DICARBOXYLIC ACIDS PLATFORM CHEMICALS FOR VALORIZATION OF
BIOREFINERY LIGNIN

By

RUOSHUI MA

A dissertation submitted in partial fulfillment of
the requirements for the degree of

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Gene and Linda Voiland School of Chemical Engineering and Bioengineering

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of RUOSHUI MA find it satisfactory and recommend that it be accepted.

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Despite decades of effort, commercial development of biomass-to-biofuel conversion processes is still not an economically viable proposition. The emerging biomass refinery industry will inevitably generate an enormous amount of lignin as the solid waste stream. Development of selective biorefinery lignin-to-bioproducts conversion processes will play a pivotal role in significantly improving the economic feasibility and sustainability of biofuel production from renewable biomass. Due to the aromatic skeleton of lignin, most previous lignin valorization studies were limited to converting lignin to monomeric phenolics. Aromatic ring opened structures such as dicarboxylic acids (DCA) have been overlooked, though commonly observed during conversion. This thesis presents a new pathway for producing DCA as a new group of platform chemicals for lignin valorization, and specifically aims to develop a systematic understanding of the reaction mechanisms and to identify the key hurdles to optimizing conversion. To achieve this goal, a variety of oxidant and catalyst combinations were screened for oxidative depolymerization and conversion. A set of representative biorefinery lignin obtained from various commercial biorefineries for testing; the lignin samples were fully characterized by a number of selected analytical techniques. Representative monomeric and dimeric model compounds were used to help
identify key intermediates and interpret the reaction mechanisms. Statistical analysis methods were
developed as a new approach for providing quantitative guidance in lignin depolymerization research by relating and predicting lignin structure and reactivity. Techno-economic analysis (TEA) was conducted to direct future work toward addressing key bottlenecks in process costs. The new insights gained in this work inspired the development of novel catalytic oxidation pathways and processes to convert biorefinery lignin to DCA as a new group of platform chemicals/intermediates, enabling sustainable biofuel production from lignocellulosic biomass. These findings also provide new direction toward lignin partial depolymerization.
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CHAPTER ONE: INTRODUCTION

1.1 Booming Modern Biorefinery

Currently, fossil fuel, especially crude oil, supplied the majority of the world’s transportation fuel need and the raw materials/chemicals consumed providing a splendid standard of living for the billions of human-beings on this planet. One of the crucial challenges human being facing is to meet the increasing demand of world’s mobility and chemical need as the population grow. The daily rising consumption and diminishing reserves of crude oil, together with the growing concern of climate change caused by over-reliance on crude oil have made a must to prepare transition from non-renewable carbon to renewable carbon bioresources. Transforming lignocellulosic biomass to liquid fuel products is one of the pathways can truly sustain the exploding demand for transportation fuel, especially considering the high potential for the annual products of 220 biollion dry tons per year of biomass from forest and agricultural land worldwide. Utilization of these lignocellulosic biomass, whose energy content is approximately five times as the total worldwide crude oil consumption, is a promising opportunity can alleviate the dependence on fossil fuel.

United States is fortunate to possess abundant and diverse agricultural and forested residues. A recent study commented that US has a very high potential capability for the sustainable production of a biomass up to 1.3 billion dry tons annually collected from forests waste and agricultural residues. Integrating these huge biomass source and the advanced biomass conversion technologies with proper land use would provide practical solution towards the nation’s demand for liquid transportation fuels without have a negative impact on food supplies and commercial by-products production. However, despite decades of effort, commercial development of biomass-to-biofuel conversion processes is still not an economically viable proposition. Identifying value-
added co-products along with the production of biofuel provides a key solution to overcoming this economic barrier.

Lignin is the second most abundant component next to cellulose in almost all plant biomass. As those polysaccharides are primarily used for bioethanol production, an enormous amount of lignin will inevitably generate by the emerging biomass refinery industry. Development of selective biorefinery lignin-to-bioproducts conversion processes will play a pivotal role in significantly improving the economic feasibility and sustainability of biofuel production from renewable biomass. The urgency and importance of this endeavor has been increasingly recognized in the last few years.

1.2 What is Lignin?

Lignin is one of the three major components representing 15 to 40% dry weight of plants in lignocellulosic biomass together with cellulose and hemicellulose. As the polysaccharides have been the primary fraction used for biofuel and chemical production, the majority of lignin are generated as waste stream combusted to recycle the minimum value and is referred to as biorefinery lignin. "Development of selective and robust catalytic processes specifically designed for lignin conversion must be a core effort in a biorefinery program" is one of the chief recommendations from a recent US Department of Energy review on the role of lignin in biomass refineries. Effectively converting lignin into valuable products at reasonable costs is a very well recognized challenge epitomized by the myth "You can make anything out of lignin... except money". Thus far, there is no strategy yet envisaged for large scale conversion of lignin to valuable products.
Structurally, lignin is the largest source of renewable material with an aromatic skeleton as the major products of the phenylpropanoid pathways. Studies of various plant species designated that lignin derives from co-polymerization of three types of monolignols: \( p \)-hydroxyphenyl (H), guaiacyl (G), and syringyl (S), based on methoxy substitution pattern on aromatic rings. The phenylpropanyl subunits are known to be interlinked by two major categories of linkages: ether linkages (\( \beta \)-O-4, \( \alpha \)-O-4, 4-O-5) and carbon-carbon linkages (5-5, \( \beta \)-\( \beta \), \( \beta \)-5, \( \beta \)-1), among which \( \beta \)-O-4 is the most abundant in protolignin (around 40-60% depending on plant species). However, the interpreting of lignin molecular architecture is quite challenging due to 1) difficulties to efficiently solubilize and characterize degradation fractions; 2) their strong electronic stabilization energies between various subunits in the adjacent polymeric chains. The artistic depiction of lignin three dimensional structure has changed remarkably over a time span, since the detection of the abundant substructures, from a three-dimensional complex to a more linear macromolecular entities in very recent studies. This fact also implies the lacking of truly systematic research on lignin primary structures, and therefore lignin depolymerization mechanisms remain not clear.

Despite the heterogeneous nature and complexity inherited from its mother plant species, the isolation processes brought in more structural complexity since most lignin extraction pathways involve harsh experimental conditions sealed chemical modification pattern on the lignin structure. This thesis work collects a variety of representative biorefinery lignin extracted from different plant species and by popular biorefining processes. (Chapter Two) The comparative characterization of the lignin structures intend to provide new insights towards the understanding of lignin depolymerization mechanisms. (Chapter Five)
1.3 Current Understanding of Lignin Depolymerization Chemistry in Paper Making

Lignin depolymerization and utilization have been traditionally championed by the pulp and papermaking industry with the aim of producing high quality paper products and generating by-product revenue. A large body of literature has already been created decades ago on the development of practical delignification chemistries, with a primary focus on sustaining the pulp and papermaking industry. Among the various delignification technologies, oxidative lignin depolymerization was demonstrated to be a selective and economically viable method that has already been widely applied as standard pulp bleaching procedures. Chlorine is one of the most selective and efficient lignin oxidation reagents (Sarkanen and Ludwig 1971, Dence 1996, Dence and Reeve 1996); however, the formation of chlorinated phenolics as end products generated significant environmental concerns in the 1960s which led to the elimination of elemental-

The depolymerization of lignin using oxygen has attracted increasing interest since it does not require the addition of stoichiometric reagents, and produces water as the main by-product. Reactions between oxygen and lignin model compounds have been performed to understand reaction mechanism. (McDonough 1983, Gratzl 1987, Gratzl 1990, Bessone, Colombato et al. 2001) Oxygen delignification proceeds predominantly through a radical reaction mechanism. (Paice, Bourbonnais et al. 1995, Tarabanko, Fomova et al. 1995, Chen and Lucia 2002, Ji and Van Heiningen 2007) The phenolic units and ring-conjugated structures of lignin are more reactive with oxygen. (Kratzl, Claus et al. 1974, Gierer 1985, Gellerstedt, Gustafsson et al. 1986, Gellerstedt, Pranda et al. 1994, Johansson and Ljunggren 1994) Due to the diradical nature of molecular oxygen, the initial autoxidation of the structures leads to the formation of peroxy radicals, which convert into peroxy anions by abstracting one electron from the phenolate ions. The peroxy anion attacks the carbonyl carbon or a vinylogous carbon in the cyclohexadienone or quinone intermediates to form the C-O-O-C cyclic peroxides. Further rearrangement of the electrons within the four-membered cyclic structure converts lignin units into oxirane structures, muconic acids (cleavage of the aromatic ring), or a mixture of aldehyde fragments (cleavage of the Cα-Cβ bond). These reactions can yield three types of main products: (1) aromatic structures from side chain removal (Scheme 1.1A); (2) dicarboxylic acids from aromatic ring cleavage (Scheme 1.1B); and (3) biphenyl derivatives from oxidative condensation (Scheme 1.1C). Several recent studies have
demonstrated the application of different catalytic processes to produce phenolic aldehydes in considerable yields (e.g. vanillaldehyde, syringaldehyde, and \( p \)-hydrobenzaldehyde) under the presence of oxygen.\(^{9-12}\) One interesting observation from these studies is that these oxygen based catalytic processes seem to be more effective at cleaving \( \alpha \)-O-4 linkages whereas lower conversion yields are obtained with dimeric model compounds with \( \beta \)-O-4 linkages. Due to the radical reaction mechanism, condensation of lignin fragments appears to be inevitable in oxygen-based lignin conversions. The presence of biphenyl structures in the product mixture verifies the condensation reaction between fragmented monomers during oxidation.\(^{13-15}\)

Scheme 1.1. Oxygen oxidation of lignin mechanisms.
Scheme 1.2. Hydrogen peroxide oxidation of lignin mechanisms.

Hydrogen peroxide is recognized as an environmentally benign chemical, which makes it a common oxidant for pulp bleaching. (Beeman and Reichert 1951, Beeman 1953, Rapson 1963, Singh 1979, Gierer 1986) Most hydrogen peroxide bleaching processes are targeted at destroying
chromophore structures in lignin (Scheme 6A) rather than removing it. (Gierer and Imsgard 1977, Agnemo and Gellerstedt 1979, Sun, Tomkinson et al. 2000) For example, the unsaturated side-chain of 3-(4-hydroxy-3-methoxyphenyl)-2-acroline (Scheme 1.2A) is oxidized to produce \( p \)-hydroxybenzaldehyde, \( p \)-hydroxybenzoic acid and \( p \)-hydroxyguaiacol. (Gellerstedt and Agnemo 1980, Nonni, Falk et al. 1982) It is also worthy to note that these fragments can also be converted to quinones by hydrogen peroxide through Dakin-like oxidation of lignin phenolic units which in turn generate new chromophore structures (Scheme 1.2B). (Gierer 1986, Crestini, Crucianelli et al. 2010) (Nonni, Falk et al. 1982, Hall 1984, Gierer 1986) Hydrogen peroxide is also a potential oxidant for aromatic ring cleavage reactions. The \( o \)- and \( p \)-quinone rings are comprised of dual enone elements which offer multiple reaction sites that can be attacked by hydroperoxide anions, leading to ring opening products (Scheme 1.2C). The ring opening reaction can also occur on quinone rings attached to lignin polymers. (Gellerstedt and Pettersson 1982, Holmbom, Ekman et al. 1991, Crestini, Crucianelli et al. 2010, Ma, Guo et al. 2014) The presence of transition metal catalysts could generate reactive free radical species from hydrogen peroxide to accelerate the degradation of lignin into \( ortho \)- and \( para \)-quinone structures; however, they can also drive the reaction towards smaller molecules and reduce product selectivity. (Zhang, Wang et al. 1996, Egami and Katsuki 2007) In oxidative conversion of lignin to high value chemicals by heterogeneous catalytic processes, hydrogen peroxide has been demonstrated to be an efficient and environmental-friendly reagent for aromatic ring cleavage. (Lange, Decina et al., Crestini, Pro et al. 2005, Crestini, Caponi et al. 2006) Most previous hydrogen peroxide delignification work was carried out under basic conditions primarily due to the need for lignin activation and cellulose fiber protection. Though conversion of lignin in neutral or acidic media has not been investigated in detail, several studies have shown that aromatic ring opening reactions are favored under mildly
acidic buffers. (Crestini, Caponi et al. 2006, Ma, Guo et al. 2014) Using mild acid and hydrogen peroxide treatment is an appealing pathway to upgrade lignin to value-added products.

