWL; however, the weight average molecular weight (M_w) and polydispersity index (PDI: M_w/M_n) were two thirds that MWL.

3.2.4. Organosolv pretreatment

Organosolv pretreatment is more feasible for biorefinery of lignocellulosic biomass, as it reduces the waste streams of the biomass components. For most organosolv processes, if the pretreatment is conducted at high temperatures (185-210°C), there is no need for acid addition, as it is believed that organic acids released from the biomass act as catalysts for the rupture of the lignin-carbohydrate complex (Duff & Murray, 1996). However, when acid catalysts are added, the rate of delignification is increased and higher yields of xylose are obtained, though the cellulose remains as solid. Mineral acids (hydrochloric acid, sulfuric acid, and phosphoric acid) are good catalysts to accelerate delignification and xylan degradation, while some organic acids such as formic, oxalic, acetylsalicylic, and salicylic acid, can also be used as catalysts (Sun & Cheng, 2002b). Regarding the type of alcohol, it was found that normal primary alcohols were better delignification agents than the secondary or tertiary alcohols, and the mixtures of n-butyl-alcohol-water appeared to be the most efficient in removing lignin from wood (Yawalata, 2001), although methanol and ethanol are more widely used because of easy solvent recovery.

Biomass is treated with the addition of catalyst at low temperatures (below 180°C) or without catalyst (auto-catalysis) at higher temperatures (185-210°C). For catalyzed alcohol delignification, many chemicals can be employed as catalysts, including mineral acids, magnesium, calcium or barium chloride or nitrate (Chang & Paszner, 1982), neutral alkali earth metal (Paszner & Cho, 1987), magnesium sulfate (Paszner, 1989), sodium hydrogen sulfate (Chum et al., 1990), and sodium hydroxide (Ray et al., 1993). After pretreatment, the main products from pretreatment are the following (Figure 1-7): (De Wild et al., 2012; Zhao et al., 2009)

1. Cellulosic fibers, which contain the relatively constant cellulose component with varying amounts of hemicellulose and residual lignin.
2. Solid lignin, obtained after removal of the organic solvent from the liquid fraction.
3. An aqueous solution of the hemicellulose sugars. This solution is the filtrate of the organic solvent-evaporated liquor.

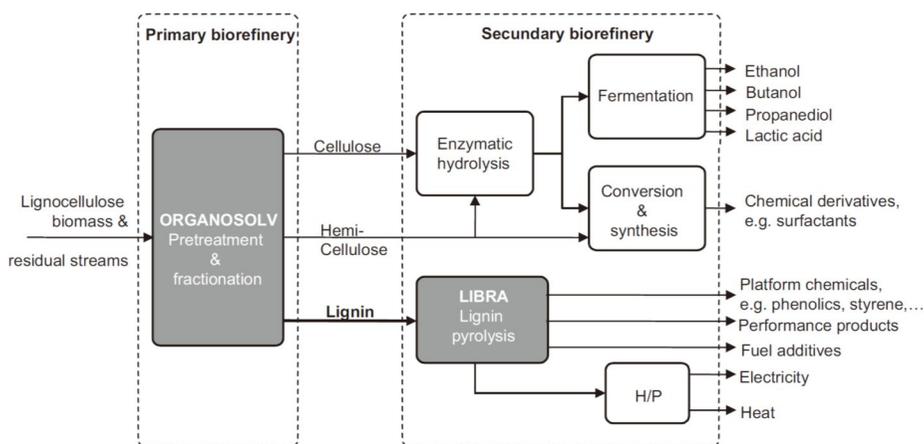


Figure 1-7. Lignin valorization in organosolv pretreatment (De Wild et al., 2012; Zhao et al., 2009).

It was found that pretreatment of pinewood with 60-80% aqueous methanol solution containing 0.2% hydrochloric acid for 45 min at 170°C removed about 75% of original lignin, whereas beech wood was about 90% delignified when pretreated with 50% aqueous methanol solution containing 0.1% hydrochloric acid for 45 min at 160°C. The hemicellulose was almost completely dissolved during these treatments. The susceptibility of residual cellulose increased with increasing delignification. For a complete hydrolysis with cellulase, the removal of lignin from pine and beech wood should be over 70% and 80%, respectively. The delignification improved with

increasing temperature, time, and acid concentration of pretreatment (Shimizu & Usami, 1978).

Koo et al., reported that lignin contents of solid fraction obtained after organosolv pretreatment with sodium hydroxide were not significantly declined depending on the pretreatment temperature increasing from 140°C to 160°C, though those of enzymatic hydrolysis yields were enhanced without significant changes of lignin contents (Koo et al., 2011a). Yields of precipitate obtained after organosolv pretreatment at relatively low temperature and short pretreatment time were less than 5% (Koo et al., 2011b).

The precipitate from organosolv pretreatment is called organosolv lignin and it has many unique properties, such as relatively high purity, sulfur free, low molecular weight, narrow distribution, low glass transition temperature, thermo fusibility, and more functional groups for diverse valuable application (El Hage et al., 2010b)

A previous study suggested in lignin hydrolysis of *Miscanthus* during ethanol organosolv pretreatment, the cleavage of α -aryl ether bonds were predominant at the severity conditions generally employed (Figure 1-8, low to medium severities, $2 < CS < 2.4$), and β -ether cleavage occurred at very high severity conditions (Figure 1-9) (El Hage et al., 2010a). However, the mechanisms for these reactions during pretreatments are very complex, depending on the pretreatment conditions (temperature, time, catalyst and concentration), and type of biomass feedstock. Moreover, the correlation between lignin degradation mechanism and enzymatic hydrolysis yield are not fully understood.

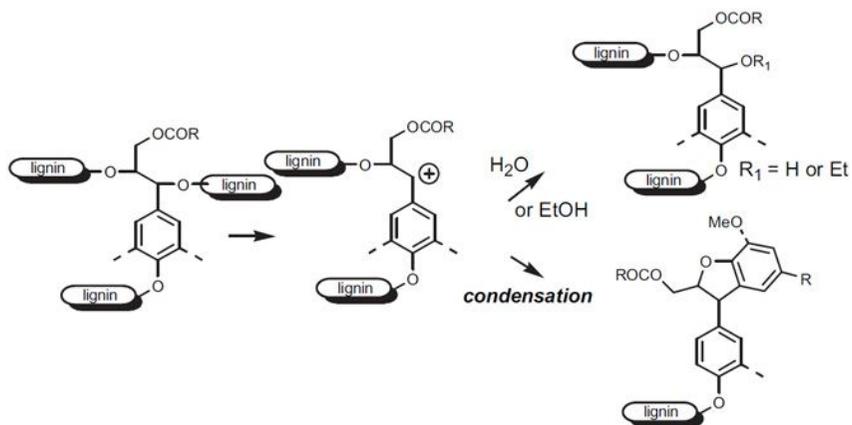


Figure 1-8. Mechanism of the solvolytic cleavage of α -aryl ether linkage (El Hage et al., 2010a).

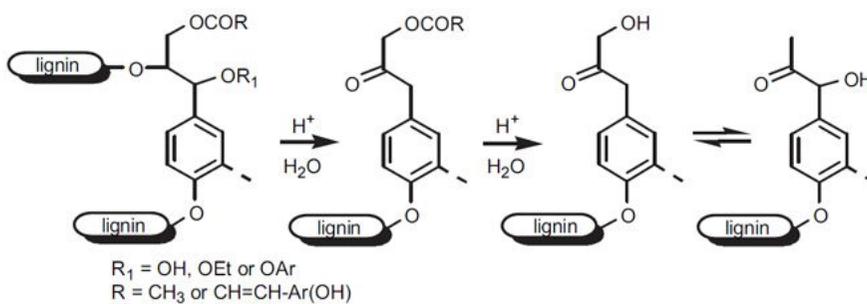


Figure 1-9. Mechanism of the solvolytic cleavage of β - and γ -aryl ether linkages (El Hage et al., 2010a).

3.3. Isolation of lignin

The isolation of lignin from lignocellulosic materials is conducted under conditions where lignin is progressively broken down to lower molecular weight fragments, resulting in changes to its physicochemical properties. Thus, taken apart from the source of the lignin, the method of isolation will have a significant influence on composition and properties of lignin. The majority of lignin isolation and delignification processes occur by either acid or base-catalyzed mechanisms. The chemistry of bond cleavage in lignin by these mechanisms has been reviewed by Gratzl & Chen-Loung (Gratzl & Chen-Loung, 2000).

3.3.1. Analytical lignin

Currently MWL is the best preparation for isolating lignin from lignocellulosic biomass with minimized chemical alteration. While MWL is directly extracted from finely ball-milled wood without any previous treatment (Bjorkman, 1957), cellulolytic enzyme lignin (CEL) is produced by removing most of the carbohydrate fractions using cellulolytic enzymes prior to dioxane extraction of ball-milled wood (Chang et al., 1975). Comparison of the chemical structures of MWL and CEL has revealed that MWL is slightly more condensed than CEL, which suggests that MWL may contain a higher proportion of lignin from the middle lamella (Ikeda et al., 2002). In general, yields increase with increased severity of milling conditions; however, a corresponding steady decrease in α -aryl ether linkages with increasing ball milling intensity has been observed (Fujimoto et al., 2005). This indicates substantial lignin depolymerization via the cleavage of α -aryl ether linkages under severe mechanical condition.

3.3.2. Kraft and sulfite lignin

The sulfite process which traditionally used to be the main pulping technology involves the reaction of a metal sulfite and sulfur dioxide (Wolfinger & Sixta, 2004). The main reactions that take place during the pulping process are: (a) the reaction between lignin and free sulphurous acid to form liginosulfonic acid; (b) the formation of the relatively soluble liginosulfonates with the cations, Mg^{2+} , Na^+ , or NH_4^+ ; and (c) the fragmentation of the liginosulfonates (Mansouri & Salvadó, 2006b). The sulfite delignification process is an acid-catalyzed process in which there is cleavage of the α -ether and β -ether linkages of lignin. Generally, less side-chain cleavage is seen under acid-catalyzed rather than alkali-catalyzed reactions. The complete breakdown of the aryl ether linkages leads to the formation of a reactive resonance-stabilised benzyl carbocation, and condensation reactions occur under these conditions. The carbocation may form a C-C bond with an electron-rich carbon atom in the aromatic ring by a S_N2 mechanism.

The kraft or sulfate process uses sodium hydroxide and sodium sulphide under strong alkaline conditions to cleave the ether bonds in lignin. The kraft process produces lignin with aliphatic thiol groups called kraft lignin (Robert et al., 1984). The kraft process occurs by alkaline hydrolysis in which the β -1,4 links in cellulose is cleaved, allowing the lignin extraction. In alkaline hydrolysis, α -aryl ether bonds are more easily broken than β -aryl ether bonds, particularly in situations where the substructures contain a free phenolic hydroxyl group in the para position (Lundquist & Kirk, 1980). In alkaline media, intermolecular condensation reactions can occur, with competition between the added nucleophiles and anionic lignin fragments. The extent of

condensation will depend on the types of structures initially formed (Chakar & Ragauskas, 2004).

Lignin recovered through extraction with sodium hydroxide is normally referred to as soda lignin. Soda lignin from non-wood sources is typically difficult to recover by filtration or centrifugation because its high carboxylic acid content, arising from oxidation of aliphatic hydroxy groups, makes it a relatively good dispersant (Ibrahim et al., 2004). Therefore, heating is required to encourage coagulation obtaining filterable material.

3.3.3. Organosolv lignin

A fine precipitate produced by dilution of liquid hydrolysate with water after organosolv pulping is called organosolv lignin. Organosolv lignin contains a trace amount of carbohydrate and is mostly composed of lignin. Inorganic compounds such as sulfur and sodium contents are significantly lower than kraft and sulfite lignins (Pan et al., 2005a). Organosolv lignin had a low molecular weight and narrow polydispersity, relative to other lignin which is important in controlling process for co-product applications (Table 1-6).

Organosolv lignin produced from organosolv pretreatment which is conducted in low temperatures over short residence times relative to organosolv pulping has higher molecular weight and polydispersity. Weight-average molecular weight of organosolv lignin obtained from yellow poplar at 140°C with sulfuric acid catalyst was over 6000 Da and that of polydispersity was almost 4.0 (Koo et al., 2011b). However it was less denaturalized compared to organosolv lignin produced in the organosolv pulping process. For example, higher phenolic hydroxyl and ether linkage contents can be expected in organosolv lignin produced from that condition.

Phenolic hydroxyls are important functional groups in lignin, affecting both physical and chemical properties (Adler, 1977). The high content of phenolic hydroxyl groups provides opportunities for modification via esterification and etherification reactions in attempts to develop novel applications for lignin.

Table 1-6. Characteristics of organosolv lignins prepared from hardwood and softwood by ethanol organosolv pulping (Creamer et al., 1997; Pan et al., 2005b)

	% Total weight	
	Mixed hardwood	Mixed softwood
Moisture	2.9	1.4
Total lignin	95.5	98.3
Klason lignin	91.4	96.7
Acid-soluble lignin	4.1	1.6
Total carbohydrate	0.25	0.08
Arabinose	ND	ND
Galactose	0.03	0.02
Glucose	0.01	0.01
Xylose	0.18	ND
Mannose	0.03	0.05
Functional groups		
Phenolic hydroxyl	4.7	5.3
Aliphatic hydroxyl	4.9	5.4
Methoxyl	19.1	16.4
Molecular weight		
M _n	1,340	1,833
M _w	1,985	2,938
M _w /M _n (polydispersity)	1.48	1.6

3.4. Depolymerization of lignin

Pyrolysis of the isolated lignins is considerably different with the pyrolysis of lignocellulosic biomass both in terms of the biooil composition and the product distribution. Therefore, the pyrolysis conditions for the conversion of isolated lignins should be separately optimized for each feedstock from different biomass species and isolation processes (Table 1-7) (Azadi et al., 2013). It has been reported that lignin pyrolysis occurs over a wider range of temperature (160-900°C) compared to polysaccharides (220-400°C) (Yang et al., 2007). The bio-oils obtained from the pyrolysis of isolated lignins are complex mixtures of many aromatic and non-aromatic compounds, each of which typically constitutes less than 1% of the total weight and with a wide molecular weight distribution from light hydrocarbons to high-molecular weight oligomers.

Isolated lignins also can be depolymerized into soluble fragments by supercritical treatment. The most widely used solvents were alcohol, including ethanol (Miller et al., 1999), methanol (Tsujino et al., 2003), and butanol (Yoshikawa et al., 2012). The depolymerization of lignin in organic solvents is typically conducted at 200–350°C. The molecular weight of the dissolved lignin ranges from about 150 Da to 2500 Da. Dissolved lignin is divided into liquid fraction (oil fraction) and solid fraction (char fraction). Lignin in liquid fraction has a molecular weight of less than 300 Da and lignin in solid fraction contains all the trimmers and larger oligomers. The individual yield of monomers can be up to few percent of the feed, but barely exceeds 5% (Azadi et al., 2013).

Table 1-7. Lignin pyrolysis product distribution (Azadi et al., 2013)

Lignin	T (°C)	Bio-oil (%)	Gas (%)	Char (%)	Ref.
Kraft	550	23	39	41	(Asmadi et al., 2011)
Kraft	700	44	18	38	(Meier et al., 1992)
Kraft	800	19	36	45	(Meier et al., 1993)
Lignosulfonate	550	40	15	45	(Zhang et al., 2008)
Soda	530	31	6	49	(Karagöz et al., 2005)
Soda	650	70	15	15	(Patwardhan et al., 2011)
Organosolv	500	16	3	81	(Zhang et al., 2012)
Organosolv	550	16	21	63	(Asmadi et al., 2011)
Organosolv	550	34	14	33	(Sasaki & Goto, 2008)
Organosolv	560	52	28	19	(Nowakowski et al., 2010)
Acid hydrolysis	500	58	10	27	(Karagöz et al., 2005)
Acid hydrolysis	650	63	20	17	(Patwardhan et al., 2011)
Steam explosion	550	33	17	50	(Zhang et al., 2008)
Pyrolytic lignin	600	32	10	58	(Heitz et al., 1995)
Milled wood lignin	600	36	16	40	(Roberts et al., 2011)
Enzymatic hydrolysis	500	24	7	69	(Zhang et al., 2012)

3.5. Lignin application

The physicochemical state of lignin dictates how and where it can be utilized in the manufacture of various products. The source from which lignin is obtained and the method of extraction has a strong bearing on its properties (Lora & Glasser, 2002). Table 1-8 gives the functional groups and molecular weight of selected lignins. The reactivities of these lignins will impact on the attributes of the end products.

Table 1-8. Molecular weight and functional groups of lignins (Doherty et al., 2010)

Lignin type	M_n (g mol ⁻¹)	COOH (%)	Phenolic-OH (%)	OMe (%)
Soda (bagasse)	2160	13.6	5.1	10.0
Organosolv (bagasse)	2000	7.7	3.4	15.1
Soda (wheat straw)	1700	7.2	23.6	16
Organosolv (hardwood)	800	3.6	3.7	19
Kraft (softwood)	3000	4.1	2.6	14

For example, lignosulfonates and kraft lignin can be used to produce a number of products (Gargulak & Lebo, 2000), such as phenol formaldehyde resins (Muller et al., 1984), vanillin (Gogotov, 2000), pesticides (Lebo Jr, 1996), dyes and pigments (Hale & Xu, 1997), water treatment (Zhuang & Walsh, 2003), emulsifiers (Gundersen et al., 2001), matrix for micronutrient fertilizers (Docquier et al., 2007), and wood preservatives (Dumitrescu et al., 2002). However the sulfur content (about 5 %) of lignosulfonates is one of the major factors restricting its use in special applications, thus, most of its lignin is currently used for energy generation.

On the other hand, organosolv lignin is high quality with high purity,

sulfur free, low molecular weight, and narrow distribution compared to other practical lignin (Pan et al., 2008), and it can be applied in the fields of adhesives, fibers, films, and biodegradable polymers (Cetin & Ozmen, 2002; Pereira et al., 2007; Vázquez et al., 1999).

After the depolymerization process, lignins could be utilized to produce low molecular weight aromatic compounds such as BTX and aliphatic aromatic compounds. In case of production of low molecular weight aromatic compounds, functional group contents and degree of condensation will be recognized as the most important factors. Therefore controlling the production conditions for manufacturing lignins having both high yield and purity should be studied.

Chapter 2

Conversion of milled wood lignin during
acid-catalyzed organosolv pretreatment

1. Introduction

Beginning with the industrial revolution, fossil fuels have become a dominant source of energy and chemicals (Amidon et al., 2008). More recently, lignocellulosic bioethanol has been recognized as a unique material for renewable energy; however the recalcitrant nature of lignocellulosic biomass is a critical barrier for economic production of bioethanol due to the requirement of pretreatment processes (Wyman, 2007). Indeed, various pretreatment methods have been studied to solve the issue of lignocellulosic biomass processing, and have identified lignin as the key factor related to the recalcitrance of lignocellulosic biomass for use in bioethanol and biorefineries.

Lignin serves as a binding agent that holds adjacent cells together and provides integrity, rigidity, and water impermeability. Thus, increased lignin content and distribution limits enzyme accessibility, and its removal increases enzymatic hydrolysis by both reducing non-productive adsorption sites for enzymes and increasing cellulose accessibility (Kim & Holtzapple, 2006).

Composition and distribution may be as important as the concentration of lignin itself. It has been reported that certain pretreatments produce different effects on the characteristics of lignin such as melting, re-distribution (steam explosion and organosolv), and disruption of lignin-carbohydrate linkages (AFEX) (Laureano-Perez et al., 2005). Likewise, even though the characteristic behaviors of lignin depend on a number of different and considerably important pretreatment conditions, the majority of pretreatments tend to focus only on its removal.

For a more accurate analysis of the characteristic changes of lignin during the pretreatment process, utilization of representative lignin isolated by various pretreatment conditions is recommended. However, isolation of lignin from lignocellulosic materials is conducted under conditions where lignin is

progressively broken down to lower molecular weight fragments, resulting in changes to its physicochemical properties. For this reason, the method of isolation has a significant influence on composition and properties of lignin isolated from the source (Gratzl & Chen-Loung, 2000).

Among the various species of lignin that have been isolated, milled wood lignin (MWL) is the best preparation for isolating lignin from lignocellulosic biomass with minimal chemical alteration. Indeed, while MWL is directly isolated from finely ball milled wood without any previous treatment (Bjorkman, 1957), cellulolytic enzyme lignin (CEL) is produced by removing most of the carbohydrate fractions with cellulolytic enzymes prior to dioxane extraction of ball milled wood (Chang et al., 1975). Comparison of the chemical structures of MWL and CEL has revealed that MWL is slightly more condensed than CEL, suggesting that MWL may contain a higher proportion of lignin from the middle lamella (Ikeda et al., 2002).

For scale-up and commercial implementation of pretreatment technologies, it is essential to understand the characteristic conversion mechanisms of lignin as a function of pretreatment conditions. In this study, the conversion characteristics of milled wood lignin as a representative material of original lignin were investigated as a function of temperature and catalyst concentration during acid-catalyzed organosolv pretreatment. The influence of these parameters on the physicochemical properties of milled wood lignin was also evaluated.

2. Materials and methods

2.1. Materials

Samples consisted of stems of twenty-year-old yellow poplar trees (*Liriodendron tulipifera*, Hwaseong-si Gyeong-ki do, Republic of Korea) provided by the Korea Forest Research Institute. Raw materials were milled and screened to less than 0.5 mm using a Cutting Mill pulverisette 15 (FRITSCH GmbH, Germany) and stored in plastic bags at room temperature after drying in air for at least 24 h air (less than 5% of the initial moisture content).

2.1.1. Milled wood lignin isolation

Milled wood lignin (MWL) was isolated according to a standard MWL isolation procedure (Björkman, 1956) with minor modifications. Briefly, raw wood meal (100 g) was placed in a porcelain jar with ceramic balls and mounted to vibratory ball mill (Pulverisette 6, FRITSCH GMBH, Germany). After 72 h of grinding, the ground wood meal was placed into a 2 l beaker with 1 l of dioxane/water (96:4, v/v), and the solution was stirred at room temperature for 24 h. The supernatant was collected by filtration using Whatman No. 42 filter paper (GE Healthcare UK Ltd., UK) and fully evaporated. The remaining solid material was once again dispersed in a 96% dioxane solution and the above procedure was repeated.

The final solid material was dissolved in 100 ml of 90% acetic acid (9:1, v/v) and added dropwise to 1 l of distilled water in a beaker under stirring to induce precipitation. The precipitated lignin was centrifuged, freeze dried, and dissolved in 30 ml of 1,2-dichloroethane/ethanol (2:1, v/v). Next, the

supernatant obtained after centrifugation was precipitated into 1 l of diethyl ether, and subjected to an additional round of washing, centrifugation, and freeze-drying. The final lignin material was weighed and stored in a conical tube at 4°C until further utilization.

2.2. Organosolv pretreatment process

As shown in Figure 2-1, organosolv pretreatment was conducted in a 500 ml stainless steel (SUS 316) reaction vessel equipped with a heating mantle and temperature controller (Hanwoul Engineering Inc., Gunpo, Gyeonggi, Republic of Korea). A Viton ring gasket was used to maintain the inner pressure, and a thermocouple and pressure gauge were located inside the reactor to measure the internal temperature and pressure during pretreatment, respectively.



Figure 2-1. The reactor for organosolv pretreatment.

For organosolv pretreatment, the reactor was loaded with 0.5 g of MWL and 5 ml of a 50:50% ethanol:water mixture (v/v) containing sulfuric acid (0.5, 1, and 2% (w/w)) as a catalyst. Pretreatment was conducted with a constant output of the heating mantle until the internal temperature reached the desired target temperature (range: 120°C to 200°C, 10°C intervals), after which the internal temperature was controlled within $\pm 2^\circ\text{C}$ for 10 min. After

pretreatment, the vessel was quenched in an ice chamber and cooled to room temperature within 10 min.

After cooling to room temperature, pretreated materials were filtered through a previously weighed 1G4 glass filter (Iwaki Glass Co. Ltd., Tokyo, Japan) and divided into pretreated solid residue and liquid hydrolysate fractions. The pretreated solid residue was washed using a double volume of distilled water to remove any surface remnants. The washed pretreated solid fraction was weighed after freeze-drying and stored until further physicochemical analyses. Finally, the pretreated materials were divided into three fractions: solid fraction, liquid hydrolysate, and washed filtrate.

2.3. Analysis of pretreated solid fractions

The water-insoluble solid (WIS) recovery rate (Eq. 1) was measured based on the initial input amount of MWL and filtered WIS weight after pretreatment.

$$\text{WIS recovery rate (\%)} = \frac{\text{water insoluble solid after pretreatment (g)}}{\text{input amount of MWL (g)}} \times 100 \quad (\text{Eq. 1})$$

For gel permeation chromatography (GPC) analysis and phenolic hydroxyl group analysis, the pretreated solid fraction was acetylated with acetic anhydride/pyridine (1:1 v/v) at 105°C for 2 h.

2.3.1. Molecular weight determination

For molecular weight determination, acetylated samples (3 mg) were dissolved in 2 ml tetrahydrofuran (THF) and subsequently passed through a 0.45 µm polytetrafluoroethylene (PTFE) filter to remove particulate matter. Molecular weight analysis was performed with a GPC max instrument (ViscotekRImax; Viscotek, UK), which consisted of a PLgel 3 µm MIXED-D column (300 × 7.5 mm, VARIAN, Inc), PLgel 3 µm MIXED-E column (300 × 7.5 mm, VARIAN, Inc), a PLgel 5µm guard column (50 × 7.5 mm, VARIAN, Inc), and UV-Vis detector (VE3210, Viscotek). THF was used as the mobile phase at a flow rate of 0.7 ml/min. A calibration curve was obtained using polystyrene standards ($M_w = 580\text{-}3,250,000$ Da).

2.3.2. Phenolic hydroxyl group

Phenolic hydroxyl group (phenolic-OH) content in pretreated MWL residue was determined as described previously (Månsson, 1983). Briefly, acetylated samples (10 mg) were dissolved in 2 ml of a dioxane/pyrrolidine

mixture (1:1 (v/v)) and 20 μl of 1-methylnaphthalene was added as an internal standard (1-methylnaphthalene, 5 μl). The aminolysis reaction was conducted in a 30°C water bath for 2 h, after which the amount of 1-acetylpyrrolidine that formed was measured using an Agilent 7890A GC equipped with a 5974C mass selective detector and FID. A DB-5 capillary column (60 m \times 0.25 mm \times 0.25 μm film thickness) was used for product separation at a split ratio of 1:20. The injector and detector temperatures were 220°C and 300°C, respectively. The oven temperature program consisted of 100°C for 5 min, followed by a heating rate of 30°C/min to 173°C for 5 min, 5°C/min to 180°C for 5 min, and finally 20°C/min to 200°C for 3 min (Kim et al., 2012b).

2.3.3. Nitrobenzene oxidation

Changes of the MWL structure after pretreatment were analyzed by measuring nitrobenzene oxidation (NBO) products. First, 30 mg of pretreated MWL residue was placed in a 10 ml glass bomb along with 4 ml of 2 M sodium hydroxide and 250 μl of nitrobenzene. The mixture was then reacted at 170°C for 2 h (Iiyama & Lam, 1990); 3-ethoxy-4-hydroxybenzaldehyde was used as an internal standard. The reaction products consisted of trimethylsilylated with 100 μl pyridine and 100 μl of N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) at 105°C for 2 h. Samples were analyzed with an Agilent 7890A GC equipped with a 5974C mass selective detector and FID. A DB-5 capillary column (60 m \times 0.25 mm \times 0.25 μm film thickness) was used for product separation at a split ratio of 1:20. The injector and detector temperatures were 200°C and 250°C, respectively. The oven temperature program consisted of 120°C for 10 min, followed by heating at a rate of 10°C/min to 280°C, which was then maintained for 20 min (Kim et al., 2012b).

2.4. Analysis of liquid hydrolysates

2.4.1. Chemical compositions

The amounts of sugars (glucose, xylose, and arabinose), sugar derived products (5-HMF and furfural), and organic acids (levulinic acid, formic acid, and acetic acid) present in liquid hydrolyzate were determined according to NREL Laboratory Analytical Procedures (Sluiter et al., 2008). Briefly, liquid hydrolysates were filtered through a 0.45 μm hydrophilic PTFE syringe filter (Advantec Co. Tokyo, Japan) and analyzed by HPLC (HP 1100 series, Agilent Technologies, Santa Clara, CA, USA) at the National Instrumentation Center for Environmental Management (NICEM, Seoul, Republic of Korea). The HPLC was equipped with an Aminex HPX-87H column (300 mm \times 7.8 mm ID \times 9 μm , Bio-Rad Laboratories, Richmond, CA, USA) and operated at a column temperature of 40°C with a mobile phase consisting of 0.01 N sulfuric acid at a flow rate of 0.5 ml/min. Products were quantified with a UV detector at 210 nm and a refractive index detector (RID).

2.4.2. Ethanol organosolv lignin recovery process

Liquid hydrolysates and the water used to wash pretreated solid residue were mixed and centrifuged. The resulting precipitate, referred to ethanol organosolv lignin (EOL), was collected and weighed after freeze-drying (Figure 2-2). The EOL yield (Eq. 2) was calculated based on the initial MWL input and weight of EOL collected after precipitation.

$$\text{EOL yield (\%)} = \frac{\text{water insoluble solid after precipitate (g)}}{\text{input amount of MWL (g)}} \times 100 \quad (\text{Eq. 2})$$

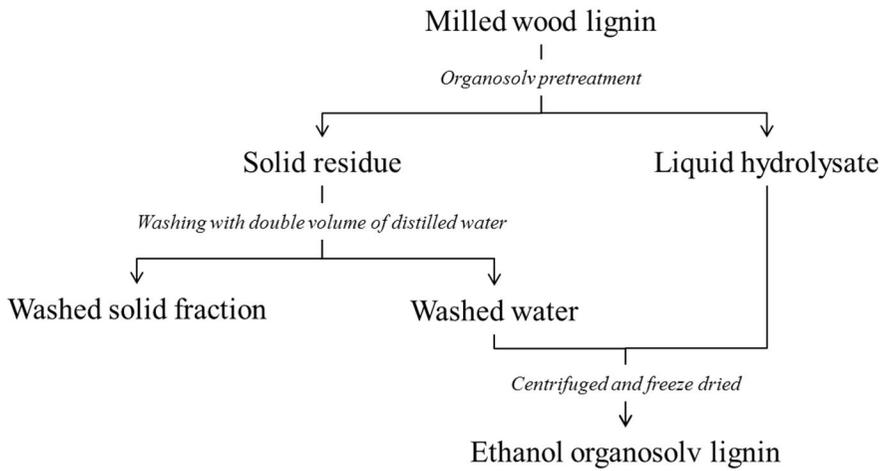


Figure 2-2. The procedure for obtaining the ethanol organosolv lignin during organosolv pretreatment of milled wood lignin.

3. Results and discussion

3.1. Conversion characteristics of milled wood lignin

The yield MWL isolated from the stems of the twenty-year-old yellow poplar trees was 10.11 ± 1.40 g / 100 g of raw material, and had a 90% Klason lignin content.

3.1.1. Water insoluble solid recovery rate of milled wood lignin

After organosolv pretreatment of MWL, the materials were divided into water insoluble solid (WIS) and liquid hydrolysate fractions. The WIS recovery rate is shown in Figure 2-3, which revealed a decrease depending on reaction temperature and acid concentration. Specifically, when MWL was organosolv pretreated with 0.5% acid, the WIS recovery rate gently decreased until reaching a temperature of 180°C. Thus, a relatively low acid concentration resulted in neither rapid degradation of MWL nor re-distribution of lignin fragments. Conversely, organosolv pretreatment with 1% or 2% acid resulted in a drastic degradation of MWL at temperatures between 130°C and 140°C. Importantly, the higher concentrations of acid significantly decreased the WIS recovery rate, the lower acid concentration had no effect. These data are consistent with a previous study showing that the lignin contents of solid fractions obtained after organosolv pretreatment are not significantly decreased when the pretreatment temperature is increased from 140°C to 160°C (Koo et al., 2011a). In this study, the WIS recovery rate of MWL with acid concentrations of 1% and 2% were maintained up to 180°C. This result could be explained by either a lack of further MWL degradation or simultaneous degradation and condensation of MWL, with the later possibility

being more likely due to my observation that the lowest WIS recovery rate was obtained using the weakest acid concentration. Furthermore, it was reported that lignin re-distribution occurs during acidic condition pretreatments such as with dilute acid (Pingali et al., 2010), steam explosion (Laureano-Perez et al., 2005), and organosolv (Koo et al., 2012). Subsequently, WIS recovery rates at each acid concentration were increased slightly after 180°C, although no increase in WIS recovery rate was observed with an acid concentration above 1%. Thus, the incremental WIS recovery rates observed in this study may be explained as condensation of small, degraded lignin fragments from the liquid fraction to the WIS fraction.

