Effect of Reaction Parameters on the Catalytic Oxidation of Poplar Lignin

by
Lin Lu

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science
in Bioengineering
(Honors Associate)

Presented April 15, 2019
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Title: Effect of Reaction Parameters on the Catalytic Oxidation of Poplar Lignin

Abstract approved: _____

Zhenglun Li

Nowadays, woody biomass is becoming increasingly significant because of its renewability and biodegradability. Lots of opportunities have been found to use excessive biological resources to derive products as diverse as fuels, heat, electricity, chemicals, food, clothing, paper, and much more. As such, biomass plays an important role in meeting demands in energy consumption, environmental protection, and economic growth.

As one of three major organic components in woody biomass, lignin is a polymer that cannot be directly incorporated in performance materials or specialty chemicals. Therefore, alkaline hydrogen peroxide (AHP) treatment - an efficient and economic lignin oxidation method - is essential to cleave chemical bonds and break large molecules into small molecules. More specifically, this type of lignin oxidation utilizes hydrogen peroxide as oxidant and an aqueous solution containing Cu-bipyridine complexes as catalyst. The purpose of this research is to optimize the preparation process of oxidized lignin at bench scale and investigate the effect of reaction parameters on the catalytic oxidation of poplar lignin.

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APPROVED:

_____
Zhenglun Li, Mentor, Food Science and Technology

_____
Kaichang Li, Committee Member, Chemical, Biological, and Environmental Engineering

_____
Joe Baio, Committee Member, Chemical, Biological, and Environmental Engineering

_____
Toni Doolen, Dean of Oregon State University Honors College

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Lin Lu, Author

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Chapter 1
Introduction

Lignin is a natural polymer with phenolic structure that exists in woody plants, herbs, and vascular plants. It is the second most abundant macromolecular material in nature, surpassed only by cellulose. As a promising alternative to fossil resources for the production of fuels and value-added chemicals [1], lignin is now widely used in building materials industry, petroleum industry, light industry, and agriculture.

In terms of physical property, lignin derived from biomass fractionation processes appears to be dark brown powder (see Figure 1.1) with relative density

Figure 1.1: Dark brown lignin powder for bench-scale experiments

between 1.35 and 1.50 [2]. It is not completely soluble in water because the hydroxyl group can form strong hydrogen bonding to prevent lignin dissolution. After further degradation, lignin becomes soluble in the mixture of water and organic solvents. Its solubility depends on many factors such as plant species, types of condensation reaction, and methods of lignin degradation or fractionation.

In terms of biological function, lignin increases mechanical strength of stems and rigidity of plant walls so that huge plants can reach heights over a hundred

meters. It also makes the cell wall impermeable to water and builds a complex

network inside living plants for water transport, which enable plants to grow taller

until reaching their maximum heights and compete for sunlight. Furthermore, lignin

functions as a physical barrier to protect plants from attacking by insects and fungi.

In terms of structure, lignin is the only macromolecule that is not composed of

carbohydrate monomers in plant cell walls. It is a randomly cross-linked polymer

consisting of many phenylpropanoid alcohols, especially coniferyl ($C_{10}H_{12}O_3$),

sinapyl ($C_{11}H_{14}O_4$), and p-coumaryl ($C_9H_{10}O_2$) alcohols (see Figure 1.2). An example

of proposed structural model for poplar lignin is shown in Appendix 3. These

p-coumaryl alcohol   coniferyl alcohol   sinapyl alcohol
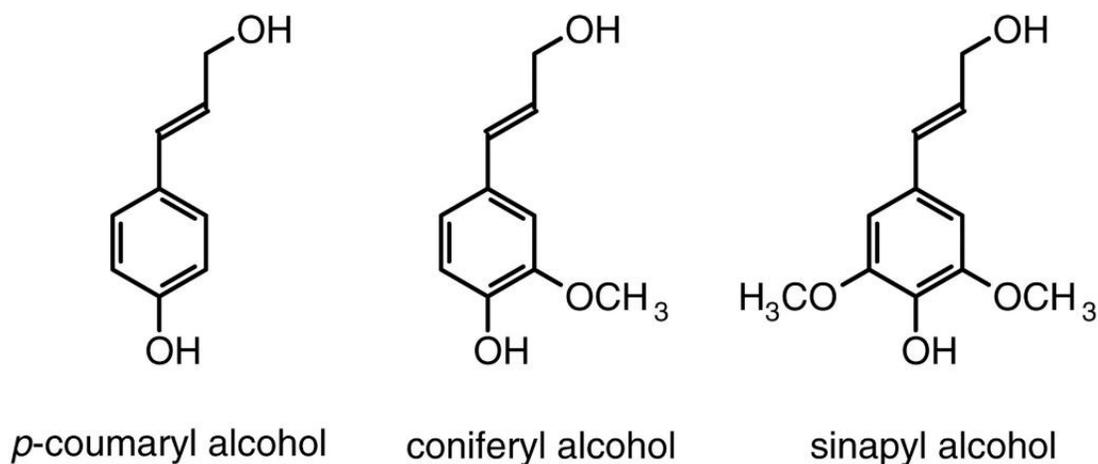
Figure 1.2: Three major phenylpropanoid alcohols found in natural plant lignin.

alcohol groups are held together by chemical bonds, including C-C bonds, C─O

bonds, and hydrogen bonds. Although no one has completely described the lignin

structure, the molecular weight of lignin in its unprocessed natural form might reach

15000 Daltons [3].

Due to the unique and complex structure, lignin degrades slowly under

natural conditions and cannot be directly utilized to derive various products.

However, lignin can be oxidatively degraded by using alkalis, oxidants, and catalysts. The oxidation aims to cleave specific bonds between different chemical groups and break large molecules into small molecules that can be further applied in lab experiments or chemical industries.

Furthermore, alkaline hydrogen peroxide pretreatment catalyzed by copper 2,2'-bypridine complexes is a promising way to achieve high yields of desired products such as sugar by overcoming cell wall recalcitrance through chemical modification or lignin cleavage that can significantly increase the solubility of lignin in alkalis [4]. It improves biomass enzymatic digestibility and provides the possibility of deriving additional coproducts.

For this research, the oxidative behavior of poplar lignin in alkalis was studied by using hydrogen peroxide as oxidant and an aqueous solution containing Cu-bipyridine complexes as catalyst. During the alkaline hydrogen peroxide (AHP) treatment, both catalyst and oxidant are sensitive to pH that changes over time. The longer the lignin oxidation takes, the lower the pH will be due to the consumption of alkalis. Once the reaction environment becomes acidic, oxidized lignin production is considered to be inefficient since the catalyst and oxidant are less active at low pH. Therefore, multiple pH adjustments are necessary to keep pH constant and maximize the extent of lignin oxidation. The purpose of this study is to optimize the preparation process of oxidized lignin at bench scale and investigate the effect of reaction parameters on the catalytic oxidation of poplar lignin.

Chapter 2
Literature Review

2.1 Oxidative Lignin Depolymerization

Oxidative lignin depolymerization is a method to break selected chemical bonds, such as carbon-carbon bonds, carbon-hydrogen bonds, carbon-oxygen bonds, aryl ether bonds or β-O-4 linkages. By applying different oxidants (such as hydrogen peroxide, oxygen gas, and metal oxides) and catalysts, lignin with high molecular weight can be broken into smaller molecules. This process can be summarized in Figure 2.1 [5]:
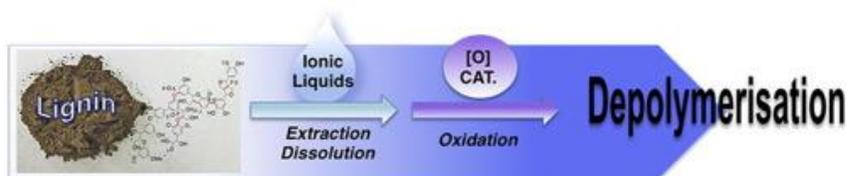
Figure 2.1 The process of oxidative lignin depolymerization

Some of the nomenclatures used in Figure 2.1 are listed below.

Ionic liquids: a molten salt utilized to dissolve biomass, which can subsequently be fractionated.

Extraction: a way to separate a substance when it is mixed with others.

Dissolution: the process by which a substance forms a solution in a solvent.

[O]: a reactant that oxidizes or removes electrons from other reactants during an oxidation reaction. e.g. hydrogen peroxide, ozone, nitric acid, sulfuric acid, halogens such as Cl_2 and F_2. In this experiment, the oxidant used was hydrogen peroxide.

CAT: a substance that increases the rate of a chemical reaction without causing mass loss and property change. In this experiment, the catalyst was an aqueous solution containing 40 mM $CuSO_4$ and 160 mM 2,2'-bipyridine.