Scheme 1.3. Mechanisms for peroxyacid oxidation of lignin.

A peroxy acid is an acid that contains a perhydroxyl group (-OOH) in the place of the hydroxyl group of its parent acid. Peroxy acid can be derived from either mineral acids (e.g. sulfuric acid) or carboxylic acids (e.g. acetic acid, formic acid). Both have shown effectiveness in the delignification of wood and pulps. (Moyer 1968, Gierer 1986, Burns and Hardy 1993) The hydroxonium ion (HO⁺) produced from heterolytic cleavage of the peroxy bond is a strong
electrophilic species which can readily react with a number of electron-rich sites in lignin, including both aromatic ring and olefinic side chain structures. (Young and Akhtar 1998, Argyropoulos 2001, Zhang, Tu et al. 2011) The application of peroxy acids for wood pulping and bleaching has attracted a considerable amount of interest in the past and at present. (Barthelemy 1934, Haney 1948, Barros, Silva et al. 2010) The peroxy acid based pulping process, MILOX, has been evaluated at pilot scale; (Sundquist and Poppius-Levlin 1997, Suchy and Argyropoulos 2001) peracetic acid bleaching was implemented in one of Södra Cell pulp mills in Sweden. (Thomasfolk, Myhrman et al. 1996) Despite significant interest in applying peroxy acid for lignin removal from lignocellulosic biomass, (Johnson 1975, Lawrence, Mckelvey et al. 1978) there has been little effort expended towards applying peroxy acid chemistry to converting biomass lignin to value-added products. As demonstrated by previous studies, aromatic ring hydroxylation by HO$^+$ substitution/addition is a predominant reaction in peroxy acid delignification. This signifies great potential for the application of peroxy acid to produce phenolic compounds from lignin. The reaction mechanisms of peroxy acid oxidation highly resemble those of chlorination. (Nimz and Schwind 1979, Gierer 1986, Zhang, Tu et al. 2011) Studies of monolignol and β-aryl ether model compounds indicate the direct production of phenolic monomeric fragments (benzaldehydes and benzoic acids) by dehydration reactions and double bond cleavage. Moreover, ether bonds are hydrolyzed in the acidic media of peroxy acid solutions. However, most of current understanding towards peroxy acid depolymerization of lignin is based on the interpretation of model compounds depolymerization products. Thus, it is important to understand the kinetics and elucidate the detailed mechanisms involved in peroxy acid reactions with biomass lignin to maximize the potential of peroxy acids for value-added chemicals production from lignin.
1.4 Economical Potential Using Oxidative Lignin Depolymerization

Chlorine dioxide, oxygen, ozone, and peroxide are commonly used in the papermaking process of bleaching pulp to improve brightness. A considerable amount of these chemicals are consumed in the bleaching process. For example, a chlorine dosage of around 2--4\% based on dry weight of pulp is typically used for commercial chemical bleaching processes. The dosage of peroxide for mechanical pulp bleaching is between 4--8\%. Therefore, the chemical loading on lignin is approximately 100--200\% on a weight basis, resulting in a large carbon footprint. The cost of bleaching for white paper production is between $30--50 per metric ton of paper. It is conceivable that LMWPC, DCAs and quinones derived from lignin will provide greater value compared to commodity paper products and can lead to a diversity of commercial end-products that have the flexibility to be integrated into existing chemical industries. In addition to providing significant economic returns, oxidative conversion of lignin will also result in sustainable chemical processes that have little adverse effect on the environment. The development of catalytic processes to minimize chemical consumption and maximize products yield/selectivity will provide impetus to the successful application of oxidative chemicals for lignin valorization.

<table>
<thead>
<tr>
<th>Oxidation Reagent</th>
<th>Reactive Species</th>
<th>Mechanisms</th>
<th>Main Products</th>
<th>Potential Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine</td>
<td>Cl⁺</td>
<td>Electrophilic</td>
<td>chlorinated Phenol</td>
<td>ortho-quinone</td>
</tr>
<tr>
<td>Chlorine Dioxide</td>
<td>ClO₂, HClO, Cl₂</td>
<td>Radical, Electrophilic</td>
<td>dicarboxylic acids, simple acids</td>
<td></td>
</tr>
<tr>
<td>Oxygen</td>
<td>O₂•, HOO•</td>
<td>Radical, Electrophilic</td>
<td>vanillaldehyde/acid syringaldehyde/acid</td>
<td>low molecular weight aromatic aldehyde/acid</td>
</tr>
</tbody>
</table>
### Hydrogen Peroxide

- **HOO-**, **HO•**: Radical, Nucleophilic
- **O2•**, **HOO•**, **HO•**: Electrophilic

<table>
<thead>
<tr>
<th>Hydrogen Peroxide</th>
<th>HOO-, HO•</th>
<th>p-hydrobenzaldehyde/acid</th>
<th>dicarboxylic acids, simple acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>vanillaldehyde</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>syringaldehyde</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-hydrobenzaldehyde</td>
<td>low molecular weight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vanillic acid</td>
<td>aromatic aldehyde/acid,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-hydrobenzoic acid</td>
<td>dicarboxylic acids,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>methoxyhydroquinone</td>
<td>simple acids</td>
</tr>
<tr>
<td></td>
<td></td>
<td>acetic acid</td>
<td></td>
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</table>

### Ozone

- **O-O-O**: Electrophilic

<table>
<thead>
<tr>
<th>Ozone</th>
<th>O-O-O</th>
<th>methanol</th>
<th>simple acids</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>p-hydroquinone</td>
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<td></td>
<td></td>
<td>methoxyhydroquinone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vanillaldehyde</td>
<td>low molecular weight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>syringaldehyde</td>
<td>phenolics,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-hydrobenzaldehyde</td>
<td>benzoquinone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vanillic acid</td>
<td></td>
</tr>
</tbody>
</table>

### Peroxyacids

- **HO•**: Electrophilic

<table>
<thead>
<tr>
<th>Peroxyacids</th>
<th>HO•</th>
<th>methanol</th>
<th>simple acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>p-hydroquinone</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>methoxyhydroquinone</td>
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<td></td>
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<td>vanillaldehyde</td>
<td>low molecular weight</td>
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<td>syringaldehyde</td>
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<td>p-hydrobenzaldehyde</td>
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<td>vanillic acid</td>
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### 1.5 Catalysts for Lignin Oxidative Conversion

Though paper making industry has input significant efforts establishing lignin depolymerization chemistry, there has been little practical methods can be directly applied on biorefinery lignin for value-added products oriented research. The thriving field of catalytic chemistry has provided a variety of novel catalytic pathways and processes that are potentially suitable for supplement the oxidative lignin depolymerization mechanisms improving the selectivity and efficiency of lignin conversion to specific products. We reviewed the most recent progresses of catalysts developed to assist oxidative lignin depolymerization. Figure 1.2
summarized the representative catalysts developed by the leading groups and their major target products. Based on their constituents, these catalysts are separated into three main groups: 1) Inorganic metal-based catalysts; 2) Organometallics; 3) Organocatalysts.

![Figure 1.2. Summary of representative catalysts developed for lignin oxidative conversion.](image)

### 1.5.1 Inorganic Metal-based Catalysts

Transition metal are generally regarded as a detrimental species during paper making, catalyzing undesired reactions leading to cellulose degradation. (Springer and Mcsweeny 1993, Axegard, Bergnor et al. 1996, Brelid, Friberg et al. 1998, Norberg, Liden et al. 2001, Granholm, Harju et al. 2010) However, it is also known that transition metals, such as Cu(II), Fe(III), Mn(II, III), Co(II), and Zr(IV), can improve the efficiency of lignin oxidative depolymerization and facilitate
benzaldehydes/benzoic acids production. Side-chain cleavage and ether bond hydrolysis are the predominant reactions during the oxidation of model compounds. (Dicosimo and Szabo 1988, Partenheimer 2009) Metal ions catalysts can promote lignin oxidation by hydrogen peroxide through a Fenton reaction mechanism. Agnemo and Gellerstedt have demonstrated that O$_2^•$ and HO•, generated from homolytic decomposition of hydrogen peroxide, are essential to attacking lignin and generating aromatic ring opening products. (Pandell 1976, Gellerstedt and Agnemo 1980, Gellerstedt and Agnemo 1980) The homogeneous nature of most simple metal ion salts may be problematic during product recovery from aqueous reaction media. Thus, there are emerging research interests in developing heterogeneous transition metal catalysts.

Scheme 1.4. Example of Mechanisms for Lignin Oxidation Catalyzed by Co and Cu based perovskite-type metal oxide composites.
Metal oxide and composite metal oxides are the most common heterogeneous catalytic materials widely used in the petroleum, chemical, and environmental industries. (Hedges and Ertel 1982, Hautala, Peuravuori et al. 1997, Hepditch and Thring 1997, Goni and Montgomery 2000, Pena and Fierro 2001, Villar, Caperos et al. 2001, Otto and Simpson 2006, Feng, Yue et al. 2011, Hosseini, Tungler et al. 2011, Sirotin, Moskovskaya et al. 2011, Song, Jiang et al. 2011, Wei, Mao et al. 2011, Chen, Zhao et al. 2012, Kaiser and Benner 2012, Zhang, Li et al. 2013) Several structures of composite metal oxides, such as perovskite-type, rutile, wurtzite and chalcopyrite, have been demonstrated as effective catalysts for oxidation reactions. (Deng, Lin et al. 2008, Deng, Lin et al. 2009, Zhang, Deng et al. 2009, Deng, Lin et al. 2010) In general, heterogeneous catalysts functioned in the reaction solutions in a similar way as metal ions. For example, lignin oxidation by oxygen in the presence Co and Cu based perovskite-type metal oxide composites proceeded in a radical pathway (Scheme 1.4). However, the heterogeneous nature allows the reaction proceeds in a milder manner. Compared to homogeneous metal ion catalysts, relatively high lignin conversion rates and aromatic aldehyde yields were observed. (Deng, Lin et al. 2008) Thus, developing a mild-condition catalytic system with controlled reactive species is key to establishing a products-driven lignin conversion process. Developing a mild-condition catalytic system with controlled reactive species is key to establishing a products-driven lignin conversion process. Thus this will be the major catalytic systems developed in this thesis work.