Based on the above results, conversion characteristics of MWL by organosolv pretreatment temperature were divided into three steps. First, up to a temperature of 140°C, degradation of MWL appeared to increase with increasing reaction temperature and acid concentration. Next, between 140°C and 180°C, both degradation and condensation reaction may have progressed simultaneously, which we designated as a re-distribution step. Finally, the condensation reaction was found to predominate at temperatures above 180°C. Interestingly, acid concentrations either accelerated or decelerated lignin behaviors. For example, with an acid concentration of 0.5%, the WIS recovery rate continued to decrease up to a temperature of 180°C without any evidence of condensation because the acid concentration was not sufficient to induce the condensation reaction. Likewise, after reaching a temperature of 180°C, the WIS recovery rate increased by only a small amount as the condensation reaction eventually took place. Conversely, compared with 0.5% and 1% acid, a vigorous condensation reaction was observed with 2% acid at temperatures above 180°C.

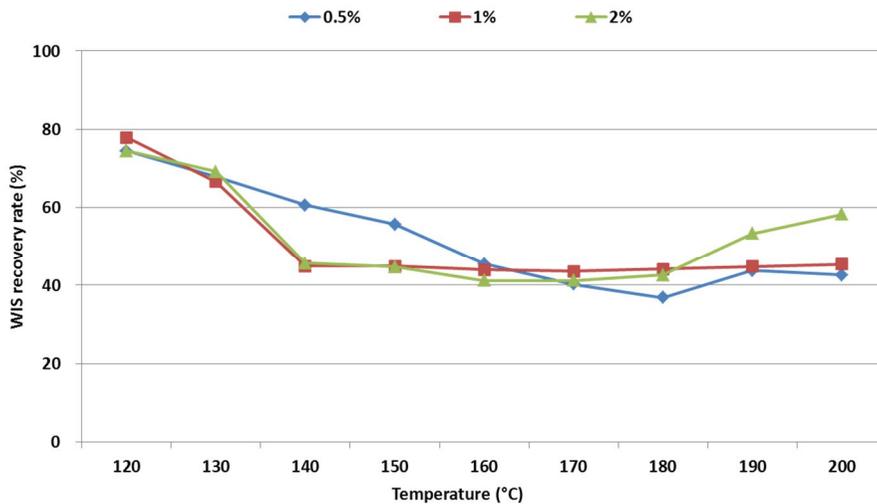


Figure 2-3. Water insoluble solid (WIS) recovery rate (%) of the solid fraction obtained by organosolv pretreatment of milled wood lignin depending on the temperature and acid concentration.

3.1.2. Molecular weight distributions of solid fraction

Table 2-1 shows the molecular weight distributions of the solid fraction obtained by organosolv pretreatment of MWL. Compared to the weight-average molecular weight (M_w) of MWL, the molecular weight of the solid fractions decreased with increasing reaction temperature and acid concentration. With respect to the 0.5% acid concentration condition, the M_w decreased rapidly between 140°C and 160°C, and more gradually thereafter. Interestingly, while M_w was not meaningfully affected by organosolv pretreatment up to 140°C, the number-average molecular weight (M_n) was significantly decreased at low temperatures compared to that of MWL. Both M_w and M_n increased slightly after 180°C, which was likely due to the condensation reaction. In addition, the polydispersity of solid fractions was higher than that of MWL during the initial pretreatment stage, and drastically declined between 140°C and 160°C (Figure 2-4). These results are consistent with previous observations that M_n is significantly decreased with increasing combined severity of pretreatment conditions, resulting in decreased polydispersity of organosolv lignin (El Hage et al., 2010a). The molecular weight distribution with an acid concentration of 1% exhibited a similar tendency as 0.5% acid, whereas an acid concentration of 2% resulted in decreased M_w and polydispersity, which was significantly increased at high temperature because of vigorous condensation.

Compared to the WIS recovery rate results, the degradation reaction reached maximal activity at approximately 140°C, while the condensation reaction initiated from that temperature. Thus, for the purpose of removing lignin from lignocellulosic biomass, organosolv pretreatment at temperatures over 150°C is not economical, although slight differences in efficiency can be obtained by varying the concentration of acid.

Table 2-1. Molecular weight distributions of the solid fraction obtained by organosolv pretreatment of milled wood lignin depending on the temperature and acid concentration

	Temp. (°C)	120	130	140	150	160	170	180	190	200
0.5%	M_w^a (Daltons)	9,631	9,749	8,825	6,685	5,541	5,047	4,330	5,295	5,126
	M_n^b (Daltons)	2,682	2,786	2,449	2,302	2,197	2,069	2,006	2,204	2,122
	M_w/M_n	3.59	3.50	3.60	2.90	2.52	2.44	2.16	2.40	2.42
1%	M_w (Daltons)	8,385	8,298	8,049	5,250	5,598	5,470	5,192	5,314	5,006
	M_n (Daltons)	2,167	2,185	2,750	2,190	2,292	2,302	2,179	2,100	1,966
	M_w/M_n	3.87	3.80	2.93	2.40	2.44	2.38	2.38	2.53	2.55
2%	M_w (Daltons)	8,494	9,068	6,832	4,824	5,278	5,051	4,934	5,778	6,576
	M_n (Daltons)	2,295	2,251	2,354	2,007	2,332	2,170	2,139	2,238	2,143
	M_w/M_n	3.70	4.03	2.90	2.40	2.26	2.33	2.31	2.58	3.07

milled wood lignin: M_w : 9919, M_n : 4240, Polydispersity: 2.34

^a weight-average molecular weight

^b number-average molecular weight

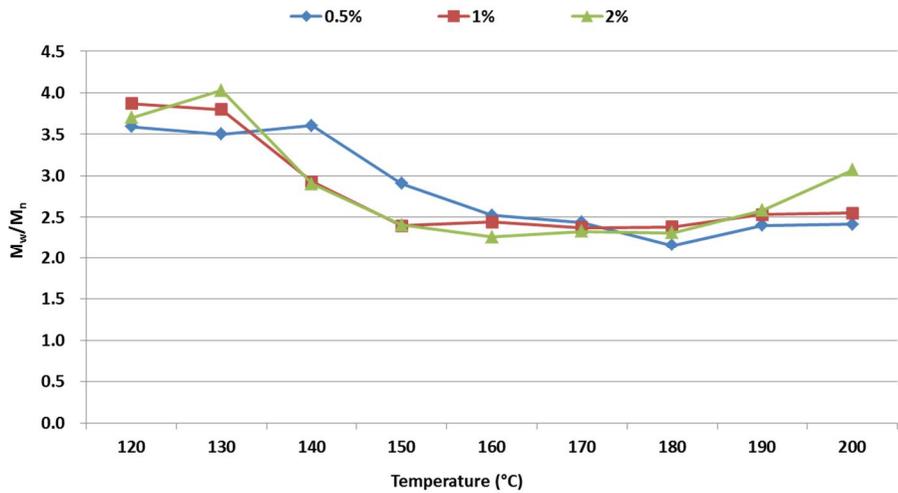


Figure 2-4. Polydispersity indexes of the solid fraction obtained by organosolv pretreatment of milled wood lignin depending on the temperature and acid concentration.

3.1.3. Phenolic hydroxyl group of solid fraction

Phenolic hydroxyl groups (phenolic-OH) are the most reactive functional group in lignin structures (Kim et al., 2012b; Sarkanen & Ludwig, 1971). The amount of phenolic-OH in MWL of yellow poplar trees was determined to be $7.00 \pm 0.45\%$. Compared to previous results, this amount was approximately 10% and 5% lower than the phenolic-OH contents of MWL and ionic liquid lignin of *populus albaglandulosa*, respectively (Kim et al., 2011b). After organosolv pretreatment of MWL, the phenolic-OH contents were held constant at each of the conditions tested (Figure 2-5). Indeed, the constant phenolic-OH content of the solid fractions indicated that lignin degradation was initiated primarily at aryl ether bonds, which can also form phenolic-OH groups. In addition, at temperatures above 140°C , the phenolic-OH content with 2% acid was slightly lower than for the other acid concentrations tested, which was likely due to an active condensation reaction.

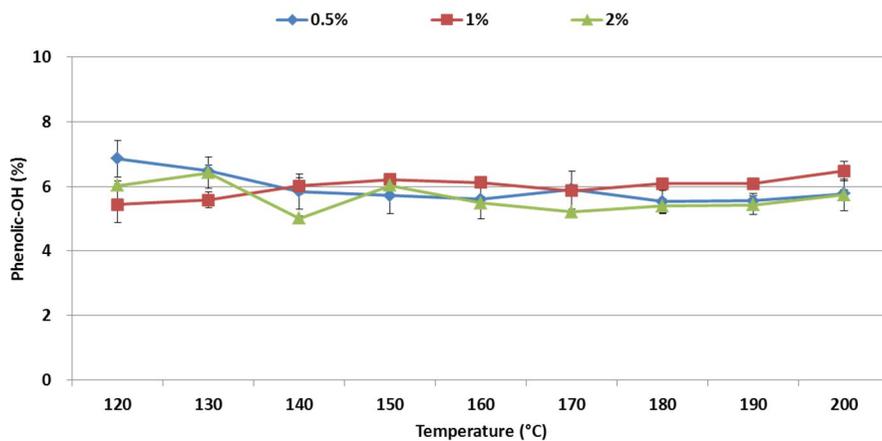


Figure 2-5. Phenolic hydroxyl (phenolic-OH) group content (%) of the solid fraction obtained by organosolv pretreatment of milled wood lignin depending on the temperature and acid concentration.

3.1.4. Nitrobenzene oxidation products of solid fraction

Nitrobenzene oxidation (NBO) can produce C6C1 phenols from lignin polymers, primarily in the form of aldehydes such as vanillin and syringaldehyde, although alcohols (guaiacol, syringol) and acids (vanillic acid, syringic acid) can be formed in minor quantities as well (Kim et al., 2012b). The total amounts of syringyl (S) and vanillyl (V) type compounds present in MWL were determined to be 2283.9 $\mu\text{mol/g}$ and 904.1 $\mu\text{mol/g}$, respectively. These results were similar to a previous study utilizing the same raw material (Kim et al., 2012b), and significantly higher than other forms of lignocellulosic biomass such as corn stover and switchgrass (Cybulska et al., 2012).

After organosolv pretreatment, the amount of NBO products in the solid fraction decreased dramatically with increasing reaction temperature and acid concentration (Table 2-2, 2-3, and 2-4). According to previous studies (Chen, 1992; Leopold & Malmström, 1951), essential NBO products are derived from oxidative degradation of ether linkages such as α -O-4 and β -O-4. Based on these results, it can be concluded that a condensation reaction took place in all of the conditions tested. However, compared with the WIS recovery rate and molecular weight distribution, it is more likely that the nitrobenzene oxidisable fractions were isolated from MWL during the early stages of organosolv pretreatment.

Because of the low amount of guaiacol in NBO products, evaluation of the S/V (syringyl to vanillyl) ratio is preferable to that of the S/G ratio (Jahan et al., 2006; Nadji et al., 2009). The S/V ratio of solid fractions obtained from MWL decreased with increasing reaction temperature, and became more pronounced as the concentration of acid increased. Indeed, low S/V ratios were obtained at 160°C with an acid concentration of 0.5% (2.04) and 1% (1.82), and reached a minimum at 140°C at an acid concentration of 2%

(1.78). Conversely, the S/V ratio obtained with 2% acid increased slightly as the reaction temperature increased. A reduction in S/V ratio indicates that more syringyl units were degraded during organosolv pretreatment compared with guaiacyl units. Furthermore, because the S/V ratio increased only slightly as the temperature increased, we were also able to conclude that syringyl units were either degraded earlier than guaiacyl units or underwent condensation to the solid fraction as the temperature increased. Previous research has suggested that guaiacyl lignin produces more recalcitrant lignin characteristics by restricting fiber swelling and enzyme accessibility compared with syringyl lignin (Ramos et al., 1992). Further, the majority of syringyl lignin is present in secondary walls of fiber and ray parenchyma, along with almost all of the cellulose and hemicellulose content. Conversely, guaiacyl units are positioned primarily in vessels and the middle lamella, which is necessary to achieve high rigidity (Saka & Goring, 1988). Building on these studies, syringyl lignins appeared to degrade earlier than guaiacyl lignins, which was likely because of characteristics including relatively low restriction of fiber swelling and an abundance of ester bonds between lignin and cellulose or hemicellulose, which have considerably lower activation energy than ether bonds.

Table 2-2. Nitrobenzene oxidation products ($\mu\text{mol/g}$ sample) of the solid fraction obtained by organosolv pretreatment with 0.5% sulfuric acid of milled wood lignin depending on the temperature

NBO products	Amount ($\mu\text{mol/g}$ sample)									Sum (V unit)	Sum (S unit)	S/V ratio
	H	V	S									
	Benzoic acid	Guaiacol	Vanillin	Vanillic acid	Aceto vanillone	Syringol	Syring aldehyde	Syringic acid				
120°C	0.6±0.9	8.0±0.2	770.0±38.9	49.8±2.3	21.7±1.6	10.1±0.0	1825.1±47.8	243.2±0.6	849.4±42.7	2078.4±48.5	2.45	
140°C	0.0±0.0	5.6±0.2	567.5±34.9	59.6±4.1	17.6±0.0	6.1±0.2	1195.1±78.1	257.9±24.1	650.3±39.0	1459.1±102.4	2.24	
0.5% 160°C	0.7±1.0	5.8±0.3	314.4±19.9	56.6±21.4	13.5±0.4	5.8±0.5	608.7±30.6	183.5±47.9	390.3±41.7	798.0±79.0	2.04	
180°C	1.1±0.3	3.1±0.2	128.6±2.6	60.8±1.3	8.9±0.2	4.8±0.3	272.2±5.9	170.6±6.1	201.4±4.1	447.6±12.3	2.22	
200°C	1.0±0.0	4.8±2.1	89.2±16.6	68.1±10.1	10.0±2.9	6.2±1.8	191.6±33.0	171.1±18.9	172.1±29.6	369.0±53.7	2.14	
MWL	0.9±0.0	9.6±1.4	754.1±27.3	119.1±1.4	21.2±2.2	8.6±1.2	1997.9±59.5	277.3±1.1	904.1±32.3	2283.9±61.8	2.53	

Table 2-3. Nitrobenzene oxidation products ($\mu\text{mol/g}$ sample) of the solid fraction obtained by organosolv pretreatment with 1% sulfuric acid of milled wood lignin depending on the temperature

NBO products	Amount ($\mu\text{mol/g}$ sample)									S/V ratio	
	H	V	S						Sum (V unit)		Sum (S unit)
	Benzoic acid	Guaiacol	Vanillin	Vanillic acid	Aceto vanillone	Syringol	Syring aldehyde	Syringic acid			
120°C	1.9±0.1	7.0±0.6	651.8±56.6	73.1±9.1	37.2±2.2	10.7±1.7	1496.2±129.3	316.7±22.8	769.1±68.6	1823.6±153.8	2.37
140°C	1.4±0.7	6.4±1.6	543.7±34.4	75.4±3.2	32.9±1.3	8.3±0.9	962.9±51.0	252.2±13.3	658.4±40.4	1223.5±65.2	1.86
1% 160°C	2.6±2.3	6.4±4.2	212.7±1.5	102.4±1.4	21.4±0.9	6.2±0.6	410.0±5.1	207.9±0.5	343.0±8.0	624.1±6.2	1.82
180°C	1.0±0.2	3.0±0.2	100.1±1.6	76.8±11.4	14.8±1.2	5.0±0.3	226.6±6.2	196.3±1.6	194.7±14.4	428.0±8.1	2.20
200°C	0.9±0.6	3.4±1.0	65.6±6.4	68.8±15.5	5.4±3.8	4.3±0.5	158.9±24.4	158.7±52.6	143.2±26.7	321.8±77.5	2.25

Table 2-4. Nitrobenzene oxidation products ($\mu\text{mol/g}$ sample) of the solid fraction obtained by organosolv pretreatment with 2% sulfuric acid of milled wood lignin depending on the temperature

NBO products	Amount ($\mu\text{mol/g}$ sample)									S/V ratio	
	H	V	S						Sum (V unit)		Sum (S unit)
	Benzoic acid	Guaiacol	Vanillin	Vanillic acid	Aceto vanillone	Syringol	Syring aldehyde	Syringic acid			
120°C	1.7±0.5	6.6±0.5	501.1±13.5	76.4±0.5	16.8±1.2	7.0±0.5	1093.8±33.5	253.8±1.3	600.9±15.7	1354.6±35.3	2.25
140°C	1.4±0.2	7.3±0.4	432.0±27.2	123.4±7.4	14.1±0.5	8.1±0.2	769.2±48.7	251.0±20.4	576.9±35.4	1028.3±69.3	1.78
2% 160°C	1.2±0.4	7.2±1.3	177.1±16.5	82.9±18.2	10.1±0.7	7.1±1.5	360.9±32.6	161.2±35.9	277.3±36.7	529.2±70.0	1.91
180°C	2.4±1.2	3.9±0.2	77.5±1.1	68.0±1.3	8.8±0.2	4.4±0.0	162.8±1.8	137.9±3.3	158.3±2.9	305.2±5.1	1.93
200°C	1.6±1.3	5.5±1.7	55.9±0.2	56.3±3.5	5.9±1.2	5.9±0.8	123.5±1.9	164.3±21.4	123.7±6.5	293.7±24.1	2.37

With respect to the WIS recovery rate, the amounts of NBO products in the solid fractions per 100 g of input are shown in Tables 2-5, 2-6, and 2-7. Approximately 44.9 g of NBO products were obtained per 100 g of MWL; however this amount decreased significantly with increasing temperature and acid concentration. Specifically, the majority of nitrobenzene oxidisable fractions were removed as the reaction temperature reached 160°C (0.5% H₂SO₄: 83.17%, 1% H₂SO₄: 86.64%, 2% H₂SO₄: 89.54%), but were removed more gradually thereafter. With respect to the 2% acid condition (Table 2-7), the amount of NBO products decreased dramatically at 120°C while that of WIS recovery rate and M_w were maintained compared to lower acid concentrations (Figure 2-3, Table 2-1). It is possible that both degradation and condensation reactions occurred simultaneously in conditions with low temperature and a high acid concentration.

To determine the conversion of MWL fragments degraded during pretreatment, the change in the total amount of NBO products and S/V ratio of the solid fraction were calculated. As shown in Figure 2-6, the amounts of NBO products decreased slowly with 0.5% acid up to a temperature of 180°C, and were maintained thereafter. However, with 1% and 2% acid, the amounts of nitrobenzene oxidisable products at a temperature of 140°C were reduced to 73.72% and 76.84% compared with 0.5% acid, respectively, and continued to decrease gradually as the temperature increased.

The S/V ratios of total reduced nitrobenzene oxidisable products are shown in Figure 2-6. The S/V ratio of the 0.5% acid concentration condition was highest at 120°C, and gradually decreased with increasing reaction temperature. On the other hand, in the case of 1% and 2% acid, the S/V ratios peaked unexpectedly at 140°C, and exhibited a sharp decrease with increasing temperatures.

Based on these results, it appears that an acid concentration of 0.5% is neither sufficient to induce a condensation reaction at relatively low reaction

temperature nor capable of facilitating selective degradation of syringyl lignin in certain conditions. Likewise, in the case of acid concentrations of 1% or 2% at a reaction temperature of approximately 140°C, the majority of nitrobenzene oxidisable products of MWL were rapidly degraded, while syringyl lignin appeared to be selectively degraded. Thus, for the purpose of lignin removal, organosolv pretreatment of lignocellulosic biomass performed at temperatures less than 140°C with 1% acid appears to be both efficient and economical. Furthermore, selective degradation of syringyl type lignin was also achieved, which may be useful for specific applications.

Table 2-5. Nitrobenzene oxidation products of the solid fraction obtained by organosolv pretreatment with 0.5% sulfuric acid of milled wood lignin depending on the temperature (on the basis of 100 g of initial oven-dried raw materials)

NBO products	Amount (g)									Sum (V unit)	Sum (S unit)	Sum (Total)
	H	V	S									
	Benzoic acid	Guaiacol	Vanillin	Vanillic acid	Aceto vanillone	Syringol	Syring aldehyde	Syringic acid				
120°C	0.0±0.0	0.1±0.0	7.8±0.4	0.5±0.0	0.3±0.0	0.1±0.0	19.2±0.5	2.7±0.0	8.6±0.4	21.9±0.5	30.6±0.9	
140°C	0.0±0.0	0.0±0.0	4.7±0.3	0.5±0.0	0.2±0.0	0.1±0.0	10.2±0.7	2.3±0.2	5.4±0.3	12.6±0.9	18.0±1.2	
0.5% 160°C	0.0±0.0	0.0±0.0	2.0±0.1	0.4±0.1	0.1±0.0	0.0±0.0	3.9±0.2	1.2±0.3	2.4±0.3	5.2±0.5	7.6±0.8	
180°C	0.0±0.0	0.0±0.0	0.6±0.0	0.3±0.0	0.1±0.0	0.0±0.0	1.4±0.0	0.9±0.0	1.0±0.0	2.4±0.1	3.4±0.1	
200°C	0.0±0.0	0.0±0.0	0.5±0.1	0.4±0.1	0.1±0.0	0.0±0.0	1.2±0.2	1.1±0.1	1.0±0.2	2.3±0.3	3.3±0.5	
MWL	0.0±0.0	0.1±0.0	10.3±0.4	1.8±0.0	0.4±0.0	0.1±0.0	28.2±0.8	4.1±0.0	12.6±0.4	32.4±0.9	44.9±1.3	

Amount (g) = nitrobenzene oxidation products of solid fraction (g) × water insoluble solid recovery rate (%) of solid fraction

Table 2-6. Nitrobenzene oxidation products of the solid fraction obtained by organosolv pretreatment with 1% sulfuric acid of milled wood lignin depending on the temperature (on the basis of 100 g of initial oven-dried raw materials)

NBO products	Amount (g)									Sum (V unit)	Sum (S unit)	Sum (Total)
	H	V		S								
	Benzoic acid	Guaiacol	Vanillin	Vanillic acid	Aceto vanillone	Syringol	Syring aldehyde	Syringic acid				
120°C	0.0±0.0	0.1±0.0	6.9±0.6	0.8±0.1	0.3±0.0	0.1±0.0	16.4±1.4	3.6±0.3	8.0±0.7	20.2±1.7	28.2±2.4	
140°C	0.0±0.0	0.0±0.0	3.3±0.2	0.5±0.0	0.1±0.0	0.1±0.0	6.1±0.3	1.7±0.1	4.0±0.2	7.8±0.4	11.8±0.7	
1% 160°C	0.0±0.0	0.0±0.0	1.3±0.0	0.7±0.0	0.1±0.0	0.0±0.0	2.5±0.0	1.3±0.0	2.1±0.0	3.9±0.0	6.0±0.1	
180°C	0.0±0.0	0.0±0.0	0.6±0.0	0.5±0.1	0.1±0.0	0.0±0.0	1.4±0.0	1.3±0.0	1.2±0.1	2.7±0.1	3.9±0.2	
200°C	0.0±0.0	0.0±0.0	0.4±0.0	0.5±0.1	0.1±0.0	0.0±0.0	1.0±0.2	1.1±0.4	1.0±0.2	2.1±0.5	3.1±0.7	

Amount (g) = nitrobenzene oxidation products of solid fraction (g) × water insoluble solid recovery rate (%) of solid fraction

Table 2-7. Nitrobenzene oxidation products of the solid fraction obtained by organosolv pretreatment with 2% sulfuric acid of milled wood lignin depending on the temperature (on the basis of 100 g of initial oven-dried raw materials)

NBO products	Amount (g)									Sum (V unit)	Sum (S unit)	Sum (Total)
	H	V		S								
	Benzoic acid	Guaiacol	Vanillin	Vanillic acid	Aceto vanillone	Syringol	Syring aldehyde	Syringic acid				
120°C	0.0±0.0	0.1±0.0	5.1±0.1	0.8±0.0	0.2±0.0	0.1±0.0	11.5±0.4	2.8±0.0	6.2±0.1	14.3±0.4	20.5±0.5	
140°C	0.0±0.0	0.0±0.0	2.7±0.2	0.8±0.0	0.1±0.0	0.1±0.0	4.9±0.3	1.7±0.1	3.7±0.2	6.7±0.5	10.3±0.7	
2% 160°C	0.0±0.0	0.0±0.0	1.0±0.1	0.5±0.1	0.1±0.0	0.0±0.0	2.1±0.2	1.0±0.2	1.6±0.2	3.1±0.4	4.7±0.6	
180°C	0.0±0.0	0.0±0.0	0.5±0.0	0.4±0.0	0.1±0.0	0.0±0.0	1.0±0.0	0.9±0.0	0.9±0.0	1.9±0.0	2.8±0.0	
200°C	0.0±0.0	0.0±0.0	0.4±0.0	0.5±0.0	0.1±0.0	0.0±0.0	1.0±0.0	1.4±0.2	1.0±0.0	2.5±0.2	3.5±0.2	

Amount (g) = nitrobenzene oxidation products of solid fraction (g) × water insoluble solid recovery rate (%) of solid fraction

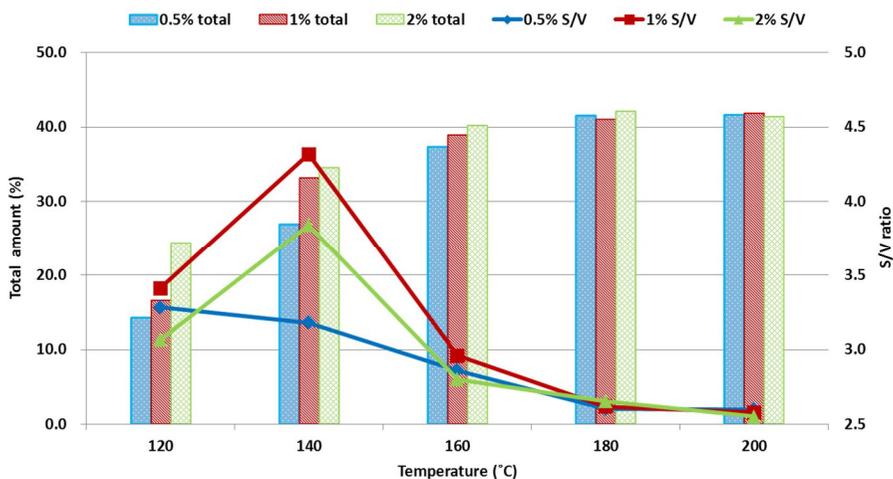


Figure 2-6. Total reduced nitrobenzene oxidisable products amounts and that of S/V ratio of the solid fraction obtained by organosolv pretreatment of milled wood lignin depending on the temperature and acid concentration. Reduction amounts of nitrobenzene oxidation products = nitrobenzene oxidation products of milled wood lignin – (nitrobenzene oxidation products of solid fraction) × (water insoluble solid recovery rate (%) of solid fraction)

3.2. Ethanol organosolv lignin and liquid hydrolysate

3.2.1. Ethanol organosolv lignin yield from liquid hydrolysate

Ethanol organosolv lignin (EOL) is produced by addition of water or removal of ethanol from the liquid fraction after organosolv pretreatment. El Hage et al., reported a 52% EOL recovery of the Klason lignin content of untreated *Miscanthus* (El Hage et al., 2009), although this amount varies with the severity of pretreatment conditions (El Hage et al., 2010a). Similar to these results, the EOL yield was enhanced as the reaction temperature increased (Figure 2-7). Further, use of 0.5% acid resulted in a gradual increase in EOL yield up to 180°C, which was well correlated with the WIS recovery rate and formation of NBO products. Interestingly, a maximum EOL yield of 26.65% was obtained at reaction temperatures up to 180°C, and decreased as the temperature increased further, which was probably related to re-condensation of EOL to the solid fraction at higher temperatures. With respect to 1% and 2% acid, EOL yield was significantly increased at 140°C but relatively stable up to 180°C. Use of 2% acid resulted in a vigorous re-condensation of EOL to the solid fraction, and thus the EOL yield decreased dramatically. This result was well correlated with the WIS recovery rate and molecular weight distribution results. On the other hand, EOL yield was not significantly changed at high temperature with 1% acid, similar to the WIS recovery rate results. Interestingly, Koo et al. reported that 1% acid causes lignin re-distribution in organosolv pretreatment of yellow poplar, and thus pretreatment conditions utilizing moderate acid concentrations may be favorable for reducing lignin re-distribution (Koo et al., 2012).

As described above, EOL is produced by precipitation of the liquid fraction, and lower molecular weight components may contribute more

towards the formation of the precipitate. Based on this assumption, an acid concentration of 0.5% was likely too low to degrade or condense EOL itself until reaching a temperature of 180°C, and thus EOL yield was stable up to that temperature, and steadily increased thereafter. Specifically, at temperatures above 180°C, it was though the condensation reaction was initiated, which in turn reduced EOL yield and increased the WIS recovery rate. In the case of 1% acid, EOL yield increased rapidly between 130°C and 140°C but remained stable as the temperature increased further, which was probably due to the degradation and condensation reactions occurring simultaneously. With respect to the 2% acid condition, there was a rapid increase in EOL yield similar to what was observed with 1% acid, but decreased significantly after 180°C, which was also likely due to vigorous re-condensation to the solid fraction.

A summary of the results of conversion of the solid fraction obtained by organosolv pretreatment of MWL as a function of temperature and acid concentration is presented in Table 2-8. The main lignin reaction was divided into three categories, namely, degradation, re-distribution (degradation and condensation), and condensation. Lignin conversion was initiated by degradation, which was followed by condensation. The condensation reaction was accelerated by the presence of a strong acid. Thus, to remove lignin from lignocellulosic biomass or to produce EOL, pretreatment conditions consisting of 0.5% acid at a temperature less than 180°C or 1% or 2% acid at a temperature of 140°C are suggested.

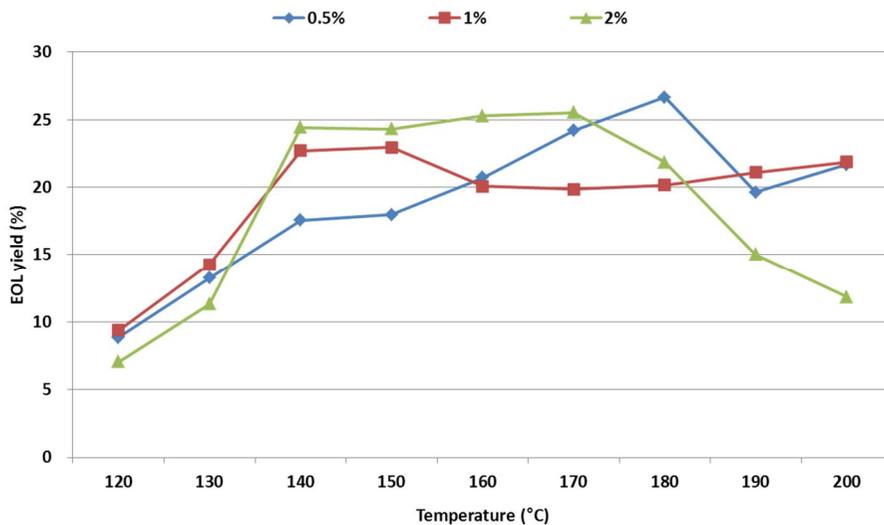


Figure 2-7. Ethanol organosolv lignin (EOL) yield from the liquid fraction obtained by organosolv pretreatment of milled wood lignin depending on the temperature and acid concentration.

Table 2-8. Conversion characteristics of the solid fraction obtained by organosolv pretreatment of milled wood lignin depending on the temperature and acid concentration

Acid conc.	Temp.	120°C	130°C	140°C	150°C	160°C	170°C	180°C	190°C	200°C
0.5%	Main reaction	←			degradation			→	condensation	
	WIS recovery rate	↓	↓	↓	↓	↓	↓	↓	↑	-
	Molecular weight	-	-	↓	↓	↓	↓	↓	↑	-
	EOL yield	↑	↑	↑	↑	↑	↑	↑	↓	-
1%	Main reaction	←	degradation	→	←	re-distribution (degradation and condensation)				→
	WIS recovery rate	↓	↓	↓↓	-	-	-	-	-	-
	Molecular weight	-	-	-	↓	-	-	-	-	-
	EOL yield	↑	↑	↑↑	-	-	-	-	-	-
2%	Main reaction	←	degradation	→	re-distribution (degradation and condensation)			condensation		
	WIS recovery rate	↓	↓	↓↓	-	-	-	-	↑↑	↑
	Molecular weight	-	-	↓	-	-	-	-	↑	↑
	EOL yield	↑	↑	↑↑	-	-	-	↓	↓↓	↓

WIS: water insoluble solid; EOL: ethanol organosolv lignin

↑: increased (p<0.05), ↑↑: significantly increased (p<0.01), ↓: decreased (p<0.05), ↓↓: significantly decreased (p<0.01), -: maintained (p>0.05)

3.2.2. Compositions of liquid hydrolysate

Even though MWL is composed primarily of lignin (approximately 90% Klason lignin), it also contains trace amounts of carbohydrates such as glucose and xylose. The compositions of liquid fractions obtained by organosolv pretreatment of MWL are presented in Tables 2-9, 2-10, and 2-11. Sugar and sugar-derived compounds were isolated from the liquid fraction in amounts ranging from 0.32% to 2.36% depending on the pretreatment condition. Although the amounts of sugar-derived compounds were low, it is likely that lignin degradation was initiated with the isolation of xylose. Moreover, the conditions during lignin condensation correlated with the degradation of xylose. Specifically, xylose was slowly isolated from the liquid fraction with 0.5% acid up to 170°C, after which it most likely underwent further dehydration to form furfural (Table 2-9). It was previously reported that the composition of furfural in liquid hydrolysates following dilute acid pretreatment is significantly increased between 160°C and 180°C, indicating a reduction in xylose content (Kim et al., 2011a). With respect to acid concentrations of 1% and 2%, xylose was rapidly isolated from the solid fraction up to 140°C, and was most likely degraded to furfural at higher temperatures (Table 2-10 and 2-11). Importantly, the xylose conversion results were comparable to the lignin conversion results.