Oxidation: the loss of electrons during a reaction by a molecule, atom or ion.

2.2 Different Types of Lignin Oxidation

In the production of simpler molecules, multiple oxidation methods have been attempted to make the process more economic and efficient. The oxidative depolymerization aims to crack C-O bonds and C-C bonds in lignin with different kinds of catalysts and oxidants, which makes aromatic aldehydes and carboxylic acids the main products from lignin oxidation. Recent study on lignin oxidation to the aromatic products is summarized in Table 2.1 [6]. Raw lignin discussed in this table include:

- Organosolv Lignin: a type of lignin derived from organosolv pulping process that uses an organic solvent to dissolve lignin from plant cell walls.

- Kraft Lignin: a type of lignin derived from Kraft pulping process by treating wood chips with a hot mixture of water, sodium hydroxide (NaOH), and sodium sulfide (Na_2S).

- Alkali Lignin: a type of lignin that is solubilized in alkali during pulping, making pH higher than 7.

- Enzymatic Hydrolysis Lignin: a type of lignin obtained from enzymatic hydrolysis process.

Main products shown in this table include:

- Vanillin: also called 4-hydroxy-3-methoxybenzaldehyde, is a phenolic aldehyde originally extracted from the vanilla bean. Its chemical structure is shown in Figure 2.2.
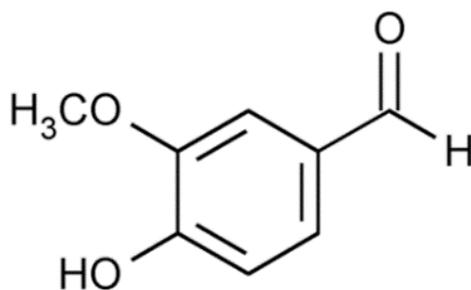
Figure 2.2: The structure of vanillin

- Vanillic acid: also called 4-hydroxy-3-methoxybenzoic acid, is a

  dihydroxybenzoic acid found in some forms of vanilla and many other plant

  extracts. Its chemical structure is shown in Figure 2.3.
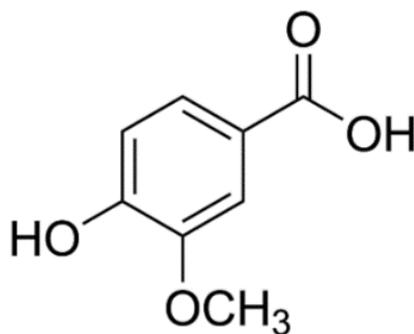
Figure 2.3: The structure of vanillic acid

- Syringic aldehyde: also called 4-hydroxy-3,5-dimethoxybenzaldehyde, is an

  organic compound often found in the wood of spruce and maple trees. Its

  chemical structure is shown in Figure 2.4.
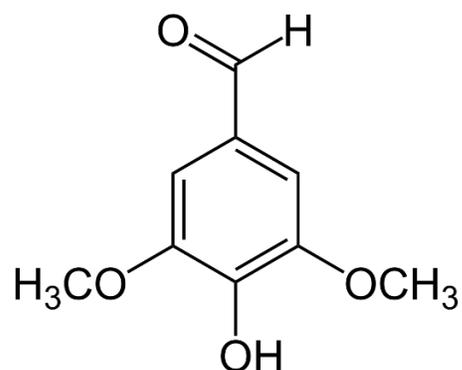
Figure 2.4: The structure of syringic aldehyde

- Syringic acid: also called 4-hydroxy-3,5-dimethoxybenzoic acid, is a component of phenolic extracts from various plants that have antioxidant and pro-oxidant activities [7].
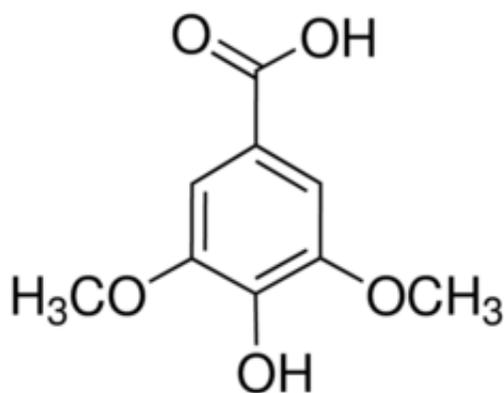
Figure 2.5: The structure of syringic acid

In addition, some of the abbreviations used in Table 2.1 are listed below.

- Tetrahydrofuran (THF): a colorless, water-miscible organic liquid mainly used as a precursor to polymers.

- Dimethyl Sulfoxide (DMSO): A by-product of the Kraft process by oxidation with oxygen or nitrogen dioxide, which can penetrate skin and other biological membranes.

- Tert-butyl hydroperoxide (TBHP): An organic peroxide widely used in a variety of oxidation processes.

- Methyltrioxorhenium (MTO): An exceptionally versatile oxygen transfer catalyst with hydrogen peroxide as terminal oxidant.

Table 2.1: Summary of oxidative depolymerization of different raw lignin

ENTRY	FEEDSTOCK	CONDITIONS	SOLVENT	CATALYST	PRODUCTS	YIELD	REFERENCE
1	Organosolv lignin from M. giganteus (an herbaceous plant)	80 °C, 24 h, air	Acetonitrile-THF or ethyl acetate-THF	Vanadium complexes bearing Schiff base ligands	Monophenolic compounds (vanillin, syringic acid, syringaldehyde)	0.78 wt %, 0.67 wt %, 0.59 wt % (Catalyst = Complex 3)	[8]
2	Organosolv lignin from mixed-hardwood (Aspen, maple and birch)	100 °C, 18 h, 0.8 MPa synthetic air	Ethyl acetate	Vanadium complexes and other organometallic catalysts	Bio-oil	Mw = 575 Da (Catalyst = Complex 2)	[9]
3	Organosolv lignin and Kraft lignin	100 °C, 8 h, H_2O_2	DMSO and acetic acid	{Fe-DABCO}	Bio-oil	/	[10]
4	Organosolv lignin	80 °C, 24 h, air	Acetonitrile-THF	Co(salen) supported on graphene oxide	Vanillin (main)	3067 g	[11]
5	Organosolv lignin and Kraft lignin	135 °C, 40 h, 1.0 MPa O_2	Pyridine	V(acac)$_3$ and Cu(NO$_3$)$_2$.3H_2O or HTc-Cu-V	Bio-oil	Mw = 300 Da	[12]
6	Organosolv lignin from hardwood (birch)	140 °C, 24 h, 0.1 MPa O_2	H_2O, tert-butyl hydroperoxide (TBHP)	Nitrogen-containing graphene material (LCN)	Bio-oil	45.8 wt %	[13]
7	Organosolv lignin from wood and bagasse	180 °C, 2 h, 13.8 MPa air	Acetic Acid-water	Co/Mn/Zr/Br mixture	Vanillin, vanillin acid, syringaldehyde, syringic acid	0.99 wt %, 2.91 wt %, 2.52 wt %, 4.51 wt %	[14]
8	Organosolv lignin from hardwood (beech)	200W, 5–30 min, H_2O_2	NaOH solution	La/SBA-15	Vanillin, syringaldehyde	9.94 wt %, 15.66 wt %.	[15]
9	Organosolv lignin	185 °C, 24 h, 0.1 MPa O_2	Methanol	Pd/CeO$_2$	Vanillin, guaiacol, 4-hydroxybenzaldehyde	5.2 wt %, 0.87 wt %, 2.4 wt %	[16]