Besides catalyzing direct oxidation reactions, some heterogeneous inorganic catalysts (e.g. TiO$_2$, ZnO) have been shown to facilitate photochemical degradation of organic compounds (Linsebigler, Lu et al. 1995, Yurdakal, Palmisano et al. 2008) as well as lignin in spent pulping liquor. (Crestini and DAuria 1997, Perez, Castellan et al. 1998, Amat, Arques et al. 2005, ...
Portjanskaja, Preis et al. 2006, Ma, Chang et al. 2008, Makhotkina, Preis et al. 2008, Portjanskaja, Stepanova et al. 2009, Ugurlu and Karaoglu 2009, Pan, Tian et al. 2012) Treatment of lignin black liquor by the TiO$_2$/UV photocatalytic system produced a mixture of aldehydes (e.g. vanillin, syringaldehyde, 3,4,5-trimethoxy benzaldehyde), acids (e.g. 4-hydroxybenzoic acid, vanillic acid) and alcohols (e.g. 2-hydroxy benzyl alcohol, catechol). (Ksibi, Ben Amor et al. 2003) A significant removal of dissolved organic carbon from black liquor (up to 40%) was observed after 30 minutes’ irradiation with UV light. Recent studies further demonstrate that $N$-doped TiO$_2$ can narrow the TiO$_2$ band gap, enabling photocatalysis to occur in the visible spectrum and potentially improving lignin oxidative degradation efficiency. (Portjanskaja and Preis 2007, Ma, Chang et al. 2008) One major challenge in applying photocatalytic oxidation to lignin for chemical production is controlling the non-selective properties of the radicals generated by electron-hole pairs in the aqueous system.

Scheme 1.5. Two steps’ reaction mechanism of Lignin aerobic oxidation catalyzed by POMs.

Polyoxometalates (POMs) is another interesting type of inorganic metal catalyst. They are structurally diverse anionic clusters consisting of $d^0$ metal cations, particularly W(VI), Mo(VI),
V(V) and Nb(V) and oxygen anions arranged in MO₆ octahedral units. The commercial availability and synthetic tractability (controllable acidity, solubility, thermal stability and redox potentials) make POMs attractive for delignification and lignin oxidative conversion. (Sonnen, Reiner et al. 1997, Weinstock, Atalla et al. 1997, Weinstock, Atalla et al. 1998, Bozell, Hoberg et al. 2000, Evtuguin, Neto et al. 2000, Pomar, Caballero et al. 2002, Yokoyama, Chang et al. 2004, Kim, Chang et al. 2006, Ruutunen, Tarvo et al. 2006, Kim, Chang et al. 2008, Kim, Chang et al. 2008, Voitl and von Rohr 2008) Generally, POMs oxidize lignin in two steps (Scheme 1.5). (Sonnen, Reiner et al. 1997, Grigoriev Vladimir, Hill Craig et al. 2001) Phenolic aldehydes are the predominant products from POM reactions on lignin samples or dimeric model compounds. (Yokoyama, Chang et al. 2004, Voitl, Nagel et al. 2010) Weinstock used [AlV⁵W¹₁O₄₀]⁶⁻ and Na₅[SiVW¹₁O₄₀] for aqueous phase aerobic oxidation of lignin/lignin model compounds under mild conditions. (Weinstock, Barbuzzi et al. 2001) A high reactivity between POMs and phenolic-type lignin model compounds was observed at room temperature. The phenolic units were mostly oxidized into para- and ortho-quinone structures. (Weinstock, Hammel et al. 1998) However, the non-phenolic type of lignin model compounds required more severe conditions to properly react. For example, a reaction temperature of 180 °C was required to achieve more than 90% degradation of 1-(3,4,5-trimethoxyphenyl)-ethanol after 6 h of reaction. (Gierer, Yang et al. 1992, Lissel, Dewal et al. 1992, Evtuguin, Daniel et al. 2000, Evtuguin, Neto et al. 2000) Heteropolyanions (HPA), one type of POMs, are soluble salts of polyoxoanions in the general formula of [X₃Mᵢₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒₒ_o) HPA-n (n being the number of vanadium atoms, n=2-6) are well-known highly selective catalysts in oxidative organic synthesis due to their quick re-oxidation by
Heterolytic cleavages of β-O-4 and alkyl-phenyl bonds were observed during the oxidation reaction catalyzed by HPA-5. As a result, methoxy-substituted phenolics and phenol ether substituted acids were formed. The non-phenolic lignin structural units are more stable towards oxidation in the HPA-5/O₂ system. Mechanism studies using dimeric model compounds suggested that hemolytic cleavage of β-O-4 and Cα-Cβ linkages are the main lignin depolymerization routes.

1.5.2 Organometallics

Inspired by peroxidase degradation (e.g. lignin peroxidase, manganese peroxidase) of lignin, applying biomimetic catalysts for lignin oxidative bleaching and delignification attracted a significant amount of interest in the past. However, there has been very little recent progress in this area. In contrast, the application of organometallics has advanced significantly (e.g. metalloporphorins) in other research fields. A comprehensive understanding of these biomimetic reagents on lignin conversion to value-added products is lacking.
Scheme 1.6. Reaction mechanism of (A) Co(Salen) complex with O₂; (B) Co(Salen) peroxo-complex oxidation of phenolic units in aqueous/polar solvent.

An early report on the reversible binding of O₂ by cobalt(II) Co(salen) complex has led to intensive research on cobalt-salen complexes and their potential for lignin oxidative depolymerization catalysts. (Tsumaki 1938, van Dort and Geursen 1967) Co(Salen) catalysts can form both monomeric Co(Salen)-superoxo complexes or dimeric-peroxo-bridged Co(Salen)-peroxo complexes (Scheme 1.6A) (Kervinen 2005) The mechanism of phenolic compounds oxidation mediated by the Co(salen)-superoxo complex is shown in the series of reactions below. (Canevali, Orlandi et al. 2002) (Satish and Ganeshpure 1986, Bozell, Hames et al. 1995, Bolzacchini, Chiavetto et al. 1996, Bolzacchini, Canevali et al. 1997, Musie, Wei et al. 2001, Kervinen, Kopi et al. 2003, Kervinen, Korpi et al. 2005) Metallosalen catalysts in combination with hydrogen peroxide has been shown to catalyze the oxidation of a wide variety of phenolics
and lignin model compounds primarily through side chain oxidation (Drago, Corden et al. 1986) to yield aldehydes and quinones (Scheme 1.6B). It is conceivable that these quinones will further undergo ring cleavage to produce DCAs. However, there has been little work done on investigating and demonstrating this potential pathway.

Metalloporphyrins is another type of organometallics have been investigated as biomimetic lignin peroxidases for lignin depolymerization. (Defrance, Meunier et al. 1992, Cui, Wijesekera et al. 1993, Kurek, Artaud et al. 1996, Keseru, Balogh et al. 1999) These biomimetic catalysts form highly oxidized metallo-oxo complexes upon reaction with the oxidant species. The metalloporphyrin complex can be activated by an oxygen donating species, including hydrogen peroxide, t-butyl hydroperoxide, sodium hypochlorite, potassium monopersulfate, and magnesium monoperoxyphthaltate. (Labat and Meunier 1989, Cui, Wijesekera et al. 1993, Cui and Dolphin 1995, Kumar, Jain et al. 2007, Zucca, Mocci et al. 2007) Metalloporphyrins typically react with the lignin molecule through a single electron transfer mechanism. The activated metallo-oxo complex abstracts an electron from the aryl group of the substrate, forming a radical cation intermediate, and concomitantly reduces the metallo-oxo complex. The reaction proceeds further through two competing pathways leading to either side chain oxidation or aromatic nuclei oxidation. (Artaud, Benaziza et al. 1993) Aromatic nuclei oxidation under higher temperature yields either quinone structures (ortho or para) or aromatic ring cleavage depending on the oxidation site on the ring which is governed by the electronic effects of the substituent groups. (Artaud, Benaziza et al. 1993, Kurek, Artaud et al. 1996, Fabbri, Aurisicchio et al. 2008) Degradation of the porphyrin ring is the main obstacle to its practical application.
Scheme 1.7. (A) General catalytic mechanism of MTO with H$_2$O$_2$; (B-D) Reaction schemes of MTO/H$_2$O$_2$ system with dimeric lignin model compounds.

A series of new organorhenium oxides such as η$_5$-(C$_5$(CH$_3$)$_3$)ReO$_3$ and (CH$_3$)ReO$_3$ have proven to be promising oxidation catalysts(Beattie and Jones 1979, Herrmann, Serrano et al. 1984) by forming monoperoxo complexes and bisperoxo complexes(Scheme 1.7A). (Herrmann, Fischer et al. 1991, Thiel, Fischer et al. 1993, Herrmann, Fischer et al. 1994, Zhu and Espenson 1995, Saladino, Neri et al. 2000, Saladino, Neri et al. 2002, Bernini, Mincione et al. 2006, Qiu, Zhang et al. 2009, Altmann, Cokoja et al. 2012, Michel, Cokoja et al. 2012, Jiang, Zhang et al. 2013, Meciarova, Mojzesova et al. 2013, Michel, Cokoja et al. 2013) etc. It has been postulated that the reactive peroxo species transfers one oxygen atom to the substrate via a concerted mechanism
without forming any radical species. (Bernini, Coratti et al. 2005) There is a series of reactions catalyzed by organorhenium in present of oxidants that include demethoxylation, ether bond hydrolysis, hydroxylation, and aromatic ring oxidation/cleavage. (Crestini, Caponi et al. 2006, Bernini, Gualandi et al. 2009, Crestini, Crucianelli et al. 2010) Based on dimeric model compounds study, the MTO/H₂O₂ system favored the cleavage of ether bonds with limited carbon-carbon bond cleavage. β-O-4 structured dimeric lignin model compounds (Scheme 1.7B) can be degraded into a mixture of (1) vanillic acids from Cα oxidation, (2) hydroxyketones from ether bonds hydrolysis and alkyl side-chain oxidation, (3) 2,6-dimethoxyphenols from ether bond cleavage, and (4) muconolactones from aromatic nuclei destruction. (Crestini, Caponi et al. 2006) However, reaction of β-β carbon bond linkages stopped at the demethoxylation step of the aromatic ring or the Cα-hydroxylation step without being further oxidatively degraded into monomeric fragments (Scheme 1.7C). (Lange, Decina et al., Ayres and Loike 1990) Oxidation of biphenyl lignin condensation is also investigated. (Scheme 1.7D). (Crestini, Caponi et al. 2006) Treatment of technical lignin samples (sugar cane lignin, kraft lignin, and hardwood lignin) with organorhenium oxides has demonstrated yielding modifications of lignin polymer structures due to the occurrence of aliphatic side-chain oxidation and aromatic ring cleavage. (Granata and Argyropoulos 1995, Jiang, Argyropoulos et al. 1995) The mild organorhenium oxidation process can be a promising and products-oriented lignin conversion method.
Similarly, the use of vanadium based complexes (Scheme 1.8A) has shown effectiveness for the selective C-O/C-C bonds cleavage of lignin model compounds that contain pinacol structures. (Geng and Zhong 2001, Hanson, Baker et al. 2010, Son and Toste 2010, Hanson, Wu et al. 2012, Zhang, Scott et al. 2012) The oxidation products from dimeric model compounds
appeared to be strongly influenced by the solvent. Dipicolinate vanadium(V) complex catalyzed aerobic oxidation of lignin model compounds in pyridine solvent (Scheme 1.8B1-B4). (Hanson, Baker et al. 2010) In a pinacol structure, the C-H bond adjacent to the alcohol moiety can break and oxidize to yield the corresponding alcohol and aldehyde (B2-B4). The reaction rate is slower when tested in DMSO solvent, and both C-H and C-C bonds cleavage products were observed from (B4). For both substrates (B3) and (B4), the C-C bonds between the alcohol and ether groups were broken during catalytic oxidation (Scheme 1.8C). For example, benzaldehyde and methanol were the major products from 1,2-diphenyl-2-methoxyethanol oxidation in DMSO, while benzoic acid and methyl benzoate were formed primarily in pyridine. These results suggested that vanadium based complexes can be a promising choice for oxidative lignin degradation for monomeric phenolic compound (mainly aromatic aldehydes/ketones) production. The composition of the resulting products could be a mixture of alcohols, aldehydes, ketones and carboxylic acids which may require further treatment before separation. Son and Toste reported a C-O bond cleavage of a pinacol structured lignin model compound by applying vanadium catalysts with a Schiff base ligand, which generated alkenes and 2-methoxyphenol as the major products. (Son and Toste 2010) It is observed that larger bite angles between the N atom and O atom (higher n value in A3) will increase the selectivity of C-O bond cleavage. The overall reaction is redox-neutral, and thus oxygen is not necessary for the redox of the catalytic complex. However, the presence of oxygen was observed to increase the reaction rate. Oxidation of similar model compounds by a 8-quinolinate vanadium complex (A4) was reported and an unusual cleavage of the C_aryl-C_phenyl bond was observed leading to the formation of a quinone structure and an acroline structure (Scheme 1.8D). (Hanson, Wu et al. 2012) It is also noted that only the phenolic units were reactive in this process. Jiang reported the selective aerobic oxidation of alcohols into their
corresponding aldehydes or ketones through a two-component system of VO(acac)$_2$/DABCO in an ionic liquid [bmim]PF$_6$, enabling the recycling and reuse of catalysts for three runs without any significant loss of catalytic activity.(Jiang and Ragauskas 2007) A variety of aromatic alcohols with different substituted groups were tested, with results indicating 90-100% conversion yields. There is a need for detailed evaluation of vanadium based catalysts for biorefinery lignin oxidation.