As described above, components of lignocellulosic biomass are intra- and inter connected by various linkages such as ether, ester, and hydrogen bonds. Among these structures, hemicellulose and lignin are interlinked by ester bonds, which have a much lower activation energy compared to ether bonds of cellulose-lignin and lignin-lignin linkages (Harmsen et al., 2010). Thus, with respect to disruption of ester bonds, lignin linked with hemicellulose is degraded during the early stages of organosolv pretreatment. Likewise,

degradation of lignin from lignin-lignin and cellulose-lignin structures takes place after ether linkages are broken.

When lignin and xylose were rapidly degraded around 140°C, the S/V ratio of the total amount of reduced nitrobenzene oxidisable products was significantly increased. This result can be explained through the difference of hardwood lignin distribution. Specifically, the majority of syringyl unit lignin is present in secondary walls of fiber and ray parenchyma along with most of the forms of hemicellulose, while guaiacyl unit lignin is present in vessels and middle lamella which demand high rigidity (Saka & Goring, 1988). Thus, syringyl unit lignin and hemicellulosic sugars were degraded together and earlier compared to guaiacyl unit lignin.

Table 2-9. Composition (g) of liquid fraction obtained by organosolv pretreatment with 0.5% sulfuric acid of milled wood lignin depending on the temperature (on the basis of 100 g of initial oven-dried raw materials)

Temp. (°C)	glucose	xylose	arabinose	formic acid	acetic acid	total
120	0.01±0.00	0.06±0.00	0.02±0.00	0.05±0.00	0.20±0.01	0.34±0.02
130	0.01±0.00	0.10±0.00	0.01±0.00	0.05±0.00	0.15±0.01	0.32±0.02
140	0.02±0.00	0.51±0.03	0.03±0.00	0.11±0.01	0.32±0.02	0.99±0.05
150	0.03±0.00	0.73±0.15	0.04±0.00	0.15±0.01	0.37±0.02	1.33±0.18
160	0.06±0.01	0.91±0.05	0.07±0.00	0.26±0.01	0.51±0.03	1.81±0.10
170	0.06±0.00	0.92±0.05	0.07±0.02	0.30±0.01	0.51±0.05	1.85±0.13
180	0.05±0.00	0.67±0.03	0.07±0.01	0.31±0.02	0.58±0.02	1.68±0.08
190	0.03±0.00	0.19±0.01	0.03±0.00	0.28±0.01	0.56±0.03	1.08±0.05
200	0.00±0.00	0.07±0.00	0.00±0.00	0.37±0.02	0.65±0.03	1.09±0.05

Table 2-10. Composition (g) of liquid fraction obtained by organosolv pretreatment with 1% sulfuric acid of milled wood lignin depending on the temperature (on the basis of 100 g of initial oven-dried raw materials)

Temp. (°C)	glucose	xylose	arabinose	formic acid	acetic acid	total
120	0.02±0.00	0.20±0.01	0.01±0.00	0.08±0.00	0.20±0.01	0.52±0.03
130	0.03±0.00	0.44±0.02	0.02±0.00	0.09±0.00	0.31±0.02	0.89±0.04
140	0.05±0.00	1.09±0.03	0.05±0.00	0.20±0.01	0.54±0.03	1.92±0.08
150	0.06±0.00	1.06±0.05	0.06±0.00	0.26±0.01	0.52±0.03	1.98±0.11
160	0.07±0.00	0.98±0.03	0.00±0.00	0.30±0.02	0.53±0.03	1.89±0.07
170	0.05±0.01	0.63±0.03	0.06±0.00	0.29±0.01	0.55±0.03	1.58±0.08
180	0.01±0.00	0.07±0.00	0.00±0.00	0.30±0.02	0.68±0.03	1.06±0.05
190	0.00±0.00	0.09±0.00	0.00±0.00	0.33±0.02	0.61±0.03	1.03±0.05
200	0.00±0.00	0.07±0.00	0.00±0.00	0.35±0.02	0.61±0.03	1.02±0.05

Table 2-11. Composition (g) of liquid fraction obtained by organosolv pretreatment with 2% sulfuric acid of milled wood lignin depending on the temperature (on the basis of 100 g of initial oven-dried raw materials)

Temp. (°C)	glucose	xylose	arabinose	formic acid	acetic acid	total
120	0.02±0.00	0.17±0.01	0.01±0.00	0.08±0.00	0.18±0.01	0.46±0.02
130	0.02±0.00	0.50±0.02	0.02±0.00	0.10±0.01	0.36±0.02	1.00±0.05
140	0.07±0.00	1.24±0.12	0.08±0.01	0.31±0.03	0.66±0.03	2.36±0.20
150	0.07±0.00	1.07±0.05	0.08±0.00	0.33±0.02	0.62±0.03	2.17±0.11
160	0.06±0.00	0.62±0.03	0.06±0.00	0.33±0.02	0.66±0.07	1.72±0.12
170	0.04±0.00	0.30±0.01	0.04±0.00	0.34±0.05	0.69±0.03	1.41±0.11
180	0.02±0.00	0.10±0.01	0.01±0.00	0.30±0.02	0.69±0.03	1.13±0.06
190	0.00±0.00	0.06±0.00	0.00±0.00	0.35±0.00	0.70±0.03	1.11±0.04
200	0.00±0.00	0.03±0.00	0.00±0.00	0.39±0.02	0.66±0.03	1.08±0.05

3.3. Lignin recovery

Figure 2-8 shows the lignin recovery results from the solid fraction and produced EOL, which ranged from 61.69 g to 87.28 g. With 0.5% acid, the recovery of lignin exhibited a relatively gradual slope up to 180°C, but was steady between 180°C and 200°C. Increased acid concentration resulted in a rapid decline of lignin recovery at 140°C, which continued up to 180°C, and was slightly increased thereafter, probably due to re-condensation.

Importantly, lignin recovery of the solid fraction and EOL was substantially lower than the initial lignin content. I presumed that a large amount of degraded lignin fragments may be present in the liquid fraction, possibly as much as the amount of produced EOL. El Hage et al. also reported that despite differences due to various pretreatment conditions, the sum of EOL lignin recovery and Klason lignin content in pulp after organosolv pretreatment is much lower than the initial lignin content (El Hage et al., 2010a). Thus, recovery of unidentified material should be performed to obtain maximum lignin recovery and confirm the conversion characteristics of lignin by organosolv pretreatment.

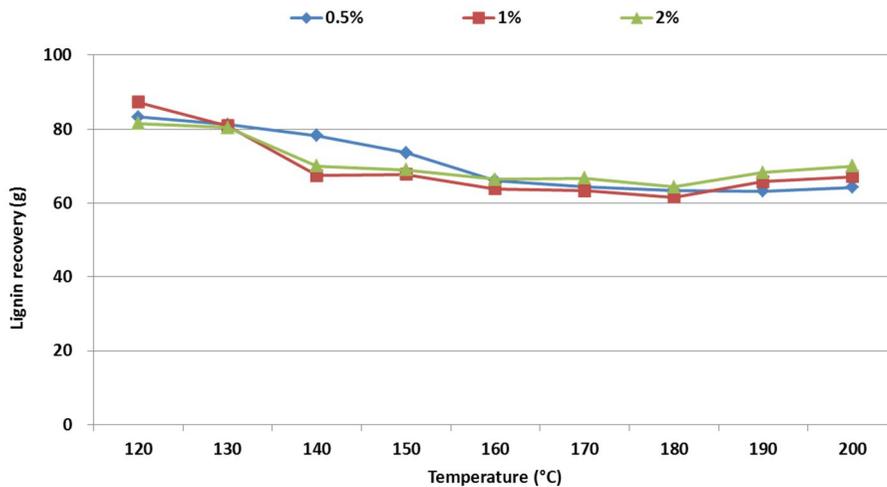


Figure 2-8. Lignin recovery (g) of the solid fraction and ethanol organosolv lignin obtained by organosolv pretreatment of milled wood lignin depending on the temperature and acid concentration. (on the basis of 100 g of initial oven-dried raw materials)

4. Conclusions

The conversion characteristics of milled wood lignin during acid-catalyzed organosolv pretreatment can be described in three steps: 1) degradation, 2) re-distribution (degradation and condensation), and 3) condensation, the precise-locations of which can vary depending on reaction temperature and acid concentration. In the present study, the degradation stage ranged from 120-180°C with 0.5% acid, and 120-140°C with 1% and 2% acid. During the degradation stage, the WIS recovery rate, M_w , and NBO products decreased while EOL yield was enhanced. Particularly, with acid concentrations of 1% and 2%, vigorous degradation of lignin in the secondary wall was expected because the S/V ratio of reduced NBO products was significantly increased.

The re-distribution (degradation and condensation) stage ranged from 140-200°C with 1% acid and 140-180°C with 2% acid. During re-distribution, lignin was simultaneously degraded and condensed, and thus the WIS recovery rate, M_w , and EOL yields were generally stable. However, the abundance of NBO products declined steadily and continuously during re-distribution. Likewise, the S/V ratio of reduced NBO products decreased during re-distribution, probably due to both degradation of G unit lignin as a result of the severe pretreatment conditions and condensation of degraded S unit lignin to the solid fraction.

At an acid concentration of 2%, the condensation reaction predominated after 180°C. Therefore, both the WIS recovery rate and M_w were increased while NBO products and EOL yield were mitigated. Likewise, the majority of lignin underwent both degradation and condensation reactions under this condition, which blocked the active site of lignin and returned the S/V ratio to that of untreated MWL. Although the WIS recovery rate did not change under

relatively less severe conditions, a small degree of condensation may have occurred. Thus, in order to remove lignin and produce EOL from lignocellulosic biomass, these results suggest that the pretreatment conditions should be controlled in the early portion of the re-distribution stage.

Chapter 3

Characteristic changes of lignin in yellow
poplar during acid-catalyzed organosolv
pretreatment

1. Introduction

Lignocellulosic bioethanol has been studied extensively in the past few years, with the US Department of Energy providing more than 1 billion dollars toward lignocellulosic bioethanol projects since 2007 in order to achieve cost competitive bioethanol (1.33 dollar per gallon) by 2012 (Slade et al., 2009). Establishing a reliable cost of lignocellulosic bioethanol requires overcoming biomass recalcitrance, which is due to accessible surface area, crystallization of cellulose, and solubilization and removal of either hemicellulose or lignin for effective enzymatic hydrolysis (Alvira et al., 2010b; Zhao et al., 2009), for which numerous pretreatment methods have been studied.

Among the various factors that contribute to biomass recalcitrance, the content and distribution of lignin has a profound effect on enzymatic hydrolysis. Previous studies have suggested that enzyme performance is reduced during enzymatic hydrolysis of lignocellulose by non-productively binding with lignin or lignin-carbohydrate complexes (LCCs) (Berlin et al., 2006; Esteghlalian et al., 2001). On the other hand, some studies have shown that enzymatic hydrolysis of lignocellulosic biomass can be significantly enhanced without a great amount of lignin removal (Kim et al., 2001; Koo et al., 2012). Such contrasting points of view can be attributed to 1) the complex structure of lignocellulosic biomass, which makes it difficult to analyze lignin separately from other components, 2) an absence of accurate observations regarding lignin recovery, and 3) a lack of understanding of lignin behavior during pretreatment processes. Furthermore, with respect to biorefineries, understanding the behavior of lignin is valuable for not only optimizing pretreatment processes, but also to maximize the economic value of biomass feedstock while utilizing produced lignin fractions (Thomsen, 2005).

Organosolv pretreatment is more feasible for biorefinery utilization of lignocellulosic biomass, because all of the biomass components can be utilized. After organosolv pretreatment of lignocellulosic biomass, the main products are divided into 1) cellulosic fibers, which contain varying amounts of hemicellulose and residual lignin, 2) organosolv lignin, which is obtained after removal of the organic solvent from the liquid fraction, and 3) an aqueous solution of hemicellulose sugars (Zhao et al., 2009). Production of organosolv lignin will be interrelated with hydrolysis of cellulosic fibers, the products of which may be further converted to chemical building blocks for additional applications (Werpy & Petersen, 2004).

In this study, I investigated the characteristic changes of lignin during acid-catalyzed organosolv pretreatment of yellow poplar. Lignin analysis of both pretreated solid fractions and organosolv lignin was performed. In addition, unidentified lignin content in liquid fraction was also analyzed. Finally, lignin recovery from all of the analyzed fractions was evaluated to support the results above.

2. Materials and methods

2.1. Materials

Stems of twenty-year-old yellow poplar (*Liriodendron tulipifera*, Hwaseong-si Gyeong-gi do, Republic of Korea) provided by the Korea Forest Research Institute were milled and screened to less than 0.5 mm using a Cutting Mill pulverisette 15 (FRITSCH GmbH, Germany) and stored in plastic bags at room temperature after air drying (less than 5% of initial moisture content).

2.2. Organosolv pretreatment process

Organosolv pretreatment was conducted in a same reaction vessel mentioned in section 2.2. of chapter 2. consisting of a heating mantle and temperature controller (Figure 2-1). The reactor was loaded with 20 g of yellow poplar and 200 ml of a 50:50% ethanol:water solution (v/v). To investigate the effect of acid concentration, sulfuric acid (0.5, 1, and 2% (w/w)) was added to the solution as a catalyst. Pretreatment time was recorded after the internal temperature reached a defined target temperature (ranging from 120°C to 200°C at 10°C intervals), after which the temperature was maintained within $\pm 2^\circ\text{C}$. All reactions were carried out with the same solid to liquid ratio (1:10), preheating time (40 min), and duration (10 min). After pretreatment, the vessel was quenched in an ice chamber and cooled to room temperature within 10 min.

After cooling to room temperature, the pretreated materials were filtered through a previously weighed filter paper (Advantec No. 2, Advantec Co.,

Tokyo, Japan), and divided into pretreated solid residue and liquid hydrolysate fractions. The pretreated solid residue was washed using two volumes of distilled water to remove degraded products and solvents present on the surface of pretreated solid residue. The washed pretreated solid fraction was then weighed after oven drying and stored until further physicochemical analysis. Finally, the pretreated materials were divided into three fractions: solid fraction, liquid hydrolysate, and wash water.

2.3. Analysis of pretreated solid fractions

The water insoluble solid (WIS) recovery rate (Eq. 1) was measured according to the method previously described in section 2.3. of Chapter 2.

2.3.1. Chemical composition analysis

Structural carbohydrates and lignin were evaluated according to the NREL Laboratory Analytical Procedures (Sluiter et al., 2008). Briefly, pretreated solid (0.3 g) fractions and 3 ml of 72% (w/w) sulfuric acid were combined in an Erlenmeyer flask and hydrolyzed at 30°C for 60 min. The hydrolysate was then diluted to an acid concentration of 4% by adding 84.0 ml of distilled water and autoclaved for 1 h at 121°C. The hydrolysate was then cooled to room temperature and filtered through a previously weighed filtering crucible (1G4, Iwaki, Tokyo, Japan). The resulting residue was used for Klason lignin analysis, while the filtrate was used for acid-soluble lignin and structural carbohydrate analysis. For Klason lignin analysis, the residue in the filtering crucible was weighed after oven drying at 105°C. For acid-soluble lignin analysis, the absorbance of the filtrate was measured at 205 nm using a UV-visible spectrophotometer (UV-1601PC, Shimadzu, Kyoto, Japan). Acid-soluble lignin content was calculated according to the following equation:

$$\text{Acid – soluble lignin (\%)} = \frac{UV_{\text{absorbance}} \times \text{Volume}_{\text{filtrate}} \times \text{Dilution}}{\epsilon \times W_{\text{initial dry biomass}}} \times 100 \quad (\text{Eq. 3})$$

(ϵ = absorptivity, equal to 110 l/g · cm)

For structural carbohydrate analysis, the filtrate was passed once more through a 0.45 μm hydrophilic PTFE syringe filter and analyzed by HPLC

(Bio-Rad Laboratories, Richmond, CA, USA) with an Aminex HPX-87H column (300 mm × 7.8 mm ID × 9 μm). The HPLC was operated at a column temperature of 40°C with a mobile phase consisting of 0.01 N sulfuric acid at a flow rate of 0.5 ml/min. A UV detector at 210 nm and refractive index detector (RID) were used to quantify products.

2.4. Analysis of liquid hydrolysate

2.4.1. Chemical compositions

Determining sugar (glucose, xylose, and arabinose), sugar derived products (5-HMF and furfural), and organic acids (levulinic acid, formic acid, and acetic acid) of the liquid fraction were described in section 2.4.1. of Chapter 2.

2.4.2. Ethanol organosolv lignin recovery process

Ethanol organosolv lignin (EOL) recovery process was described in section 2.4.2. of Chapter 2. The EOL yield (Eq. 2) was calculated based on the initial input of wood meal and weight of EOL collected after precipitation.

2.4.3. Chemical compositions of ethanol organosolv lignin

The chemical composition of EOL was determined as described in section 2.3.1.

2.5. Confirmation of unidentified lignin

To identify and confirm lignin fragments obtained during the EOL recovery process, the supernatant was extracted by dichloromethane (Figure 3-1). The extraction process was performed in a 250 ml separatory funnel with 30 ml of supernatant and 50 ml of dichloromethane at 250 rpm for 15 min. After extraction, the dichloromethane phase was collected and the above-described process was repeated three times. The resulting collected solvent was evaporated, and the extracts were weighed and subjected to further physicochemical analysis. Finally, the supernatants were divided into two fractions: extracts and extracted supernatant.

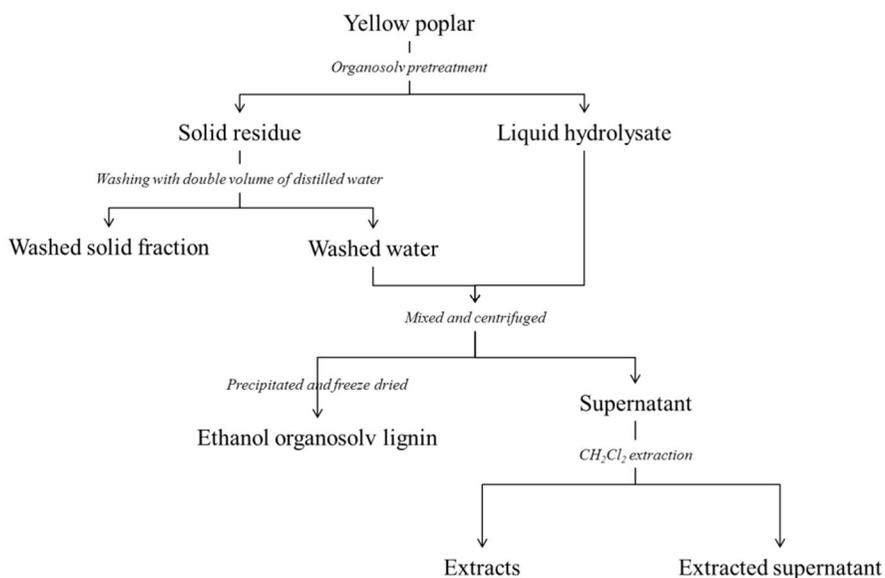


Figure 3-1. The procedure for obtaining the extracts of supernatant during ethanol organosolv lignin recovery process.

2.5.1. Analysis of supernatant and extracted supernatant fractions

Chemical compositions

Determination of sugars (glucose, xylose, and arabinose), sugar derived products (5-HMF and furfural), and organic acids (levulinic acid, formic acid, and acetic acid) present in the supernatant and extracted supernatant was performed as described in section 2.4.1.

Total phenol

The amounts of total phenols in supernatant and extracted supernatant fractions were measured colorimetrically by the Folin-Ciocalteu method with little modification (Singleton & Rossi, 1965). Briefly, the supernatant and extracted supernatant fractions were diluted 100 fold with water to obtain a final absorbance between 0.2 and 0.7. Next, 1 ml of Folin-Ciocalteu reagent (Aldrich F9252, Sigma-Aldrich Co., Yongin, Gyeonggi-do, Republic of Korea, diluted 10 fold with water) and 0.8 ml of a sodium carbonate solution (17.5% (w/w)) were added to 0.2 ml of diluted supernatant along with extracted supernatant fractions. Sodium carbonate was added 1 min after addition of the Folin-Ciocalteu reagent, and the assay tubes were kept in the dark for 2 h. Results are expressed as vanillin equivalents per amount of supernatant or extracted supernatant; a calibration curve was generated with aqueous solutions of vanillin (2-200 mg/l).

2.5.2. Analysis of extracts from supernatant

The yields of supernatant extracts (Eq. 4) were calculated based on the initial input amount of wood meal (yellow poplar) before pretreatment and weights of final extracts.

Extracts yield (%) =

$$\frac{\text{extracts from supernatant of ethanol organosolv lignin recovery process (g)}}{\text{input amount of wood meal (g)}} \times 100 \quad (\text{Eq. 4})$$

Molecular weight distribution

Molecular weight of extract was determined according to the methods described in section 2.3.1. of Chapter 2.

¹³C-NMR

To identify the types of carbon present in extracts, a ¹³C-NMR spectrometer (Jeol NM-LA400 with LFG, JEOL, Japan) was used. Extracts (50 mg) were dissolved in 0.5 ml deuterated dimethyl sulfoxide-d₆ (DMSO, Aldrich 156914, Sigma-Aldrich Co., Yongin, Gyeonggi-do, Republic of Korea) at room temperature. Scans were performed for at least 6 h.

3. Results and discussion

3.1. Conversion characteristics of lignocellulosic biomass

3.1.1. Water insoluble solid recovery rate

After organosolv pretreatment of yellow poplar, pretreated materials were divided into a water insoluble solid (WIS) fraction and liquid fraction. The WIS recovery rates, which are shown in Figure 3-2, ranged from 13.77% to 90.97%. In general, WIS recovery decreased with increasing reaction temperature and acid concentration. Specifically, a higher WIS recovery rate was obtained at lower concentrations of acid vs higher concentrations of acid compared across different reaction temperatures. Likewise, WIS recovery rates continuously decreased with increasing reaction temperature, however, prior to reaching 150°C, approximately half of the components of yellow poplar had degraded from the solid fraction, indicating that the major components were hydrolyzed before reaching 150°C and were in the process of undergoing further conversion to smaller fragments. Previous studies using yellow poplar reported WIS recovery rates of 60.7% and 47.3% following organosolv pretreatment with 1% sulfuric acid at 140°C (Koo et al., 2011b) and 1% sulfuric acid at 150°C (Kim et al., 2012a), respectively, which are similar to the results of the present study. Specifically, the WIS recovery rate continued to decrease with 0.5% acid even after the reaction temperature reached 200°C. Conversely, higher acid concentrations led to an increased WIS recovery rate, which was probably due to predominant condensation activity with higher temperature conditions. At temperatures under 120°C with 2% acid, the WIS recovery rate declined rapidly compared to the MWL

WIS recovery results, which may have been due to degradation of hemicellulose and hemicellulose related structures.

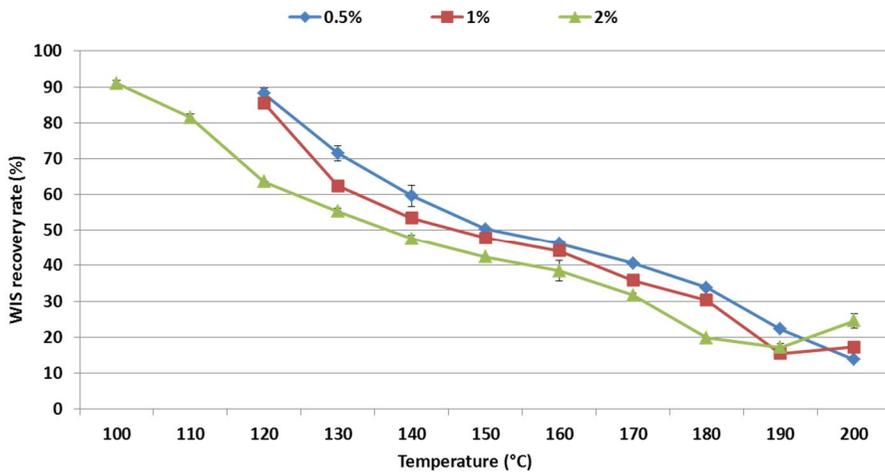


Figure 3-2. Water insoluble solid (WIS) recovery rate (%) of the solid fraction obtained by organosolv pretreatment of yellow poplar depending on the temperature and acid concentration.

3.1.2. Chemical composition of solid fraction

During pretreatment, the major components of yellow poplar are converted and hydrolyzed to the solid fraction. Table 3-1 shows the composition of the yellow poplar solid fraction obtained by organosolv pretreatment with 0.5% acid as a function of temperature. As the temperature increased, the glucan composition increased to 77.29% upon reaching 170°C, but exhibited a sudden decomposition at higher temperatures. Conversely, xylan content was reduced early during pretreatment, with almost all xylan content being degraded from the solid fraction prior to the reaction temperature reaching 170°C. Total lignin content was determined according to the sum of Klason lignin and acid soluble lignin (ASL) content, and rapidly decreased at approximately 140°C. However, at temperatures above 140°C, total lignin composition in the solid fraction increased. Similar results have been reported with respect to the Klason lignin content of the solid fraction after organosolv pretreatment, with a combined severity ranging from 1.75 to 2.3 (El Hage et al., 2010a). However, under the most severe conditions, Klason lignin content exhibits further increases due to the decomposition of cellulose in the solid fraction (El Hage et al., 2010a).

Interestingly, there was only a small decrease of Klason lignin content between 120°C and 130°C, while xylan and ASL content were significantly reduced. This result may have been due to the fact that most of the lignin fragments in ASL were derived from lignin-carbohydrate complexes, which are linked via ester bonds and are thus degraded earlier than the lignin fraction of Klason lignin. While degradation of total lignin and xylan was strongly correlated, Klason lignin content exhibited temperature delayed degradation compared to xylan and ASL. A previous study reported that xylan and lignin are simultaneously hydrolyzed and degraded in the same pattern, which is due

to hemicellulose and lignin in lignocellulosic biomass forming lignin-carbohydrate complexes via ether and ester bonds (Salvi et al., 2010).

The composition of the solid fraction obtained by organosolv pretreatment with 1% acid as a function of temperature is shown in Table 3-2. Glucan composition increased to 77.08% at 160°C and decomposed at higher temperatures. A similar result was obtained with 0.5% acid, and because of the severe conditions, glucan and xylan contents were completely removed from the solid fraction, with the total lignin content reaching 100%. Further, xylan and lignin were found to degrade simultaneously under these conditions.

Under the most severe pretreatment conditions tested (2% acid), xylan and lignin degradation occurred at relatively low temperatures (Table 3-3). This observation can be explained in terms of the previous results, whereby the xylose and total phenol contents in the liquid hydrolysate after organic acid pretreatment increased as the acid concentration increased, despite relatively low pretreatment temperatures (Kim et al., 2011a). The highest glucan composition (75.56%) was obtained at 150°C, and decreased in the same manner as observed with lower concentrations of acid. However, with higher concentrations of acid, a significant portion of the degradation of structural sugars occurred prior to the temperature reaching 190°C.

Total lignin content was reduced due to degradation of both xylan and lignin up to 150°C; however, the total amount did not decrease past 12% (Figure 3-3). According to the degradation of cellulose and condensation of lignin after 180°C, lignin contents were significantly increased. Based on these results, I attributed the characteristic changes of lignin in yellow poplar to three steps, namely, degradation (under 150°C), re-distribution (degradation and condensation, between 150°C and 180°C), and condensation (over 180°C).

Table 3-1. Composition of solid fraction obtained by organosolv pretreatment with 0.5% sulfuric acid of yellow poplar depending on the temperature

Temp. (°C)	Structural sugars (%)			Lignins (%)			Total (%)
	Glucan	Xylan	Total	Klason lignin	Acid soluble lignin	Total	
Unpret.	37.13±0.07	16.94±0.03	54.08±0.10	22.86±1.40	3.71±0.12	26.57±1.52	80.64±1.42
120	40.30±0.14 (95.67)	17.21±0.46 (89.57)	57.51±0.32 (93.58)	21.21±0.14 (81.79)	3.02±0.02 (71.83)	24.23±0.13 (80.40)	81.74±0.90
130	48.83±0.68 (94.04)	13.58±0.16 (57.33)	62.42±0.52 (82.39)	20.65±0.36 (64.60)	2.28±0.01 (43.94)	22.93±0.38 (61.71)	85.35±0.60
140	59.07±1.65 (94.89)	9.79±0.09 (34.49)	68.86±1.56 (75.83)	15.07±2.16 (39.33)	1.69±0.11 (27.16)	16.76±2.15 (37.63)	85.62±1.32
150	68.60±2.34 (93.04)	8.02±0.46 (23.84)	76.62±1.88 (71.23)	10.89±0.45 (23.98)	1.22±0.11 (16.51)	12.10±0.56 (22.94)	88.72±0.68
160	74.17±2.01 (92.29)	5.00±0.10 (13.64)	79.17±2.11 (67.52)	11.55±1.41 (23.34)	1.01±0.01 (12.58)	12.56±1.43 (21.84)	91.73±0.63
170	77.29±0.84 (84.43)	2.62±0.12 (6.26)	79.91±0.72 (59.83)	13.33±1.37 (23.65)	1.03±0.01 (11.21)	14.35±1.35 (21.91)	94.26±0.63
180	74.03±0.86 (67.27)	0.00±0.00 (0.00)	74.03±0.86 (46.11)	15.84±1.49 (23.37)	1.29±0.02 (11.73)	17.12±1.51 (21.75)	91.16±0.65
190	56.98±1.89 (34.05)	0.00±0.00 (0.00)	56.98±1.89 (23.34)	34.58±2.24 (33.57)	1.26±0.21 (7.53)	35.84±2.02 (29.93)	92.82±0.13
200	30.99±0.26 (11.49)	0.00±0.00 (0.00)	30.99±0.26 (7.88)	63.78±0.57 (38.42)	0.96±0.00 (3.56)	64.74±0.57 (33.55)	95.73±0.84

Table 3-2. Composition of solid fraction obtained by organosolv pretreatment with 1% sulfuric acid of yellow poplar depending on the temperature

Temp. (°C)	Structural sugars (%)			Lignins (%)			Total (%)
	Glucan	Xylan	Total	Klason lignin	Acid soluble lignin	Total	
Unpret.	37.13±0.07	16.94±0.03	54.08±0.10	22.86±1.40	3.71±0.12	26.57±1.52	80.64±1.42
120	45.28±0.91 (104.30)	15.69±0.40 (79.23)	60.98±0.50 (96.26)	22.42±1.25 (83.88)	2.93±0.05 (67.64)	25.36±1.20 (81.61)	86.33±1.71
130	62.29±0.40 (104.68)	8.85±0.49 (32.60)	71.14±0.09 (81.95)	17.60±2.10 (48.03)	1.74±0.03 (29.33)	19.34±2.08 (45.42)	90.48±1.99
140	69.20±0.78 (99.42)	6.65±0.14 (20.92)	75.85±0.92 (74.69)	15.76±1.13 (36.77)	1.34±0.05 (19.33)	17.10±1.18 (34.33)	92.95±0.27
150	75.06±0.08 (96.64)	4.04±0.44 (11.41)	79.10±0.37 (69.81)	12.75±1.33 (26.66)	1.13±0.03 (14.51)	13.87±1.36 (24.97)	92.97±0.99
160	77.08±0.12 (91.53)	2.43±0.15 (6.32)	79.51±0.03 (64.71)	14.49±0.02 (27.95)	1.08±0.01 (12.88)	15.58±0.02 (25.85)	95.08±0.05
170	76.47±1.13 (73.76)	0.00±0.00 (0.00)	76.47±1.13 (50.55)	14.78±0.79 (23.15)	1.23±0.02 (11.90)	16.01±0.77 (21.58)	92.48±0.36
180	68.43±1.61 (55.73)	0.00±0.00 (0.00)	68.43±1.61 (38.20)	22.19±2.03 (29.35)	1.37±0.05 (11.15)	23.56±1.98 (26.81)	91.99±0.37
190	41.36±2.84 (17.26)	0.00±0.00 (0.00)	41.36±2.84 (11.83)	53.55±2.48 (36.29)	0.91±0.06 (3.81)	54.47±2.54 (31.75)	95.83±0.30
200	0.51±0.08 (0.24)	0.00±0.00 (0.00)	0.51±0.08 (0.16)	99.76±1.62 (75.32)	0.84±0.09 (3.89)	100.60±1.52 (65.35)	101.11±1.45

Table 3-3. Composition of solid fraction obtained by organosolv pretreatment with 2% sulfuric acid of yellow poplar depending on the temperature

Temp. (°C)	Structural sugars (%)			Lignins (%)			Total (%)
	Glucan	Xylan	Total	Klason lignin	Acid soluble lignin	Total	
Unpret.	37.13±0.07	16.94±0.03	54.08±0.10	22.86±1.40	3.71±0.12	26.57±1.52	80.64±1.42
100	40.71±0.40 (99.73)	18.35±0.22 (98.52)	59.05±0.62 (99.17)	23.05±0.50 (91.74)	3.45±0.15 (84.67)	26.51±0.65 (90.75)	85.56±1.27
110	44.89±1.24 (98.53)	16.16±0.19 (77.77)	61.05±1.05 (91.86)	22.89±0.53 (81.60)	3.09±0.10 (67.94)	25.98±0.63 (79.69)	87.04±0.41
120	56.08±0.11 (96.02)	11.35±0.44 (42.60)	67.43±0.56 (79.14)	18.94±1.77 (52.67)	2.04±0.01 (34.98)	20.98±1.76 (50.20)	88.40±2.32
130	65.45±0.30 (97.48)	8.20±0.15 (26.78)	73.66±0.15 (75.19)	14.68±1.34 (35.51)	1.33±0.03 (19.76)	16.01±1.37 (33.31)	89.66±1.22
140	71.00±1.34 (90.85)	5.91±0.67 (16.57)	76.91±0.67 (67.46)	12.40±0.92 (25.76)	1.01±0.08 (12.90)	13.40±0.85 (23.97)	90.32±1.52
150	75.56±1.15 (86.21)	3.31±0.15 (8.27)	78.87±0.99 (61.67)	11.75±1.84 (21.78)	0.88±0.04 (10.03)	12.63±1.88 (20.14)	91.50±0.89
160	75.52±1.65 (78.26)	1.93±0.56 (4.39)	77.45±1.09 (55.02)	14.14±0.36 (23.80)	0.99±0.06 (10.32)	15.14±0.42 (21.92)	92.59±0.67
170	72.37±1.26 (61.73)	0.74±0.07 (1.39)	73.11±1.19 (42.74)	19.90±0.70 (27.57)	1.16±0.00 (9.94)	21.07±0.71 (25.11)	94.18±1.90
180	49.86±0.53 (26.59)	0.00±0.00 (0.00)	49.86±0.53 (18.22)	44.26±1.18 (38.34)	0.82±0.01 (4.35)	45.08±1.18 (33.59)	94.94±0.65
190	4.26±0.75 (1.97)	0.00±0.00 (0.00)	4.26±0.75 (1.35)	95.78±0.18 (71.94)	0.81±0.12 (3.76)	96.59±0.30 (62.42)	100.85±1.05
200	0.00±0.00 (0.00)	0.00±0.00 (0.00)	0.00±0.00 (0.00)	98.68±1.91 (106.07)	1.03±0.16 (6.84)	99.72±1.75 (92.21)	99.72±1.75

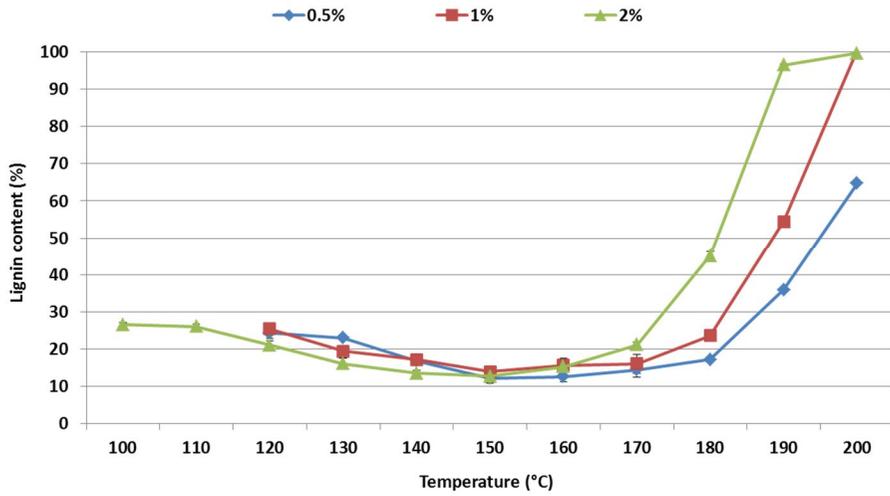


Figure 3-3. Total lignin contents (%) of the solid fraction obtained by organosolv pretreatment of yellow poplar depending on the temperature and acid concentration.