10	Kraft lignin	170 °C, 0.3 h, 0.5 MPa O_2	Methanol-water and H_2SO_4	$H_3PMo_{12}O_{40}$	Vanillin, methyl vanillate	5.2 wt %	[17]
11	Kraft lignin	170 °C, 0.3 h, 1.0 MPa O_2	Methanol-water and H_2SO_4	$H_3PMo_{12}O_{40}$	Vanillin, methyl vanillate	4.6 wt %, 4.2 wt %	[18]
12	Kraft lignin	170 °C, 1 h, 1.0 MPa O_2	Methanol-water and H_2SO_4	$CuSO_4$; $FeCl_3$; $CuCl_2$; $CoCl_2$	Vanillin, methyl vanillate	6.3 wt %	[19]
13	Kraft lignin	45 °C, 1 h, H_2O_2	Acetone-water	Metal salt catalysts	Vanillin-based monomers	0.51 wt %	[20]
14	Enzymatic hydrolysis lignin from steam-explosive cornstalk	120 °C, 0–3 h, 0.5 MPa O_2	NaOH solution	$LaMnO_3$	Vanillin, p-hydroxybenzyl aldehyde, syringaldehyde	4.32 wt %, 2.03 wt %, 9.33 wt %	[21]
15	Enzymatic hydrolysis lignin	120 °C, 0–3 h, 0.5 MPa O_2	NaOH solution	$LaCoO_3$	Vanillin, p-hydroxybenzyl aldehyde, syringaldehyde	4.55 wt %, 2.23 wt %, 9.99 wt %	[22]
16	Hydrolytic sugar cane lignin and red spruce (softwood) Kraft lignin	25 °C, 24 h, H_2O_2	Acetic acid	MTO or poly(4-vinylpyridine)/MTO or polystyrene/MTO	Bio-oil	/	[23]
17	Alkali lignin	175–225 °C, 0–1 h, 0.5–1.5 MPa O_2	Water	NaOH	Formic acid, acetic acid, succinic acid, oxalic acid, glutaconic acid	44.0 wt %	[24]
18	Wheat alkali lignin	150 °C, 1 h, H_2O_2	Water/methanol/1,4-dioxane/ tetrahydrofuran/ethanol	CuO, $Fe_2(SO_4)_3$ and NaOH	Monophenolic compounds	17.92 wt % (in methanol-water)	[25]
19	Softwood (Loblolly pine) lignin	80 °C, 24 h, 0.27/1.24 MPa O_2	Methanol	Copper-phenanthroline complex and NaOH	Vanillic acid, vanillin	3.5 wt %, 12.6 wt %	[26]

2.3 H_2O_2 Oxidation Catalyzed by Cu - 2,2,'-bipyridine Complexes

Oxidative degradation occurs at many sites in lignin structure, which makes its products often complex. It can be achieved by treatment with an oxidizing agent such as peracetic acid (CH_3COOOH), periodate (IO_4^-), chlorine dioxide (ClO_2), hypochlorite salts, hydrogen peroxide (H_2O_2), ozone (O_3), and oxygen (O_2). Among these oxidants, hydrogen peroxide is the most common oxidizing agent employed in the alkaline dissolution of lignin since alkaline hydrogen peroxide (AHP) treatment is quite effective for woody biomass, although this approach requires high H_2O_2 loadings on the lignin, ranging from 250 mg to 2000 mg H_2O_2 per gram of biomass [4]. Studies have shown that hydrolysis of hydrogen peroxide causes the formation of hydroxyl radicals, which can degrade lignin efficiently and produce low molecular weight monomers [27]. The near-optimal pH for this reaction to occur was determined to be 11.5 later by Correia et al (2013) [28].

Li and co-workers discovered that the alkaline hydrogen peroxide (AHP) pretreatment catalyzed by Cu (II) – 2,2'-bipyridine complexes could substantially improve the enzymatic hydrolysis of hybrid poplar [29]. In their study, they performed both uncatalyzed and catalyzed AHP pretreatment to investigate how the addition of Cu (II) – 2,2'-bipyridine complexes would affect the lignin removal and hydrolysis yields. It turned out that uncatalyzed AHP pretreatment of hybrid poplar using 125 – 500 mg H_2O_2 / g hybrid poplar resulted in only 10–36 % lignin removal and 18–30 % hydrolysis yields but AHP pretreatment catalyzed by the complexes with shorter pretreatment time and less H_2O_2 loadings caused at most 40% lignin removal and more than 60% glucose hydrolysis yields. Therefore, by adding small

amount of the complexes, not only did the product yields significantly increase but also the H_2O_2 loadings reduced to 25 - 100 mg H_2O_2 per gram of biomass.

According to the research conducted by Korpi et al. (2006) [30], the structure of active species that played a crucial role in the AHP pretreatment catalyzed by copper salts and 2,2′-bipyridine was [Cu(2,2'-bipyridine)$_2$OH] $^+$. They found that the existence of [Cu(2,2'-bipyridine)$_2$OH] $^+$ was closely related to reaction conditions and the excess of copper salts and 2,2'-bipyridine would protect this active species from destructive hydroxide ions, meaning that the reaction conditions and the amount of catalysts must be controlled precisely in order to obtain more products.

Furthermore, Bhalla et al. (2016) [4] presented two strategies to improve alkaline hydrogen peroxide (AHP) pretreatment of hybrid poplar catalyzed by Cu (II) – 2,2'-bipyridine complexes. One strategy is to utilize H_2O_2 more efficiently. They added the H_2O_2 in small batches (without changing total amount of H_2O_2 and pretreatment time) rather than added all the H_2O_2 at the beginning of the pretreatment. This modification resulted in an increase in glucose yields (obtained from enzymatic hydrolysis of Cu-AHP pretreated poplar wood) from 63% to 77%. The other strategy is to perform an alkaline extraction step prior to the AHP pretreatment. This step aimed to remove easily extracted hemicellulose and lignin so that the Cu(bpy) complexes could readily penetrate the plant cell wall. The results showed that this strategy increased glucose yields from 63 to 86%. By combining these two strategies, glucose and xylose yields could reach 96% and 94% (theoretical maximum).

AHP pretreatment is a harsh treatment that requires appropriate reaction conditions, strong reaction vessels, and precise chemical control. It often leads to

lignin removal and high yields of reducing sugar. The major disadvantage of the process is that it is costly to use hydrogen peroxide during the pretreatment. More studies are being conducted to make this process cost-efficient and obtain more products.

Chapter 3
Methods

3.1 Materials

1. Hybrid poplar lignin

The poplar lignin was obtained from a commercial biorefinery operated by ZeaChem, which used biochemical conversion to produce biofuels from hybrid poplar. It was recovered from the biorefining process as a coproduct.

2. $CuSO_4$ and 2,2'-bipyridine

An aqueous solution containing 40 mM $CuSO_4$ and 160 mM 2,2'-bipyridine was used as the catalyst for lignin oxidation.

3. Hydrogen peroxide (H_2O_2)

The oxidant for this experiment was 500 mL hydrogen peroxide, 30% solution, AR Select (ARS) manufactured by Macron Fine Chemicals.

3.2 Experimental Procedures

1. Wash the hybrid poplar lignin obtained from ZeaChem with deionized water and dry at 50 °C. After that, grind lignin cakes into fine powders.
2. Weigh out 10 g of ground poplar lignin.

   Note: Make sure the bag containing ground poplar lignin is well-sealed to prevent moisture absorption.

3. Mix 2.5 mL catalyst solution with 80 mL aqueous solution of NaOH at pH 11.5 and pour the mixture into a plastic bottle that contains ground poplar lignin.

4. Stir gently. Make sure ground lignin and solution are mixed well.

5. Measure pH of the solution. If the pH is lower than 11.5, add 5M NaOH solution to bring pH back to 11.5 (first adjustment) and record the volume of 5M NaOH used.

6. Add 1.5 mL 30% hydrogen peroxide solution to the mixture and stir gently.

7. Measure the pH of the solution. If the pH is lower than 11.5, add 5M NaOH to bring pH back to 11.5 (second adjustment) and record the volume of 5M NaOH used.

8. After 30 minutes, add another 1.5 mL 30% hydrogen peroxide solution to the solution and stir gently.

9. Measure the pH of the solution. If the pH is lower than 11.5, add 5M NaOH to bring pH back to 11.5 (third adjustment) and record the volume of 5M NaOH used.

10. After 30 minutes, measure the pH of the solution again. If the pH is lower than 11.5, add 5M NaOH to bring pH back to 11.5 (final adjustment) and record the volume of 5M NaOH used.

11. Cap the plastic bottle and incubate the reaction mixture at room temperature for seven days.

12. After seven days, recheck the pH. If the pH is lower than 11.5, add 5M NaOH to bring pH back to 11.5 (additional adjustment) and record the volume of 5M NaOH used.

13. Filter the solution to get rid of insoluble solids and collect the flow through.

14. Wash the solids with the aqueous solution of NaOH at pH 11.5 until the filtrate becomes clear (solid washing). An experimental schedule is shown in Figure 3.1.

15. Measure and record the volume of black liquor.

16. Add dilute HCl solution (concentrated HCl: H_2O =1:2) to the black liquor until the pH reaches 2.0 and record the volume of diluted HCl solution added.

17. Filter the solution again, then collect and dry the solid.

18. Weigh the dry solid and record its mass. Label the sample clearly for FTIR analysis.

Note: The experiment described above is performed under a total pressure of 1 atm for a temperature at 25 °C.

	Trial 1, 2, 3, 4, 7,8 (replicates)	Trial 5	Trial 6	Trial 9
Step 1-11 Step 13 Step 15-18	X	X	X	X
Step 12		X		X
Step 14			X	X

X	Performed		Not Performed

Figure 3.1: An experimental schedule that shows treatment differences among all trials.