1.5.3 Organocatalysts

Organocatalysts is comprised of a large family of organic compounds typically containing nitrogen, sulfur, or phosphor as active site constituents to accelerate chemical reactions.(Schreiner 2003, Pozzi, Cavazzini et al. 2004) The novel use of this catalyst has gained increasing attention in the last few years largely due to the high selectivity and catalytic efficiency of organocatalysts in many reactions, including the Mannich reaction, the Aldol reaction, and oxidation.(Adam, Saha-Moller et al. 2001, Dalko and Moisan 2004) While showing great potential, very few of these reactions have been investigated in detail for biorefinery lignin conversion. Among these organocatalysts, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) has been investigated by several groups for the chemoselective oxidation of alcohols that are likely present in lignin structures.(Pozzi, Cavazzini et al. 2004, Pozzi, Cavazzini et al. 2004) This section will be focused on literature related to the mechanisms and potential products from TEMPO mediated oxidation of lignin model compounds with the objective of illustrating potential applications of organocatalysts for biorefinery lignin conversion.

Conversion of primary and secondary alcohols to aldehydes, ketones, or carboxylic acids under mild conditions using TEMPO with a series of oxidants (including HNO$_3$, bis(acetoxy)iodobenzene, trichloroisocyanuric acid, and NaOCl) has been demonstrated.(Verhoef, Peters et al. 1999, Jiang, Drouet et al. 2000, Tashino and Togo 2004, Barbier, Breton et al. 2006)
As shown in Scheme 1.9A, a simplified two-cycle mechanism has been proposed based on reactions with monomeric lignin model compounds. (Cui, Wijesekera et al. 1993, Crestini and DAuria 1997, Cyr, Chiltz et al. 2000, Herrmann, Weskamp et al. 2000) Electron(s) in the oxidant are transferred through NO/NO$_2$ and TEMPO/TEMPOH pairs to the alcohol, leading to the oxidation of the alcohol to its corresponding aldehyde/ketone structure. The TEMPO system primarily functions as a mediator and can be regenerated during the reaction. It has been speculated that TEMPO oxidation can weaken C-C/C-O bonds adjacent to the C$_\alpha$ hydroxyl which may lead to potential applications in lignin depolymerization. This hypothesis was tested by using TEMPO to oxidize dimeric lignin model compounds which share the pinacol structure (Scheme 1.9B). (Rahimi, Azarpira et al. 2013) The oxidation of the C$_\alpha$ hydroxyl to the corresponding aldehyde/ketone was confirmed for the dimers, but cleavage of the C-C or C-O bond was not observed by TEMPO treatment alone. However, the addition of alkaline or reductive catalysts did promote the formation of monomeric phenolic products due to either C-C or C-O cleavage (Scheme 1.9C). (Nguyen, Matsuura et al. 2013, Rahimi, Azarpira et al. 2013)

So far there has been no success in applying the TEMPO system to macromolecular lignin for chemical production. It appears that TEMPO mediated oxidation is hampered by its limited selectivity towards the pinacol structure, which may only constitute a small fraction of most biorefinery lignin samples. Similar to MOFs, organocatalysts are likely to offer selective oxidation of alcohols to ketones or carboxylic acid structures. This method can be applied to refine and modify phenolic compound mixtures obtained from other lignin oxidation methods to improve phenolic aldehyde or acid yields. Besides the TEMPO system, a few other organocatalysts have also been reported as effective mediators in related fields, such as chiral dioxiranes for epoxidation,
planar-chiral bisflavin for Baeyer-Villiger reaction, and so on. (Dalko and Moisan 2004) Future work on related catalytic processes for lignin oxidative conversion may yield promising results.

Scheme 1.9. (A) Proposed two-cycles oxidation mechanisms of metal free catalyzed alcohol oxidation; (B) Selective oxidation of lignin dimeric model compounds by metal free catalytic system; (C) Further depolymerization of oxidized B.
1.6 Thesis Objectives

Lignin depolymerization and aromatic ring cleavage has long been observed in delignification and paper bleaching process. However, the dicarboxylic acids generated during aromatic ring cleavage reaction has been overlooked as potential value-added platform chemicals. Thus, the main objective of this dissertation is to develop selective oxidation pathways to produce dicarboxylic acids as value-added platform chemicals from biorefinery lignin, establishing a comprehensive understanding of the reaction mechanisms and identify the key hurdles to optimize the process.

In order to achieve the objective, the research will start by preparing a number of representative lignin substrate isolated from popular pretreatment methods applied in current biorefinery. The lack of existing theory interpreting exact lignin macromolecular structure is one of the major hurdle in understanding the lignin oxidative conversion mechanisms and as well limit the development of new chemistry and pathways. Thus, the lignin substrate structural characteristics will be further investigated integrating a selected group of analytical method including traditional chemistry methods (such as acidolysis, thioacidolysis, alkaline nitrobenzene oxidation, CHNO elemental analysis), spectroscopic methods (e.g. FT-IR, NMR), and other instrumentation methods (e.g. Gel Permeation Chromatography, scanning electron microscopy, x-ray diffraction). Oxidative delignification has long been applied as the practical method in paper-making industry to remove the lignin selectively. During the bleaching processes, sometimes the aromatic rings were readily to cleave when attacked by reactive oxidative species resulting in the formation of dicarboxylic acids. Transition metal derived catalysts were screened to explore the synergetic effect together with peroxxygen oxidant (hydrogen peroxide and peroxy acids) to depolymerize lignin to DCAs. GCMS together with other color metric method (Folin-phenol assay)
were used to quantitatively analyze the lignin products yield. Representative monomeric and
dimeric model compounds were used to further investigate the reaction mechanisms. The results
from model compounds study provides the key evidences for the interpretation of the lignin
conversion mechanisms. During the study, it was found the lignin degraded at a sequential pathway:
lignin degradation from macromolecular to LMWPC by side-chain removal and the subsequent
aromatic ring cleavage of LMWPC to DCA. Improving the first step lignin depolymerization to
LMWPC is likely a key to improve the overall DCA production, and thusly was studied. Statistical
analysis was conducted using the structural information from the representative lignin
characterization and their reactivity toward lignin depolymerization and aromatic ring opening.
This step allows us to provide a tool for the advanced research in lignin re-engineering and
valorization. Results from techno-economic analysis were also adapted as guidance to help
improve the strategy and propose the perspective to future study.

1.7 Organization of the dissertation

This dissertation represents the major manuscripts that I have written during my Ph.D
research. There are seven chapters in total:

Chapter One consisted of an overview introduction, covering the essential information
such as backgrounds, hypothesis and objectives. The backgrounds introduction and literature
review part in this chapter has been expanded and published as a review paper. The major part of
the manuscript is written by me with the guidance of Dr. Xiao Zhang.

Chapter Two described the source of materials and the experimental details used in this
study. The detailed mother biomass sources and the pretreatment procedure were presented in this
chapter. I also briefly reviewed the specific feature of each pretreatment method and their potential
impact on lignin structure. The summary of theory and experimental procedure of analytical methods applied in this study are also integrated and presented in this chapter. I categorize the analytical methods into four sections, including 1) methods used for lignin composition analysis; 2) spectroscopic methods used for lignin characterization; 3) instrumentation used to characterize lignin physical properties related to its conversion reactivity; 4) chromatography and colorimetric methods used to separate, identify and quantify the low-molecular weight degradation products and model compounds in this study.

Chapter Three is a published manuscript about a new reaction pathway selectively converting biorefinery lignin to dicarboxylic acids (DCA). Oxidative aromatic ring cleavage to yield muconic acid and its derivatives is a well-recognized reaction. Although DCA and their derivatives have been detected in the reaction mixture from oxidative delignification of wood pulp, there has not been successful attempt to produce DCA from biomass lignin. The goal of this work is to introduce a new chemistry pathway producing lignin to dicarboxylic acids to expand the potential products platform in lignin valorization. Such goal is achieved by studying chalcopyrite/H\textsubscript{2}O\textsubscript{2} catalyzed lignin depolymerization under a mild acid condition. Lignin was found to degrade at a sequential manner which can involves two major steps: 1) lignin depolymerization to LMWPC; and 2) aromatic ring cleavage of LMWPC to DCA. Further investigation using hydromatairesinol and a group of monomeric model compounds confirmed the sequential degradation mechanism and the identified intermediates helped proposed a preliminary mechanism for aromatic ring cleavage. By adding α-tocopherol, the radical scavenger reagent, to the guaiacol oxidation provides additional evidence that two different reaction mechanisms occurred during lignin oxidative depolymerization and aromatic nuclei cleavage. The results from
Chapter Four is a published manuscript investigating peroxy acid depolymerization of biorefinery lignin to produce selective monomeric phenolic compounds (MPC), in order to improve the overall conversion of lignin to DCA. Study in Chapter Three suggested a two stage reaction mechanisms for lignin conversion to DCAs, and the reaction yields was not satisfied might due to the non-sufficient depolymerization of lignin to LMWPC. Thus, the goal of this research is to bridge the advanced oxidative delignification chemistry developed by paper-making industry, exploring the opportunity to use peracetic acid (PAA, a type of peroxy acid) to effectively depolymerize lignin to MPCs. After extensively review of the chemistry proposed in previous preliminary research, I screened a number of transition metal/metal oxides catalysts to find a proper synergy effect promoting the lignin depolymerization and products yield. Representative model compounds, iso-eugenol and hydromatairesinol, were utilized as model compounds to illustrate the key chemistry mechanisms during PAA treatment of lignin. Further evidence from this mechanism study showed the potential existence of a special type of reactive species that lead to the high reaction selectivity. Dr. Xiaowen Chen provides the raw corn stover lignin substrate for the research. Dr. Vincent R. Hebert, Dr. Jinwen Zhang and Dr. Michael P. Wolcott provides valuable suggestions and instrumentation supports on substrate and products characterization. The manuscript is written with the guidance of Dr. Xiao Zhang.

Chapter Five is a ready-to-submit manuscript about to investigate and establish a correlation between biorefinery lignin structural features and their oxidative conversion reactivity. Currently, there still lacks an agreed depiction of lignin structure. Most of previous lignin research are conducted at a trial-and-error style without a guidance. The goal of this work is to provide
systematic methodologies to characterize selected representative biorefinery lignin, and utilize the statistics method to investigate the correlation between the structure features and lignin conversion reactivity. In order to obtain as much details of lignin characteristics, I integrate the state-of-art instrumentation (CHNO elemental analysis, $^{13}$C NMR, $^{1}H$/$^{13}$C HSQC NMR, FT-IR, GPC, SEM, B.E.T.) in lignin chemistry together with traditional wet chemistry (acidolysis, thioacidolysis, alkaline nitrobenzene oxidation). The reactivity of these lignin towards oxidative depolymerization and aromatic ring opening were investigated. R Statistics was used as a tool to help evaluate the linear correlation between these lignin structural features themselves, and to explore their correlation to conversion reactivity by conducting multiple-variable linear estimation(MVLE). The manuscript is written by me with the guidance of Dr. Xiao Zhang.