3.1.3. Actual total lignin content in solid fraction

Analysis of the composition of pretreated solid fractions is important, as it allows a better understanding of how the solid fraction may be utilized for various processes such as enzymatic hydrolysis for fermentable sugar production. However, WIS recovery rates should be considered when precisely evaluating the composition of solid fractions. Figure 3-4 shows the actual total lignin content of the solid fraction obtained by organosolv pretreatment as a function of both temperature and acid concentration. For each of the acid concentrations tested, actual total lignin content decreased as the reaction temperature increased up to a certain temperature, and was maintained for some time until it again started to increase; higher acid concentrations accelerated this tendency.

The majority of lignin was degraded from the solid fraction before reaching 150°C (from 26.57 g to 5.35 g). Actual total lignin content was sustained between 150°C and 180°C for 0.5% and 1% acid, but increased between 140°C and 170°C with 2% acid due the increased severity of conditions. This set of observations was designated as the re-distribution stage, and corresponded with previous studies suggesting that lignin is re-distributed during acidic pretreatment (Koo et al., 2012; Pingali et al., 2010). At temperatures above 180°C, the actual total lignin content increased dramatically except for the 0.5% acid condition, for which only a slight increase was noted. Conversely, 2% acid resulted in an abundant amount of degraded lignin fragments in the liquid hydrolysate re-condensing to the solid fraction during this stage, eventually reaching a lignin content equal to that of the raw material.

Based on these results and consistent with the data shown above, lignin conversion of yellow poplar by organosolv pretreatment can be clearly divided into three stages consisting of 1) degradation (until 150°C), re-

distribution (degradation and condensation, from 150°C to 180°C) and condensation (after 180°C). At temperatures above 150°C, there was no further removal of lignin from the solid fraction, but rather generation of undesirable reaction products due to condensation of lignin and excessive degradation of structural sugars. Thus, for the purpose of lignin removal from yellow poplar, organosolv pretreatment should be conducted under moderate conditions (1% acid concentration at 150°C).

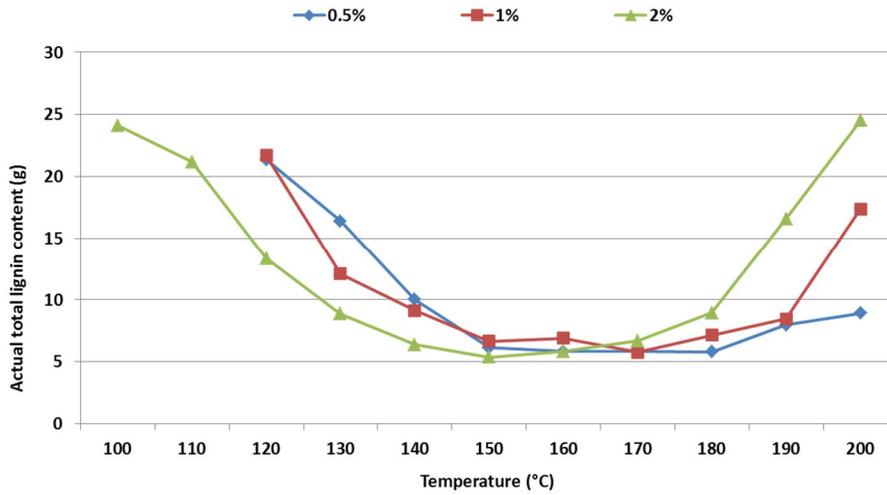


Figure 3-4. Actual total lignin content (g) of the solid fraction obtained by organosolv pretreatment of yellow poplar depending on the temperature and acid concentration. (on the basis of 100 g of initial oven-dried raw materials)

3.1.4. Chemical composition of liquid hydrolysate

The major components of yellow poplar were degraded and converted into liquid hydrolysates after organosolv pretreatment. Table 3-4 shows the composition of liquid hydrolysate obtained by organosolv pretreatment with 0.5% acid. As expected, isolation of xylose and acetic acid from hemicellulosic xylan took place earliest among the components. This tendency has also been reported even with different pretreatment methods (Liu et al., 2009; Lloyd & Wyman, 2005). The amount of xylose gradually increased up to 170°C, corresponding with the chemical composition analysis results of the solid fraction. At higher temperatures, xylose was converted to furfural by a dehydration reaction. Previous studies have also reported that furfural is the main product of acid-catalyzed dehydration of xylose and xylan (Yemis & Mazza, 2011). Glucose was hydrolyzed in earnest after 170°C and after a short while, the dehydration product of glucose, 5-HMF, as well as its rehydration products levulinic acid and formic acid, were formed.

Most of the reactions described above generally occurred with a lower reaction temperature in the presence of 1% or 2% acid (Table 3-5, 3-6). However, glucose, arabinose, and furfural contents were decreased at the end of the pretreatment condition. The decrease in glucose and arabinose content can be easily explained as further degradation to 5-HMF and furfural; however, the decreased abundance of furfural was difficult to clarify. It has suggested previously that cross-polymerization reactions between furfural and intermediates of xylose, as well as self-polymerization reactions of furfural, are likely the cause of decreased furfural content during acid-catalyzed conversion processes (Zeitsch, 2000). Likewise, several other studies have reported that acid catalyzed dehydration of carbohydrates is responsible for the formation of pseudo-lignin (Hu et al., 2012; Sannigrahi et al., 2011), and

together were likely responsible for the reduction of furfural in liquid hydrolysates.

Table 3-4. Composition of liquid hydrolysate obtained by organosolv pretreatment with 0.5% sulfuric acid of yellow poplar depending on the temperature (on the basis of 100 g of initial oven-dried raw materials)

Temp. (°C)	Sugars (g)			Sugar derived products (g)					Total (g)
	Glucose	Xylose	Arabinose	Furfural	5-HMF	Levulinic acid	Formic acid	Acetic acid	
120	0.07 ±0.01	0.34 ±0.06	0.20 ±0.01	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.31 ±0.02	0.92 ±0.05
130	0.24 ±0.08	2.30 ±0.16	0.24 ±0.06	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.85 ±0.03	3.63 ±0.33
140	0.53 ±0.02	5.22 ±0.11	0.39 ±0.01	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	1.31 ±0.06	7.45 ±0.20
150	0.83 ±0.11	7.97 ±0.84	0.59 ±0.06	0.14 ±0.07	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	1.64 ±0.22	11.18 ±1.29
160	1.34 ±0.01	9.87 ±0.12	0.92 ±0.00	0.64 ±0.01	0.07 ±0.02	0.00 ±0.00	0.00 ±0.00	2.04 ±0.10	14.87 ±0.20
170	2.23 ±0.08	9.51 ±0.09	1.40 ±0.03	2.07 ±0.04	0.19 ±0.05	0.00 ±0.00	0.00 ±0.00	2.23 ±0.02	17.63 ±0.00
180	4.04 ±0.06	7.52 ±0.12	2.12 ±0.05	4.60 ±0.31	0.39 ±0.07	0.08 ±0.00	0.00 ±0.00	2.26 ±0.08	21.01 ±0.45
190	8.08 ±0.13	4.90 ±0.33	3.56 ±0.09	7.56 ±0.02	1.11 ±0.10	0.72 ±0.12	0.67 ±0.06	2.23 ±0.02	28.84 ±0.00
200	9.50 ±0.73	3.78 ±0.34	3.63 ±0.28	8.69 ±0.06	1.94 ±0.04	2.40 ±0.30	1.71 ±0.29	2.53 ±0.23	34.17 ±0.42

Table 3-5. Composition of liquid hydrolysate obtained by organosolv pretreatment with 1% sulfuric acid of yellow poplar depending on the temperature (on the basis of 100 g of initial oven-dried raw materials)

Temp (°C)	Sugars (g)			Sugar derived products (g)					Total (g)
	Glucose	Xylose	Arabinose	Furfural	5-HMF	Levulinic acid	Formic acid	Acetic acid	
120	0.12 ±0.00	0.70 ±0.11	0.23 ±0.01	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.72 ±0.10	1.76 ±0.21
130	0.53 ±0.01	5.67 ±0.24	0.44 ±0.01	0.07 ±0.01	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	1.77 ±0.03	8.49 ±0.25
140	0.77 ±0.06	7.21 ±0.25	0.58 ±0.06	0.19 ±0.01	0.03 ±0.04	0.00 ±0.00	0.00 ±0.00	1.73 ±0.02	10.53 ±0.43
150	1.26 ±0.09	8.74 ±0.33	0.85 ±0.07	0.57 ±0.09	0.08 ±0.00	0.00 ±0.00	0.00 ±0.00	2.04 ±0.12	13.53 ±0.70
160	1.96 ±0.05	8.73 ±0.02	1.27 ±0.02	1.56 ±0.05	0.13 ±0.00	0.00 ±0.00	0.00 ±0.00	2.06 ±0.09	15.72 ±0.70
170	3.47 ±0.04	7.24 ±0.03	1.97 ±0.00	3.68 ±0.05	0.30 ±0.00	0.04 ±0.00	0.00 ±0.00	2.10 ±0.05	18.81 ±0.02
180	6.54 ±0.04	5.03 ±0.28	3.17 ±0.04	6.13 ±0.13	0.68 ±0.01	0.47 ±0.04	0.64 ±0.03	2.05 ±0.01	24.71 ±0.42
190	9.30 ±0.15	3.99 ±0.01	4.04 ±0.07	7.69 ±0.28	1.47 ±0.04	2.14 ±0.13	1.81 ±0.16	2.46 ±0.07	32.90 ±0.19
200	3.13 ±0.07	1.76 ±0.01	1.33 ±0.01	6.77 ±0.17	1.06 ±0.00	6.02 ±0.29	3.68 ±0.02	2.95 ±0.23	26.69 ±0.14

Table 3-6. Composition of liquid hydrolysate obtained by organosolv pretreatment with 2% sulfuric acid of yellow poplar depending on the temperature (on the basis of 100 g of initial oven-dried raw materials)

Temp (°C)	Sugars (g)			Sugar derived products (g)					Total (g)
	Glucose	Xylose	Arabinose	Furfural	5-HMF	Levulinic acid	Formic acid	Acetic acid	
100	0.07 ±0.00	0.32 ±0.04	0.20 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.50 ±0.04	1.09 ±0.08
110	0.19 ±0.02	1.62 ±0.32	0.25 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	1.07 ±0.11	3.14 ±0.45
120	0.42 ±0.01	5.20 ±0.15	0.36 ±0.02	0.05 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	1.71 ±0.01	7.75 ±0.17
130	0.70 ±0.01	7.30 ±0.07	0.55 ±0.02	0.19 ±0.01	0.05 ±0.00	0.00 ±0.00	0.00 ±0.00	1.86 ±0.08	10.65 ±0.14
140	1.05 ±0.17	8.42 ±0.76	0.75 ±0.08	0.47 ±0.18	0.09 ±0.02	0.00 ±0.00	0.00 ±0.00	1.88 ±0.08	12.65 ±1.28
150	1.68 ±0.11	9.40 ±0.33	1.10 ±0.04	1.45 ±0.17	0.13 ±0.02	0.00 ±0.00	0.00 ±0.00	2.14 ±0.02	15.90 ±0.05
160	2.92 ±0.27	7.81 ±0.25	1.64 ±0.12	3.35 ±0.57	0.22 ±0.02	0.05 ±0.01	0.00 ±0.00	2.12 ±0.31	18.12 ±1.06
170	5.21 ±0.27	5.37 ±0.43	2.59 ±0.14	5.77 ±0.23	0.44 ±0.02	0.44 ±0.07	0.56 ±0.03	2.24 ±0.02	22.62 ±0.20
180	8.44 ±0.27	4.15 ±0.60	3.81 ±0.14	7.45 ±0.38	1.02 ±0.03	1.94 ±0.70	1.60 ±0.55	2.61 ±0.02	31.01 ±0.10
190	4.54 ±0.23	2.19 ±0.08	1.99 ±0.11	6.00 ±0.18	1.00 ±0.00	5.16 ±0.20	3.87 ±0.33	2.96 ±0.01	27.72 ±0.08
200	0.11 ±0.02	0.85 ±0.01	0.19 ±0.01	4.07 ±0.14	0.00 ±0.00	8.16 ±0.09	5.16 ±0.11	3.17 ±0.04	21.70 ±0.21

3.2. Characteristics of ethanol organosolv lignin

3.2.1. Ethanol organosolv lignin yield

Ethanol organosolv lignin (EOL) is produced by precipitation of liquid hydrolysates following the addition of two volumes of washing liquid to solid residue. Contrary to dilute acid pretreatment, Evtuguin et al. insisted that addition of organosolv during pretreatment decreases both acid catalyzed and radical side reactions, which causes lignin condensation, and together these reactions guide the formation of EOL (Evtuguin et al., 1997). Figure 3-5 shows the EOL yields based on the initial input amount, which varied from 1.50% to 12.87%. These results were similar to those of a previous study that reported an EOL yield of 1.2% after organosolv pretreatment with 1% sulfuric acid at 120°C, which was enhanced by increasing the reaction temperature (Koo et al., 2011b). Specifically, as temperature increased, EOL yield was enhanced significantly up to 150°C, which was further increased by raising the acid concentration. Between 150°C to 190°C, EOL yield was relatively constant regardless of acid concentration. El Hage et al. also reported that the EOL yield of *Miscanthus* is significantly increased when the combined severity of organosolv pretreatment is increased from 1.7 to 2.5, while further increases (up to 2.9) do not meaningfully change EOL yield (El Hage et al., 2010a). However, at temperatures above 200°C, I observed a drastic decrease in EOL yield with an acid concentration of 2%. This result was connected with the augmentation of WIS recovery and lignin content in the solid fraction at very high reaction temperatures, and might have been caused by condensation of lignin fragments to the solid fraction. In summary, the EOL production process can be separated into three steps, namely, rapid isolation step, constant step, and slight mitigation step, and are very closely correlated

to the lignin characteristic changes of the solid fraction. Thus, for production of EOL from yellow poplar, pretreatment during the early stage of the constant step (approximately 150°C) is both efficient and economic. According to the results of lignin characteristic changes of the solid fraction, EOL may have possibly undergone a condensation reaction at the active site of lignin structures at higher temperatures, which includes phenolic hydroxyl groups and/or alkyl aryl ether linkages and which may have resulted in reduced functionality. Furthermore, negative reactions also took place, including rapid degradation of cellulosic fibers and production of inhibitory fermentation compounds (i.e. furfural, 5-HMF, and organic acids).

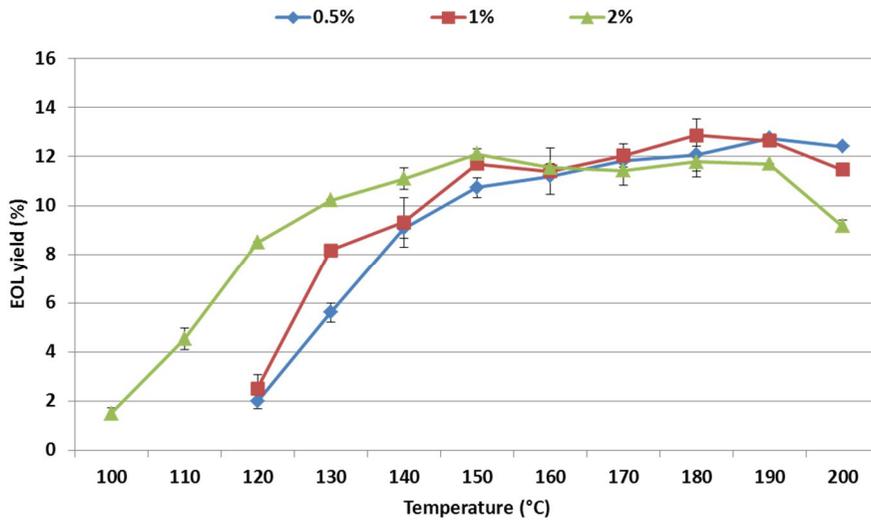


Figure 3-5. Ethanol organosolv lignin (EOL) yield (%) obtained by organosolv pretreatment of yellow poplar depending on the temperature and acid concentration.

3.2.2. Chemical composition of ethanol organosolv lignin

The composition of EOL obtained by organosolv pretreatment with 0.5% acid concentration as a function of temperature is shown in Table 3-7. EOL was composed mainly of lignin and ranged from 69.96% to 82.71%. The lignin content of EOL increased up to 150°C, but exhibited little change thereafter up to 200°C. A small amount of xylose was also present in EOL at low reaction temperatures, but was removed from EOL at temperatures above 170°C. Likewise, a small amount of glucose persisted in EOL. This result can be explained simply as the conversion characteristics of the structural sugars described above. Because the total abundance of identified products was less than 87.48%, it may also be possible that oligosaccharides were also present in EOL. Thus, it can be concluded that the isolated EOL contained only a small amount of carbohydrates, which subsequently formed lignin-carbohydrate complexes.

The lignin contents of EOL produced using 1% acid concentration ranged from 73.99% to 93.58% (Table 3-8). As the severity of pretreatment conditions increased, lignin composition increased compared to the 0.5% acid condition. However, the content of monomeric sugars did not change, suggesting decreased inclusion of other components. This result may have been due to the disruption of lignin-carbohydrate complexes at higher concentrations of acid.

In general, the composition of EOL was similar between the 1% and 2% acid conditions (Table 3-9). Interestingly however, in the case of the 2% acid condition, the lignin composition at both the lowest temperature (100°C) and highest temperature (200°C) were somewhat decreased. The former observation was probably due to the low severity of the pretreatment condition, which was approximately the same result observed between 0.5%

and 1% acid. Conversely, the latter observation was attributed to the formation of pseudo-lignin at high temperatures. (Hu et al., 2012; Sannigrahi et al., 2011); a very high ASL content ($> 8\%$) in the severe condition supported this hypothesis.

With respect to the composition of EOL, the actual lignin recovery of EOL is shown in Figure 3-6. Lignin recovery from EOL ranged from 0.94 g to 11.78 g, which varied with different pretreatment conditions. Lignin recovery of EOL was similar to the results of the EOL yield, but was slightly decreased in both low and high severity conditions. In addition, lignin recovery of EOL was the highest with 1% acid, which was a moderate condition and considered good for EOL production.

Table 3-7. Composition of ethanol organosolv lignin obtained by organosolv pretreatment with 0.5% sulfuric acid of yellow poplar depending on the temperature

Temp. (°C)	Structural sugars (%)			Lignins (%)			Total (%)
	Glucan	Xylan	Total	Klason lignin	Acid soluble lignin	Total	
120	0.98 ±0.13	2.25 ±0.08	3.23 ±0.22	67.50 ±3.68	2.46 ±0.05	69.96 ±3.62	73.19 ±3.41
130	0.35 ±0.00	4.23 ±0.04	4.58 ±0.04	70.23 ±0.14	2.44 ±0.08	72.67 ±0.06	77.25 ±0.02
140	0.36 ±0.09	4.16 ±0.08	4.52 ±0.01	74.79 ±2.66	2.16 ±0.01	76.95 ±2.66	81.46 ±2.67
150	0.53 ±0.10	4.46 ±0.14	4.98 ±0.24	78.59 ±1.52	2.24 ±0.24	80.83 ±1.76	85.81 ±1.52
160	0.62 ±0.03	4.06 ±0.37	4.67 ±0.34	80.27 ±0.29	2.12 ±0.06	82.39 ±0.34	87.06 ±0.01
170	1.36 ±0.14	3.32 ±0.24	4.68 ±0.39	76.72 ±0.91	3.08 ±0.14	79.80 ±1.05	84.49 ±1.43
180	2.35 ±0.64	2.09 ±0.59	4.44 ±1.24	78.26 ±0.47	3.53 ±0.10	81.79 ±0.38	86.23 ±1.61
190	4.02 ±0.21	0.75 ±0.06	4.78 ±0.15	78.80 ±0.29	3.91 ±0.05	82.71 ±0.24	87.48 ±0.09
200	3.99 ±0.69	0.27 ±0.07	4.27 ±0.76	75.17 ±0.59	4.43 ±0.22	79.59 ±0.37	83.86 ±0.39

Table 3-8. Composition of ethanol organosolv lignin obtained by organosolv pretreatment with 1% sulfuric acid of yellow poplar depending on the temperature

Temp. (°C)	Structural sugars (%)			Lignins (%)			Total (%)
	Glucan	Xylan	Total	Klason lignin	Acid soluble lignin	Total	
120	0.55 ±0.18	3.37 ±0.28	3.92 ±0.46	70.25 ±0.40	3.74 ±0.19	73.99 ±0.59	77.91 ±1.05
130	0.40 ±0.15	3.99 ±0.13	4.39 ±0.28	80.16 ±0.42	2.59 ±0.19	82.75 ±0.23	87.14 ±0.52
140	0.50 ±0.02	4.28 ±0.17	4.78 ±0.18	80.93 ±1.22	2.43 ±0.28	83.36 ±0.94	88.14 ±0.76
150	0.80 ±0.21	4.12 ±0.12	4.92 ±0.33	80.30 ±1.37	2.95 ±0.63	83.25 ±0.74	88.17 ±0.41
160	1.18 ±0.05	3.81 ±0.26	4.99 ±0.31	80.57 ±0.90	3.60 ±0.48	84.17 ±0.42	89.17 ±0.11
170	1.46 ±0.08	1.77 ±0.12	3.23 ±0.03	89.60 ±1.42	3.98 ±0.16	93.58 ±1.58	96.81 ±1.62
180	2.86 ±0.86	0.77 ±0.31	3.62 ±1.17	85.78 ±2.20	4.53 ±0.50	90.31 ±1.69	93.93 ±2.87
190	3.59 ±0.07	0.28 ±0.03	3.87 ±0.04	89.37 ±1.88	3.78 ±0.34	93.15 ±1.53	97.01 ±1.58
200	1.56 ±0.07	0.00 ±0.00	1.56 ±0.07	85.14 ±1.76	5.49 ±0.11	90.62 ±1.87	92.18 ±1.80

Table 3-9. Composition of ethanol organosolv lignin obtained by organosolv pretreatment with 2% sulfuric acid of yellow poplar depending on the temperature

Temp. (°C)	Structural sugars (%)			Lignins (%)			Total (%)
	Glucan	Xylan	Total	Klason lignin	Acid soluble lignin	Total	
100	0.48 ±0.02	1.81 ±0.13	2.30 ±0.15	60.61 ±2.67	2.24 ±2.62	62.85 ±2.62	65.14 ±2.77
110	0.34 ±0.00	3.28 ±0.15	3.62 ±0.15	69.54 ±0.65	2.31 ±0.60	71.85 ±0.60	75.47 ±0.75
120	0.28 ±0.07	3.75 ±0.50	4.02 ±0.57	70.61 ±1.23	4.14 ±1.60	74.74 ±1.60	78.77 ±2.18
130	0.37 ±0.01	3.79 ±0.16	4.15 ±0.17	73.18 ±0.59	3.97 ±0.66	77.15 ±0.66	81.30 ±0.49
140	0.53 ±0.02	3.99 ±0.19	4.52 ±0.17	73.60 ±1.40	3.67 ±1.59	77.27 ±1.59	81.79 ±1.42
150	0.95 ±0.09	3.78 ±0.02	4.74 ±0.11	73.58 ±1.42	4.64 ±0.36	78.22 ±1.06	82.96 ±1.17
160	1.66 ±0.26	2.65 ±0.04	4.31 ±0.31	76.28 ±0.04	4.01 ±0.07	80.29 ±0.02	84.60 ±0.33
170	3.07 ±0.06	1.10 ±0.11	4.18 ±0.05	81.21 ±1.81	4.52 ±0.54	85.73 ±1.27	89.91 ±1.32
180	3.52 ±0.25	0.28 ±0.02	3.80 ±0.27	81.67 ±1.89	5.56 ±0.24	87.23 ±1.64	91.02 ±1.91
190	1.92 ±0.08	0.17 ±0.03	2.08 ±0.05	82.27 ±0.95	5.39 ±0.47	87.66 ±0.48	89.74 ±0.52
200	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	66.99 ±0.94	8.16 ±0.08	75.15 ±0.86	75.15 ±0.86

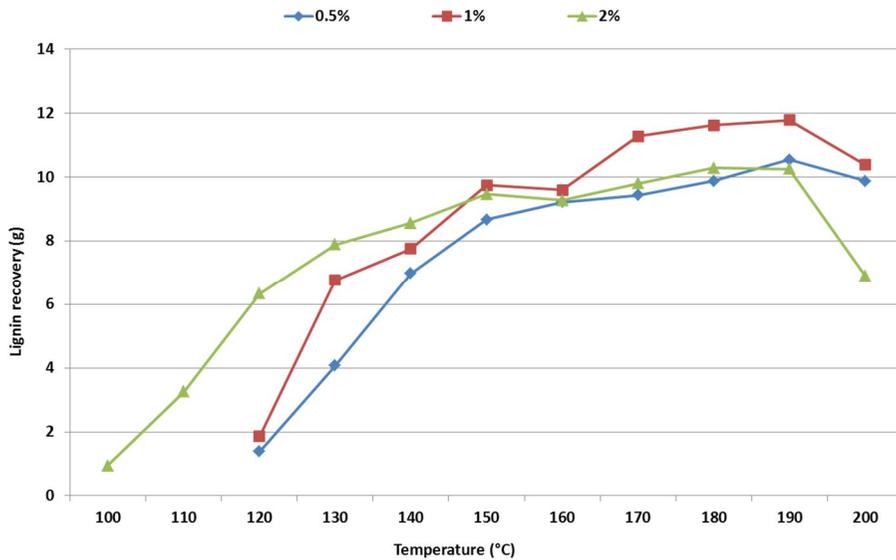


Figure 3-6. Lignin recovery (g) from ethanol organosolv lignin (EOL) obtained by organosolv pretreatment of yellow poplar depending on the temperature and acid concentration. (lignin recovery (g) = total lignin composition of EOL (%) × EOL yield (g), on the basis of 100 g of initial oven-dried raw materials)

3.2.3. Lignin recovery from solid fraction and ethanol organosolv lignin

Lignin recovery from solid fractions and EOL as a function of temperature and acid concentration is shown in Figure 3-7. Lignin recovery decreased with increasing reaction temperature, and appeared to plateau before again significantly increasing at higher temperatures. The lowest lignin recovery (14.77 g) was achieved at 140°C with 0.5% acid concentration, and represented only 55.69% of the initial lignin content. Conversely, lignin recovery with severe conditions, including 190°C with 2% acid and 200°C with 1% or 2% acid, resulted in a lignin recovery in excess of the original amount. This result can be explained by the possibility of pseudo-lignin formation (Hu et al., 2012; Sannigrahi et al., 2011), and corresponded with a previous study demonstrating the generation of pseudo-lignin from holocellulose and α -cellulose with 0.1 M sulfuric acid pretreatment at 180°C (Hu et al., 2012). Conversely, in the case of 0.5% acid, lignin recovery was very low even at high reaction temperatures, indicating that very little condensation of lignin fragments and/or production of pseudo-lignin took place. In addition, lignin recovery was significantly decreased with the moderate temperature condition, possibly because most of the small lignin fragments were still present in supernatant following EOL production. This lack of lignin recovery provided a motivation to confirm and characterize the unidentified constituents in the supernatant.

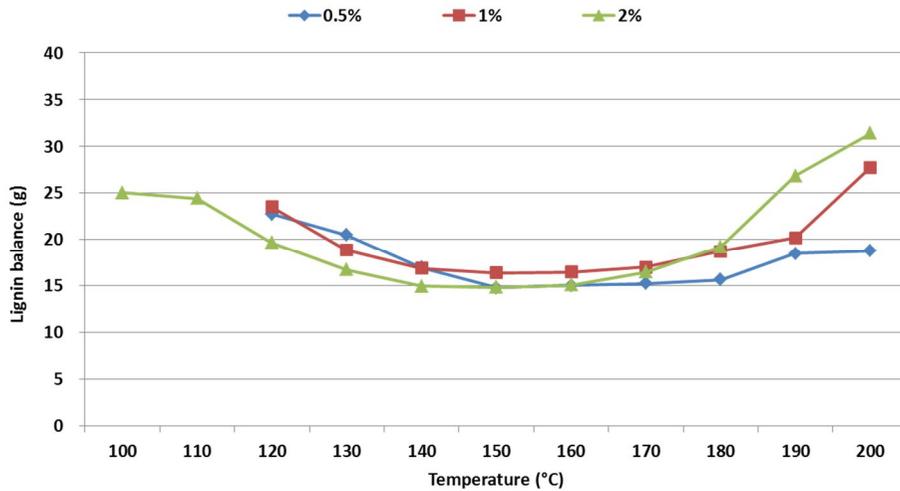


Figure 3-7. Lignin recovery (g) of the solid fraction and ethanol organosolv lignin obtained by organosolv pretreatment of yellow poplar depending on the temperature and acid concentration. (on the basis of 100 g of initial oven-dried raw materials)

3.3. Characterization of unidentified constituents in supernatant

3.3.1. Chemical composition of supernatant after dichloromethane extraction

Supernatants obtained after production of EOL (0.5% H₂SO₄ at 140°C, 1% H₂SO₄ at 140°C, 1% H₂SO₄ at 180°C) were extracted by dichloromethane to isolate lignin-derived hydrophobic compounds. The compositions of supernatants before and after extraction by dichloromethane are shown in Table 3-10. The total compositions of supernatant obtained from 0.5% and 1% acid concentration at 140°C before and after extraction were equivalent despite a small concentration of sugars, which may have been due to water solubilized in dichloromethane. However this result was less than that of the extracted supernatant obtained after pretreatment with 2% acid at 180°C due to the presence of sugar degradation products such as furfural and 5-HMF.