3.3 Analytical Methods

1. Control Parameters and Experimental Parameters

In general, there are ten parameters for the catalytic oxidation of poplar lignin: reaction temperature, reaction pressure, reaction time, mass of raw poplar lignin, volume of NaOH solution at pH 11.5 used to dissolve the poplar lignin, volume of catalyst, volume of 30% hydrogen peroxide (H_2O_2) solution, volume of 5M sodium hydroxide (NaOH) solution for each adjustment, volume of NaOH solution at pH 11.5 used to wash the solids, and volume of dilute hydrogen chloride solution. In order to determine if additional pH adjustment and solid washing have significant effects on the yield of oxidized lignin, these parameters must be controlled so that repeatable results can be obtained on lignin recovery. If more than one parameter changes at a time, the cause and effect is unclear. Therefore, it is essential to only allow the parameter of interest to change and other parameters should be held constant to ensure that any effect on the outcome is due to the changed parameter.

These parameters are classified into two categories: control parameter (the parameter that doesn't change in an experiment) and experimental parameter (the parameter that is manipulated by the researcher). As shown in Figure 3.2, parameters including the reaction temperature, the reaction pressure, the reaction time, the reaction pH, the mass of raw poplar lignin, the volume of NaOH solution at pH 11.5 used to dissolve the poplar lignin, the volume of catalyst, and the volume of 30% hydrogen peroxide (H_2O_2) solution are held constant. By manipulating pH control and washing condition, a relationship between experimental parameters and yields of oxidized lignin can be determined through simple comparison.
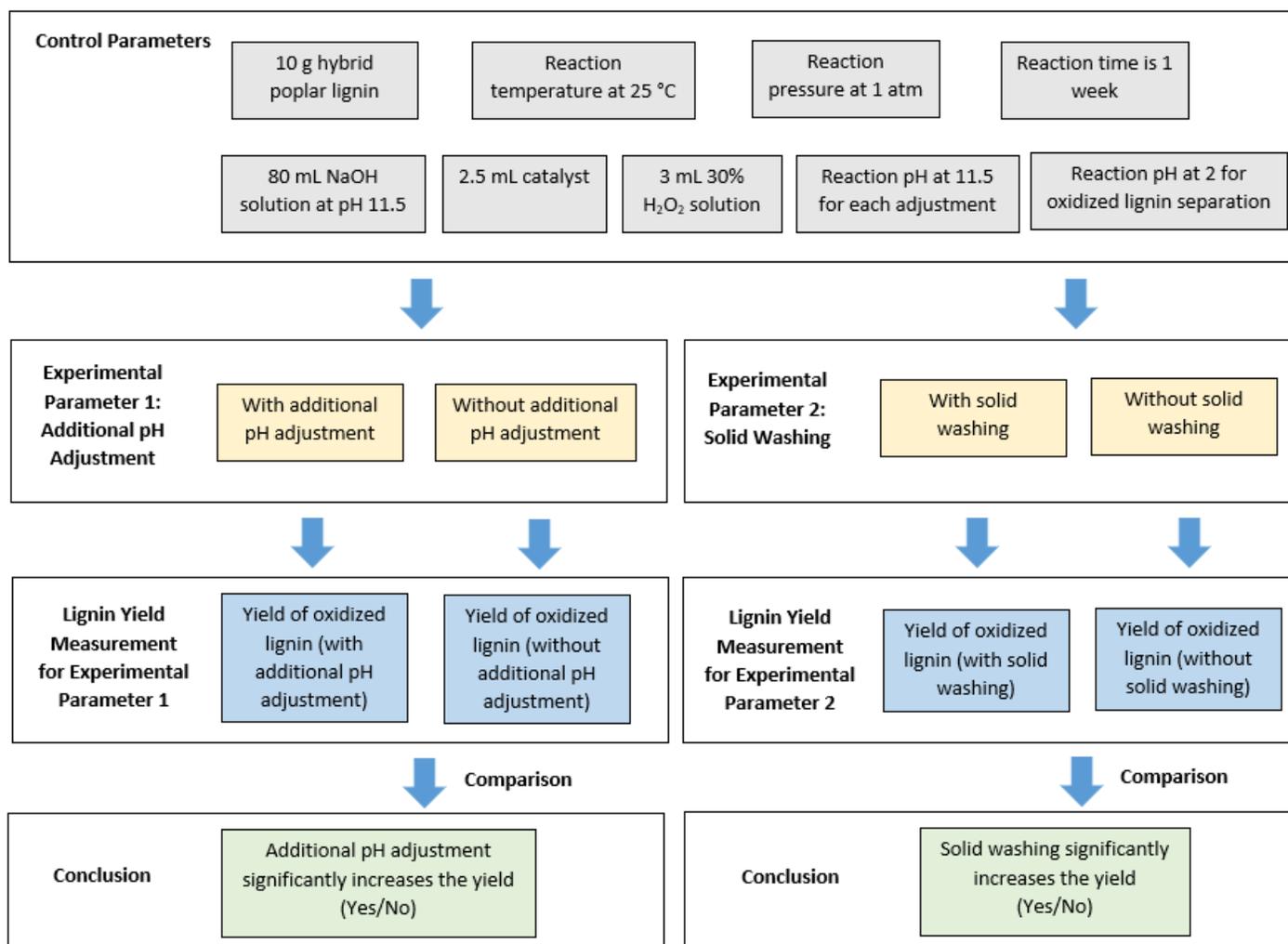
Figure 3.2: The process to determine if additional pH adjustment and solid washing have significant effects on the yield of oxidized lignin

2. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform infrared spectroscopy, an analytical technique that utilizes infrared radiation to characterize various compounds, is widely applied in organic synthesis and polymer science. When IR radiation passes through a sample, some radiation is absorbed by the sample and some passes through (is transmitted)[31]. By using an interferometer to modulate the wavelength of light from an infrared source, the spectrometer can measure the intensity of transmitted or reflected light as a function of its wavelength [32].

For this research, FTIR analysis was carried out to investigate changes in the structure of poplar lignin due to catalytic oxidation. More specifically, since the wavelength of light absorbed by the lignin reflects its molecular structure, infrared spectroscopy table (see Appendix 1) can help identify functional groups represented by abnormal regions in IR spectrums for raw lignin and oxidized lignin.

In general, raw lignin contains the following functional groups: phenolic hydroxyl, primary and secondary aliphatic hydroxyl, ketone and aldehyde groups. Moreover, chemical treatment may facilitate the formation of new functional groups such as carboxyl groups and carbonyl groups [31]. Most of these functional groups can be detected by FTIR spectrometer, which makes it a useful tool for lignin characterization.

Chapter 4
Results and Discussion

4.1 Quantification for Oxidation of Hybrid Poplar Lignin

In order to study the oxidation of hybrid poplar lignin, reactions were performed in aqueous solution of NaOH at pH 11.5 under a total pressure of 1 atm for a temperature at 25 °C. After multiple pH adjustments, reaction mixture was filtered, and a black liquor was collected. Later, the black liquor was acidified with dilute hydrogen chloride (HCl) solution and the precipitates were collected. During this process, the volume of 5M NaOH solution used for each adjustment and the volume of dilute hydrogen chloride (HCl) solution were monitored. The results are shown in Figure 4.1 and Appendix 2.

Although reaction conditions are held constant, the volume of 5M NaOH solution used for the same pH adjustment step varies depending on many factors,