In order to evaluate the economic viability to produce DCAs as new platform chemicals from biorefinery lignin, we conducted a techno-economic analysis using Aspen Plus® in order to identify the key technical or economic hurdle during the commercialization. (**Chapter Six**) Based on the results obtained from the TEA study, I propose a new strategy to partially depolymerize lignin(PDL) to oligomeric size for further conversion to DCA to improve the overall economics. The hydromatairesinol was used as a model to demonstrated the viability to selective convert oligomeric PDL to DCA. The TEA of the process is finished under assistance from Mond Guo. The work presented in this chapter is being prepared for publication.

Finally, I summarized my thesis work and the major conclusions. I also provide recommendation for the future studies in **Chapter Seven**. The results from Chapter Six suggested a promising strategy to produce partial depolymerized lignin (PDL) for the further conversion to aromatic ring opening DCAs, which can significantly lower the dosage of oxidant consumed. It leaves the key of the process on identify proper lignin partial depolymerization approach. In this
chapter, I presented the preliminary results obtained using deep eutectic solvent to produce ether-linkage free PDL from woody biomass.
1.8 List of Publications

The thesis is based on the following original research papers which are referred to in the text as Chapter three to Chapter Five. The publications are reproduced with kind permission from the publishers. Additional unpublished material is also presented.


CHAPTER TWO: MATERIALS & METHODOLOGY

In this thesis study, we have evaluated a wide selection of biorefinery lignin which covers a broad variety according to their mother biomass and isolation processes. The heterogeneous nature and the structural complexity of lignin significantly limit the accuracy of lignin structure determination. In another word, not a single method can fully interpret the lignin structure representatively. Thus, integration of a set of lignin characterization methods to understand the lignin structure at monomeric, inter-unit and also molecular level is the base to identify efficient valorization pathways. In this chapter, we will have a brief review of the main lignin characterization methodologies applied in my thesis study.

2.1 Preparation of Biorefinery lignin

a. **Diluted acid corn stover lignin (DACSL)** was collected from the corn stover-to-bioethanol conversion process via diluted acid pretreatment carried out in the pilot plant of the National Renewable Energy Laboratory (NREL, Golden, CO). (Pretreatment 2011) Diluted acid (e.g., \( \text{H}_2\text{SO}_4, \text{HCl} \)) pretreatment has been applied on a variety of lignocellulosic materials. Diluted acid pretreatment is typically carried out at a temperature above 160 °C with mineral acids as catalyst. During this acid treatment process, the majority of lignin remains as a solid residue after enzymatic hydrolysis. Previous work has shown that diluted acid lignin contains low carbohydrate content. The ether and ester linkages are partially cleaved during diluted acid pretreatment, and thus may also destroy the matrix structure and generate low molecular-weight lignin fragments with increased hydroxyl group content. However, the detailed structural information of the lignin samples treated with diluted acid still needs further investigation. Crude lignin was collected as solid residue after the hydrolysis and fermentation process. The crude residual lignin was purified by diluted alkaline extraction to produce DACSL.
b. Crude steam explosion spruce lignin was collected from spruce-to-ethanol conversion process via steam explosion pretreatment carried out in the pilot plant of FPInnovations, Forintek Division (Quebec, QC). Steam explosion is one of the leading biomass deconstruction/pretreatment methods providing substrates for enzymatic hydrolysis. Similar to
diluted acid lignin, steam exploded lignin (SEL) remains as a solid residue after enzymatic hydrolysis. A number of previous studies have characterized the softwood lignin separated from the steam explosion process. Typical SEL has higher phenolic hydroxyl group content, slightly lower methoxy group content and more frequent carbon--carbon interunit linkages compared to its native form. High mass recovery of lignin can be readily attained from steam-explosion-based biomass conversion processes. Crude lignin was collected as solid residue after enzymatic hydrolysis of steam exploded spruce. (Fang, Deng et al. 2011) The crude residual lignin was purified by diluted alkaline extraction to produce SESPL.

c. Soda-WSL was collected in the lab scale sodium hydroxide solution treatment of wheat straw. Soda treatment is a typical type of alkaline process commonly applied in paper making and biorefinery. The alkaline conditions likely resulted in the breakdown of the protolignin and thus extracted lignin by dissolution of lignin fragments. The crude lignin was extracted and remain in the extraction solution. The lignin was precipitated by neutralizing the pH of the black liquor to a pH of 2-4. The thorough wash of lignin with excessive amount of water was required to remove the salt after neutralization. The labile cleavage of lignin-carbohydrate complex resulted in a significant amount of residual saccharide in the isolated alkaline lignin. In addition, the residue mineral is higher compare to other isolation processes due to the neutralization step for lignin collection.

d. Milled wood lignin of D. Fir was collected from the mechanical milling of D. Fir wood to ethanol processes. Mechanical refining is an industrial process used to produce fibrous pulp from biomass, predominantly wood, by a combination of heat and mechanical force. This is the existing commercial process to make either paper products such as newsprint or fiber board products such as medium density fiber board. With minimized structural modification by chemical treating, the
milled wood lignin is considered the most resemble to native lignin existing in biomass. The crude lignin was collected as a solid residue after enzymatic hydrolysis of a lab scaled ball-milled D. Fir wood. The crude residual lignin was further purified by a common dioxane/water/HCl extraction to produce MWL for this study.

e. SPORL lignin of D. Fir was provide by Dr. Zhu from FPL/USDA. SPORL (sulfite pretreatment to overcome recalcitrance of lignocellulose) is a biomass pretreatment modified from sulfite pulping. SPORL yields sulfonated lignin (lignosulfonates) both dissolved in spent pretreatment liquor and retained in the solid substrate. The existing commercial products from lignin are predominantly derived from lignosulfonates. Compared to other types of biorefinery lignin, lignosulfonates have higher average molecular weight and hydrophilicity.[23c] SPORL lignin also likely contains a high sulfur content compared to other biorefinery lignin.

f. DES lignin was a novel type of lignin developed in our lab applying deep eutectic solvent as alternative to ionic liquid to extract high purity lignin with minimum loss of saccharides. The crude lignin was collected soluble in the DES solution and could be recovered by water/ethanol mixture precipitation. Simply wash the collected lignin with water/ethanol mixture multiple times could obtain a high purity lignin (>95% klason lignin purity) with little present of ash or sugar residue. Moreover, this process not only extract lignin in high purity, as well in a high recovery yield (~60% of original lignin determined by comparing lignin content of residue before and after treating). Our previous study has shed some lights on the characterization of the DES lignin meant to elucidate the extraction chemistry and mechanisms, and this study will provide detailed insights compare it to other biorefinery lignin.

g. Alkaline lignin was purchased from Sigma-Aldrich, a standardized commercial lignin as a control in this study. It is another type of lignosulfonate isolated from gynomsperm wood species.
2.2 Characterization of Biorefinery lignin

2.2.1 Lignin Composition Analysis

a. Acid hydrolysis determination of lignin purity.

The determination of the lignin content is an analysis performed routinely for characterizing lignocellulosic material, and as well the basic to evaluate the quality of the lignin isolation processes. The general meaning of determination of lignin involves the assessing the impact of the chemical, physical or biological treatment of the wood/grass. In the development of wood chemistry, various methods were proposed for this purpose, but however hardly get everyone agreed on. Thus there still lacks a definitive structural formula of lignin currently. Determining the lignin content in an isolated lignin samples or pretreatment residue employs sulfuric acid to promote carbohydrate hydrolysis is a widely accepted method. Basically carbohydrate component of the lignified materials was hydrolyzed and solubilized, leaving the lignin as a solid residue to be determined gravimetrically. The remaining solid collected afterwards called acid-insoluble lignin, or more commonly as Klason lignin, name after the pioneer in wood chemistry. This provide a quick screening of the lignin content in a lignocellulosic material, no matter whether it has undergoing a pretreatment or not.

**Materials:** Sulfuric acid, 72% H2SO4 solution is prepared by carefully pouring 665 ml of concentrated H2SO4 (95.5 to 96.5%) into 300 ml of water and, after cooling, diluting to 1000 ml. The strength is adjusted to 24 ± 0.1 N after titration with standard alkali, measurement of specific gravity. A variation of 0.1 % in the strength of acid at this concentration causes a change of 0.0012 in specific gravity. The acid is cooled to 10-15°C before use.

**Acid Hydrolysis Protocol:** Biomass should be free of extractives and with adequate particle diameter using mesh 20 (850μm) and 80 (180μm). Weigh 300.0 ± 10.0 mg of the sample into a
tared pressure tube. Add $3.00 \pm 0.01$ mL (or $4.92 \pm 0.01$ g) of 72% sulfuric acid to each pressure tube, incubate at 30°C during 60 minutes. Stir the sample every five to ten minutes without removing the sample from the bath. Dilute the acid to a 4% concentration by adding $84.00$ mL deionized water. Mix the sample by inverting the tube several times. Prepare a set of sugar recovery standards (SRS). Weigh out the required amounts of each sugar (glucose, xylose, galactose, arabinose and mannose) and add $10.0$ mL deionized water. Add $348$ μL of 72% sulfuric acid. Transfer the SRS to a pressure tube and cap tightly. Autoclave the sealed samples and sugar recovery standards for one hour at 121°C. After completion allow the hydrolyzates to slowly cool to near room temperature before removing the caps. Vacuum filter the autoclaved hydrolysis solution through one of the previously weighed filtering crucibles. Capture the filtrate in a filtering flask. Transfer an aliquot, approximately 50 mL, into a sample storage bottle. This sample will be used to determine acid soluble lignin as well as carbohydrates, and acetyl if necessary. Acid soluble lignin determination must be done within six hours of hydrolysis. If the hydrolysis liquor must be stored, it should be stored in a refrigerator for a maximum of two weeks.

**Acid insoluble lignin was determined as follows:** Use deionized water to quantitatively transfer all remaining solids out of the pressure tube into the filtering crucible. Rinse the solids with a minimum of 50 mL fresh deionized water. Hot deionized water may be used in place of room temperature water to decrease the filtration time. Dry the crucible and acid insoluble residue at 105 + 3 °C until a constant weight (6h). Remove the samples from the oven and cool in a desiccator. Record the weight of the crucible and dry residue to the nearest 0.1 mg.

**Acid soluble lignin was determined by following procedure:** On a UV-Visible spectrophotometer, run a background of deionized water or 4% sulfuric acid. Using the hydrolysis liquor aliquot measure, the absorbance of the sample at an appropriate wavelength. Dilute the
sample as necessary to bring the absorbance into the range of 0.7 – 1.0, recording the dilution. Deionized water or 4% sulfuric acid may be used to dilute the sample, but the same solvent should be used as a blank.