The weights of the supernatants extracted by dichloromethane are shown in Figure 3-8. The total extracts obtained from the supernatant of 0.5% acid at 140°C, 1% acid at 140°C, and 1% acid at 180 °C were 7.19, 8.30, and 14.07 g, respectively, compared to 100 g of initial oven-dried raw materials. As shown in Table 3-10, the amounts of sugar and sugar-derived products in the extracts were 0.11, 0.24, and 7.93 g respectively. Thus, the difference between the weights of extract and amount of sugar (7.08, 8.07, and 6.14 g, respectively) for each extract were attributed to lignin-derived compounds.

Table 3-10. Composition (g) of supernatant obtained from the production of ethanol organosolv lignin after the dichloromethane extraction (on the basis of 100 g of initial oven-dried raw materials)

		glucose	xylose	arabinose	furfural	5-HMF	levulinic acid	formic acid	acetic acid	total
Supernatant (before extraction)	0.5% H ₂ SO ₄ at 140°C	0.66±0.04	6.10±0.06	0.51±0.01	0.07±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.75±0.06	9.09±0.02
	1% H ₂ SO ₄ at 140°C	0.96±0.02	8.87±0.04	0.71±0.03	0.24±0.01	0.00±0.00	0.00±0.00	0.00±0.00	2.46±0.16	13.25±0.14
	1% H ₂ SO ₄ at 180°C	7.17±0.04	4.92±0.03	3.44±0.03	7.00±0.02	0.77±0.01	0.85±0.05	0.95±0.06	2.73±0.10	27.82±0.30
Supernatant (after extraction)	0.5% H ₂ SO ₄ at 140°C	0.73±0.01	6.48±0.09	0.52±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.25±0.02	8.99±0.13
	1% H ₂ SO ₄ at 140°C	1.03±0.01	9.38±0.13	0.75±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.85±0.03	13.01±0.18
	1% H ₂ SO ₄ at 180°C	7.80±0.11	5.30±0.08	3.70±0.05	0.00±0.00	0.09±0.00	0.34±0.00	0.67±0.01	1.99±0.03	19.89±0.28
Differences (before-after)	0.5% H ₂ SO ₄ at 140°C	0.00±0.00	0.00±0.00	0.00±0.00	0.07±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.50±0.05	0.56±0.11
	1% H ₂ SO ₄ at 140°C	0.00±0.00	0.00±0.00	0.00±0.00	0.24±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.61±0.18	0.86±0.33
	1% H ₂ SO ₄ at 180°C	0.00±0.00	0.00±0.00	0.00±0.00	7.00±0.02	0.68±0.00	0.51±0.04	0.28±0.06	0.74±0.07	9.21±0.02

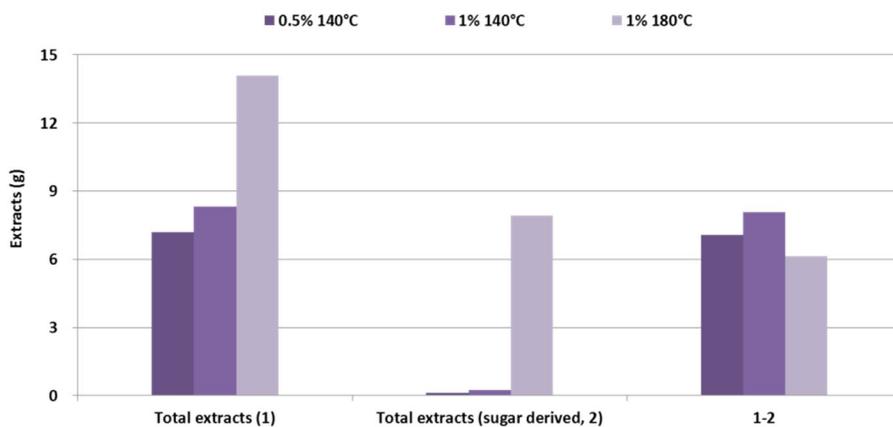


Figure 3-8. Extracts (g) of the supernatant obtained from the production of ethanol organosolv lignin by dichloromethane. (on the basis of 100 g of initial oven-dried raw materials)

3.3.2. ^{13}C -NMR analysis

^{13}C -NMR spectra of extracts from supernatants are presented in Figure 3-10. Aromatic and phenolic signals at 105-150 ppm and methoxyl signals at 55 ppm were observed in the extracts of supernatants obtained from each condition (Kim et al., 2011b). Especially, in the case of 0.5% and 1% H_2SO_4 at 140°C , clear lignin derived signals were obtained with the less condensed form. However at 180°C , lignin signals were further distinguishable due to a large amount of furfural (110 ppm, (Fawcett & Dadamba, 1982)) and 5-HMF (150 ppm, (Kimura et al., 2013), which were extracted simultaneously.

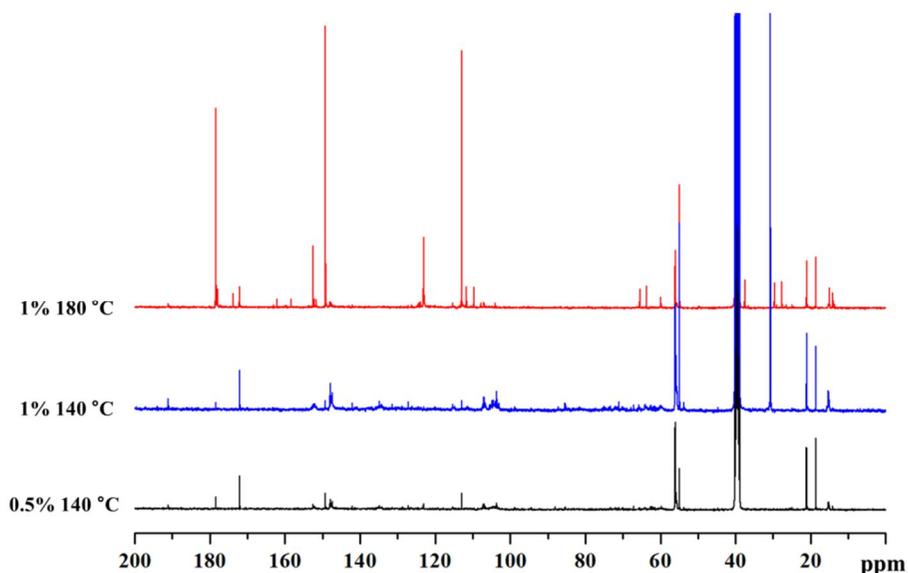


Figure 3-9. ^{13}C -NMR spectrum of extracts of the supernatant obtained from the production of ethanol organosolv lignin by dichloromethane.

3.3.3. Determination of total phenol in supernatant after dichloromethane extraction

To more precisely confirm the unidentified components of the extracts, quantitative analysis of total phenol content of the supernatants was performed (Figure 3-9). Importantly, total phenol was not detected in any of the dichloromethane-extracted supernatants (data not shown). Conversely, the total phenol content was 5.02 (0.5% H₂SO₄ at 140°C), 5.43 (1% H₂SO₄ at 140°C), and 6.73 g (1% H₂SO₄ at 180°C) before dichloromethane extraction. Compared with the results of total extracts, the amounts of unidentified compounds appeared to be similar to total phenol contents. However, in the case of supernatant obtained with 1% acid at 180°C, quantitated total phenols were significantly higher than the phenol content of the total extract.

I assumed that a large amount of extracted sugar degradation products, including furfural and 5-HMF, may have affected total phenol quantitation, as the UV absorption of furfural and 5-HMF are measured in the same manner as for total phenol quantitation. After adjusting for furfural content, the total phenol content of supernatant obtained from 1% acid at 180°C was reduced from 6.73 g to 5.67 g. The total phenol contents were next determined by radical reaction of phenolic compounds (Månsson, 1983; Muller et al., 1984). Thus furfural also might be forming radical formation in acidic circumstance. Importantly, as described previously, furfural is responsible for the formation of pseudo-lignin (Hu et al., 2012; Sannigrahi et al., 2011) through self-polymerization reactions (Zeitsch, 2000), together which provided a connection between radical-mediated formation of furfural.

Similar to furfural, the total amount of phenol was reduced from 5.67 g to 5.37 g after taking into consideration 5-HMF content. Compared to the effect of furfural, the influence of 5-HMF was significantly decreased due to its low abundance in supernatants, despite a similar activation. A final determination

of 4.68 (0.5% H₂SO₄ at 140°C), 5.05 (1% H₂SO₄ at 140°C), and 5.37 g (1% H₂SO₄ at 180°C) of phenolic compounds in the supernatant per 100 g of initial oven-dried raw materials was established. In the context of total extracts results, the total phenol contents were slightly lower than that of the total extracts. Of course, this result may have been due to the total extracts present in other components of the lignocellulosic biomass used in this study, including wood extractives. Conversely, it is also possible that the differences in phenol contents were due to the standard used for analysis. Specifically, vanillin is a C₆C₁ phenolic compound, and has a molecule weight of only 152.15 g mol⁻¹. Thus, if the unidentified lignin-derived compounds present in supernatant consisted primarily of C₆C₃ phenol structures, the total phenolic contents may have been approximately 20% higher.

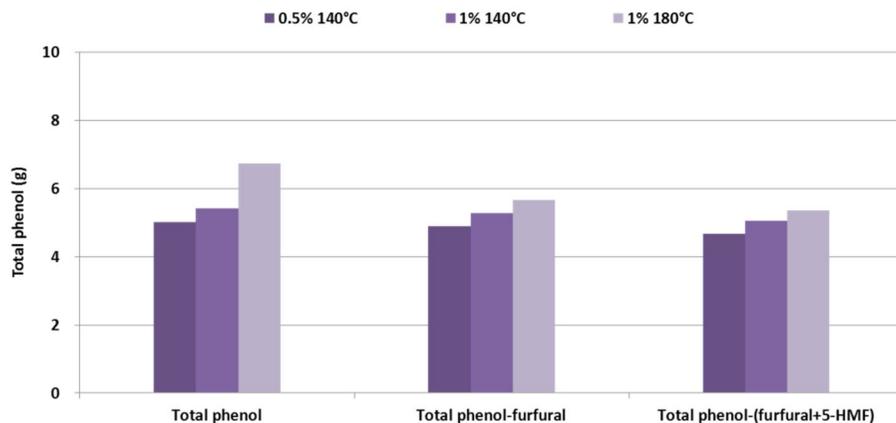


Figure 3-10. Total phenol contents (g) of the supernatant obtained from the production of ethanol organosolv lignin. (on the basis of 100 g of initial oven-dried raw materials)

3.4. Total lignin recovery

3.4.1. Lignin recovery from liquid hydrolysate

Lignin recovery from EOL and supernatant obtained after production of EOL is shown in Figure 3-11. After production of EOL, a maximum lignin content of 43.74% (1% H₂SO₄ at 180°C) based on the initial lignin content of raw material was recovered. Lignin recovery under mild pretreatment conditions was below 30%; however, with respect to the extracts from the supernatant, lignin recovery from the liquid hydrolysates was approximately doubled, with 17.77 g of lignin obtained after pretreatment with 1% acid at 180°C. Moreover, mitigation of the differences in lignin recovery of EOL between severe and mild condition was attributed primarily to re-condensation of small lignin fragments in supernatants.

Following the two-step lignin recovery from liquid hydrolysates, lignins with extremely different characteristics were obtained. Specifically, the weight-average molecular weights (M_w) of EOLs were 5490 Da (0.5% H₂SO₄ at 140°C), 5240 Da (1% H₂SO₄ at 140°C), and 2214 Da (1% H₂SO₄ at 180°C). In contrast, those of extracts were 921 Da (0.5% H₂SO₄ at 140°C), 849 Da (1% H₂SO₄ at 140°C), and 595 Da (1% H₂SO₄ at 180°C), indicating that the lignin oligomers consisted of 3 to 5 lignin monomers. The M_w of extracts were distinctly lower than that of EOL, despite the similar tendency of M_w to decrease with increasing condition severity. Previous research has shown that acid soluble lignin consists of relatively low molecular weight lignin fragments (Kaar & Brink, 1991). Similar to these findings, lignin oligomers were dissolved in the liquid hydrolysate after acid-catalyzed organosolv pretreatment. Furthermore, these results confirmed my assumption described

in section 3.2.1. of Chapter 2, in that there may be a minimum molecular weight of lignin fragments required for precipitation during EOL production.

The vastly different characteristics of lignin formed during similar conditions can be explained as followed. Lignin isolated from the solid fraction by cleavage of ester bond of lignin-hemicellulose consisted mainly of EOL lignin, which has a relatively high M_w and contained only a slight amount of hemicellulosic sugars. Conversely, lignin isolated from solid fractions was due to cleavage of ether bonds of lignin-lignin complexes or degradation of EOL, and consisted primarily of extracts from the supernatant due to a failure to re-condense to EOL or to the solid fraction. It is thus important to discern the characteristics of lignins in order to take advantage of its potential applications for further specific utilization.

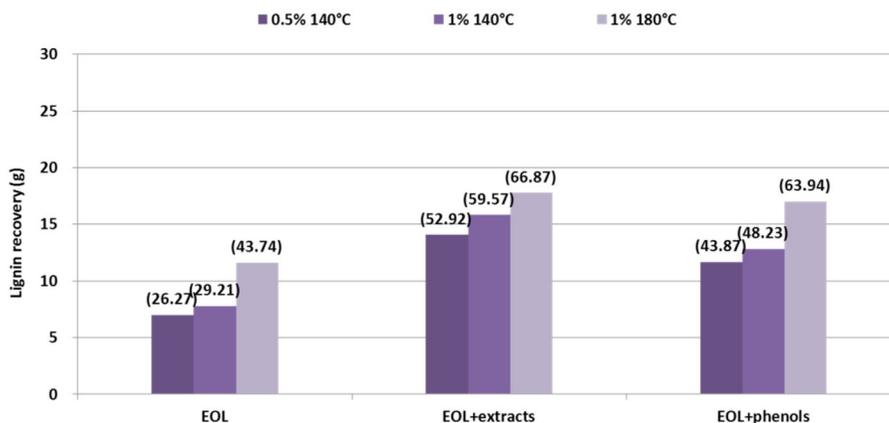


Figure 3-11. Lignin recovery (g) from the ethanol organosolv lignin and supernatant obtained after the production of ethanol organosolv lignin. (on the basis of 100 g of initial oven-dried raw materials) Numbers in parenthesis indicate the percentage of lignin based on the initial lignin content of raw materials

3.4.2. Lignin recovery from all fractions

The main purpose of this chapter was to examine the conversion characteristics of lignin in lignocellulosic biomass during organosolv pretreatment. Thus, accomplishing lignin recovery is an essential prerequisite to support the results of lignin characteristic changes. Lignin recoveries of the solid fraction and EOL barely exceeded 70% based on the initial lignin content of raw materials (Figure 3-12). Similar results have also been reported for the ammonia recycled percolation process (Wu & Lee, 1997) and organosolv pretreatment (El Hage et al., 2010a; Koo et al., 2011b). However, lignin recovery was considerably enhanced after including extracts or total phenol content from the supernatant. Specifically, lignin recoveries increased from 63.91% to 90.56% (0.5% H₂SO₄ at 140°C), from 63.53% to 93.89% (1% H₂SO₄ at 140°C) and from 70.53% to 93.66% (1% H₂SO₄ at 180°C) when extracts were included. With respect to the total phenol content, between 81.51% and 90.73% of lignin were identified depending on the pretreatment condition.

Finally, a lignin recovery of over 90% was achieved with selected pretreatment conditions, and could be clearly explained by a three-step conversion process (degradation, re-distribution, and condensation), the results of which varied depending on pretreatment conditions. Therefore, these results strongly support a characteristic changes three-step conversion of lignin in yellow poplar by organosolv pretreatment.

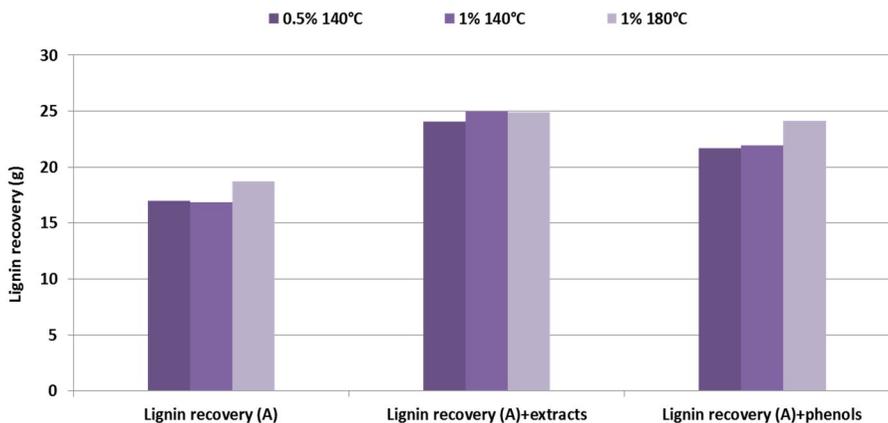


Figure 3-12. Lignin recovery (g) from the solid fraction and ethanol organosolv lignin (A) extracts and total phenol amounts in supernatant. (on the basis of 100 g of initial oven-dried raw materials)

4. Conclusions

The characteristic changes of lignin in yellow poplar during acid-catalyzed organosolv pretreatment are highly similar to those of MWL. Specifically, lignin underwent 1) degradation (under 150°C), 2) redistribution (degradation and condensation, between 150°C and 180°C), and 3) condensation (over 180°C), with recovery varying depending on reaction temperature, while higher concentrations of acid accelerated this process.

At temperatures above 150°C, there was no further removal of lignin from solid fractions. Rather, higher temperatures resulted in undesirable reactions such as condensation of lignin and excessive degradation of structural sugars. Furthermore, EOL yield was also significantly increased up to 150°C, and was relatively constant as the temperature increased further until it drastically decreased as the conditions became more severe. Thus, for efficient removal of lignin and production of EOL, processing with organosolv pretreatment under 150°C is desirable.

The actual total lignin content of solid fraction and EOL of some of the severe conditions exceeded the initial lignin content, which provided evidence for the possible formation of pseudo-lignin. Furthermore, lignin recovery from the solid fraction and EOL was considerably enhanced (greater than 90%) after including the extracts and total phenol contents from the supernatant. Likewise, the M_w values of extracts were distinctly lower than that of EOL, despite the similar tendency of M_w to decrease with increasing condition severity.

Production of contrasting M_w results of lignins processed under the same conditions can be explained as follows. EOL was formed by the cleavage of ester bonds of lignin-hemicellulose, and thus had a relatively high M_w and contained a slight amount of hemicellulosic sugar. On the other hand, extracts

isolated by the cleavage of ether bonds in lignin-lignin structures and lignin-carbohydrate complexes present in EOL resulted in low M_w and polydispersity. Importantly, utilization of the unique characteristics of lignins may be useful for particular applications.

High lignin recovery was obtained with selected pretreatment conditions, and could be clearly explained by a three-step conversion (degradation, redistribution, and condensation) as a function of pretreatment conditions. Based on these results, the characteristic changes of lignin in yellow poplar by organosolv pretreatment can be better understood.

Chapter 4

Characterization of ethanol organosolv
lignin depending on the acid-catalyzed
organosolv pretreatment conditions

1. Introduction

Bioethanol is produced universally by fermentation of glucose from cellulose. The cellulose compositions of hardwoods, softwoods, cornstalks, and wheat straw are only about 40-50% and contain considerable amounts of lignin, and are simply combusted to produce heat or electricity (Demirbaş, 2002). Thus, lignocellulosic bioethanol is not economically feasible at this time.

In order to overcome the limitations of the bioethanol production process for lignocellulosic bioethanol, the biorefinery concept continues to gain momentum, especially with emerging research trends for production of bio-based products. A biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power, materials, and chemicals from biomass (FitzPatrick et al., 2010). The biorefinery concept is analogous to today's petroleum refinery, which produces multiple fuels and products from petroleum (Kaparaju et al., 2009). Importantly, biorefineries have the potential to maximize the economic value of biomass feedstock while simultaneously reducing waste streams (Thomsen, 2005) by converting them to useful by-product streams.

Lignin is nature's most abundant aromatic polymer, and is a major component in waste streams of lignocellulosic bioethanol and biorefineries (Lora & Glasser, 2002). Lignin is a cross-linked racemic macromolecule with molecular mass in excess of 10,000 Da. In addition, lignin contains various functional groups including methoxyl, phenolic hydroxyl, aliphatic hydroxyl, noncyclic benzyl ether, and carbonyl groups. Importantly, these functional groups afford lignin with abundant chemical reactivity (Azadi et al., 2013).

Reliable biorefinement of lignocellulosic biomass requires a feasible pretreatment process. Among the various pretreatment methods that have been

developed, organosolv pretreatment is vastly superior to other pretreatments for biorefinement of lignocellulosic biomass, as it allows for utilization of all the components of biomass, especially lignin. Lignin isolated by organosolv pretreatment is called organosolv lignin and has many unique properties including relatively high purity, zero sulfur content, low molecular weight, narrow distribution, low glass transition temperature, and an abundance of functional groups that make it useful for a diverse array of important and valuable applications (Sannigrahi et al., 2009). Ethanol organosolv lignin (EOL) refers to lignin isolated with ethanol as the organosolv. Previous studies have proposed that EOL is formed by the cleavage of α -aryl ether bonds under relatively less severe pretreatment conditions (El Hage et al., 2010a), and cleavage of β -aryl ether bonds under more severe conditions (El Hage et al., 2009). However the formation mechanisms of EOL are very complex, and vary according to both pretreatment conditions (temperature, time, catalyst and concentration) as well as specific source of biomass feedstock.

In this study, the characteristics of EOL as a function of acid-catalyzed organosolv pretreatment conditions (pretreatment time and concentration of acid catalyst) were investigated. After characterization of EOL by qualitative analysis, optimal organosolv pretreatment conditions of yellow poplar for utilization of EOL are suggested.

2. Materials and methods

2.1. Materials

Ethanol organosolv lignin (EOL) was produced from organosolv pretreatment of yellow poplar according to the methods described in section 2.2. and 2.4.2 of Chapter. EOL was categorized depending on pretreatment temperature (from 120°C to 200°C at intervals of 10°C) and sulfuric acid concentration (0.5%, 1%, and 2%).

2.2. Analysis of ethanol organosolv lignin

Physicochemical analysis was used to investigate the effect of pretreatment conditions on the mechanism of EOL formation.

2.2.1. Molecular weight

Molecular weight of EOL was determined according to the methods described in section 2.3.1. of Chapter 2.

2.2.2. Methoxyl group

The amount of methoxyl (OMe) groups in EOL was measured as described by Baker (Baker, 1996). Briefly, 40 mg of EOL was mixed with 4 ml of 57% (w/w) hydroiodic acid (Aldrich 210013, Sigma-Aldrich Co., Yongin, Gyeonggi-do, Republic of Korea) in a vial with a screw cap, and vials were reacted at 130°C for 30 min with agitation. After cooling the vials in an ice bath, 200 µl of ethyl iodide as an internal standard and 3 ml of HPLC grade pentane (Aldrich 34956, Sigma-Aldrich Co., Yongin, Gyeonggi-do, Republic of Korea) was added. The vials were then shaken for 1 min and an aliquot of the pentane phase was passed through a 0.45 µm hydrophobic PTFE filter for GC analysis.

2.2.3. Phenolic hydroxyl group

Analysis of phenolic hydroxyl (phenolic-OH) group amount of EOL was carried out according to the methods described in section 2.3.2. of Chapter 2.

2.2.4. Nitrobenzene oxidation

Nitrobenzene oxidation (NBO) products of EOL were determined according to the methods described in section 2.3.3. of Chapter 2.

2.2.5. Pyrolysis GC/MS

Extracts (1 mg) were combined with 1 μ l of internal standard (IS, 1.3 mg of fluoranthene/ml of methanol) and introduced to a quart tube with glass wool. Samples were then vacuum dried in an air-evacuated desiccator for 12 h and pyrolyzed using the Pt coil of a CDS Pyroprobe 2000 (CDS Analytical Inc., Oxford, PA, USA). The pyrolyzer interface was set to 250°C, and the set time for the temperature to increase to a final pyrolysis temperature of 600°C was set as 50-60 ms (residence time: 20 s in an inert atmosphere (>99.9% He)). The released volatile products were on-line transferred to a split injector (320°C) of a gas chromatograph (Agilent Technologies 7890A) and pyrolysis products were separated by an DB-5 capillary column (60 m \times 0.25 mm ID \times 0.25 μ m film thickness, split ratio of 1:200) using the following temperature program: initial temperature of 50°C for 1 min followed by heating at 3°C/min to 130°C, 1.5°C/m to 180°C, and 6°C/min to 280°C, where it was held for 5 min before heating to 320°C for 10 min. A mass selective detector (Agilent Technologies 5975A) was used to obtain the mass to charge (m/z) ratio for each compound at 70 eV. The mass spectra were identified using NIST MS Search 2.0 (NIST/EPA/NIH Mass Spectral Library; NIST 02) as described previously (Faix et al., 1990). For quantitative analysis, I utilized established response factors of 20 authentic compounds purchased from Sigma-Aldrich with an IS (Eom et al., 2012).

2.3. Further analysis of chosen ethanol organosolv lignin

Ethanol organosolv lignins (EOL) produced by pretreatment conditions of 0.5% H₂SO₄ at 140°C, 1% H₂SO₄ at 140°C, and 1% H₂SO₄ at 180°C were subjected to further analysis.

2.3.1. Elemental analysis

Carbon, hydrogen, and nitrogen contents were determined with an elemental analyzer (Flash EA 1112, Thermo Electron Corporation, USA). Briefly, carbon, hydrogen, and nitrogen contents were simultaneously determined as gaseous products such as carbon dioxide, water vapor, and nitrogen, while the oxygen content of EOL was obtained as the difference.

2.3.2. TGA

Thermo-gravimetric analysis (TGA) of EOL was performed using a thermo gravimetric analyzer (Q-5000 IR, TA Instruments, New Castle, USA). Analysis was carried out under nitrogen gas atmosphere, with a temperature rise of 10°C per min to 800°C (Gupta & Lee, 2010).

2.3.3. FT-IR

Chemical structures and types of bonds present in the produced EOLs were analyzed with an FT-IR spectrophotometer equipped with a universal ATR (Nicolet 6700, Thermo Scientific, Waltham, USA). Samples were pressed uniformly and tightly against a diamond surface using a spring-loaded anvil. Mid-IR spectra were obtained by averaging 16 scans from 4,000 cm⁻¹ to

400 cm⁻¹ at a resolution of 2 cm⁻¹.

2.3.4. ¹³C-NMR

To identify the types of carbon present in EOL, I used a high resolution NMR spectrometer (AVANCE 600, Bruker, Karlsruhe, Germany). Briefly, 50 mg of extracts were dissolved in 0.5 ml of dimethyl sulfoxide-d₆ (DMSO, Aldrich 156914, Sigma-Aldrich Co., Yongin, Gyeonggi-do, Republic of Korea) at room temperature. Scans were then performed for at least 12 h (Park et al., 2010).

3. Results and discussion

3.1. Characteristics of ethanol organosolv lignin

3.1.1. Molecular weight distribution

Table 4-1 shows the molecular weight distributions of EOL obtained by organosolv pretreatment of yellow poplar. Compared to the weight-average molecular weight (M_w) of MWL, the results of EOL were much lower than that of MWL. The M_w was slightly increased between 130°C and 140°C and reached peak values with 0.5% H_2SO_4 at 140°C (5490 Da), 1% H_2SO_4 at 140°C (5240 Da), and 2% H_2SO_4 at 130°C (4504 Da). It is likely that the EOL produced during the very low acid pretreatment condition consisted primarily of low molecular lignin fragments, which can be explained as active isolation of both high and low molecular weight lignin up to 140°C, after which M_w significantly declined and was maintained at less than 2000 Da at temperatures above 180°C. A similar result was reported previously, whereby the M_w of EOL at 130°C (9818 Da) was increased compared with that of EOL at 120°C (7448 Da), but declined significantly to 6395 Da at 140°C (Koo et al., 2011b). In general, the molecular weight distribution of EOL as a function of increasing acid concentration exhibited similar tendencies, although the changes in M_w were observed at temperatures approximately 10°C higher. Consistent with these observations, a previous study reported that the molecular weight of organosolv lignin from *Miscanthus* varies from 6500 Da to 3200 Da depending on the severity of pretreatment conditions (El Hage et al., 2010a).

Polydispersity is a measure of the heterogeneity in sizes of molecules or particles in a mixture, and is an important factor for utilization of polymers.

The polydispersity of EOLs obtained by organosolv pretreatment of yellow poplar is shown in Figure 4-1. The initial polydispersity of EOL was as same as that of MWL, but increased dramatically with increasing M_w . Specifically, the polydispersity with 0.5% and 1% acid was highest at 140°C (3.51 and 2.94, respectively), while in case of 2% acid, the highest polydispersity point was obtained at 130°C but was not significantly higher than for the other temperatures (2.59). As described previously, under harsh pretreatment conditions, higher molecular weight lignin fragments are isolated along with hemicellulose, and relatively low molecular lignin fragments are formed simultaneously leading to high polydispersity. Koo et al. also reported that the polydispersity of EOL peaks at 130°C, and decreases with increasing reaction temperature (Koo et al., 2012). In this study, the polydispersity of EOL was found to decrease up to 180°C, where it was maintained at approximately 1.7. Compared to previous research showing that polydispersity is maintained at 1.4 following a combined pretreatment severity of 2.39 (El Hage et al., 2010a), these results indicated that the majority of the EOL specimens were ultimately degraded into small molecular weight fragments with a narrow distribution.

Table 4-1. Molecular weight distributions of ethanol organosolv lignin obtained by organosolv pretreatment of yellow poplar depending on the temperature and acid concentration

Acid conc.	Temp. (°C)	120	130	140	150	160	170	180	190	200
0.5%	M_w^a (Daltons)	4279	5399	5490	4376	2720	2046	1929	1727	1767
	M_n^b (Daltons)	1874	1830	1565	1522	1317	1151	1152	1052	1054
	M_w/M_n	2.28	2.95	3.51	2.88	2.07	1.78	1.67	1.64	1.68
1%	M_w (Daltons)	4439	4747	5240	3196	2587	1785	2214	1941	1913
	M_n (Daltons)	1926	1691	1783	1468	1406	1088	1239	1107	1090
	M_w/M_n	2.30	2.81	2.94	2.18	1.84	1.64	1.79	1.75	1.76
2%	M_w (Daltons)	3943	4504	3126	2660	2138	2057	1900	1876	1778
	M_n (Daltons)	1648	1738	1504	1452	1203	1184	1084	1098	1072
	M_w/M_n	2.39	2.59	2.08	1.83	1.78	1.74	1.75	1.71	1.66

milled wood lignin: M_w : 9919, M_n : 4240, Polydispersity: 2.34

^a weight-average molecular weight

^b number-average molecular weight

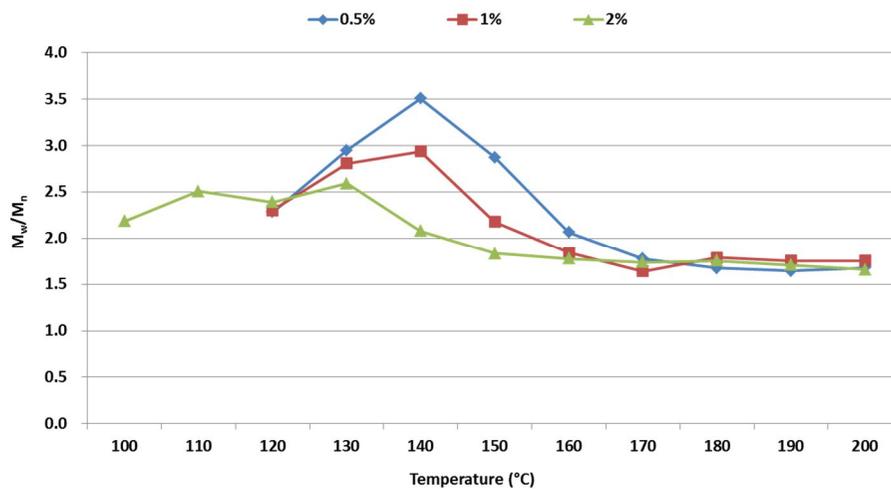


Figure 4-1. Polydispersity indexes of ethanol organosolv lignin obtained by organosolv pretreatment of yellow poplar depending on the temperature and acid concentration.

3.1.2. Functional group determination

Methoxyl group

Determination of the amount of methoxyl groups (OMe) can provide evidence of the formation mechanism of EOL, because it is related to the ratio of lignin type (S/G ratio). Methoxyl group content of EOL ranged from 9.88% to 13.71% with 0.5% acid, 8.54% to 13.34% with 1 % acid, and from 6.91% to 12.41% with 2% acid (Figure 4-2). Methoxyl group content increased slightly with increasing pretreatment temperature, and was not altered significantly thereafter. Increasing methoxyl group content was related to 1) high lignin content in EOL and 2) greater isolation of S unit lignin rather than G unit lignin, which coincided with my previous results for MWL described in Chapter 2. Previous research of EOL obtained from hybrid poplar indicated that methoxyl group content ranges from 7.82 mmol/g to 8.63 mmol/g of lignin, and similar to the results of this study, are highest at intermediate pretreatment temperatures (Pan et al., 2006b).