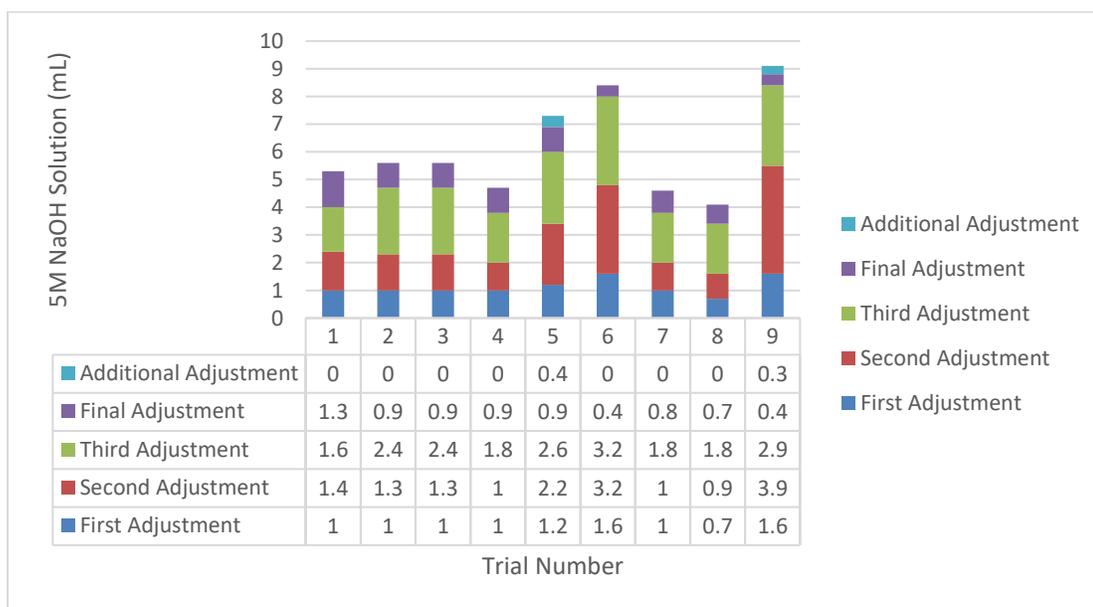|	1	2	3	4	5	6	7	8	9
Additional Adjustment	0	0	0	0	0.4	0	0	0	0.3
Final Adjustment	1.3	0.9	0.9	0.9	0.9	0.4	0.8	0.7	0.4
Third Adjustment	1.6	2.4	2.4	1.8	2.6	3.2	1.8	1.8	2.9
Second Adjustment	1.4	1.3	1.3	1	2.2	3.2	1	0.9	3.9
First Adjustment	1	1	1	1	1.2	1.6	1	0.7	1.6

Figure 4.1: The volume of 5M NaOH solution used for each trial

such as human errors, air humidity, and how lignin powder is stored. Figure 4.1

shows that the amount of 5M NaOH solution used to restore reaction pH are 0.7 – 1.6

mL for the first adjustment, 0.9 – 3.2 mL for the second adjustment, 1.6 – 3.2 mL for

the third adjustment, 0.4 – 1.3 for the final adjustment, and 0.3 – 0.4 for the additional

adjustment. In addition, as shown in Appendix 2, the volume of dilute hydrogen

chloride (HCl) solution is approximately 0.06 mL / 1 mL black liquor.

The amount of chemicals needed for the oxidation of hybrid poplar lignin is

summarized in Table 4.1. This table is a good reference when similar experiments are

conducted in the future.

Table 4.1: Summary of the amount of chemicals needed for the oxidation of hybrid poplar lignin

Mass of Hybrid Poplar Lignin (g)	10	/
Volume of aqueous solution of NaOH at pH 11.5 (mL)	80	/
Volume of Catalyst (mL)	2.5	/
Volume of H$_2$O$_2$ (mL)	3	/
First Adjustment (Volume of 5M NaOH solution in mL)	0.7 – 1.6	Add 0.7 mL first, then perform small increments
Second Adjustment (Volume of 5M NaOH solution in mL)	0.9 – 3.2	Add 0.9 mL first, then perform small increments
Third Adjustment (Volume of 5M NaOH solution in mL)	1.6 – 3.2	Add 1.6 mL first, then perform small increments
Final Adjustment (Volume of 5M NaOH solution in mL)	0.4 – 1.3	Add 0.4 mL first, then perform small increments
Additional Adjustment (Volume of 5M NaOH solution in mL)	0.3 – 0.4	Add 0.3 mL first, then perform small increments
Volume of Dilute HCl Solution / 1 mL Black Liquor	0.06	/

4.2 Parameter Investigation

Multiple trials with different pH controls and washing conditions were

performed to determine if additional pH adjustment and solid washing have

significant effects on the yield of oxidized lignin. The product yield for control was

calculated by taking the average of five replicates (trial 2, 3, 4, 7, and 8), the product

yield for additional pH adjustment was obtained from trial 5, and the product yield for

solid washing was obtained from trial 6. The results are shown in Figure 4.2.
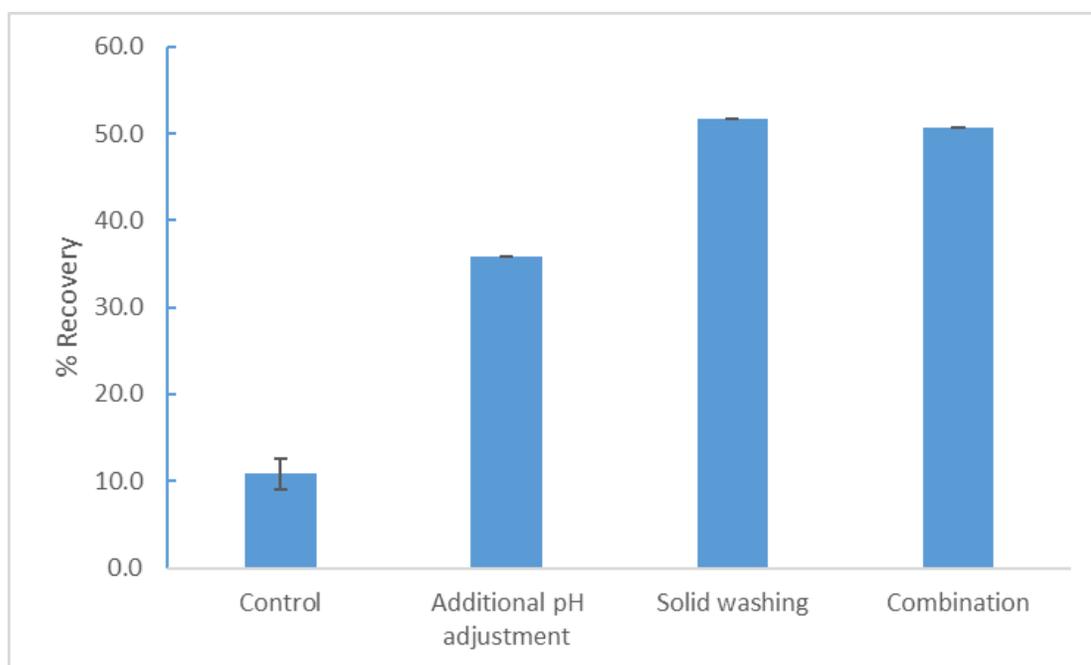
Figure 4.2: Product yield for each treatment (% recovery = the mass of products / the mass of raw hybrid poplar lignin). Standard error of mean for the control is 1.81 g.

Additional pH adjustment

By comparing % recovery for control and additional pH adjustment, the effect of

additional pH adjustment on the yield of oxidized lignin can be determined. Control

parameters were held constant for all trials, but the additional pH adjustment was

performed only for trial 5. Figure 4.2 shows that product yield for the additional

pH adjustment is 35.8%, which is 25% higher than that for the control. Therefore, it is reasonable to conclude that the additional pH adjustment can significantly increase the yield of oxidized lignin. This makes sense because adding more 5M NaOH solution will digest undissolved poplar lignin in the reaction mixture and more products will precipitate out during the acidification.

Solid Washing

Five replicates and trial 6 were studied to figure out the effect of solid washing on the yield of oxidized lignin. Similarly, the control parameters were held constant for all trials but the solid washing was performed only for trial 6. The result shows that product yield for the solid washing is 51.6%, which is 40.8% higher than that for the control. This washing step further removes concentrated black liquor in the residue, so more products are recovered from black liquor obtained after the filtration.

Combining additional pH adjustment and solid washing

For trial 9, both additional pH adjustment and solid washing were performed with all control parameters held constant. Meanwhile, the reaction pH was raised to 12 to dissolve more lignin. The result shows that the product yield for trial 9 is 50.7%, which is similar to that for trial 6. As such, the maximum yield of oxidized lignin is achieved by employing these two strategies.

4.3 Structural Changes Associated with Lignin Oxidation

As mentioned before, Fourier-transform infrared spectroscopy (FTIR) is a useful tool to investigate the interactions of molecules with various forms of electromagnetic radiation. In order to characterize structural changes associated with

lignin oxidation, IR spectroscopy analysis were performed on the raw hybrid poplar lignin and the product derived from it. The results are recorded as spectra in the 500 – 4000 cm^{-1} region shown in Figure 4.3 and Figure 4.4, from which a wealth of structural details can be observed.

Figure 4.3 shows all IR spectra have similar trends, meaning that the products obtained from trial 2, 5, 6, 7, and 9 have similar chemical structures. They didn't experience any structural change due to the additional pH adjustment, the solid washing, and the pH increase. On the contrary, the oxidized lignin went through three significant structural changes compared to the raw hybrid poplar according to Figure 4.4. Each difference in the spectra corresponds to a change of functional group or chemical bonding. The major structural changes are summarized in Table 4.2.