**Structural carbohydrates are determined by following procedure:** Prepare a series of calibration standards containing the compounds that are to be quantified, suggested concentration range is 0.1-4 mg/ml for each compound. Using the hydrolysis liquor, transfer an approximately 20 mL aliquot of each liquor to a 50 mL Erlenmeyer flask. Use calcium carbonate to neutralize each sample to pH 5 – 6 (slowly after pH of 4). Allow the sample to settle, and decant off the supernatant. The pH of the liquid after settling will be approximately 7. Filter the decanted liquid through a 0.2 μm filter into an autosampler vial. (4) Analyze the calibration standards, CVS, and samples by HPLC using a Biorad Aminex HPX-87P column equipped with the appropriate guard column. HPLC analysis of sugar composition are conducted under the following instrument setting ups. (Injection volume: 10 – 50 μL, dependent on concentration and detector limits; Mobile phase: HPLC grade water, 0.2 μm filtered and degassed; Flow rate: 0.6 mL / minute; Column temperature: 80 - 85°C; Detector temperature: as close to column temperature as possible; Detector: refractive index; Run time: 35 minutes) Note: The deashing guard column should be placed outside of the heating unit and kept at ambient temperature. This will prevent artifact peaks in the chromatogram. Quantitative analysis of all component in the lignocellulosic sample was determined follow the equations listed below:

\[
\text{%AIR} = \frac{\text{Weight}_{\text{crucible plus AIR}} - \text{Weight}_{\text{crucible}}}{\text{ODW}_{\text{sample}}} \times 100
\]
Acid Insoluble Lignin (AIL)  
\[ \%\text{AIR} = \frac{(\text{Weight}_{\text{crucible plus AIR}} - \text{Weight}_{\text{crucible}}) - (\text{Weight}_{\text{crucible plus AIL}})}{\text{ODW}_{\text{sample}}} \times 100 \]

Acid Soluble Lignin (AIL)  
\[ \%\text{ASL} = \frac{\text{UVabs} \times \text{Volume}_{\text{filtrate}} \times \text{Dilution}}{\varepsilon \times \text{ODW}_{\text{sample}} \times \text{Pathlength}} \times 100 \]

Where: \( \text{Volume}_{\text{filtrate}} \) = 86.73 and Dilution E.g 500/1

Total Lignin  
\[ \%\text{Lignin}_{\text{ext free}} = \%\text{AIL} + \%\text{ASL} \]

b. Nitrobenzene oxidation

Nitrobenzene oxidation of lignin in alkaline solution is one of the most significant lignin subunits analysis methods converting GHS units to the benzaldehyde structures respectively. In general, the yield and molar ratio of the phenolic aldehydes, depend on the plant species being investigated. Thus, the nitrobenzene oxidation of lignin is not only important in terms of the characterization of lignin, by providing information on the minimal quantities and the relative amounts of the uncondensed GSH units present in a lignin. Moreover, the method is relatively simple compared with the potassium permanganate oxidation of lignin. However, the reaction mechanisms is not clear though there is some preliminary work on the model compounds study. Thus, it is hard to speculate the possible side-chain structures based on nitrobenzene oxidation results.

Materials: 10 mg of lignin sample, 50 mL test tube, .25mL 99% nitrobenzene, 4mL 2M NaOH, pipette and tips (glass and plastic), Teflon tube and chamber, oil bath, 18mL MTBE, pH
paper, 28% HCl (v/v), vortex mixer, 100 mL beaker, Pasteur glass pipette, mass balance, mass paper, spatula, GC-MS vial, syringe filter.

Figure 2.2. Potential low molecular weight products identified from nitrobenzene oxidation.

Protocol: Measure 10mg of lignin sample then transfer it to a Teflon tube. Add 4mL of 2M NaOH and .25mL 99% nitrobenzene to the tube. Gently swirl the Teflon tube then cap and tightly seal it in its chamber. Use a wrench to ensure tightness. Preheat oil bath to 190°C. Heat reaction mixture in oil bath for 4 hrs. Remove Teflon chamber from oil bath and allow to cool for at least 60 minutes. Transfer the content of the Teflon tube to a 50mL test tube using a Pasteur pipette. Add 3mL MTBE to the Teflon tube and use the Pasteur pipette to aid in rising the tube before transferring its content to the test tube. Vortex test tube and allow the mixture to separate before removing and dumping organic layer. Repeat this extraction two more times (add MTBE directly to the test tube). Adjust solution to pH 3-4 by slowly adding 28% HCl to the mixture with a Pasteur glass pipette while gently vortexing. Use pH paper to test for an orange-red color. The formation of precipitates is an indication that the mixture is close to the desired pH. Add 3mL MTBE to the now acidic content of the test tube and mix with a vortex mixer. Allow separation
and transfer organic layer to an 8 mL glass vial using a Pasteur glass pipette. Repeat this extraction two more times. Dry organic layer under air until no flowing solvent remains then place in desiccator for 24hrs.

GC-MS was applied to analysis the yielded products and quantify the GHS ratio. Dried organic residue was re-dispersed into 5 ml of ethyl acetate solution. 200 µL aliquot was added to 700 µL ethyl acetate and 100 µL sylation solution (10% trimethylchlorosilane with N, O-Bis(trimethylsilyl) trifluoroacetamide) using an appropriate pipette and cap vial immediately after.

Vortex mixture then place in an incubator at 60 °C for 30 minutes. Using a Pasteur glass pipette, transfer the vial’s content to a syringe filter which has been positioned over a GC-MS vial and slowly filter mixture. N-docosane was added as internal standards. The silylated EAE fraction was analyzed by a GC-MS (Agilent 7890A/5975C) equipped with a DB-5 capillary column. The following oven program was used: injection temperature 100 °C hold 2 minutes, 5°C/min ramp to 200 °C hold 1 min, then 20 °C/min ramp to 280 °C hold 3 min. The MS detector was run on positive EI mode. The temperature of the MS was set to: MS Source Setpoint: 230 °C; MS Quad Setpoint: 150 °C. Solvent delay was set to 6 minutes and the compounds between 6-38 minutes were identified and quantified. Scanning ion range was set from m/z 50–450. The quantification of MPC by GC/MS analysis was conducted based on TIC peak area by using vanillic acid as a standard to convert peak area to concentration.

c. **Thioacidolysis**

Thioacidolysis is an acid-catalyzed solvolysis in dioxane-thanethiol with boron trifluoride etherate reaction which results in the depolymerization of lignin. The method may be used to estimate the amount and composition of uncondensed alkyl aryl ether structures, without affecting methoxyl group on aromatic ring, and of several minor structural types in both isolated and in situ
lignin. Scheme 2.1 depict the major mechanisms occurred during the thioacidolysis reaction, in which prominent arylglycerol-β-aryl ether linkages in lignin are primarily cleaved. This reaction combines the synergy of boron trifluoride etherate functioned as a hard Lewis acid, and the ethanethiol, a soft nucleophile. The selective modification of propanyl side chain by the thioacidolysis provides unambiguous evidence for the occurrence of arylglycerol aryl ether structures (the most characteristic ones in lignin) and allow a precise quantitative analysis of each units based on the specific mass spectroscopy. On this basis, thioacidolysis may be viewed as a diagnostic test for the presence of lignin where other chemical or spectroscopic methods fail for lack of specificity or sensitivity, respectively. The high sensitivity of the thioacidolysis made it is possible to characterize unambiguously as little as 20 mg of lignin with a standard deviation commonly less than 10%.

**Scheme 2.1. Main mechanism of thioacidolysis.**

**Materials:** 10 mg of lignin sample, 15 mL capped test tubes, 3mL thioacidolysis solution, 12 µL docosane solution, pipette and tip, heating block, 6mL MTBE, pH paper, 28% HCL,
5mL 0.4mol/L Na$_2$CO$_3$, vortex mixer, 100 mL beaker, Pasteur glass pipette, mass balance, mass paper, spatula, GC-MS vial, syringe filter, 8ml glass vial.

**Protocol:** Measure 10mg of lignin sample then transfer it to a 15mL glass test tube. Add 3mL of freshly prepared thioacidolysis solution and 12 µL docosane solution to the test tube using an appropriate pipette then tightly cap the test tube. Heat reaction mixture in heating block at 100°C for 4 hrs. Mix the mixture every 30-45 minutes using a vortex mixer. To ensure the temperature in on point throughout the reaction, place a test tube with about 4mL of oil into the heating block. Insert an oil thermometer into the oil then seal the test tube with aluminum foil. Check the thermometer periodically. Place capped test tube with reaction mixture into a 100 mL beaker half filled with ice and leave standing for 15 minutes. Stop reaction by adding 5mL of 0.4mol/L Na$_2$CO$_3$ (NaHCO$_3$ According to official protocol (ATOP)) to the test tube. Adjust solution to pH 3-4 by slowing adding 28% HCl (6N HCl ATOP) to the mixture with a Pasteur glass pipette while gently vortexing. Use pH paper to test for an orange-red color. The formation of precipitates is an indication that the mixture is close to the desired pH. Add 2ml of MTBE to the now acidic content of the test tube with an appropriate pipette and mix with a vortex mixer. Allow separation and transfer organic layer to an 8 mL glass vial using a Pasteur glass pipette. Repeat this extraction two more times. Dry organic layer under air until no flowing solvent remains then place in desiccator for 24hrs. N-docosane was added as internal standards. The silylated EAE fraction was analyzed by a GC-MS (Agilent 7890A/5975C) equipped with a DB-5 capillary column. The following oven program was used: injection temperature 100 °C hold 2 minutes, 5°C/min ramp to 200 °C hold 1 min, then 20 °C/min ramp to 280 °C hold 3 min. The MS detector was run on positive EI mode. The temperature of the MS was set to: MS Source Setpoint: 230 °C; MS Quad Setpoint: 150 °C. Solvent delay was set to 6 minutes and the compounds between 6-38 minutes
were identified and quantified. Scanning ion range was set from m/z 50–450. The quantification of MPC by GC/MS analysis was conducted based on TIC peak area by using vanillic acid as a standard to convert peak area to concentration.

d. CHNO Elemental Analysis

Elemental analysis, for organic chemistry, always refers to CHNO analysis (might include some other elements, such as sulfur, halogens) of a sample. Most commonly, the CHN analysis is accomplished by combustion method, in which a macromolecular sample is burned in the presence of excessive amount of oxygen and various traps was used to collect the combustion products: carbon dioxide, water and nitric oxide. The products are quantitatively captures and analyzed to determine the composition of the unknown sample. Specifically when analyze lignin samples, the elemental analysis is integrated with methoxyl analyses to predict the empirical formula of lignin samples on a C9 basis. These formulas emphasize the C9 carbon skeletons of lignin monomeric units while leverage the influence of GSH monolignol ratio on methoxy content. However, the estimation of empirical formula is based on the assumption side chain is not extensively chemical degraded, thus in some cases such as in kraft pulping, the calculation on a C9-basis may not be justified.

**CHNO Elemental Analysis Protocol:** The CHNO-elemental analyses were performed on DACSL and SESPL to calculate their formulas. The CHNO elemental analyses were performed with 2400 Series II CHNO Elemental Analyzer (Perkin Elmer®, Boston, MA) according to the following procedure: The sample is burned in a reaction column at 1000 °C, in which helium gas flows. The halogen is removed with silvered cobalt oxides. The excess oxygen is subsequently removed with copper, and nitrogen oxides are reduced to nitrogen. Nitrogen, carbon dioxide and water are separated on a gas chromatograph fitted with a Porapak QS column at 120 °C and
detected with a katharometer (thermal conductivity detector). O-analysis: The sample is pyrolyzed in a reaction column at 1060 °C through which helium gas flows. The arising gases are conducted over nickel activated carbon resulting in carbon monoxide production. The pyrolysis gases are conducted over an anhydrous ascarite column to remove carbon dioxide, subsequently separated on a Porapak QS column at room temperature and detected with a katharometer.