Methoxyl group content decreased at high temperatures (0.5% H₂SO₄ at 180°C, 1% H₂SO₄ at 170°C, and 2% H₂SO₄ at 160°C), which is likely due to 1) degradation of S unit lignin from EOL, 2) degradation of G unit lignin from the rigid parts of cell walls such as the middle lamella and cell corner, and 3) condensation of degraded G unit lignin to EOL. It is also possible that demethylation or demethoxylation occurred in the lignin fraction; however, the majority of lignin was present as determined by pyrolysis (Kaal et al., 2012) and biological condition (Ander et al., 1983; Niemenmaa et al., 2008). Additionally it has been reported that the demethylation of dimethoxy groups occurs between 350°C and 450°C (Murwanashyaka et al., 2001), and that no further demethylation or demethoxylation occurs after 450°C (Ralph & Hatfield, 1991). Building on previous results, although organosolv

pretreatment was carried out in acidic condition, I felt it more logical to consider that the differences in methoxyl group content in EOL were related to the specific type of isolated lignin at low temperatures (below 200°C).

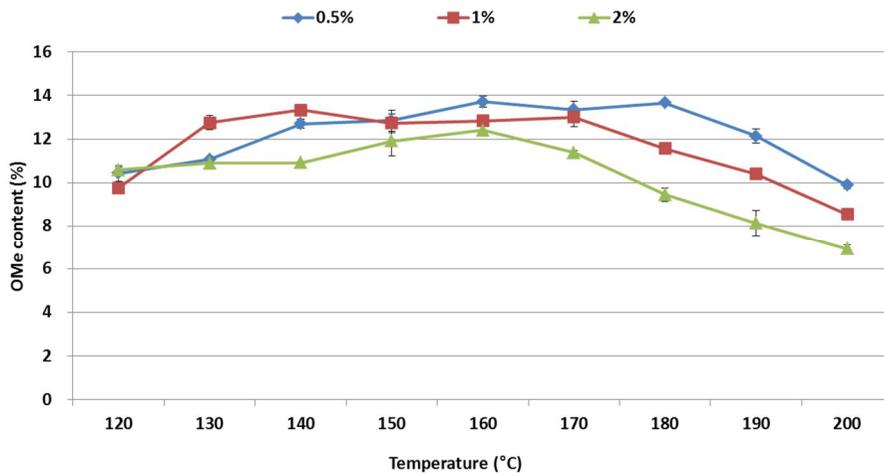


Figure 4-2. Methoxyl group (OMe) content of ethanol organosolv lignin obtained by organosolv pretreatment of yellow poplar depending on the temperature and acid concentration.

Phenolic hydroxyl group

With respect to the structure of lignin, phenolic hydroxyl groups (phenolic-OH) are the most reactive sites. (Kim et al., 2012b; Sarkanen & Ludwig, 1971). Compared to the amounts of phenolic-OH in the MWL of yellow poplar ($7.00\pm 0.45\%$), phenolic-OH content was lower in the majority of isolated EOL (Figure 4-3). Conversely, phenolic-OH was much higher than that of other practical lignins such as kraft lignin (4.50%), soda lignin (4.40%), and liginosulfonate (2.00%) (Mansouri & Salvadó, 2006a). As higher acid concentrations lead to increased reduction of phenolic-OH during the production of EOL, this result could be expected. Interestingly, phenolic-OH contents of EOL produced at approximately 140°C using 0.5% acid concentration were extremely higher than any other condition ($9.40\pm 0.55\%$), indicating that EOL produced under such conditions consisted of more free-phenolic moieties that were likely released as a result of isolation of lignin fragments from lignin-hemicellulose structures. In contrast to the results above, the steady increase in phenolic-OH content of EOL, which was measured by NMR spectroscopy, was explained according to increasing reaction temperature, time, and acid concentration (Pan et al., 2006b). Therefore, further analysis of phenolic-OH should be carried out to understand its exact behavior following organosolv pretreatment.

Phenolic-OH contents remained constant at every condition after 160°C , with the exception of 2% acid, where phenolic-OH content declined slightly at temperatures above 180°C . This result was attributed to the vigorous condensation reaction associated with the more severe condition.

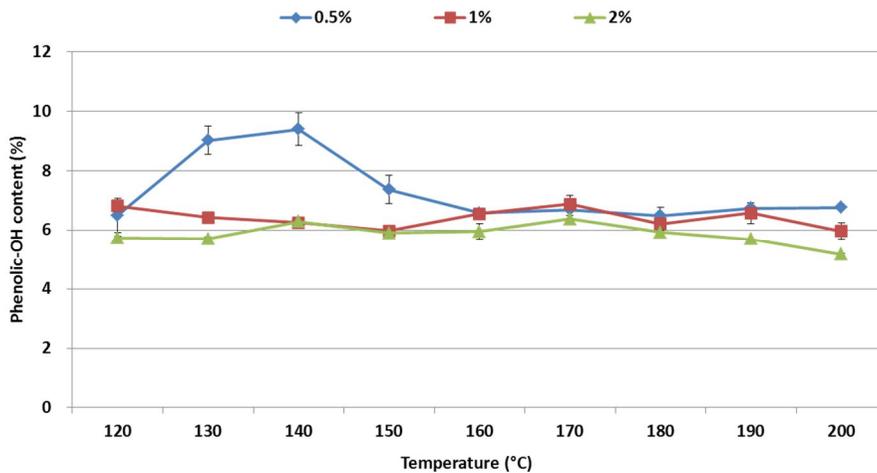


Figure 4-3. Phenolic hydroxyl (phenolic-OH) group content of ethanol organosolv lignin obtained by organosolv pretreatment of yellow poplar depending on the temperature and acid concentration.

3.1.3. Nitrobenzene oxidation product

The formation of nitrobenzene oxidation (NBO) products of EOL obtained from yellow poplar as a function of temperature and acid concentration are shown in Tables 4-2, 4-3, and 4-4. In the case of 0.5% acid, the total NBO products of syringyl (S) and vanillyl (V) type compounds consisted primarily of syringaldehyde and vanillin, and varied from 1156.7 $\mu\text{mol/g}$ and 812.2 $\mu\text{mol/g}$ to 179.6 $\mu\text{mol/g}$ and 71.6 $\mu\text{mol/g}$, respectively (Table 4-2). Compared with the NBO products of MWL (S type: 2283.9 $\mu\text{mol/g}$, V type: 904.1 $\mu\text{mol/g}$), those of EOL were generally lower, and varied according to organosolv pretreatment condition. Cybulska et al. previously reported that the vanillin yields of organosolv lignin produced from the prairie cord grass, corn stover, and switchgrass are 4.8%, 3.9%, and 6.0%, respectively (Cybulska et al., 2012). In comparison with the results of yellow poplar processes under similar pretreatment conditions, EOL produced from woody biomass may have a more condensed structure than that from herbaceous plants.

Noticeably, the sum of S type and V type of NBO products were highly consistent between 120°C and 140°C, suggesting that the condensation reaction was less prominent during organosolv pretreatment between these two temperatures (Chen, 1992; Leopold & Malmström, 1951). However, as the temperature increased, NBO products steadily decreased as pretreatment temperatures increased. Indeed, the S/V (syringyl to vanillyl) ratio at 120°C was considerably lower than other temperature, including for MWL. This observation accounted for a small amount of the low molecular lignin fragments released under less severe conditions, which consisted primarily of V type lignin. The S/V ratio rose from 2.43 at 140°C and remained steady thereafter. In support of these findings, a previous study reported that the S/G ratio of formosolv and ethanosolv lignin produced by reflux treatment at 90°C

is barely 1.8 (Wang et al., 2011). A similar pattern was observed with 1% acid (Table 4-3). Specifically, the total NBO product amount of S and V type compounds ranged from 1174.8 $\mu\text{mol/g}$ and 656.9 $\mu\text{mol/g}$ to 200.0 $\mu\text{mol/g}$ and 87.8 $\mu\text{mol/g}$, respectively, and the sums of S type and V type NBO products at 140°C were not significantly decreased compared to 120°C, although they exhibited a drastic decrease after 140°C. The S/V ratio at 120°C was significantly lower than any of the other temperatures, and dramatically increased to 3.03 at 140°C, similar to the 0.5% acid condition. Previously, it was shown that the vanillin yield of kraft lignin (*Pinus spp*) changes with increasing reaction time, indicating that the condensation reaction may occur at severe conditions (Silva et al., 2009).

In comparison with the above results, use of 2% acid resulted in a somewhat different profile of NBO product that were released. Specifically, due to the high concentration of acid, a decrease in the amount of NBO products was observed at temperatures below 140°C. Interestingly, because the decrease in S/V ratio was initiated below 140°C, a minimum was reached at 160°C (2.37), but increased again as the temperature increased past 160°C.

The S/V ratio of NBO products and OMe content results of EOL were affected depending on the temperature. Even though NBO products cannot be derived from condensed aryl-aryl linkages, which resulted in a minor discordance of data at higher temperatures, the tendency of the S/V ratio was highly similar to the methoxyl content results.

Table 4-2. Nitrobenzene oxidation products ($\mu\text{mol/g}$ sample) of ethanol organosolv lignin obtained by organosolv pretreatment with 0.5% sulfuric acid yellow poplar depending on the temperature

NBO products	Amount ($\mu\text{mol/g}$ sample)										S/V ratio
	H	V	S						Sum (V unit)	Sum (S unit)	
	Benzoic acid	Guaiacol	Vanillin	Vanillic acid	Aceto vanillone	Syringol	Syring aldehyde	Syringic acid			
120°C	2.5±0.3	6.2±0.3	757.2±24.3	38.8±1.6	16.2±1.6	5.6±0.7	1017.2±0.3	134.0±12.7	812.2±27.5	1156.7±13.7	1.42
140°C	0.0±0.0	4.5±0.7	462.5±0.5	38.6±1.1	10.6±0.7	6.2±0.9	1095.7±36.2	142.6±13.4	511.8±2.3	1244.6±50.5	2.43
0.5% 160°C	0.0±0.0	3.2±0.0	245.5±12.8	37.1±0.5	8.2±0.4	5.0±0.0	593.1±28.7	122.6±4.6	290.7±13.6	720.8±33.3	2.48
180°C	0.0±0.0	2.4±0.6	101.1±10.2	34.9±4.9	7.5±0.5	4.6±0.9	246.0±19.0	105.8±8.9	143.5±15.6	356.4±28.9	2.48
200°C	0.0±0.0	0.0±0.0	48.0±2.9	17.7±13.0	5.9±0.8	2.9±0.1	115.6±9.7	61.0±36.6	71.6±16.8	179.6±46.4	2.51
MWL	0.9±0.0	9.6±1.4	754.1±27.3	119.1±1.4	21.2±2.2	8.6±1.2	1997.9±59.5	277.3±1.1	904.1±32.3	2283.9±61.8	2.53

Table 4-3. Nitrobenzene oxidation products ($\mu\text{mol/g}$ sample) of ethanol organosolv lignin obtained by organosolv pretreatment with 1% sulfuric acid yellow poplar depending on the temperature

NBO products	Amount ($\mu\text{mol/g}$ sample)										S/V ratio
	H	V	S						Sum (V unit)	Sum (S unit)	
	Benzoic acid	Guaiacol	Vanillin	Vanillic acid	Aceto vanillone	Syringol	Syring aldehyde	Syringic acid			
120°C	1.2±1.6	4.1±1.1	600.1±18.4	38.0±4.0	18.8±8.3	4.7±0.5	1030.5±77.2	139.7±5.7	656.9±30.7	1174.8±83.4	1.79
140°C	0.0±0.0	3.6±0.1	308.8±3.8	33.7±9.0	9.6±0.0	3.9±0.0	907.2±13.7	156.6±28.0	352.0±12.8	1067.7±41.7	3.03
1% 160°C	0.0±0.0	4.1±0.1	158.9±5.2	28.9±24.7	15.6±2.8	4.5±0.1	413.4±24.5	118.0±88.4	203.4±32.7	535.9±113.0	2.63
180°C	1.5±0.0	3.6±0.2	84.2±4.1	39.6±0.8	14.9±2.2	4.0±0.1	201.8±7.2	118.5±2.3	138.7±7.1	324.3±9.6	2.34
200°C	1.7±0.6	2.6±1.0	45.4±11.1	31.7±8.7	10.8±0.1	2.7±1.0	101.0±25.7	96.3±44.8	87.8±20.0	200.0±71.5	2.28

Table 4-4. Nitrobenzene oxidation products ($\mu\text{mol/g}$ sample) of ethanol organosolv lignin obtained by organosolv pretreatment with 2% sulfuric acid yellow poplar depending on the temperature

NBO products	Amount ($\mu\text{mol/g}$ sample)									S/V ratio	
	H	V	S						Sum (V unit)		Sum (S unit)
	Benzoic acid	Guaiacol	Vanillin	Vanillic acid	Aceto vanillone	Syringol	Syring aldehyde	Syringic acid			
120°C	4.5±0.6	4.2±0.4	415.9±48.9	49.1±5.3	17.0±8.9	4.6±0.4	1189.0±130.4	185.7±31.7	482.1±63.1	1379.4±162.4	2.86
140°C	4.8±0.2	3.0±0.4	188.5±1.5	38.9±4.6	16.7±0.2	3.0±0.3	502.3±1.4	134.3±6.8	244.1±6.3	639.7±8.5	2.62
2% 160°C	2.1±0.7	3.3±0.8	102.5±13.2	45.3±6.8	17.3±3.5	3.8±0.6	257.1±32.5	130.7±14.8	165.1±23.5	391.6±48.0	2.37
180°C	1.9±0.1	2.7±0.3	50.6±3.9	32.7±2.3	11.9±1.2	3.3±0.1	112.9±7.9	118.7±9.1	95.2±7.4	234.9±17.1	2.47
200°C	10.1±0.5	2.6±0.1	30.0±2.0	26.1±1.3	9.5±0.8	3.1±0.3	67.5±4.9	122.1±21.4	65.6±4.1	192.8±26.6	2.94

With respect to the EOL yield and lignin composition, the NBO products from EOL based on an input of 100 g are shown in Figure 4-4. The majority of NBO products were released before 140°C, except for the 2% acid condition, where they were released at 120°C. At temperatures above 140°C, the amounts of NBO products released were drastically decreased until reaching 200°C. Consistent with my previous observations, a significant amount of lignin content of EOL was released below 140°C, and underwent re-distribution and condensation as the temperature increased.

Figure 4-5 shows the sum of syringyl type (S) and vanillyl type (V) NBO products analyzed from EOL based on a 100 g input. As described above, the S/V ratio of EOL was very small during the early stages of pretreatment, where temperatures were low, while the sum of S type lignin increased significantly between 120°C and 140°C. On the other hand, the sum of V type lignin was relatively even compared to S type lignin. Indeed, the sum of V type lignin was only slightly increased at 140°C, but in comparison with S type lignin, was relatively constant at every condition. Together, these results indicated that the enormous enhancement of S type lignin was the main contributor to the drastic changes in the S/V ratio of EOL. Interestingly, the possibility of increased amounts of V type lignin in EOLs with more condensed structure has been proposed (Chen, 1992), however this assumption should be considered in light of the fact that V type lignin is primarily isolated from the middle lamella or cell corners, which have highly rigid structures (Saka & Goring, 1988).

Based on the above results, the following mechanism is proposed: 1) EOL is mainly formed by cleavage of lignin-hemicellulose structures in secondary walls below 140°C, resulting in a high S/V ratio; 2) after which the total NBO products from EOL decrease due to condensation reactions, and lastly 3) V type lignin is isolated differently from S type lignin.

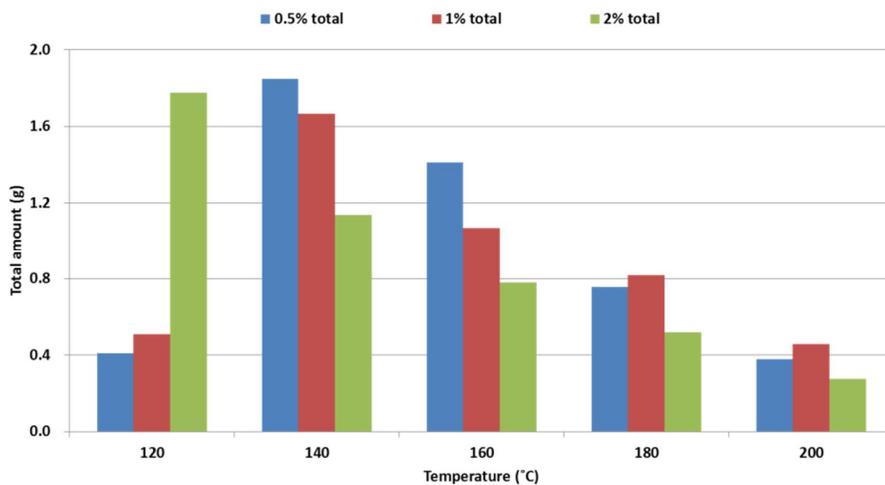


Figure 4-4. Nitrobenzene oxidation products (g) of ethanol organosolv lignin obtained by organosolv pretreatment of yellow poplar depending on the temperature. (on the basis of 100 g of initial oven-dried raw materials)

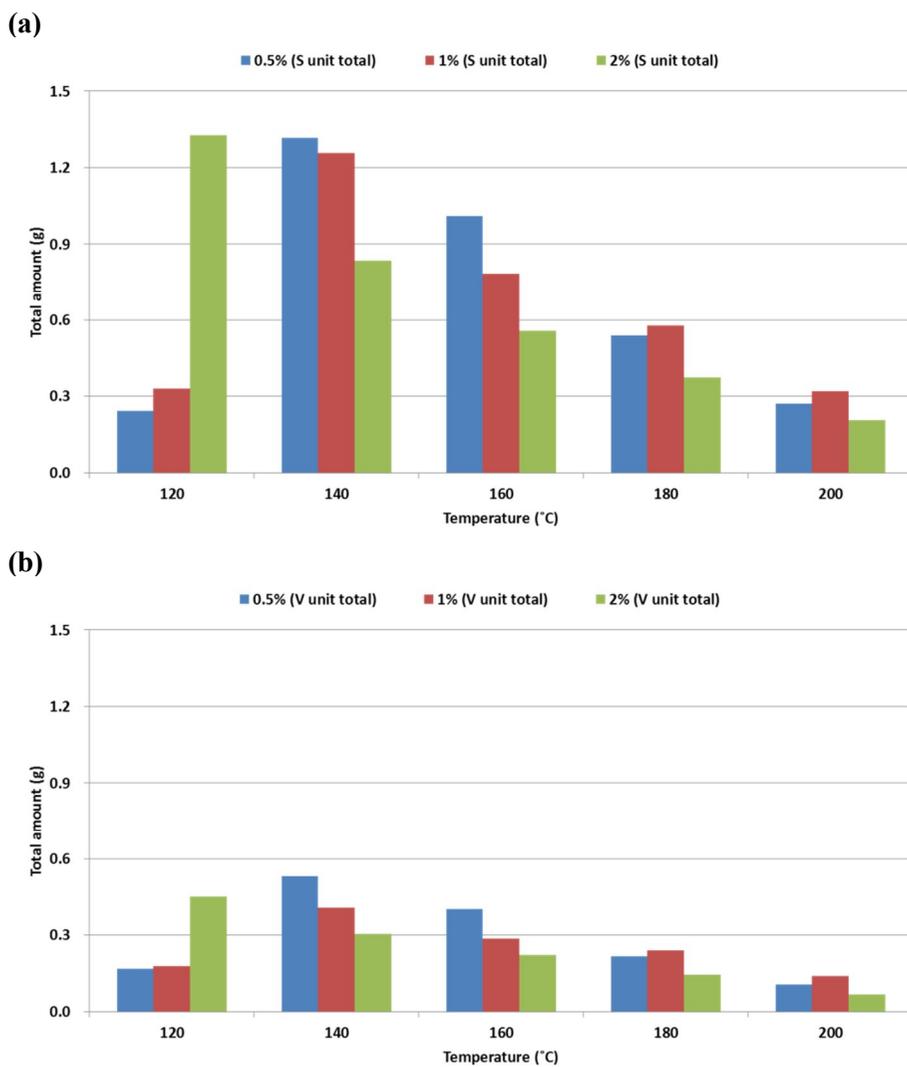


Figure 4-5. Sum of total nitrobenzene oxidized syringyl type (a) and vanillyl type (b) lignin of ethanol organosolv lignin obtained by organosolv pretreatment of yellow poplar depending on the temperature. (on the basis of 100 g of initial oven-dried raw materials)

3.1.4. Pyrolysis GC/MS analysis

Figure 4-6 shows a pyrolysis GC/MS chromatogram of yellow poplar and isolated EOL. Analysis of yellow poplar revealed the presence of both sugar-derived compounds and lignin-derived monomers. Compared to yellow poplar, sugar derived compounds are rarely present in MWL, whereas large amounts of lignin derived monomers are typically detected. Likewise, a significant amount of aliphatic phenols such as 4-methyl syringol, 4-vinyl syringol, 4-propenyl syringol, syringaldehyde, and sinapaldehyde were present along with guaiacol and syringol. These findings were similar to the results of a previous study of beech wood MWL analyzed by pyrolysis GC/MS (Scholze & Meier, 2001).

A pyrolysis GC/MS chromatogram of EOL obtained by organosolv pretreatment with 0.5% sulfuric acid of yellow poplar is shown in Figure 4-7. At retention times less than 25 min, both sugar derived compounds and unknown compounds were primarily detected. In addition, seven major compounds from lignin were detected as follows: guaiacol, 4-methoxy guaiacol, 3-methoxy catechol, syringol, 4-methyl syringol, syringyl acetone, and propiosyringone. The peak heights of the major compounds were highest at 120°C, and were maintained until 180°C. At temperatures above 180°C, the amount of major compounds decreased slightly with increasing temperature, and syringyl acetone and propiolactone were not detected at 200°C. Consistent with previous results, a large amount of phenolic compounds were identified, and were present at relatively stable levels until reaching higher temperatures, although aliphatic phenols detected at retention times of 50 min and 75 min were significantly reduced compared to MWL. Furthermore, it was previously reported that fewer aliphatic phenols are present in highly condensed kraft lignin compared with spruce milled wood lignin and degraded lignin (Sáiz-Jiménez & De Leeuw, 1984). Taken together, these

results strongly suggest an absence of EOL condensation reaction prior to 180°C with 0.5% acid pretreatment.

The amounts of the seven major compounds described above decreased with 1% acid pretreatment (Figure 4-8). Specifically, the majority of aliphatic phenol compounds were rapidly reduced compared with 0.5% acid, and the quantities of all peaks were significantly decreased at temperatures above 160°C, which was probably due to the condensed structure of the generated EOL. Specifically, due to the higher acid concentration, condensation of EOL occurred earlier than for 0.5% acid.

Finally, when the acid concentration was increased to 2%, the amounts of lignin monomer compounds were significantly lowered compared with the 0.5% acid and 1% acid results (Figure 4-9). Specifically, the quantities of all of the lignin monomer compounds, including guaiacol and syringol, were considerably reduced after 160°C, and were barely detected due to the high degree of condensation. On the other hand, significant amounts of sugar-derived compounds including furfural, levulinic acid, and ethyl levulinate were detected because of the severe conditions used during EOL production.

Comparison of the above results obtained by pyrolysis GC/MS analysis with those obtained by other methods revealed a tendency of the major compounds to decrease with increasing severity of pretreatment conditions, similar to the NBO product results. However, minor compounds derived from lignin, such as aliphatic phenols, were not meaningfully detected except with 0.5% acid. Thus, in order to produce aliphatic phenols from EOL, organosolv pretreatment should be conducted at 140°C with an acid concentration of 0.5%.

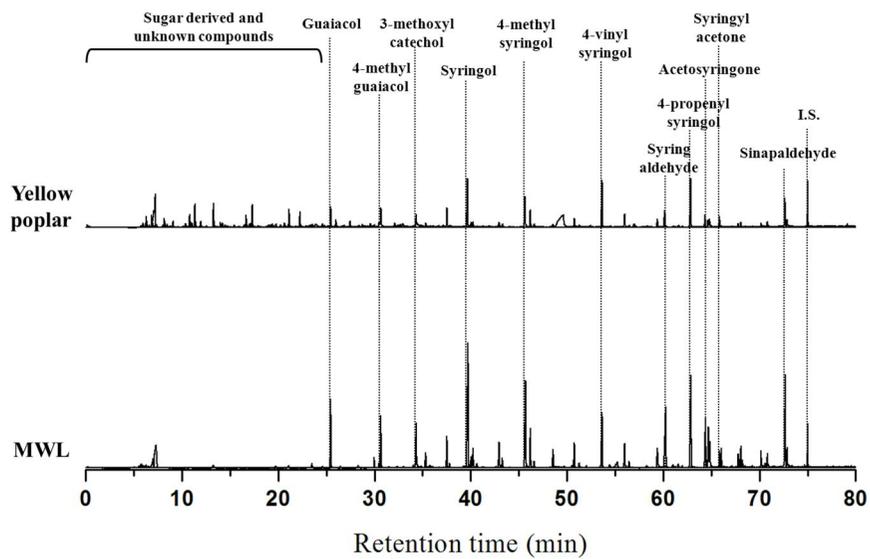


Figure 4-6. Pyrolysis GC/MS chromatogram of yellow poplar and milled wood lignin (MWL).

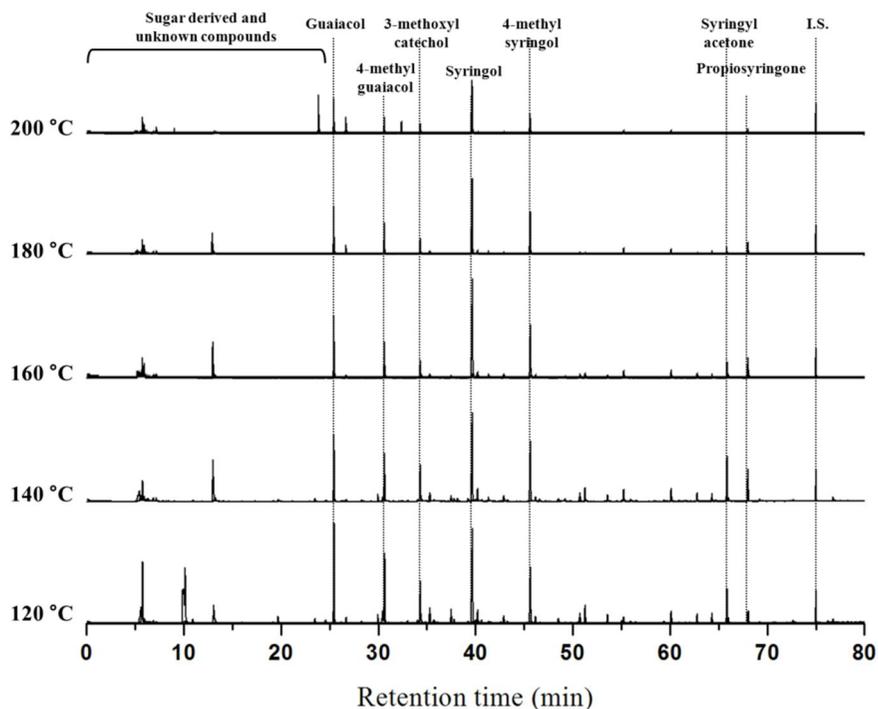


Figure 4-7. Pyrolysis GC/MS chromatogram of ethanol organosolv lignin obtained by organosolv pretreatment with 0.5% sulfuric acid of yellow poplar depending on the temperature.

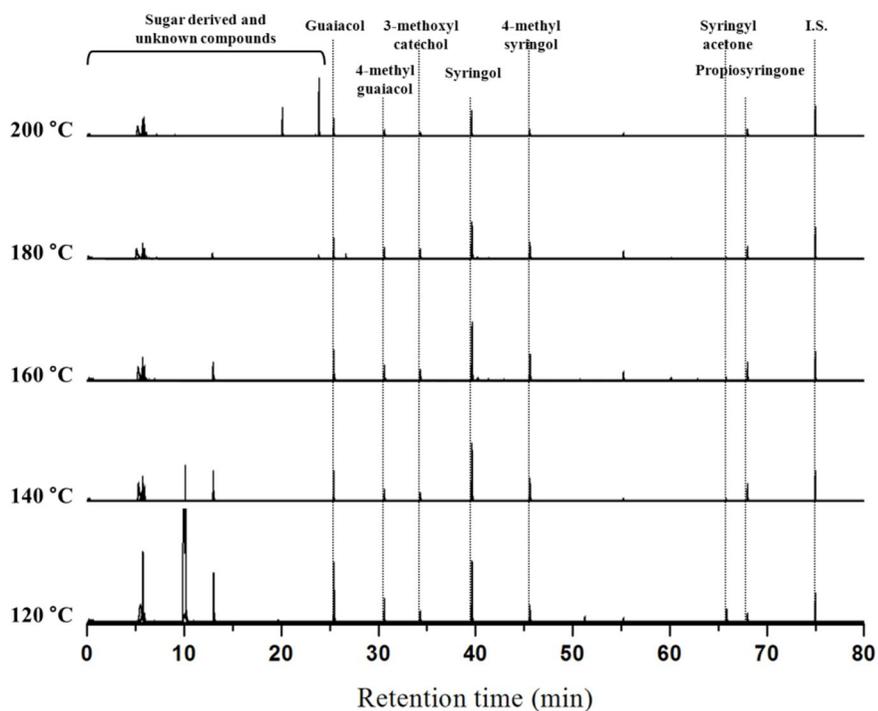


Figure 4-8. Pyrolysis GC/MS chromatogram of ethanol organosolv lignin obtained by organosolv pretreatment with 1% sulfuric acid of yellow poplar depending on the temperature.

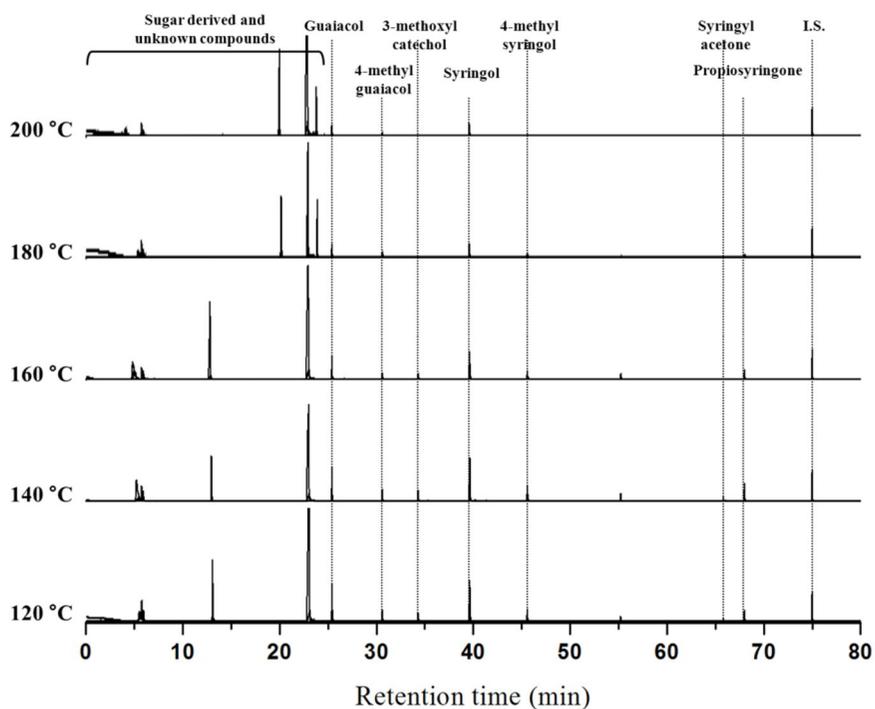


Figure 4-9. Pyrolysis GC/MS chromatogram of ethanol organosolv lignin obtained by organosolv pretreatment with 2% sulfuric acid of yellow poplar depending on the temperature.

3.2. Further analysis of chosen ethanol organosolv lignin

EOL produced by pretreatment with 0.5% H₂SO₄ at 140 °C, 1% H₂SO₄ at 140 °C, and 1% H₂SO₄ at 180°C were selected for further analysis.

3.2.1. Chemical structural characteristics

The chemical structure characteristics of EOL are shown in Table 4-5. The average number of double bond equivalents (DBE) was determined by Robert's method (Robert et al., 1984). Compared to MWL, EOL produced with 0.5% acid at 140°C and 1% acid at 140°C exhibited similar chemical structural characteristics with slight evidence of condensation and slightly increased abundance of aromatic structures. Conversely, EOL produced with 1% acid at 180°C exhibited a high degree of condensation and had considerably more aromatic structures.