The first significant difference is the region located in the 997 – 1055 cm^{-1} wavenumber range. This sharp absorption band with strong intensity is caused by the presence of ethers. According to Figure 4.4, the sharp band only presents in the spectrum for raw poplar lignin, which means that some ethers are broken down during the process of oxidative depolymerization.

The second significant difference is the region located in the 1585 – 1700 cm^{-1} wavenumber range. Figure 4.4 shows that absorption band of the raw poplar lignin in this region is a little broad, but when it comes to the oxidized lignin, absorption band in the same region becomes too broad with no strong peak. This is due to the breakdown of carbon – carbon double bond (C = C) in alkenes and the oxidation of carbon – oxygen double bond (C = O) in ketones during the process of oxidative depolymerization.

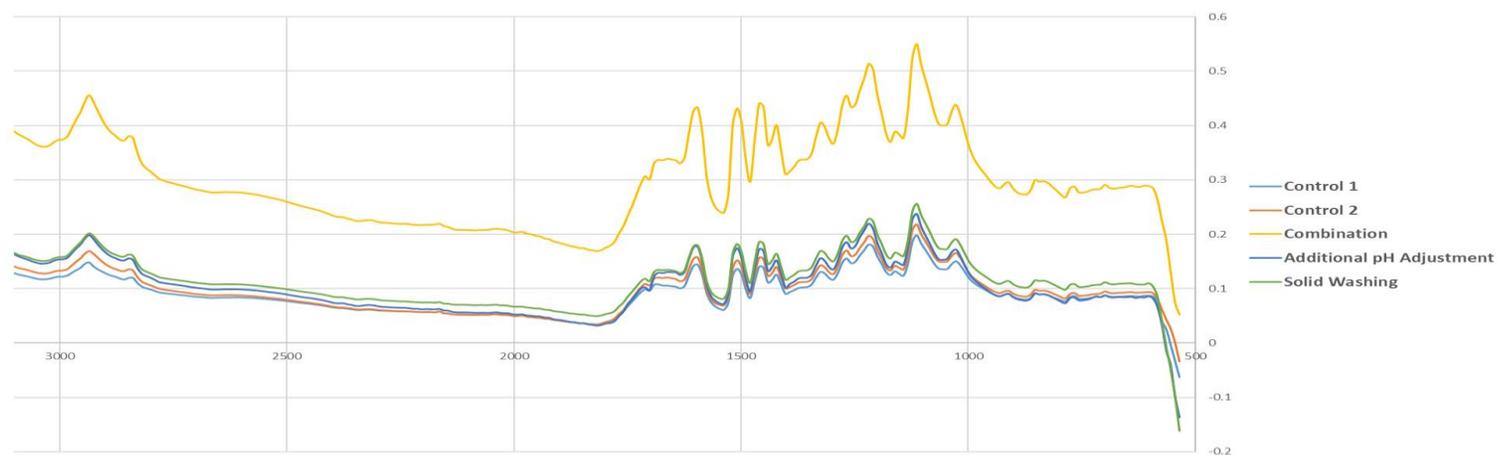**Figure 4.3**: Overlay of the FTIR spectra for trial 2, 5, 6, 7, and 9
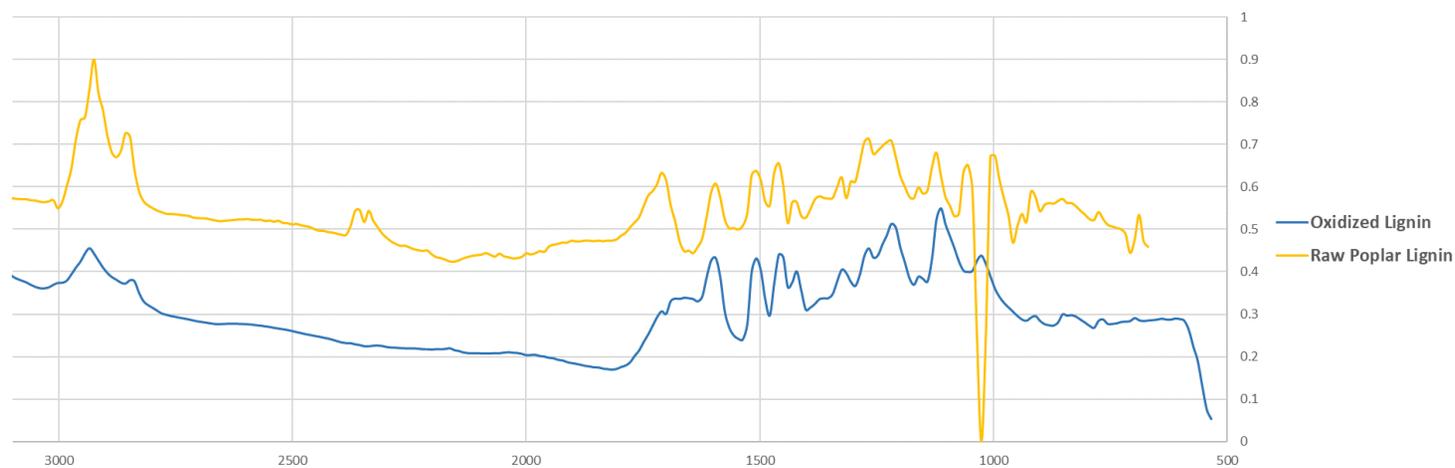
Figure 4.4: Overlay of the FTIR spectra for raw hybrid poplar lignin (up) and the product derived from it (down)

Table 4.2: Summary of structural changes

Structural Difference	Wavenumber Range	Breakdown of Functional Groups
R – O – R'	997 – 1055 cm^{-1}	Ether R–O–R'
C = C and C = O	1585 – 1700 cm^{-1}	Alkene C=C and Ketone R–C(=O)–R'
O – H	2848 – 3000 cm^{-1}	Carboxylic Acid R–C(=O)–OH
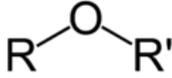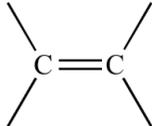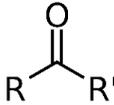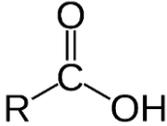
The third significant difference is the region located in the 2848 – 3000 cm-1 wavenumber range. As shown in Figure 4.4, this absorption band with strong intensity is much broader in the spectrum for the oxidized lignin, indicating the presence of oxygen – hydrogen single bond (O – H) in carboxylic acids.

4.4 Sources of Variability

Random Measurement Errors

Every measurement is a source of random error. For instance, the mass of oxidized lignin was measured three times by using the same balance, but the three values were slightly different. These errors cannot be avoided but they can be evaluated through

statistical analysis and can be reduced by averaging over a large number of measurements. Since the value differences caused by random measurement errors are too small, these errors won't have a significant effect on the results.

Storage Conditions of Lignin Powder

In order to make poplar lignin powder, the hybrid poplar lignin obtained from ZeaChem is dissolved in deionized water, filtered, and then dried in an isothermal drying oven at 50 °C. For this research, two batches of poplar lignin powder were utilized. One batch was properly stored in a food bag, but the other batch was exposed to the air for a long time. Moisture in the air absorbed by the dry lignin powder would make the actual weight of lignin powder lower than 10 g. Consequently, the yield of oxidized lignin would be significantly reduced.

Instruments

Two pH meters manufactured by different companies were used for the experiment. They might show different readings although correct calibration had been performed on both pH meters before the pH measurement. In addition, these two pH meters were not calibrated regularly to maintain accuracy, so they could drift from their calibrated settings. This would directly affect the amount of 5M NaOH solution added to the reaction mixture.

Chapter 5
Conclusion

Lignin conversion to renewable resources is a promising way to improve the economic value of lignocellulosic biorefineries and reduce greenhouse gas emissions. Importantly, oxidative degradation of lignin is never a simple process because it not only involves lot of chemicals but also requires appropriate reaction conditions. This research mainly focused on the alkaline hydrogen peroxide (AHP) treatment of hybrid poplar lignin catalyzed by Cu (II) 2,2′-bipyridine complexes. In general, three significant conclusions were made by analyzing the experimental data.

First, the AHP treatment of hybrid poplar lignin can be quantified to simplify future experiments. The minimum amount of 5M NaOH solution utilized to dissolve the poplar lignin are 0.7 mL, 0.9 mL, 1.6 mL, 0.4 mL, and 0.3 mL for each pH adjustment and the minimum amount of dilute HCl solution utilized to separate oxidized lignin is 0.06 mL / 1 mL black liquor. Second, the employment of additional pH adjustment and solid washing results in a significant improvement in the yield of oxidized lignin. More specifically, the maximum percent recovery was achieved by combining these two strategies. Third, the comparison of spectra shows that all the products have similar chemical structure while there are three significant structural differences between raw poplar lignin and oxidized lignin due to the breakdown of ethers, alkenes, ketones and carboxylic acids.