2.2.2 Spectroscopic Methods

a. Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectroscopy is a versatile and rapid bench-top technique for lignin characterization. Fourier transform infrared spectroscopy (FT-IR) can obtain an infrared spectrum (wavelength range from 2.5 μm to 15 μm; wavenumber range 4000 cm⁻¹ to 660 cm⁻¹) of absorption or transmission of lignin sample or lignin treatment residue to obtain the structural information, especially very powerful in qualitative understanding the functionality on the lignin macromolecule. Since early 1950s, the IR spectroscopy method has been identified a routine analytical method for lignin chemistry. FT-IR has a relative high signal-to-noise ratio, partially because a broad wavelengths are observed simultaneously during the entire test. Table XX lists the band assignments for the FT-IR spectra which are mostly used in lignin analysis. In results analysis and structure interpretation, the O-H, C-H, and C=O stretching modes above 1600 cm⁻¹ and the aromatic skeletal vibration around 1510 cm⁻¹ are normally assigned with little argument. However, some bands, for instance, 1600 cm⁻¹ aromatic skeletal vibration band are sometimes broadened by the C=O stretching. Interpretation of the bands below 1430 cm⁻¹ are mostly difficult.

**FT-IR Sample Preparation and Test:** Dry, spectroscopy-grade potassium bromide is used as suitable embedding media. The potassium bromide is pre-dried at 120°C in oven for at least 1 h, followed by cooling in a desiccator to room temperature every time before use. All lignin samples
are supposed to dry in the desiccator to a minimum moisture. A standard 13-mm diameter pellet is prepared by pressing a 1-2 mg sample in 250 mg of KBr in an evacuated die. The KBr should be ground in a mortar to a proper fineness since unnecessarily long time would cause high water uptake. A blank fresh-prepared KBr pellet is used as standard to collect the background of the test. Residue water has signals at about 1625 and 3700 cm\(^{-1}\), while CO\(_2\) always shows signals at about 2500 cm\(^{-1}\). The IR spectrum was collected at a range of wavenumber from 4000 to 800 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\) for 64 cycles. In this study, the FT-IR spectroscopy is used to compare the methoxyl, carbonyl, carboxylic, phenolics OH and aliphatic OH groups existing on lignin aromatic rings and propanyl side-chains.

<table>
<thead>
<tr>
<th>Wavenumbers (cm(^{-1}))</th>
<th>Band origin, short comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3460-3412</td>
<td>O-H stretch</td>
</tr>
<tr>
<td>3000-2842</td>
<td>C-H stretch in methyl and methylene groups</td>
</tr>
<tr>
<td>1738-709</td>
<td>C=O stretch in unconjugated ketones, carbonyl, and in ester groups (frequently of carbohydrate origin)</td>
</tr>
<tr>
<td>1675-1655</td>
<td>C=O stretch in conjugated p-units aryl ketones; strong electronegative substituents lower the wavenumber</td>
</tr>
<tr>
<td>1605-1593</td>
<td>Aromatic skeletal vibration plus C=O stretch; S&gt;G, Gcondensed&gt; G etherified</td>
</tr>
<tr>
<td>1515-1505</td>
<td>Aromatic skeletal vibrations; G&gt;S</td>
</tr>
<tr>
<td>1470-1460</td>
<td>C-H deformations; asym, in –CH(_3) and –CH(_2)-</td>
</tr>
<tr>
<td></td>
<td>Aromatic skeletal vibrations combined with C-H in-plane deform</td>
</tr>
<tr>
<td>1370-1365</td>
<td>Aliphatic C-H stretch in CH(_3), not in OMe</td>
</tr>
</tbody>
</table>
b. **UV-Vis Determination of Benzoquinone Formation in Lignin Oxidation**

Lignin is the largest natural resource with aromatic skeleton, and thus a component adsorbs more ultraviolet light much stronger than the polysaccharides and exhibits characteristic maxima in the ultraviolet light region. Thus, UV-vis spectroscopy is not surprisingly a useful tool in lignin chemistry for a history longer than half century. It is among the most convenient methods for the qualitative and quantitative analysis of lignin and products in solution. The location and intensity of the maxima absorption highly depend on the type of lignin, and as well those distinct structural patterns related to its chemical modification. Though it can hardly provide an accurate structure of lignin, the sensitive response of structural change on the shift of wavelength provide unique information interpreting reaction mechanism. Thus, this thesis study, we identified a new method to quantify the formation of benzoquinone structure, a significant intermediates formed during lignin oxidation. The benzoquinone was commonly proposed in previous studies as a key intermediates, but only a few studies presented the detection of them using complicated instrumentations such as NMR. However, the stability of benzoquinone and their separation are still a myth. Not like the phenolics structures which has a sharp response towards UV lights, the benzoquinone structures are likely to absorb UV lights and yield a bump in the Intensity-wavelength plot. Adding benzenediamine to a mixture in which benzoquinone formed can generate
phenazine structure, which has a unique maximum absorption of UV-vis at around 400 nm with. We adapt this chemistry and applied in capturing benzoquinone to help interpreting reaction chemistries in this study.

**Detection of Benzoquinone:** Separate ethyl acetate extractions were conducted to obtain samples for quinone analysis. Then O-phenylenediamine and glacial acetic acid were added into the extracts. The mixture was reacted for 4 hours at 60 °C. The reaction solution was dried under nitrogen atmosphere and was diluted with ethyl acetate to desired concentration prior to UV-vis measurement. The UV-Vis measurements were carried out using a UV-Vis spectrophotometer (SpectraMax M5) with a scanning range from 200 to 800 nm of wavelength. As o-benzoquinone derivatives have a known absorption in the range of 350-450 nm according to their structural variation(Zhu, Olmstead et al. 1995), the quinone concentration in this study was determined by its peak absorption wavelength at 398nm.

c. **Nuclear Magnetic Resonance (NMR) Spectroscopy**

The current development and recent advance in NMR spectroscopy provides the most possibility of detailed insight into lignin molecular structure both qualitatively and quantitatively, which normally applied but not limit to analyze a broader range of properties including monomeric units (estimate GHS ratio, functional group, side-chain functionality, free phenolic content) and inter-subunit linkages of lignin macromolecules. In lignin chemistry, 13C, and 2D[1H; 13C] heteronucler single quantum coherence NMR are the two types of NMR used most often. 13 C NMR spectroscopy has been shown of significant potential towards quantitative analysis of lignin structure, in which the aromatic and methoxyl signals are used as internal standards in expressing the functionalities per aromatic ring or per methoxy units, respectively. Such quantitative method benefits from the wide spread chemical shift compare to the narrow frequency range render of
proton NMR signals. The assignments of the chemical shift value matching to a specific structure is based on the model compounds study. Thus, the accuracy of assignments is also limited by the availability of relevant model compounds for spectral measurements and by spectrometer resolution. Upon efforts in recent years, 2D $[^1\text{H}; ^{13}\text{C}]$ HSQC NMR has proven to be extremely powerful for refining the interpretation of lignin inter-units linkages. A library of the linkage structures is currently available thanks to the efforts on model compounds study. The 2D NMR also provide a certain level of quantitative insights since the guaiacyl and syringyl aromatic signals are usually referred as internal standards. Technically, the common NMR analysis are conducted in liquid phase, thus the improvement of lignin solubility is necessary. The acetylation of biorefinery lignin can significantly improve the solubility of most lignin in organic solvents such as DMSO, pyridine. Lignosulfonate cannot be acetylated but can be solubilized in deuterated water.

**Acetylation Protocol:** The acetylation was conducted based on previous study. A certain amount of lignin is weighed (here is an example with 100 mg of sample) and was acetylated overnight at room temperature with 1-1.5 ml of pyridine-acetic anhydride (1: 1, v/v) mixture. The reaction was quenched by addition of methanol-ice water. After evaporation of the mixture to dryness, the residue is suspended in toluene and again evaporated.

**$^{13}\text{C NMR Protocol:}$** The structural information of lignin were determined by the $^{13}\text{C}$ NMR spectra of acetylated lignin recorded on a Bruker 500MHz NMR spectrometer with a 90$^\circ$ pulse width, a 1.4 s acquisition time, and a 1.7 s relaxation delay following standard procedures for lignin analysis.(Lin and Dence 1992) All lignin were analyzed under identical conditions using DMSOd6 as solvent (100mg sample/0.6ml DMSOd6). A small amount of relaxation agent (Cr(aca)3, 2 mg) was added during sample preparation to facilitate the relaxation of the magnetization.(Xia, Akim et al. 2001)
**HSQC NMR Protocol:** The inter-units linkage information of lignin were analyzed by HSQC NMR spectra of acetylated recorded on a Bruker 500MHz NMR spectrometer used spectral widths of 5000 Hz (from 10 to 0 ppm) and 20,843 Hz (from 200 to 0 ppm) for the 1H- and 13C dimensions. For 1H, the number of collected complex points was 2048 with a recycle delay of 1.5 s. For 13C, the number of transients was 64, and 256 time increments were always documented. The 1JCH used was 145 Hz. The central solvent peak was used as an internal reference (δC 39.5; δH 2.49). Long range J-coupling evolution times of 66 and 80 ms were used in different heteronuclear multiple bond correlation acquisition experiments. Analysis of HSQC peaks were following the literature qualitatively.

2.2.3 **Analysis of Physical Properties**

a. **Gel Permeation Chromatography**

Gel permeation chromatography was applied in this thesis work to analyze the molar mass distribution of those biorefinery lignin. The Gel permeation chromatography entails the chromatographic separation and fractionation of the molecular based on their molecular size, and have been commonly applied in macromolecules’ characterization. In early literatures, GPC is also sometimes referred to as size exclusion chromatography (SEC) or gel filtration chromatography (GFC). Since the difficulty to identify proper lignin standards, the estimation of the molar mass distribution of the biorefinery lignin are usually providing relative molar mass value. The test of GPC is commonly conducted on a liquid chromatography, in which case certain procedures are necessary to solubilize lignin before experiments. Generally there are two options: 1) derived hydroxyl and carboxyl group on lignin with acetylation/methylation to improve the solubility of lignin in organic solvent; 2) using alkaline water as the eluent. The objective of GPC analysis in this study is to obtain the unbiased molecular weight distribution of the seven biorefinery lignin
under identical conditions and to assess the molecular level complexity of the lignin macromolecules. Herein, we are running the GPC on Perkin Elmer HPLC with a three column GPC separation system. Seven polystyrene polymers and one verotrole was used as the calibration standards. The solubility of biorefinery lignin samples are always varied and cannot completely soluble, and any data thus obtained will not be representative. Acetylation of the hydroxyl groups on the biorefinery lignin were conducted to improve the lignin solubility in the organic eluents, such as THF.

To be fair, a couple of difficulties encountered applying GPC to determine molecular weight distribution should be noticed: 1) acidic functional groups (e.g. phenolic hydroxyl, carboxyl, and sulfonic acids) can affects the swell of lignin in different eluent system, and thus the separation pattern is highly depending on the ionic strength of the eluent; 2) the separation behavior can be influenced by the presence of charged groups in the gel or on the sample; 3) Hydrogen bonding or hydrophobic interactions might cause the biased interaction of samples to gel columns. Thus, it needs to be clarified each comparative GPC study of a group of lignin carries the pattern of testing conditions.

Acetylation Protocol: The acetylation was conducted based on previous study. A certain amount of lignin is weighed (here is an example with 100 mg of sample) and was acetylated overnight at room temperature with 1-1.5 ml of pyridine-acetic anhydride (1: 1, v/v) mixture. The reaction was quenched by addition of methanol-ice water. After evaporation of the mixture to dryness, the residue is suspended in toluene and again evaporated.