The EOL products investigated above exhibited several particular features. Firstly, EOL produced with 0.5% acid at 140°C showed a high abundance of phenolic-OH content with few condensed structures. EOL produced with 1% acid at 140°C exhibited a considerably higher OMe content, supporting a high S/V ratio with less condensation, whereas EOL produced with 1% acid at 180°C exhibited a highly condensed aromatic structure. Such variation in the characteristics of EOL obtained from different organosolv pretreatments condition may be a useful for specific applications. For example, EOL produced by pretreatment with 0.5% H₂SO₄ at 140°C may be advantageous for producing aliphatic phenols by depolymerization, while EOL produced by pretreatment with 1% H₂SO₄ at 140°C may be useful for applying S unit lignin isolation such as syringol. Lastly, EOL produced by

pretreatment with 1% H₂SO₄ at 180°C may be useful for films, filling agent, and other applications that require a narrow molecular weight distribution.

Figure 4-10 shows the atomic ratios of the EOL products (Van Krevelen, 1950). All of the EOL samples generated by various pretreatment conditions exhibited low atomic H:C and O:C ratios compared to MWL. Specifically, the atomic H:C and O:C ratios of EOL produced with 0.5% acid at 140°C and 1% acid at 140°C were slightly decreased compared with MWL, while those of EOL produced with 1% acid at 180°C were decreased considerably. For comparison purposes, the atomic H:C and O:C ratios of alkali lignin were also determined, and were found to be significantly lower than all of the EOL samples with the exception of the H:C ratios of EOL produced with 1% acid at 180°C, one of the more severe conditions tested. Therefore, EOLs produced by pretreatment with 0.5% and 1% H₂SO₄ at 140°C, the degradation stage of lignin, have a relatively less condensed structure, whereas EOL produced by pretreatment with 1% H₂SO₄ at 180°C, which is at the boundary of re-distribution and condensation stages of lignin, exhibits a considerably condensed structure.

Table 4-5. Chemical structural characteristics of ethanol organosolv lignin, milled wood lignin, and alkali lignin

Lignin	Elemental composition (wt%)				Functional groups (wt%)		C ₉ formula	M _w /C ₉ (g/mol)	DBE ^d
	C	H	N	O ^a	OMe ^b	Phe-OH ^c			
0.5%, 140°C	59.7	6.2	0.3	33.8	12.7±0.2	9.4±0.6	C ₉ H _{8,68} O _{2,06} (OCH ₃) _{0,58} (OH) _{1,44}	192.2	4.4
1%, 140°C	58.7	5.9	0.2	35.2	13.4±0.1	6.3±0.1	C ₉ H _{9,82} O _{3,70} (OCH ₃) _{0,48} (OH) _{1,53}	193.5	4.6
1%, 180°C	61.4	5.4	0.1	33.1	11.6±0.1	6.2±0.3	C ₉ H _{7,47} O _{2,18} (OCH ₃) _{0,37} (OH) _{1,25}	183.0	5.3
MWL ^e	57.2	6.1	0.0	36.7	12.2±0.8	7.0±0.5	C ₉ H _{9,24} O _{2,68} (OCH ₃) _{0,45} (OH) _{1,42}	198.4	4.3
Alkali lignin	63.2	5.9	0.7	30.2	-	-	-	171.0	5.0

^a determined by difference

^b methoxyl group

^c phenolic hydroxyl group

^d double bonds equivalent (Robert et al., 1984)

^e milled wood lignin

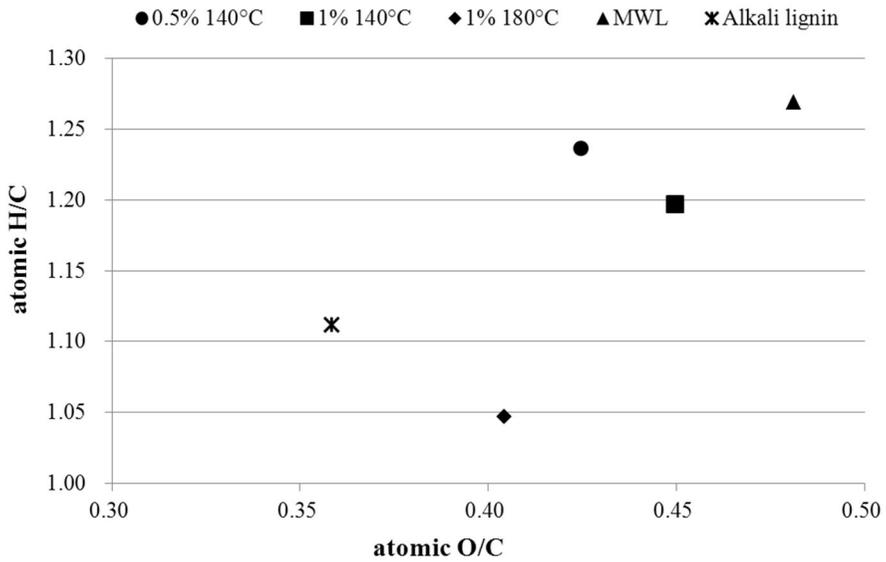


Figure 4-10. Van Krevelen diagram of ethanol organosolv lignin (0.5% H₂SO₄ at 140°C, 1% H₂SO₄ at 140°C, 1% H₂SO₄ at 180°C), milled wood lignin (MWL), and alkali lignin.

3.2.2. Physicochemical properties

TGA

The results of thermogravimetric and differential thermal analysis (TGA and DTG, respectively) of EOL, MWL, and alkali lignin are shown in Figures 4-11. EOL obtained by pretreatment with 0.5% acid at 140°C was converted into 24.8% of condensed char at the final temperature (800°C). Likewise, the amount of condensed char generated from 1% acid at 140°C increased to 30.75% and 31.98% with 1% acid at 180°C. The differences in condensed char results of MWL (23.94%) and alkali lignin (42.47%) were attributed to the degree of condensation of each lignin (Krieger-Brockett, 1994).

As observed in DTG curves, some evidence of degradation of EOL was present before 200°C, possibly due to a small inclusion of hemicellulose (Yang et al., 2007). Successive thermal degradation of lignin from other species was as follows: MWL, EOL obtained with 0.5% acid at 140°C, EOL obtained with 1% acid at 140°C, EOL obtained with 1% acid concentration at 180°C, and alkali lignin. Together, these results suggest that EOL produced under less severe conditions are similar to MWL with respect to thermal conversion characteristics, while EOL produced under more severe conditions is more similar to alkali lignin.

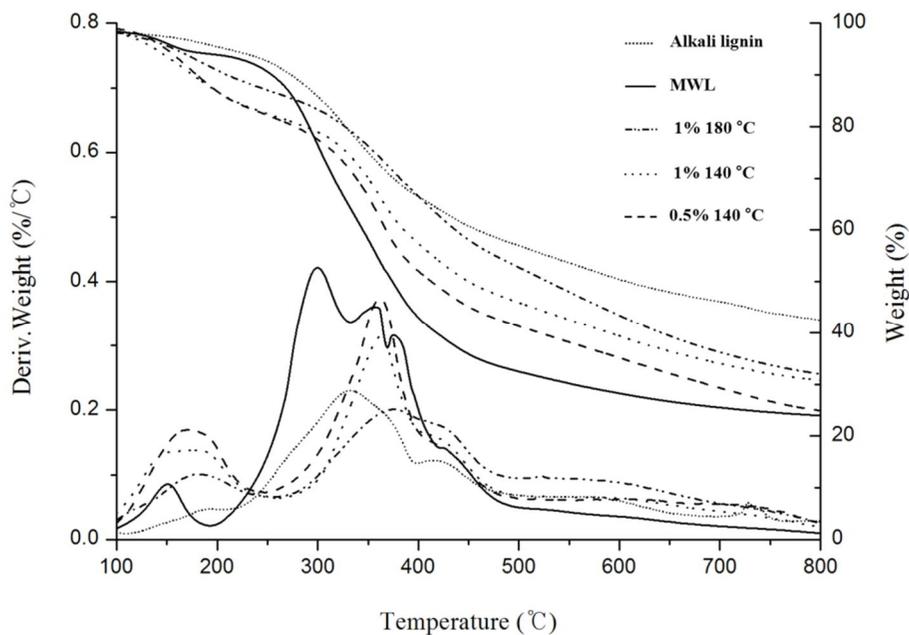


Figure 4-11. Thermogravimetric and differential thermal analysis of ethanol organosolv lignin (0.5% H₂SO₄ at 140°C, 1% H₂SO₄ at 140°C, 1% H₂SO₄ at 180°C), milled wood lignin (MWL), and alkali lignin.

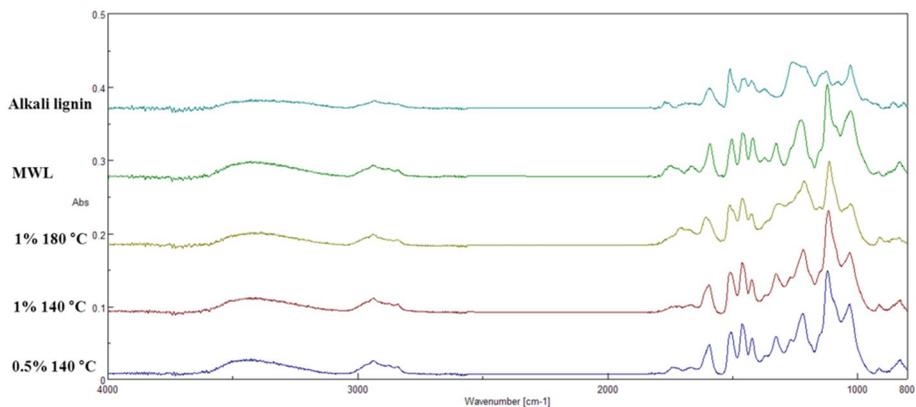
FT-IR

Changes in bonding types and chemical structures of EOL were investigated by FT-IR (Figure 4-12). FT-IR spectra of EOL products clearly identified lignin related peaks at $1,590\text{ cm}^{-1}$, $1,502\text{ cm}^{-1}$, and $1,420\text{ cm}^{-1}$, which were attributed to aromatic skeletal vibrations of lignin (Chundawat et al., 2007a), while a peak at $1,460\text{ cm}^{-1}$ was attributed to C-H deformations and aromatic ring vibrations of lignin (El Hage et al., 2009). Analysis of the different EOLs indicated that the characteristic peaks did not change significantly, despite the different severity of pretreatment conditions.

On the other hand, ether linkage-associated peaks changed significantly depending on the production conditions of EOL. Specifically, the peak heights at $1,328\text{ cm}^{-1}$, $1,230\text{ cm}^{-1}$, and $1,120\text{ cm}^{-1}$ attributed to ring breathing C-O stretching of lignin (El Hage et al., 2009) decreased considerably as the severity of EOL pretreatment conditions increased. Consequently, these results suggested that degradation of EOL progressed mainly by cleavage of ether linkages, which is consistent with the results of previous studies (El Hage et al., 2009; El Hage et al., 2010a).

Lastly, the spectra of EOL produced at 140°C was identical to that of MWL, in that it contained a large amount of functional groups, while that of EOL produced at 180°C was quite different due to the condensation reaction. In addition, the highly condensed structure of EOL produced with 1% acid concentration at 180°C was predicted to affect lignin monomerization, even though the production yield of EOL was greater compared with the more mild condition.

(a)



(b)

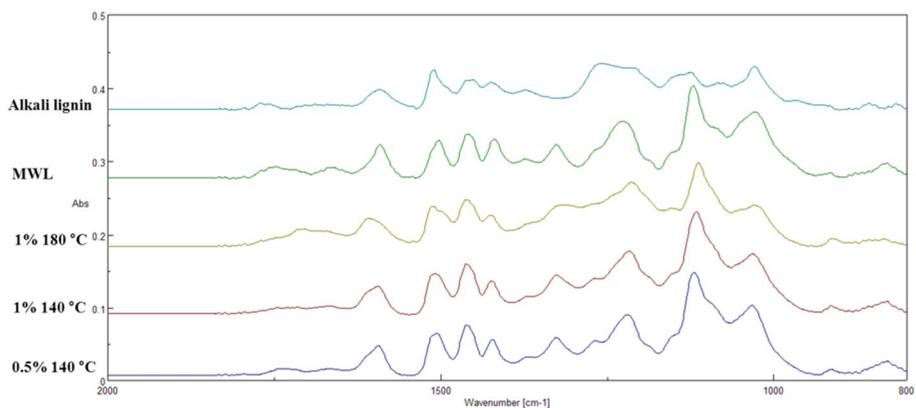


Figure 4-12. FT-IR spectra of ethanol organosolv lignin (0.5% H₂SO₄ at 140°C, 1% H₂SO₄ at 140°C, 1% H₂SO₄ at 180°C), milled wood lignin (MWL), and alkali lignin (a: 4,000-800 cm⁻¹; b: 2,000-800 cm⁻¹).

¹³C-NMR analysis

To investigate the carbon types of EOL, I performed ¹³C-NMR analysis. As shown in Figure 4-13, analysis of EOL revealed aromatic and phenolic signals at 105-150 ppm, S type C3/C5 of β-O-4 signals at 150-155 ppm, S type C2/C6 signals at 104 ppm, C-α signals at 70-80 ppm, C-β signals at 80-90 ppm, C-γ signals at 60-70 ppm, and a methoxyl signal at 55 ppm (Hallac et al., 2010; Kim et al., 2011b). The ¹³C-NMR spectrum of EOL produced with 0.5% acid at 140°C was much closed to that of MWL. Consistent with the results thus far, increasing acid concentration and temperature during EOL production resulted in a significant decrease in S type C3/C5 of β-O-4, S type C2/C5, and C-β signals. Interestingly, the ¹³C-NMR spectrum of EOL produced with 1% acid at 180°C was more similar to that of alkali lignin.

The ¹³C-NMR analysis of EOL detected a significant peak for R-CH₂CH₃, which was relatively small for MWL. Previous studies have reported that aryl ether linkages of lignin are cleaved by addition of H or ethyl groups during ethanol organosolv pretreatment (El Hage et al., 2010a). Further, the high R-CH₂CH₃ peak present in EOL was not observed in MWL or alkali lignin. In the case of alkali lignin, the active site signals and other aliphatic carbon site signals were significantly decreased compared to those of EOL, confirming the low degree of condensation of EOL produced in this study.

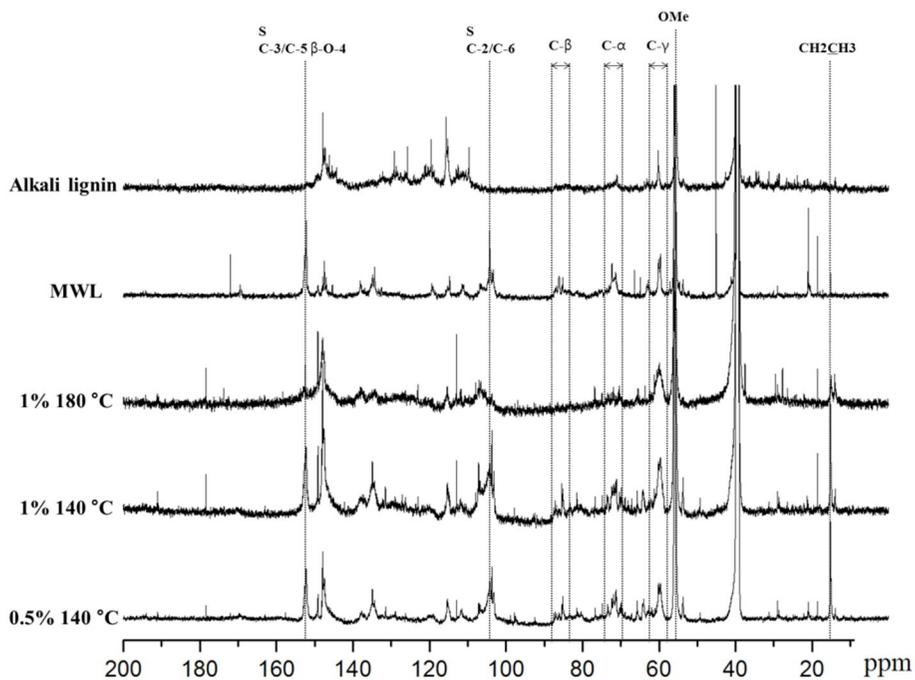


Figure 4-13. ^{13}C -NMR spectrum of ethanol organosolv lignin (0.5% H_2SO_4 at 140°C , 1% H_2SO_4 at 140°C , 1% H_2SO_4 at 180°C), milled wood lignin (MWL), and alkali lignin.

4. Conclusions

The mechanism of EOL formation during acid-catalyzed organosolv pretreatment can be divided into three stages: 1) isolation of S unit lignin, 2) degradation with condensation of S unit lignin, and 3) maintenance of lignin structure. Importantly, the order of these stages can vary depending on reaction temperature and acid concentration.

The isolation stage of S unit lignin ranges from between 120°C and 140°C with 0.5% and 1% acid, and between 120°C and 130°C with 2% acid. During the isolation stage of S unit lignin, EOL yield, M_w , polydispersity, OMe contents, phenolic-OH contents (0.5% acid concentration only), and NBO products of S unit lignin are critically enhanced. EOL is formed primarily by cleavage of lignin-hemicellulose structures in the secondary wall below 140°C, and thus exhibits a high S/V ratio.

The stage of degradation with condensation of S unit lignin ranged from 140°C to 170°C with 0.5% acid, from 140°C to 160°C with 1% acid, and from 130°C to 150°C with 2% acid. During this stage, the M_w , polydispersity, and NBO products of S unit lignin decrease drastically while EOL yield, OMe content, and phenolic-OH content are largely unchanged. Specifically, EOL underwent both cleavage of lignin structural linkages and condensation. In all of the conditions described above, EOL appeared to be stable, and was maintained without significant changes.

Based on further analysis of EOL products, the results revealed that the chemical structure characteristics of EOL produced under less severe conditions, including functional groups, atomic ratios, and C9 molecular weight were very similar to that of MWL, whereas EOL produced under more severe conditions was much closer to alkali lignin. Noticeably, all of the

produced EOLs shared a high R-CH₂CH₃ peak on ¹³C-NMR spectra, which was attributed to the addition of ethyl groups during the EOL isolation process. Interestingly, various characteristics of EOL may be exploited using different organosolv pretreatment conditions to obtain lignin for specific applications. For example, EOL produced with 0.5% acid at 140°C had significantly high phenolic-OH content and less condensation, and thus could be useful for production of aliphatic phenols by depolymerization. On the other hand, EOL produced with 1% acid at 140°C exhibited a considerably higher S/V ratio with less condensation, and thus may be useful for isolation of S unit lignins such as syringol.

The characteristics of EOL formed during acid-catalyzed organosolv pretreatment can be better understood based on these results. Importantly, the characteristics of EOL were significantly altered by the pretreatment conditions, and thus controlling the pretreatment process is necessary for efficient EOL production. Furthermore, several special characteristics of EOL make it potentially useful for a number of applications as well as lignin depolymerization. Further analyses of EOL products are necessary to establish their usefulness and potential economic impact.

Chapter 5

Advancement of ethanol organosolv lignin
procedure for application to biorefinery
process

1. Introduction

During the lignin pretreatment process, especially acidic pretreatment, it is thought that lignin undergoes severe chemical modification including condensation. After steam explosion treatment, the phenolic hydroxyl group content of lignin is significantly increased (from 23 to 47 per 100 aromatic units) while β -O-4 ether linkages are decreased (from 64 to 36 per 100 aromatic units) (Martin-Sampedro et al., 2011). Furthermore, based on the severity of steam explosion conditions, both the average molecular weight and heterogeneity of lignin structures can increase (Li et al., 2007). These observations can be explained by condensation reactions. In addition, it was previously shown that lignin droplets are produced on pure cellulosic fibers during pretreatment under acidic pH at temperatures above 130°C by re-distribution (Selig et al., 2007). Furthermore, acid pretreatment results in increased condensation of aromatic C groups and decreased abundance of β -O-4 ether linkages due to cleavage-mediated depolymerization.

To reduce condensation reactions of lignin, metal catalysts that encourage hydrogenation reactions, including palladium and nickel, have been widely utilized in pyrolysis processes (Ali, 2012; Sergeev & Hartwig, 2011; Zakzeski et al., 2010). However, such approaches have rarely been used for pretreatment. Importantly, reducing the frequency of lignin re-condensation may influence and minimize non-productive binding of cellulolytic enzymes to lignin, leading to enhanced enzymatic hydrolysis. Furthermore, the yield of ethanol organosolv lignin produced from the organosolv pretreatment process may be increased if the degree of condensation and re-distribution of lignin to the solid fraction can be reduced by the addition of a metal catalyst.

Practical lignins such as lignosulfonates and kraft lignin can be used to produce a number of products (Gargulak & Lebo, 2000) including phenol

formaldehyde resins (Muller et al., 1984) and vanillin (Gogotov, 2000); however, most of the forms of lignin are simply used for energy production due to their high sulfur content. On the other hand, organosolv lignin has a high quality, high purity, is sulfur free, has a low molecular weight, and exhibits a narrow molecular weight distribution compared with other sources of practical lignin (Pan et al., 2008). Indeed, organosolv lignin has a number of potential applications including adhesives, fibers, films, and biodegradable polymers (Cetin & Ozmen, 2002; Pereira et al., 2007; Vázquez et al., 1999).

Towards realizing a more valuable utilization of isolated lignin, depolymerization remains an attractive goal. Of the existing depolymerization methods, supercritical treatment has received a great deal of attention as of late. Supercritical treatment of lignin with organic solvents is typically conducted in a temperature range of 200-350°C using alcohols such as ethanol (Miller et al., 1999), methanol (Tsujino et al., 2003), and butanol (Yoshikawa et al., 2012). The molecular weight of depolymerized lignin ranges from approximately 150 Da to 2500 Da, and can be divided into a liquid oil fraction and solid char fraction. Lignin isolated from the liquid fraction has a molecular weight less than approximately 300 Da, while the solid char fraction contains lignin trimers and larger oligomers. The individual yield of monomers can comprise up to few percent of the total amount, but rarely exceeds 5% (Azadi et al., 2013).

In this study, organosolv pretreatment of yellow poplar was modified for application during the biorefinery process. Specifically, palladium was applied to the organosolv pretreatment process as a metal catalyst to facilitate hydrogenation and prevent lignin condensation. The productivity of fermentable sugars and organosolv lignin produced by palladium-assisted acid pretreatment were evaluated. And depolymerization of organosolv lignin was performed to verify the possibility of producing monomeric lignin compounds.

2. Materials and methods

2.1. Yield enhancement of ethanol organosolv lignin

2.1.1. Materials

Yellow poplar powder (pass through 0.5 mm sieve; less than 5% the initial moisture content) was prepared according to the methods described in section 2.1. of Chapter 3.

2.1.2. Organosolv pretreatment process with palladium

Organosolv pretreatment was performed in a reaction vessel consisting of an electric band heater, a magnetic drive with a paddle type impeller, and a control box (HR-8300, Hanwoul Engineering Inc., Gunpo, Gyeonggi, Republic of Korea) (Figure 5-1). The vessel was manufactured from stainless steel (SUS 316) and had a capacity of 1,000 ml. A Teflon gasket was used to maintain internal pressure. A thermocouple and pressure gauge were located inside the reactor to measure internal temperature and pressure during pretreatment, respectively. The Teflon impeller located inside the reactor was used for regular stirring, and the control box was used to regulate both the temperature of the vessel via the heater and the stirring rate of the impeller.

The reaction vessel was loaded with 50 g of yellow poplar and 500 ml of a 50% solution of ethanol (v/v) with 1% (w/w) sulfuric acid as a catalyst. Pretreatment times were recorded after the internal temperature reached the desired target temperature (145°C, 160°C). All reactions were carried out with the same solid to liquid ratio (1:10), preheating time (40 min), and residence time (10 min). Palladium (0.5 g) on carbon (Aldrich 205699, Sigma-Aldrich

Co., Yongin, Gyeonggi-do, Republic of Korea) was added to prevent condensation of lignin fragments. After pretreatment, the reactor was quenched in an ice chamber and cooled to room temperature within 10 min.

After cooling to room temperature, the pretreated materials were filtered through a previously weighed filter paper (Advantec No. 2, Advantec Co., Tokyo, Japan) and divided into pretreated solid residue and liquid hydrolysate fractions. The pretreated solid residue was then washed with two volumes of distilled water to remove degradation products and solvents on the surface of pretreated solid residues. The washed pretreated solid fraction was stored in 4°C until enzymatic hydrolysis. Finally, the pretreated materials were divided into three fractions: a solid fraction, liquid hydrolysate fraction, and washed filtrate fraction.



Figure 5-1. The reactor for organosolv pretreatment with palladium.

2.1.3. Analysis of pretreated solid fraction

The water insoluble solid (WIS) recovery rate (Eq. 1) was measured according to the method previously described in section 2.3. of Chapter 2.

2.1.3.1. Chemical compositions

Chemical compositions of pretreated solid fractions were determined according to the methods previously described in section 2.3.1. of Chapter 3.

2.1.3.2. Enzymatic hydrolysis

The commercial cellulase mixture (cellulase complex NS50013 and β -glucosidase NS50010) used in this study was kindly provided by Novozyme Korea Ltd (Seoul, Korea). The enzymatic activity of NS50013 was 15 FPU/g dry biomass and NS50010 was supplemented at 30% of the weight of the added cellulase complex. Samples were prepared with 2% solid loading by mixing the biomass equivalent of 1 g of dry mass (determined by moisture content) with 50 ml of 50 mM sodium acetate buffer (pH 5.0). Enzymatic hydrolysis was performed in a 250 ml Erlenmeyer flask at 50°C and 150 RPM for 72 h. The hydrolysate was sampled after 72 h to determine the amount of glucose and xylose that had been released.

The contents of glucose and xylose were determined by HPLC (Ultimate 3000 series, Dionex, Sunnyvale, CA, USA) using an Aminex HPX-87P column (300 mm \times 7.8 mm ID \times 9 μ m, Bio-Rad Laboratories, Richmond, CA, USA). The HPLC was operated at a column temperature of 75°C with HPLC-grade water as the mobile phase at a flow rate of 0.5 ml/min. A refractive index detector (RID) was used for detection.

2.1.4. Analysis of liquid hydrolysate

2.1.4.1. Chemical compositions

Sugar (glucose, xylose, and arabinose), sugar derived products (5-HMF and furfural), and organic acids (levulinic acid, formic acid, and acetic acid) of the liquid fraction were determined as described in section 2.4.1. of Chapter 2.

2.1.4.2. Ethanol organosolv lignin recovery process

EOL recovery process was described in section 2.4.2. of Chapter 2. The EOL yield (Eq. 2) was calculated based on the initial input of amount of yellow poplar wood meal for pretreatment and collected EOL weight after precipitate.

2.2. Depolymerization of ethanol organosolv lignin

2.2.1. Materials

Ethanol organosolv lignin chosen for further analysis as described in section 2.3. of Chapter 4 was used for supercritical-ethanol mediated depolymerization.

2.2.2. Supercritical treatment

Depolymerization of EOL by supercritical treatment was carried out in a hydrothermal device consisting of a heater capable of generated a temperature of 750°C and three stainless steel reaction vessels (SUS316, capacity: 50 ml). Each reactor included a stirrer, pressure gauge, and thermocouple. A total of 0.3 g of sample and 20 ml of ethanol were mixed in the reactor, which was then purged for 2 min with nitrogen gas (10 l/min) to remove any reactive gas from the reactor. Supercritical treatment conditions were then performed at 350°C for 40 min. During supercritical treatment, the heating, cooling, and stirring rates were maintained at 10°C/min, -30°C/min, and 200 RPM, respectively. After the reaction, recoverable products (oil and char) were collected and the yields of the three main products (oil, gas, and char) were determined according to the following equation:

$$\text{Oil yields (\%)} = \frac{\text{the weight of oil fraction}}{\text{input amount of supercritical treatment}} \times 100 \quad (\text{Eq. 5})$$

$$\text{Gas yields (\%)} = 100 - (\text{Oil yield} + \text{Char yield}) \quad (\text{Eq. 6})$$

$$\text{Char yields (\%)} = \frac{\text{the weight of solid fraction}}{\text{input amount of supercritical treatment}} \times 100 \quad (\text{Eq. 7})$$

2.2.3. Analysis of oil fraction

2.2.3.1. GC/MS

GC/MS analysis of oil was performed on an Agilent HP7890A GC equipped with an Agilent HP5975A mass selective detector (MSD). The oil samples were diluted with 3 ml of acetone and 1 ml of diluted sample was mixed with 100 μ l of fluoranthene (9.9 mg/10 ml in acetone) as an internal standard. A 1 μ l diluted sample was separated with a DB-5 capillary column (30 m \times 0.25 mm ID \times 0.25 μ m film thickness) using the following temperature program: initial oven temperature 50°C for 5 min, heating at 3°C/min up to 140°C for 10 min, followed by heating at 2°C/min up to 280°C for 10 min. The injector and detector temperatures were set to 220°C and 300°C, respectively. The carrier gas was He of 99.99% purity. A mass selective detector (Agilent Technologies 5975A) was used to obtain the mass to charge (m/z) ratios for each compound at 70 eV, and mass spectra were identified as described in section 2.2.5. of Chapter 4.

2.2.3.2. Molecular weight

Molecular weight of the oil was determined according to the methods described in section 2.3.1. of Chapter 2.

2.2.3.3. ¹³C-NMR

To identify the carbon type of the oil, NMR spectrometer (Jeol-LA400 with LFG, JEOL, Japan) was used according to the methods previously described in section 2.5.2. of Chapter 3.

3. Results and discussion

3.1. Yield enhancement of ethanol organosolv lignin

3.1.1. Composition of solid fraction

The compositions of the solid fraction obtained by organosolv pretreatment with palladium of yellow poplar are shown in Table 5-1. As the pretreatment temperature increased, the WIS recovery rate (from 52.01% to 42.46%), glucan (from 37.50% to 34.16%), xylan (from 3.58% to 0.79%), and total lignin (from 6.04% to 3.73%) contents were moderately decreased. This result was similar that of a previous study reporting that the total yields of residual cellulose are between 40% and 60% (Zhao et al., 2009). However, compared to the lignin contents described in Chapter 3, the results of the present study were slightly reduced, which was probably due to the reaction vessel used in this study, which had a Teflon impeller located inside the reactor for controlled stirring.

Addition of palladium during the organosolv pretreatment did not significantly affect the composition of the solid fraction. However, a slight reduction in Klason lignin content was observed, and the amount of acid soluble lignin increased. Further, it appeared that the condensation reaction was somewhat abrogated because of palladium-induced hydrogenation (Taillades-Jacquín et al., 2008). Importantly, the decrease in the amount of lignin in solid fraction was expected to enhance the yield of enzymatic hydrolysis.

Table 5-1. Composition of solid fraction obtained by organosolv pretreatment with palladium of yellow poplar (g on the basis of 100 g of initial oven-dried raw materials)

Temp. (°C)	Time (min)	Acid (%)	Palladium (g)	WIS ^a recovery rate	Glucan	Xylan	Klason lignin	ASL ^b	Total lignin ^c	Total
145	10	1	0	52.01±0.08	37.50±0.55	3.58±0.12	5.42±0.32	0.63±0.02	6.04±0.34	47.12±0.32
145	10	1	0.5	51.81±0.16	36.80±0.16	3.73±0.04	4.97±0.38	0.74±0.07	5.71±0.31	46.25±0.51
160	10	1	0	42.46±0.56	34.16±0.01	0.79±0.01	3.34±0.31	0.38±0.03	3.73±0.34	38.67±0.34
160	10	1	0.5	42.20±0.14	34.93±0.12	0.94±0.04	2.67±0.07	0.45±0.04	3.13±0.03	39.00±0.19

^a water insoluble solid residue

^b acid soluble lignin

^c sum of Klason lignin and ASL

3.1.2. Composition of ethanol organosolv lignin

The composition of EOL obtained by organosolv pretreatment with palladium is shown in Table 5-2. Consistent with the slight decrease in the lignin content of the solid fraction following addition of palladium, EOL yield was significantly increased by addition of palladium. Specifically, the EOL yield was increased from 12.02 g to 13.35 g at 145°C and from 13.36 g to 16.30 g at 160°C. Moreover, the purity of EOL was also enhanced. The lignin contents of EOL were increased from 94.82% to 95.96% at 145°C and from 92.90% to 96.93% at 160°C. In the absence of palladium, the EOL yield in this study was similar to that of previous studies (Cybulska et al., 2012; El Hage et al., 2009; El Hage et al., 2010a); however, the addition of palladium to the pretreatment process significantly enhanced the EOL yield.

The impact of the addition of palladium became more evident at higher pretreatment temperatures, primarily because the condensation reaction of lignin became more vigorous as the reaction conditions became more severe. The enzymatic hydrolysis of the solid fraction was expected to increase due to decreased surface condensation of lignin, which would otherwise form non-productive complexes with enzymes. Likewise, EOL yield and purity were enhanced, and losses of fermentable sugars in the liquid hydrolysate were reduced.