Undoubtedly, the alkaline hydrogen peroxide (AHP) treatment catalyzed by Cu (II) 2,2′-bipyridine complexes is an efficient method for recovering alkali-soluble lignin from enzymatic lignin. However, the major drawback of the AHP treatment is

that this method entails high consumption of chemicals such as oxidants and alkalis, which makes it uneconomical for industrial use. Therefore, further research in this area needs to be conducted to either develop a new efficient treatment method or improve the current treatment method.

References

[1] A. Xu, X. Guo, Y. Zhang, Z. Li, and J. Wang, "Efficient and sustainable solvents for lignin dissolution: aqueous choline carboxylate solutions," *Green Chem.*, vol. 19, no. 17, pp. 4067–4073, Aug. 2017.

[2] A. J. Stamm, "Density of Wood Substance, Adsorption by Wood, and Permeability of Wood," *J. Phys. Chem.*, vol. 33, no. 3, pp. 398–414, Jan. 1928.

[3] "Enzymes in Human and Animal Nutrition - 1st Edition." [Online]. Available: https://www.elsevier.com/books/enzymes-in-human-and-animal-nutrition/nunes/978-0-12-805419-2. [Accessed: 07-Feb-2019].

[4] A. Bhalla *et al.*, "Effective alkaline metal-catalyzed oxidative delignification of hybrid poplar," *Biotechnol. Biofuels*, vol. 9, no. 1, p. 34, Feb. 2016.

[5] J. Dai, A. F. Patti, and K. Saito, "Recent developments in chemical degradation of lignin: catalytic oxidation and ionic liquids," *Tetrahedron Lett.*, vol. 57, no. 45, pp. 4945–4951, Nov. 2016.

[6] C. Cheng *et al.*, "Catalytic Oxidation of Lignin in Solvent Systems for Production of Renewable Chemicals: A Review," *Polymers*, vol. 9, no. 6, p. 240, Jun. 2017.

[7] M. Cotoras, H. Vivanco, R. Melo, M. Aguirre, E. Silva, and L. Mendoza, "In vitro and in vivo evaluation of the antioxidant and prooxidant activity of phenolic compounds obtained from grape (Vitis vinifera) pomace," *Mol. Basel Switz.*, vol. 19, no. 12, pp. 21154–21167, Dec. 2014.

[8] J. M. W. Chan, S. Bauer, H. Sorek, S. Sreekumar, K. Wang, and F. D. Toste, "Studies on the Vanadium-Catalyzed Nonoxidative Depolymerization of Miscanthus giganteus-Derived Lignin," *ACS Catal.*, vol. 3, no. 6, pp. 1369–1377, Jun. 2013.

[9] C. Díaz-Urrutia, W.-C. Chen, C.-O. Crites, J. Daccache, I. Korobkov, and R. T. Baker, "Towards lignin valorisation: comparing homogeneous catalysts for the aerobic oxidation and depolymerisation of organosolv lignin," *RSC Adv.*, vol. 5, no. 86, pp. 70502–70511, Aug. 2015.

[10] J. Mottweiler, T. Rinesch, C. Besson, J. Buendia, and C. Bolm, "Iron-catalysed oxidative cleavage of lignin and β-O-4 lignin model compounds with peroxides in DMSO," *Green Chem.*, vol. 17, no. 11, pp. 5001–5008, Nov. 2015.

[11] X.-F. Zhou and X.-J. Lu, "Co(salen) supported on graphene oxide for oxidation of lignin," *J. Appl. Polym. Sci.*, vol. 133, no. 44, 2016.

[12] J. Mottweiler *et al.*, "Copper- and Vanadium-Catalyzed Oxidative Cleavage of Lignin using Dioxygen," *ChemSusChem*, vol. 8, no. 12, pp. 2106–2113, 2015.

[13] Y. Gao, J. Zhang, X. Chen, D. Ma, and N. Yan, "A Metal-Free, Carbon-Based Catalytic System for the Oxidation of Lignin Model Compounds and Lignin," *ChemPlusChem*, vol. 79, no. 6, pp. 825–834, 2014.

[14] W. Partenheimer, "The Aerobic Oxidative Cleavage of Lignin to Produce Hydroxyaromatic Benzaldehydes and Carboxylic Acids via Metal/Bromide Catalysts in Acetic Acid/Water Mixtures," *Adv. Synth. Catal.*, vol. 351, no. 3, pp. 456–466, 2009.

[15]   X. Gu, C. Kanghua, H. Ming, Y. Shi, and Z. Li, "La-modified SBA-15/H2O2 systems for the microwave assisted oxidation of organosolv beech wood lignin," *Maderas Cienc. Tecnol.*, vol. 14, no. 1, pp. 31–41, 2012.

[16]   W. Deng, H. Zhang, X. Wu, R. Li, Q. Zhang, and Y. Wang, "Oxidative conversion of lignin and lignin model compounds catalyzed by CeO2-supported Pd nanoparticles," *Green Chem.*, vol. 17, no. 11, pp. 5009–5018, Nov. 2015.

[17]   T. Voitl and P. Rudolf von Rohr, "Oxidation of Lignin Using Aqueous Polyoxometalates in the Presence of Alcohols," *ChemSusChem*, vol. 1, no. 8–9, pp. 763–769, 2008.

[18]   "Demonstration of a Process for the Conversion of Kraft Lignin into Vanillin and Methyl Vanillate by Acidic Oxidation in Aqueous Methanol - Industrial & Engineering Chemistry Research (ACS Publications)." [Online]. Available: https://pubs.acs.org/doi/abs/10.1021/ie901293p. [Accessed: 10-Feb-2019].

[19]   H. Werhan, J. M. Mir, T. Voitl, and von R. P. Rudolf, "Acidic oxidation of kraft lignin into aromatic monomers catalyzed by transition metal salts," *Holzforschung*, vol. 65, no. 5, pp. 703–709, 2011.

[20]   F. Napoly, N. Kardos, L. Jean-Gérard, C. Goux-Henry, B. Andrioletti, and M. Draye, "H2O2-Mediated Kraft Lignin Oxidation with Readily Available Metal Salts: What about the Effect of Ultrasound?," *Ind. Eng. Chem. Res.*, vol. 54, no. 22, pp. 6046–6051, Jun. 2015.

[21]   H. Deng *et al.*, "Perovskite-type Oxide LaMnO3: An Efficient and Recyclable Heterogeneous Catalyst for the Wet Aerobic Oxidation of Lignin to Aromatic Aldehydes," *Catal. Lett.*, vol. 126, no. 1, p. 106, Aug. 2008.

[22]   H. Deng *et al.*, "Activity and Stability of Perovskite-Type Oxide LaCoO3 Catalyst in Lignin Catalytic Wet Oxidation to Aromatic Aldehydes Process," *Energy Fuels*, vol. 23, no. 1, pp. 19–24, Jan. 2009.

[23]   C. Crestini, M. C. Caponi, D. S. Argyropoulos, and R. Saladino, "Immobilized methyltrioxo rhenium (MTO)/H2O2 systems for the oxidation of lignin and lignin model compounds," *Bioorg. Med. Chem.*, vol. 14, no. 15, pp. 5292–5302, Aug. 2006.

[24]   A. G. Demesa, A. Laari, I. Turunen, and M. Sillanpää, "Alkaline Partial Wet Oxidation of Lignin for the Production of Carboxylic Acids," *Chem. Eng. Technol.*, vol. 38, no. 12, pp. 2270–2278, 2015.

[25]   "Effect of solvent on hydrothermal oxidation depolymerization of lignin for the production of monophenolic compounds - ScienceDirect." [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0378382015302691?via%3Dihub. [Accessed: 10-Feb-2019].

[26]   "Catalytic Alkaline Oxidation of Lignin and its Model Compounds: a Pathway to Aromatic Biochemicals | SpringerLink." [Online]. Available: https://link.springer.com/article/10.1007%2Fs12155-013-9348-x. [Accessed: 10-Feb-2019].

[27]   V. Chaturvedi and P. Verma, "An overview of key pretreatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value added products," *3 Biotech*, vol. 3, no. 5, pp. 415–431, Oct. 2013.

[28]   J. A. da C. Correia, J. E. M. Júnior, L. R. B. Gonçalves, and M. V. P. Rocha, "Alkaline hydrogen peroxide pretreatment of cashew apple bagasse for ethanol production: Study of parameters," *Bioresour. Technol.*, vol. 139, pp. 249–256, Jul. 2013.