Table 5-2. Composition of ethanol organosolv lignin obtained by organosolv pretreatment with palladium of yellow poplar
(g on the basis of 100 g of initial oven-dried raw materials)

Temp. (°C)	Time (min)	Acid (%)	Palladium (g)	Yield	Glucan	Xylan	Klason lignin	ASL ^a	Total lignin ^b	Total
145	10	1	0	12.02±0.06	0.06±0.00	0.58±0.03	11.21±0.14	0.33±0.03	11.54±0.17	12.18±0.19
145	10	1	0.5	13.35±0.17	0.06±0.00	0.55±0.00	12.35±0.14	0.31±0.01	12.66±0.13	13.27±0.14
160	10	1	0	13.36±0.32	0.14±0.00	0.49±0.01	12.47±0.28	0.48±0.03	12.95±0.31	13.58±0.30
160	10	1	0.5	16.30±0.12	0.19±0.00	0.69±0.01	14.63±0.18	0.51±0.01	15.14±0.17	16.03±0.16

^a acid soluble lignin

^b sum of Klason lignin and ASL

3.1.3. Enzymatic hydrolysis of solid fraction

Solid fractions obtained after organosolv pretreatment with palladium were enzymatically hydrolyzed for 72 h, the results of which are shown in Figure 5-2. Enzymatic digestibility of the solid fraction was increased from 82.20 g to 92.27 g as the temperature increased. At 145°C, enzymatic digestibility was not affected by the addition of palladium; however, there was a slight enhancement at 160°C, possibly due to decreased total lignin content and subsequent prevention of lignin re-distribution on the surface of cellulosic fiber.

With respect to the WIS recovery rate and composition of glucan in the solid fraction, glucose production approached almost 100% in every condition tested. It has been reported previously that enzymatic hydrolysis yields can reach approximately 100% following organosolv pretreatment of hybrid poplar (Pan et al., 2006a) and beetle-killed lodgepole pine (Pan et al., 2008). Thus, it may be useful to optimize these pretreatment conditions to increase process throughput and improve economic feasibility. For example, for glucose production alone, these results suggest pretreatment at 145°C without palladium. On the other hand, when considering both glucose and EOL production, pretreatment at 160°C with palladium may be superior if the cost of palladium is economical.

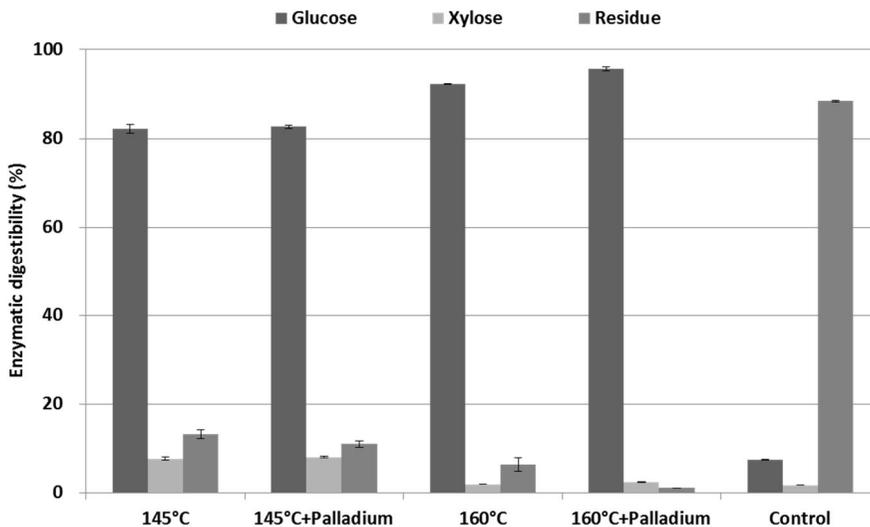


Figure 5-2. Enzymatic digestibility of the solid fraction obtained by organosolv pretreatment with palladium of yellow poplar.

3.1.4. Mass balance

To investigate the quantitative conversion characteristics of yellow poplar by organosolv pretreatment with palladium, mass balance analysis was performed, the results of which are shown in Figure 5-3. The majority of glucose was recovered after enzymatic hydrolysis of the solid fraction. Conversely, a greater amount of xylose was recovered in liquid hydrolysates with dehydrated furfural, while a partial amount of xylose was recovered after enzymatic hydrolysis at 145°C. At 160°C, detection of components other than glucose were rare, and their abundance after enzymatic hydrolysis was very small. This result strongly suggested that re-distribution of lignin to the solid fraction was reduced by addition of palladium, and that the use of palladium may be help to mitigate the lignin waste stream after enzymatic hydrolysis.

At higher reaction temperatures, the glucose content of the solid fraction and xylose content of liquid fraction was slightly increased according after the

addition of palladium. Importantly, the EOL recovery yield was enhanced from 11.04% at 145°C to 21.99% at 160°C. Lignin recovery from the solid fraction and EOL was ordered as follows: 160°C with 0.1wt% palladium > 145°C with 0.1wt% palladium > 160°C without palladium > 145°C without palladium. However, even the recovery of lignin at 160°C in the presence of 0.1wt% palladium was only 18.92%, which was much lower than the initial lignin content of 26.57%. Thus, recovery of lignin fragments from liquid hydrolysate is important for higher lignin recovery, as discussed in section 3.3. of Chapter 3.

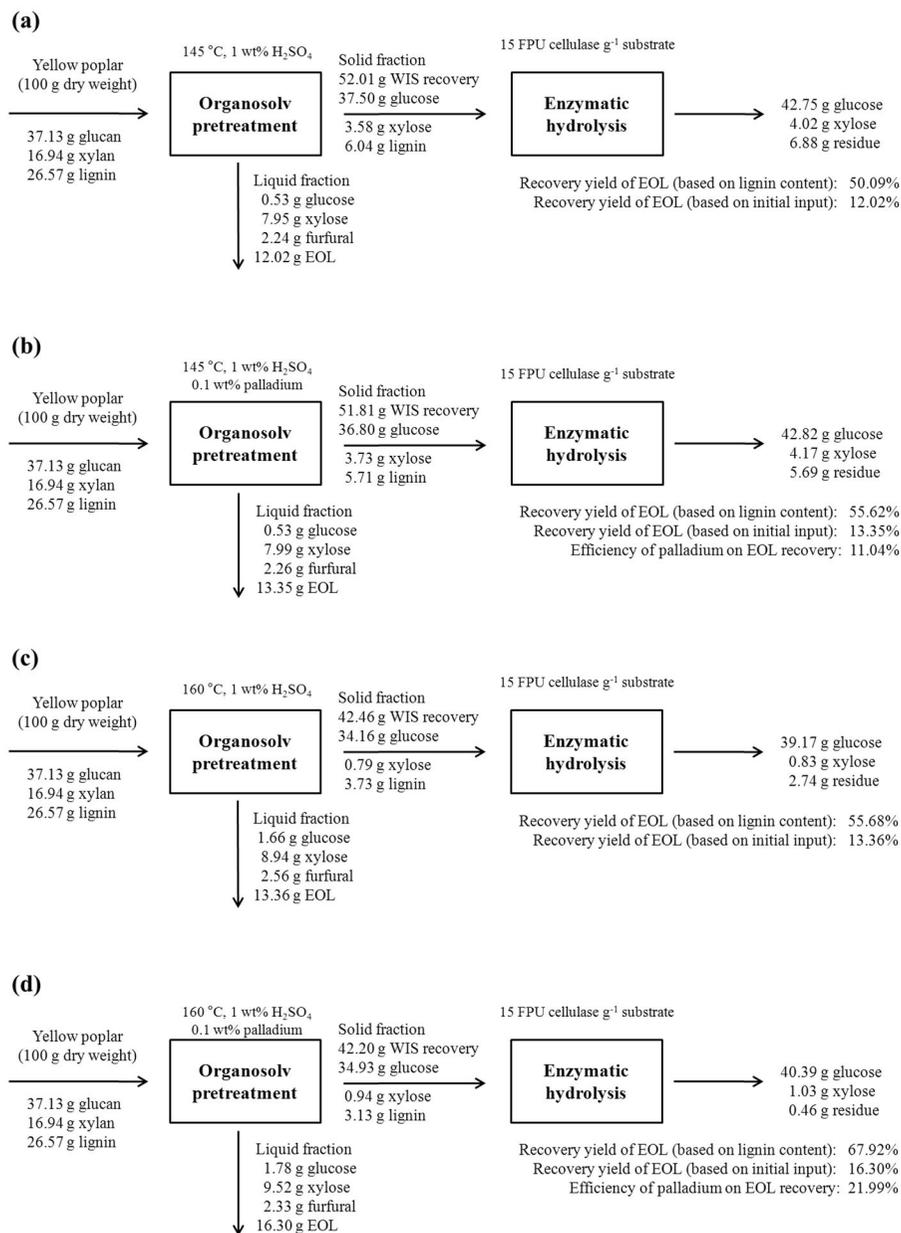


Figure 5-3. Mass balance for organosolv pretreatment with palladium of yellow poplar. (a: 145°C, b: 145°C with 0.1wt% palladium, c: 160°C, d: 160°C with 0.1wt% palladium)

3.2. Depolymerization of ethanol organosolv lignin

3.2.1. Mass balance of depolymerized products

Depolymerization of EOL was conducted with supercritical ethanol at 350°C. The yields of depolymerized products (char, oil, and gas) are shown in Figure 5-4. Depending on the conditions used to produce EOL, the yields of char, oil, and gas ranged from 38.32% to 42.25%, 23.79% to 36.67%, and 33.97% to 36.15%, respectively. It was difficult to more clearly define the yields.

Previous studies have reported an increase in the oil yield of Jack pine powder from 19% at 200°C (sub-critical ethanol) to 44% at 350°C (supercritical ethanol) (Xu & Etcheverry, 2008) as well as a maximum yield from bio-oil of 39.7% with supercritical 2-propanol:water (Yuan et al., 2007). Thus, the yield of oil in this study was slightly lower than expected. Furthermore, the yield of char was considerably higher than the results reported in previous studies (Yuan et al., 2007). It is possible that conditions for supercritical ethanol mediated depolymerization of EOL may have favored a high formation of char due to re-polymerization. The yields of depolymerized products were closely related to those of lignin monomer in oil, and thus further optimization of the depolymerization process of EOL for lignin monomer production should be carried out.

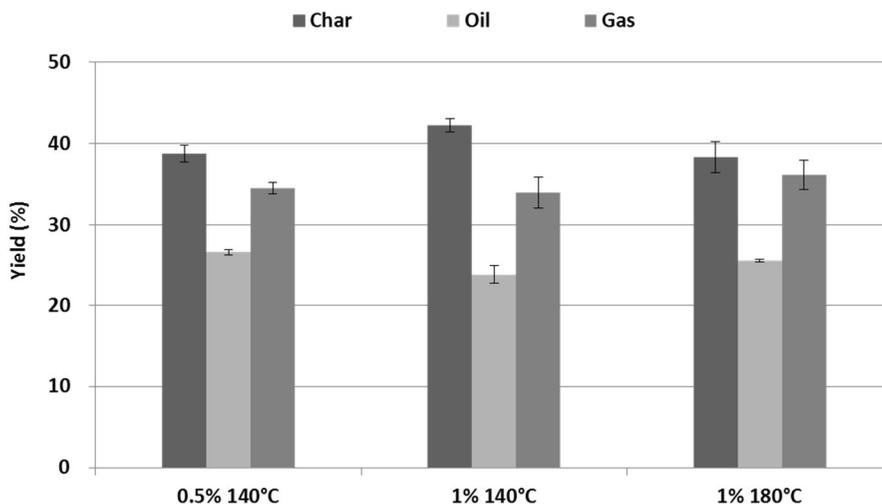


Figure 5-4. The yield of depolymerized products of ethanol organosolv lignin. (0.5% H₂SO₄ at 140°C, 1% H₂SO₄ at 140°C, 1% H₂SO₄ at 180°C)

3.2.2. GC/MS analysis

GC/MS analysis of the extracted oils was carried out to identify the phenolic monomer compounds present after depolymerization. Table 5-3 lists the compound names, retention times, molecular weights, sources, and amount of phenolic monomers that were identified. Phenolic monomers were detected between retention times of 35 min and 75 min. Fluoranthene (M_w : 202.26) was used as an internal standard (IS), and a response factor between each of the compounds and the IS was used to quantify each compound in the oil.

Following supercritical ethanol treatment of EOL, 17 monomeric phenolic compounds were identified using NIST MS Search 2.0 (NIST/EPA/NIH Mass Spectral Library; NIST 02) and the literature (Faix et al., 1990). Interestingly no H unit phenolic monomers were detected after supercritical ethanol treatment of EOL. A total of 40.4 mg/g of phenolic monomers were obtained from EOL generated via pretreatment with 0.5%

acid at 140°C. The top five compounds present were as follows: 4-methyl syringol (8.2 mg/g), syringol (6.3 mg/g), 4-methyl guaiacol (6.0 mg/g), guaiacol (4.3 mg/g), and syringyl acetone (3.2 mg/g). C6 and C1C6 structure were predominantly identified by GC/MS analysis, whereas C2C6 and C3C6 structures were rare. The yield of monomeric oil observed in this study were consistent with the results of a previous study reporting that lignin monomer content rarely exceeds 5% (Azadi et al., 2013),

A total of 28.2 mg/g of phenolic monomers were produced from EOL generated from pretreatment with 1% acid at 140°C, with 4-methyl syringol (5.2 mg/g), syringol (4.8 mg/g), 4-methyl guaiacol (3.8 mg/g), syringyl acetone (3.4 mg/g), and guaiacol (2.6 mg/g) comprising the top five compounds that were identified. Interestingly, the total amount of phenolic monomers was decreased compared with the 0.5% acid pretreatment condition, whereas the main compounds that were identified were similar. This result was probably due to the higher acid concentration used during EOL production. For EOL obtained with 1% acid at 180°C, a total of 23.3 mg/g of phenolic monomers were produced, the top five of which were 4-methyl syringol (4.8 mg/g), syringol (4.5 mg/g), 4-methyl guaiacol (3.3 mg/g), guaiacol (2.7 mg/g), and 3-methoxy catechol (2.4 mg/g).

With respect to the production of monomeric compounds, these results indicated that the degree of EOL condensation affected the production of lignin monomers. Therefore, it may be more feasible to produce EOL under mild condition for production of lignin monomeric compounds, despite the ability to achieve a slightly higher yield of EOL using more severe pretreatment conditions. The maximum yield of monomeric compounds was only 4.04% of EOL, however it should be noted that for the purpose of this study, supercritical ethanol treatment of EOL was conducted only to ascertain whether production of lignin from EOL was feasible. Specifically, I did not utilize methods to enhance yields, including addition of hydrogenase (Sharma

& Bakhshi, 1993) or phenolic compounds (Okuda et al., 2004). Likewise, the conditions for supercritical treatment (reaction temperature, retention time, solid to liquid ratio) were not optimized, leaving open the possibility of producing large quantities of low molecular weight lignin monomeric compounds and oils.

Table 5-3. Chemical compounds in oil identified by GC/MS

No.	Phenolic monomers	RT	MW	Source	Amount of phenolic monomers derived from ethanol organosolv lignin (mg/g)		
					0.5% H ₂ SO ₄ at 140°C	1% H ₂ SO ₄ at 140°C	1% H ₂ SO ₄ at 180°C
1	Guaiacol	36.05	124.1	G	4.3	2.6	2.7
2	3-methyl guaiacol	41.16	138.2	G	0.6	0.3	0.3
3	4-methyl guaiacol	41.95	138.2	G	6.0	3.8	3.3
4	3-methoxy catechol	46.79	140.0	S	3.1	2.1	2.4
5	4-ethyl guaiacol	48.05	152.2	G	1.8	0.9	0.9
6	Syringol	53.32	154.2	S	6.3	4.8	4.5
7	4-propyl guaiacol	54.61	154.2	G	0.7	0.4	0.2
8	4-methyl syringol	60.03	168.2	S	8.2	5.2	4.8
9 ^a	Acetoguaiacone	63.13	166.0	G	0.3	0.1	0.1
10 ^a	3-ethyl syringol	63.86	182.0	S	0.2	0.1	0.0
11 ^a	4-ethyl syringol	65.28	182.2	S	1.1	0.7	0.7
12 ^a	guaiacyl acetone	65.81	180.2	G	2.5	2.3	0.9
13	cis-4-propenyl syringol	70.16	194.2	S	0.4	0.2	0.0
14	4-propyl syringol	70.62	196.2	S	1.0	0.6	0.5
15	trans-4-propenyl syringol	68.40	194.2	S	0.3	0.3	0.3
16	Acetosyringone	70.70	196.2	S	0.5	0.4	0.4
17 ^a	Syringyl acetone	72.55	210.2	S	3.2	3.4	1.4
Sum of H-unit (mg/g of EOL)					-	-	-
Sum of G-unit (mg/g of EOL)					16.1	10.4	8.5
Sum of S-unit (mg/g of EOL)					24.2	17.8	14.9
Total phenolic monomer					40.4	28.2	23.3

^a Quantified by assuming response factor as 1

All phenolic compounds were identified by MS and reference (Faix et al., 1990)

3.2.3. Molecular weight distribution

The molecular weight distributions of oils obtained by supercritical ethanol treatment of EOL are shown in Table 5-4. The weight-average molecular weights (M_w) of oils ranged between 357 Da to 376 Da, and consisted only of dimer structures, which was a major factor responsible for the low yield of monomeric compounds. These results were similar to those reported previously for the oil fraction M_w , which was approximately 300 Da (Azadi et al., 2013). In addition, the smallest molecular weight polystyrene standard used in this study was 580 Da, and thus an exact molecular weight could not be determined. A chromatogram of the molecular weight distributions of oils obtained by supercritical ethanol treatment of EOL is presented in Figure 5-5. All of the molecular weight distributions of oils were present between retention times of 25 min and 30 min, and the shapes of the chromatograms for each sample were nearly identical. The oils produced in this study by supercritical ethanol treatment of EOL consisted primarily of dimers. Thus, further analysis should be conducted to determine whether the oils produced by the methods described here could be utilized for lignin-dimer specific applications.

Table 5-4. Molecular weight distributions of oils obtained by supercritical ethanol treatment of ethanol organosolv lignin

Temp. (°C)	0.5% H ₂ SO ₄ at 140°C	1% H ₂ SO ₄ at 140°C	1% H ₂ SO ₄ at 180°C	MWL ^c
M _w ^a (Daltons)	366	357	376	9919
M _n ^b (Daltons)	140	132	139	4240
Polydispersity (M _w /M _n)	2.61	2.70	2.71	2.34

^a weight-average molecular weight

^b number-average molecular weight

^c milled wood lignin

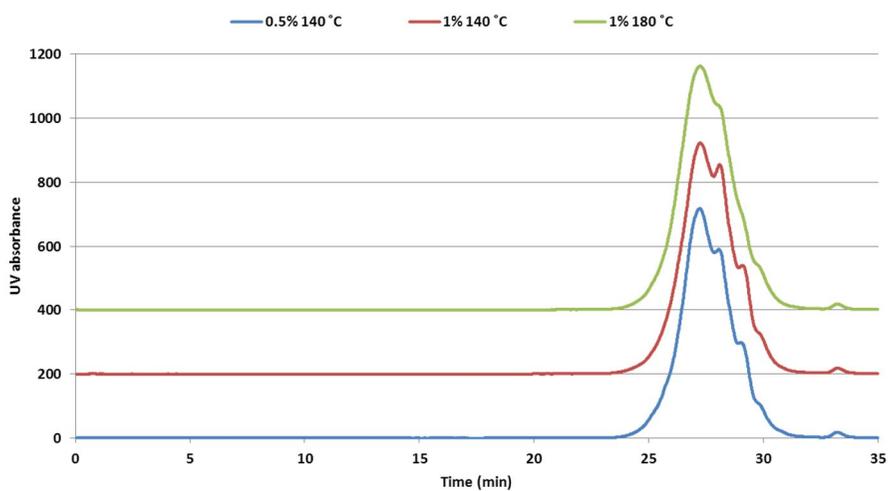


Figure 5-5. Molecular weight distributions of oils obtained by supercritical ethanol treatment of ethanol organosolv lignin.

3.2.4. ^{13}C -NMR analysis

To investigate the types of carbons present in the oils generated from EOL by supercritical ethanol treatment, I utilized ^{13}C -NMR analysis. As shown in Figure 5-6, aromatic and phenolic signals at 105-150 ppm, S type C2/C6 signals at 104 ppm, C- α signals at 70-80 ppm, C- γ signals at 60-70 ppm, and a methoxyl signal at 55 ppm were clearly observed in the oils (Hallac et al., 2010). In comparison with EOL, the signals belonging to S type C3/C5 of β -O-4 signals at 150-155 ppm were absent, indicating that the oils had a highly condensed structure. The ^{13}C -NMR spectra of the different oils generated in this study were very similar to each other, with the exception of minor aliphatic differences. Based on these results, it is clear that the oils consisted primarily of dimeric lignin compounds.

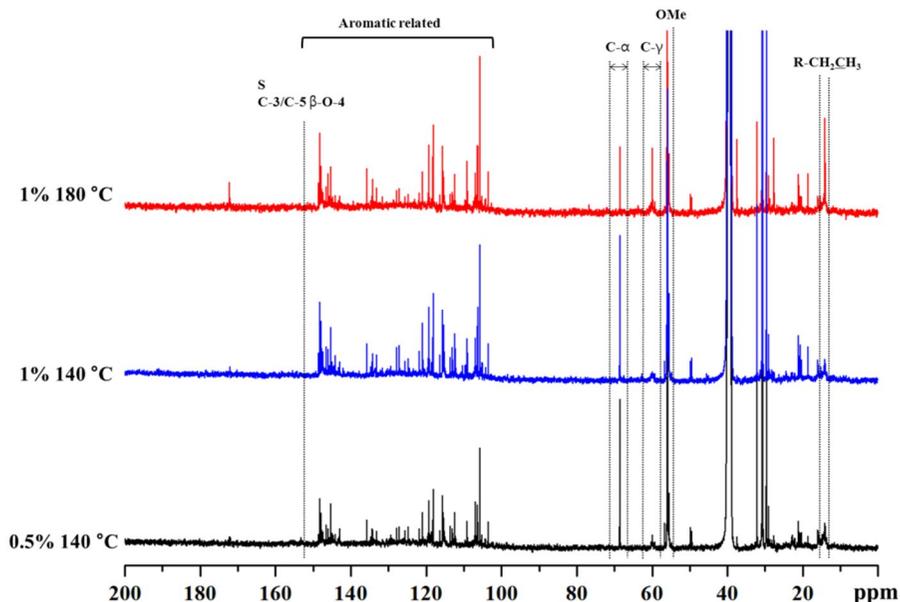


Figure 5-6. ^{13}C -NMR spectrum of oils obtained by supercritical ethanol treatment of ethanol organosolv lignin.

4. Conclusions

Introduction of palladium affords numerous advantages to several aspects of organosolv pretreatment. First, enzymatic hydrolysis of the solid fraction was encouraged by decreased re-distribution of lignin on the surface of the solid fraction. Secondly, the yield and purity of EOL was enhanced due to the decreased re-distribution of lignin fragments present in the liquid hydrolysate. Lastly, loss of fermentable sugars in the liquid hydrolysate was slightly reduced.

Addition of palladium in organosolv pretreatment should be a deliberate decision based on the intended downstream purpose. Specifically, if glucose production is the only desired outcome of organosolv pretreatment, it is sufficient to conduct pretreatment at less than 145°C without palladium. However, for the production of both glucose and EOL, pretreatment with 160°C in the presence of palladium might be a useful option. Despite its remarkable efficacy, the cost of palladium remains very high. Thus, it may be useful to substitute palladium with another catalyst such as zeolite, although further studies are needed to confirm this approach. Lastly, the increased lignin recovery obtained by addition of palladium was still less than the initial lignin content, indicating that it is important to recover lignin fragments from liquid hydrolysates.

Supercritical ethanol treatment at 350°C for 40 min was used to depolymerize EOL, which resulted in an oil yield ranging from 23.79% to 26.67%. A correlation between the conditions used to produce EOL and depolymerized products was not readily apparent; however, there did appear to be an effect on the formation and yield of specific monomeric compounds. The maximum yield of monomeric compounds obtained from EOL produced by pretreatment with 0.5% acid at 140°C was 4.04%, which decreased with

increasing severity of EOL production conditions. In addition, more S unit lignin monomers were generally produced from supercritical treatment compared with G unit lignin monomers, while H unit lignin monomers were not detected. The S/G ratio of lignin monomeric compounds was lower than that of NBO products, indicating that more S typed lignin underwent condensation. Therefore depolymerization of EOL by supercritical ethanol treatment could be further optimized to economically produce low molecular weight lignin derived compounds. The yield of monomeric compounds was significantly lower than expected, because supercritical treatment of EOL was not optimized. Thus, optimization of supercritical treatment conditions (reaction temperature, retention time, solid to liquid ratio) and use of yield-enhancement methods (i.e. addition of catalyst) may have huge implications for production of large amounts of monomeric lignin compounds.

Chapter 6

Concluding remarks

Lignin is recognized as the key factor related to the lignocellulosic biomass recalcitrance in bioethanol processing. Thus most pretreatment processes have aimed to remove lignin for enhancing hydrolysis yield of cellulose since. However, lignin itself has various functional groups, including methoxyl, phenolic hydroxyl, noncyclic benzyl ether, and carbonyl. These functional groups allow the use of lignin in various valuable applications. Organosolv pretreatment is more feasible for biorefinery of lignocellulosic biomass, because it can produce organosolv lignin with unique properties, such as relatively high purity, sulfur free, low molecular weight, narrow distribution, and more functional groups compared to other practical lignin.

In this study, to understand the formation mechanisms of ethanol organosolv lignin (EOL) produced in acid-catalyzed organosolv pretreatment, representative lignin (milled wood lignin (MWL) pre-isolated from yellow poplar) was organosolv pretreated at various conditions (temperature and catalyst concentration). Based on the results, characteristic changes of lignin in yellow poplar (*Liriodendron tulipifera*) and formation mechanisms of EOL during organosolv pretreatment were investigated. After determining the formation mechanisms of the EOL, a metal catalyst (palladium) was introduced in the pretreatment process and low-molecular lignin-derived compounds were produced through the depolymerization of isolated EOL.

Conversion of MWL after organosolv pretreatment was represented into three steps: 1) degradation, 2) re-distribution (degradation and condensation), and 3) condensation. In the degradation stage (0.5% H₂SO₄ at 120-180°C, 1% and 2% H₂SO₄ at 120-140°C) syringyl unit lignin was mainly degraded with hemicellulose. Condensation was initiated with the presence of degradation in the re-distribution stage (1% H₂SO₄ at 140-200°C, 2% H₂SO₄ at 140-180°C). Water insoluble solid (WIS) recovery rate, weight-average molecular weight (M_w), and EOL yield were maintained; however, the amount of NBO products declined, while S/V (syringyl to vanillyl) ratio re-rose due to condensation of

degraded syringyl unit lignin to solid fraction. At severe conditions (2% H₂SO₄ at 180-200°C), the condensation reaction was predominant, along with associated phenomena.

Characteristic changes of lignin in yellow poplar during acid-catalyzed organosolv pretreatment were close to those of MWL. Above 150°C lignin was no longer removed from solid fraction; undesirable reactions, such as re-distribution of lignin and excessive degradation of structural sugars occurred instead. Despite recovering EOL from liquid hydrolysates, lignin recovery was significantly lower than initial lignin contents; that was solved by determining the unidentified compounds in supernatant obtained after EOL production. One third of initial lignin content of lignin-derived phenolic compounds was dissolved in supernatant mainly composed of lignin oligomer.

Similar to behavior of MWL and lignin in yellow poplar, formation mechanisms of EOL were also divided into three stages: 1) isolation of S unit lignin, 2) degradation and condensation of EOL, and 3) maintenance of lignin structure. EOL was mainly formed by cleavage of lignin-hemicellulose structure in the secondary wall, thus having high M_w and S/V ratio. As pretreatment temperature and acid concentration increased, M_w, polydispersity, and NBO products of S unit lignin were significantly decreased due to both cleavage of lignin structure and condensation reaction. EOL produced at relatively low severe condition (0.5% and 1% H₂SO₄ at 140°C) was analogous to MWL in functional groups, atomic ratios, and C9 molecular weight. However EOL produced at more severe conditions (1% H₂SO₄ at 180°C) was rather closer to alkali lignin, which has a considerably condensed structure. Thermogravimetric analysis, FT-IR analysis, and ¹³C-NMR analysis also supported the above results.

To reduce re-distribution of lignin fragment on the surface of solid residue, palladium was introduced as a catalyst to induce hydrogenation reaction. This offered multiple positive advantages to every fraction.

According to less re-distribution of lignin to solid residue, 1) enzymatic hydrolysis of solid residue was encouraged, 2) yield and purity of EOL were enhanced, and 3) simultaneously, monomeric sugars in liquid hydrolysate were slightly preserved.

Supercritical ethanol treatment of EOL was carried out to produce depolymerized products, and from 23.79% to 26.67% of oils based on the EOL and from 2.33% to 4.04% of lignin monomeric compounds were produced. M_w of oils was between 357 Da and 376 Da which is only about dimer structure. Production yield of lignin monomer was influenced by the degree of condensation of EOL thus optimization of both EOL production and the depolymerization process should be required.

Based on these results, it is recommended to control the organosolv pretreatment conditions within the early part of the re-distribution stage. If this is not done, condensation reactions can occur invisibly, reducing the pretreatment efficiency and quality of isolated EOL. For the purpose of end-use application such as low molecular weight aromatic compounds, producing less condensed lignin more functional group will be key point.

In this study, the characteristic changes of lignins in MWL, yellow poplar, and EOL were investigated. Lignins were simply undergoing degradation and condensation reactions, however, the re-distribution stage caused by simultaneous occurrence of those reactions resulted in various interesting phenomena. Conversion characteristics of lignins and formation mechanisms of EOL could be evaluated by an investigation of the lignin recovery of the total process. Furthermore, through understanding the characteristic changes of lignin, appropriate pretreatment conditions of organosolv pretreatment process for biorefinery application are indicated.

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초 록

산촉매 유기용매 전처리에 의한 백합나무 리그닌 특성 변화

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Milled wood lignin (MWL)의 산촉매 유기용매 전처리에 따른 전환은 분리, 재배치, 재중합의 세 단계로 구분될 수 있는데 분리 단계 (0.5% 황산 120–180°C, 1%와 2% 황산 120–140°C)에서 다량의 리그닌이 (56.44–63.16%) syringyl (S) type의 형태로 헤미셀룰로오스와 함께 분리되었다. 재배치 단계 (1% 황산 140–200°C, 2% 황산 140–180°C)에서는 전처리 고형분 잔여율, 중량평균분자량 및 ethanol organosolv lignin (EOL) 수율은 유지되었으나, 분해된 S type 리그닌의 축합반응으로 인하여 S/V 비율은 다시 증가하였다. 재중합 단계 (2% 황산 180–200°C)에서는 축합 반응의 일반적인 현상이 주로 일어났으며, 특히 EOL 수율이 감소함에 따라 전처리 고형분 잔여율이 증가하였다.

백합나무 리그닌의 특성 변화는 MWL과 유사하였으며, 150°C 이전에 대부분의 리그닌이 분해되었다. 이후로 반응온도가 증가하여도 리그닌이 더 분해되지 않다가 매우 높은 온도조건에서

전처리 고품상에 리그닌의 재중합과 가상리그닌 (pseudo-lignin)의 생성이 예상되었다. 리그닌의 분해를 통해 액상 가수분해물로부터 최대 12.87%의 EOL을 생산할 수 있었으나, 여전히 초기 시료의 리그닌 함량대비 44.41% 리그닌이 확인되지 않았으며, 이는 EOL생산시 얻어지는 상층액의 분석을 통해 대부분 회수되었다.

EOL 생성 조건 별 특성은 MWL과 백합나무 리그닌 전환 특성을 통해 해석 가능하였다. 반응초기에 대부분의 EOL은 헤미셀룰로오스의 분해와 함께 생성되어 높은 분자량과 S/V 비율을 나타내지만, 전처리 온도가 증가함에 따라 축합반응이 일어나기 시작하여 분자량, 다분산성 및 NBO산물이 크게 감소하였고 EOL수율은 유지되었다. 0.5% 황산 140°C에서 생산된 EOL은 MWL과 관능기, 원소비율, C9 분자량 등이 유사하였으며, TGA, FT-IR, ^{13}C -NMR 분석 결과가 이를 뒷받침 해주었다.

리그닌의 재중합 반응을 방지하기 위하여 유기용매 전처리에 palladium을 투입하였으며, 재배치 단계 (1% 황산 160°C)에서 전처리 고품분의 효소당화 (3.73%)와 EOL 수율 (22.00%)이 향상되었다. EOL의 초임계 에탄올 처리를 통해서 낮은 분자량을 가지는 오일 (23.79-26.67%)과 리그닌 단량체 (2.33-4.04%)를 생산할 수 있었으며, EOL의 축합 정도가 이에 영향을 끼침을 확인할 수 있었다.

본 연구에서는 산촉매 유기용매 전처리 공정에서의 MWL의 전환을 분석하였고, 이러한 전환 특성의 이해를 통해 백합나무 리그닌의 특성 변화와 생성된 EOL의 특성을 구명하였다. 이를 바탕으로 유기용매 전처리 조건을 최적화하여 당화효율과

EOL수율의 향상이 동시에 가능하였으며 리그닌 단량체 생산을 통해 바이오피파이너리 공정으로의 적용을 확인하였다.

키워드 : 리그닌 특성 변화, 산촉매 유기용매 전처리, 백합나무,
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