[29]   Z. Li *et al.*, "Chemical and structural changes associated with Cu-catalyzed alkaline-oxidative delignification of hybrid poplar," *Biotechnol. Biofuels*, vol. 8, no. 1, p. 123, Aug. 2015.

[30]   H. Korpi *et al.*, "Copper-2,2′-bipyridines: Catalytic performance and structures in aqueous alkaline solutions," *Appl. Catal. Gen.*, vol. 302, no. 2, pp. 250–256, Apr. 2006.

[31]   I. Bykov, *Characterization of natural and technical lignins using FTIR spectroscopy*. 2008.

[32]   "Fourier Transform Infrared Spectroscopy | FTIR Failure Analysis | FTIR Material Analysis." [Online]. Available: https://www.mee-inc.com/hamm/fourier-transform-infrared-spectroscopy-ftir/. [Accessed: 13-Feb-2019].

[33]   "(PDF) Palladium-catalyzed lignin valorization: Towards a lignin-based biorefinery." [Online]. Available: https://www.researchgate.net/publication/318570690_Palladium-catalyzed_lignin_valorization_Towards_a_lignin-based_biorefinery. [Accessed: 13-Feb-2019].

Appendices

Appendix 1: Infrared Spectroscopy Table

CHARACTERISTIC INFRARED ABSORPTION BANDS OF FUNCTIONAL GROUPS

Class of Compounds	Absorption, cm⁻¹	Intensity	Assignment	Class of Compounds	Absorption, cm⁻¹	Intensity	Assignment
Alkanes and	2850-3000	s	C-H stretch	**Carboxylic Acids**	2500-3500	s, broad	O-H stretch
Alkyls	1450-1470	s	C-H bend	R-C(O)-OH	1710-1715	s, broad	C=O stretch
	1370-1390	m	CH_3 C-H bend	C=C-C(O)-OH or	1680-1710	s	C=O stretch
	1365 + 1395	m	-CH(CH_3)$_2$ or	Ar-C(O)-OH			
	(two bands)		-(CH_3)$_3$ bend				
	715-725	w	-(CH_2)$_n$ bend	**Esters**	aliphatic 1160-1210	s-vs	O=C-O-C
					acetates ~1240		stretch
					aromatic 1250-1310		
Alkenes	3020-3140	w-m	=C-H stretch	R-C(O)-O-R	1735-1750	s	C=O stretch
	1640-1670	vw-m	C=C stretch	C=C-C(O)-O-R or	1715-1730	s	C=O stretch
RCH=CH$_2$	910 + 990	m + s	=C-H bend	Ar-C(O)-O-R			
	(two bands)			R-C(O)-O-Ar	1760-1790	s	C=O stretch
RR'C=CH$_2$	885-895	s	=C-H bend				
cis-RCH=CHR'	665-730	m-s, broad	=C-H bend	**Acyl Chlorides**			
trans-RCH=CHR'	960-980	s	=C-H bend	R-C(O)-Cl	1785-1815	s	C=O stretch
RCH=CR'R"	790-840	s	=C-H bend	Ar-C(O)-Cl	1770-1800	s	C=O stretch
Alkynes				**Anhydrides**			
R-C≡C-H	3265-3335	s, sharp	≡C-H stretch	R-C(O)-O-C(O)-R	~1750 + ~1815	s,s	C=O symmetric
	2100-2140	m	C≡C stretch	Ar-C(O)-O-C(O)-Ar	~1720 + ~1775	s,s	& asym. stretch
	610-700	s, broad	≡C-H bend		(both two bands)		
R-C≡C-R'	2190-2260	vw-w	C≡C stretch	**Nitriles**			
				R-C≡N	2240-2260	m-s	C≡N stretch
Alkyl halides				C=C-C≡N or	2220-2240	s	C≡N stretch
				Ar-C≡N			
R-F	1000-1350	vs	C-F stretch				
R-Cl	750-850	s	C-Cl stretch	**Amines**			
R-Br	500-680	s	C-Br stretch	R-NH$_2$	~3400 + ~3500	w	N-H symmetric
R-I	200-500	s	C-I stretch		(two bands)		& asym. stretch
					1580-1650	w-m	N-H bend
Alcohols	3300-3400	s, broad	O-H stretch	RR'N-H	3310-33350	w	N-H stretch
C=C-CH$_2$-OH	1035-1050	m-s	C-O stretch				
R-CH$_2$-OH (1°) or	1050-1085	m-s	C-O stretch	**Amides**			
C=C-CH(R)-OH				R-C(O)-NH$_2$	3200-3400 and	w-m	N-H symmetric
RR'CH-OH (2°) or	1085-1125	m-s	C-O stretch		3400-3500		& asym. stretch
C=C-CRR'-OH					(two bands)		
RR'R"C-OH (3°)	1125-1205	m-s	C-O stretch		1650-1690	s, broad	C=O stretch
Ar-O-H	1180-1260	m-s	C-O stretch		1590-1655	m-s	N-H bend
				R-C(O)-NH-R	3400-3500	w-m	N-H stretch
Ethers					1640-1690	s, broad	C=O stretch
R-O-R'	1085-1150	s	C-O-C stretch		1510-1560	m-s	N-H bend
Ar-O-R	1020-1075 and	m-s	=C-O-C sym.	R-C(O)-NR'R"	1630-1680	m-s	C=O stretch
	1200-1275		& asym. stretch				
	(two band)			**Nitro Compounds**			
				R-NO$_2$	~1550 and	s	N-O symmetric
Aldehydes	2700-2725	m	H-C=O stretch		~1370	s	& asym. stretch
R-CH=O	1720-1740	s	C=O stretch	C=C-NO$_2$ or	~1525 and	s	N-O symmetric
C=C-CH=O or	1685-1710	s	C=O stretch	Ar-NO$_2$	~1335	s	& asym. stretch
Ar-CH=O					(both two bands)		
Ketones				**Aromatic Compounds**	3010-3100	m	Ar C-H stretch
RR'C=O	1710-1720	s	C=O stretch		1450-1600	m-s	ring C=C stretch
C=C-C(O)-R	1665-1685	s	C=O stretch		(two to four bands)	sharp	
Ar-C(O)-R	1675-1695	s	C=O stretch	monosubstituted	730-770 and	s	C-H bend
four member	1770-1780	s	C=O stretch		690-710	s	C-H bend
cyclic					(two bands)		
five member	1740-1755	s	C=O stretch	o-disubstituted	735-770	s	C-H bend
cyclic				m-disubstituted	750-810 and	s	C-H bend
six member	1710-1720	s	C=O stretch		690-710	s	C-H bend
cyclic				p-disubstituted	810-840	s	C-H bend

Intensity abbreviations: vw = very weak, w = weak, m = medium, s = strong, vs = very strong

Appendix 2: Raw Data of Chemical Dosage during Lignin Oxidation

	Trial 1	PH	Trial 2	PH	Trial 3	PH	Trial 4	PH	Trial 5	PH	Trial 6	PH	Trial 7	PH	Trial 8	PH	Trial 9	pH
First adjustment (mL)	1	11.52	1	11.52	1	11.54	1	11.58	1.2	11.54	1.6	11.51	1	11.58	0.7	11.5	1.6	11.99
Second adjustment (mL)	1.4	11.52	1.3	11.53	1.3	11.41	1	11.53	2.2	11.48	3.2	11.52	1	11.52	0.9	11.55	3.9	11.99
Third adjustment (mL)	1.6	11.5	2.4	11.53	2.4	11.54	1.8	11.54	2.6	11.51	3.2	11.55	1.8	11.57	1.8	11.56	2.9	12.01
Final adjustment (mL)	1.3	11.53	0.9	11.5	0.9	11.25	0.9	11.52	0.9	11.54	0.4	11.51	0.8	11.52	0.7	11.56	0.4	12.01
Additional Adjustment (mL)	0		0		0		0		0.4	11.55	0		0		0		0.3	12.01
Volume of PH 11.5 H2O used (washing) (mL)											80						90	
Volume of Black Liquor (mL)			65		58		67		74		152		72		69		162	
Volume of HCl used (mL)			4.20		3.60		4.20		4.60		17.70		7.8		4.10		13.90	
mL HCl added/1 mL Black Liquid			0.06		0.06		0.06		0.06		0.12		0.11		0.06		0.09	
Mass of Oxidized Lignin (g)			1.20		0.55		0.86		3.58		5.16		1.63		1.18		5.07	
g Oxidized Lignin/1 mL Black Liquid			0.02		0.01		0.01		0.05		0.03		0.02		0.02		0.03	
% recovery			11.96		5.53		8.60		35.83		51.63		16.34		11.77		50.70	

Appendix 3: A proposed structural model for poplar lignin [33]