GPC Protocol: Approximately 2 mg of sample was dissolved in tetrahydrofuran and then filtered through a 0.45 um filter. A Perkin-Elmer High performance liquid chromatography (HPLC) equipped with a diode array detector (DAD) was used to conduct gel permeation chromatograph
(GPC) analysis of lignin molecular weight distribution. A series of three Agilent ZORBAX PSM columns (60S, 300S and 1000S) was used to elute different lignin molecular weight fractions by tetrahydrofuran at a flow rate of 0.6 ml per minute for 60 minutes. Polystyrene standards (Alfa Aesar) with molecular weights ranging from 1,300 to 123,000 g/mol as well as veratrol (138 g/mol) were used to calibrate the molecular weight based on retention time.

b. Thermalgravimetric Analysis (TGA)

Thermal analysis is an important technique providing the direct analysis of lignin physical properties and also an indirect way to allude the chemical structural properties. It contains a set of method such as thermogravimetry (TG), differential thermal analysis (DTA)m differential scanning calorimetry (DSC) techniques. Herein, we primarily focusing thermogravimetric properties of lignin. TGA is a technique that measures the weight change of a sample versus a programmed function of temperature. A TG curve is recorded by plotting the weight W or weight ratio W/W0 (Wo is the original weight, W is the sample weight at certain temperature) against temperature (T). The plateau in the TG curves suggests a range of temperature that the material is thermally stable. TG is commonly used to understand the degradation, oxidation, reduction, evaporation, sublimation, and other heat-related changes occurring on the lignin. In my thesis work, TGA is employed to understand the thermal stability of lignin isolated from different pretreatment methods, and thus give some hint on the ratio of ether linkages versus carbon-carbon linkages. The obtained comparative discussion are meant to give leads of lignin valorization as both chemicals and precursors for new materials.

**TGA Protocol:** The thermogravimetric analysis of lignin and lignin degradation samples are tested on a Thermogravimetric Analyzer with small furnace produced from Mettler-Toledo. Approximately 10 mg of each lignin samples was used in the test. The temperature program was
set from 25 °C to 400 °C with an aluminum weight microplate, at a heating ramp of 10 °C/min. Both W and W/Wo was recorded, but W/Wo was used to discuss the thermal stability of each biorefinery lignin.

2.2.4 Separation of Identification of Low-Molecular Weight Fragments and Model Compounds

a. Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography integrated with mass spectrometry is one of the most powerful separation and analytical techniques applied in lignin chemistry, mainly used for analysis of low molecular weight volatile fragments in mixture solution collected from chemical degradation of lignin. In general, the objectives of GC-MS analysis is to identify the structure of the low molecular weight compounds based on its retention time chromatographically together with further confirmation of the structure by analysis the mass fragments in mass spectrometry. The separation of the volatile compounds is primarily the results of different boiling point together the interaction of those compounds with the column based on polarity. The combination of GC and MS in a package has shown noteworthy advantages: 1) relative short test time; 2) high sensitivity to a traceable level; 3) products separation and characterization are proceeded simultaneously; 4) qualitative and quantitative analysis can be finished straightforward with computer software assistance. Certainly, it also has the limitation when analyzing products not synthesized or included in the mass library, the structure determination cannot be tentative. Anyway, the GC-MS is still the most sensitive and reliable method for the analysis of lignin depolymerization products. GC-MS can normally analyse the phenolics having a molecular weight up to 300 without further derivation when the GC is equipped with a DB-5 column (low-polarity column). Presence of more hydroxyl and carboxylic group will significantly increase the products boiling point and sometimes
make the compounds has a boiling point higher than its decomposition temperature. Thus, chemical derivation of the hydroxyl/carboxylic group in the compounds with trimethylsilyl group could improve the products volatility and the completeness of the products profile detected.

**GC-MS Protocol:** To analyze the phenolic compounds in mixture in detail, ethyl acetate was applied to extract the phenolic compounds from the aqueous reaction solution. (Örsa and Holmbom 1994, Mitchell, Taylor et al. 2014) 0.5-1 mL of aqueous samples (depending on the concentration) were extracted with 2 mL ethyl acetate (HPLC grade) three times. The ethyl acetate fractions were combined and dried in a 100 ± 5°C oven for 2 hours to evaporate solvent and obtain the dry matter of reaction compounds. The total dry weight of the ethyl acetate extract (EAE) was reported based on the initial dry weight of the lignin samples. Separate ethyl acetate extractions were conducted to obtain samples for GC/MS analysis. The combined ethyl acetate extracts were dried under a stream of nitrogen using a Pierce ReactiVap apparatus (Thermo Scientific, Fremont, CA) to remove solvent prior to trimethylsilylation, which used 100 µl of 10% Trimethylchlorosilane with N,O-bis(Trimethylsilyl) trifluoroacetamide (Fisher Scientific, Fremont, CA). The silylated EAE fraction was analyzed by a GC-MS (Agilent 7890A/5975C) equipped with a DB-5 capillary column. The following oven program was used: injection temperature 100 °C hold 2 minutes, 5°C/min ramp to 200 °C hold 1 min, then 20 °C/min ramp to 280 °C hold 3 min. The MS detector was run on positive EI mode. The temperature of the MS was set to: MS Source Setpoint: 230 °C; MS Quad Setpoint: 150 °C. Solvent delay was set to 6 minutes and the compounds between 6-38 minutes were identified and quantified. Scanning ion range was set from m/z 50–450. The quantification of MPC by GC/MS analysis was conducted based on TIC peak area by using vanillic acid as a standard to convert peak area to concentration.

b. **High Performance Liquid Chromatography (HPLC)**
HPLC is a well-developed technique in chromatography over the last decade, and primarily used for analytical separation. As regards its particular development in lignin, it still lags behind that has been developed in other fields, mainly because of the complexity of lignin derived mixtures which are obtained from lignin isolation and lignin depolymerization methods. However, it is remaining a very useful tool applied in a variety of field in lignin chemistry. N.G. Lewis reviewed the potential application of HPLC in lignin chemistry. It can be used for analysis of monomeric size low molecular weight phenolics such as monolignols, monolignol glucosides and hydroxycinnamic acids which have proper standards. It is also an alternative used to quantify sugar residue and lignin-derived fragments in some of lignin depolymerization reactions such as sulphonomethyl monophenyl, thioacidolysized monomer, aromatic alcohols, benzaldehyde, phenolics acids, and lignin model compounds. Compare to GC-MS, the HPLC has relative low capability of separation of low molecular weight compounds, mainly due to difficulty of proper eluent composition However, it provides a potential method to identify the oligomeric phenols derived from lignin depolymerization since GC-MS separation capability is limited by the boiling point of the compounds.

The specific protocols of HPLC analysis is not described since it will be modified based on specific objective of each experiment, and thus will be described

c. Rapid High-throughput Folin-Ciocalteu Assay

The Folin-Ciocalteu (FC) reagent, also called Folin’s Phenol Reagent, is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric quantification of soluble low molecular weight phenolic compounds (LMWPC) in vitro assay. A facile preparation procedure made it a high throughput method applied in lignin chemistry to quantify the depolymerization phenolics compound. Adding the Folin-Ciocalteu reagent to a soluble phenolics solution and aging
for a period of time will generate distinct blue color solution, and thus can be quantified at a wavelength in visible area. In my thesis study, a rapid 96 well microplate high-throughput methodology was used for the quantitative estimation of total phenolics yields from lignin depolymerization chemistries.

**96 Well Microplate FC Protocol:** Folin-Ciocalteu reagent (FCR) were purchased from Sigma-Aldrich. FCR was diluted 1:5 (v/v) with water. Sodium carbonate 6% (w/v) was prepared as reaction buffer. A number of monomeric model compounds (e.g. phenol, catechol, guaiacol, eugenol) were prepared containing a phenolic concentration varied from 1 to 10 mg/L. The high-throughput assay assessment was performed on a microplate reader using spectrophotometric detection and microtiter 96-well plates. 50 μL of sample/standard solution and 50 μL of FCR (1:5, v/v) were placed in each well. After that, 100 μL of Sodium carbonate 6% (w/v) was added. The plate is aged at ambient temperature for two hours. The absorbance at 760 nm of each well of the plate was measured to quantify the LMWPC in the solution calculated with proper monomeric phenol standard solution.
CHAPTER THREE: SELECTIVE CONVERSION OF BIOREFINERY LIGNIN TO DICARBOXYLIC ACIDS

3.1 Abstract

The emerging biomass-to-biofuel conversion industry has created an urgent need for identifying new biorefinery lignin valorization strategies. This paper demonstrates a novel route to producing dicarboxylic acids from biorefinery lignin via chalcopyrite catalyzed oxidation in a highly selective process. Up to 95% selectivity of stable dicarboxylic acids was obtained from several types of biorefinery lignin and model compounds under mild, environmentally friendly reaction conditions. The findings from this study paved a new avenue to biorefinery lignin conversions and applications.

3.2 Introduction

Lignin is a ubiquitous component in almost all plant biomass (Fengel and Wegener 1984, Sjostrom 1993). Large quantities of industrial lignin are already produced annually as a waste product of the pulp and paper industry, where the vast majority is burned as a low cost fuel to provide energy for the chemical pulping process(Zhang, Tu et al. 2011). The emerging biomass refinery industry will further introduce an enormous amount of lignin.(Holladay, Bozell et al. 2007) Thus, there is an urgent need to develop technologies that can create new applications for biorefinery lignin.

Lignin is the largest source of renewable material with an aromatic skeleton. Depolymerizing lignin to low molecular weight aromatic compounds (LMWAC) offers a promising route to generating value added products and has been the focus of the majority of previous researches (Lange, Decina et al., Zakzeski, Bruijnincx et al. 2010, Zhang, Tu et al. 2011,
Hanson, Wu et al. 2012, Rahimi, Azarpira et al. 2013). However, one potential group of chemicals has been overlooked among oxidative conversion products: dicarboxylic acids (DCA). DCAs, such as muconic, maleic, and succinic acids, are important and highly valuable industrial chemicals and intermediates used in many industries including the biopolymer, pharmaceutical, and food additives industries (Sato, Aoki et al. 1998, Dugal, Sankar et al. 2000, Lee, Hong et al. 2004, Yu, Peng et al. 2011). Current commercial dicarboxylic acids are all produced from petroleum based feedstocks (Castellan, Bart et al. 1991, Sato, Aoki et al. 1998). Developing a green route to produce DCA from renewable biomass lignin will be of a prime interest to both chemical and biomass conversion industry. (Niemelä 1990, Werpy, Petersen et al. 2004, Dodds and Gross 2007, Gallezot 2012) Oxidative aromatic ring cleavage to yield muconic acid and its derivatives is a well-recognized reaction. (Demmin, Swerdloff et al. 1981, Lin, Reid et al. 2001, Ran, Zhao et al. 2008). Although DCA and their derivatives have been detected in the reaction mixture from oxidative delignification of wood pulp, (Gierer 1986) there has not been successful attempt to produce DCA from biomass lignin. Discovering a selective route to convert lignin to DCA will pave a new avenue towards lignin utilization.

In this chapter we reported a method of selective production of DCA via chalcopyrite (CuFeS₂) catalysed oxidation of biorefinery lignin including diluted acid corn stover lignin (DACSL) and steam exploded spruce lignin (SESPL) (See details in Chapter Two.), in the presence of hydrogen peroxide (H₂O₂). Chalcopyrite is a di-transition metal catalysts belong to the family of transition metal dichalcogenides. (Wang, Kalantar-Zadeh et al. 2012) The unique structure of chalcopyrite with complex metal oxidation states, including Cu⁺, Cu²⁺, Fe²⁺, and Fe³⁺, allows for highly effective electron transfer on the surface of and inside the chalcopyrite crystals (Fujisawa, Suga et al. 1994, Jayadevan and Tseng 2005). This opens the potential for
application of chalcopyrite as an effective mediator of catalytic oxidation reactions. Though H$_2$O$_2$ can generate oxidation products on its own, chalcopyrite aided catalysis produced successful reactions at conditions mild enough where controls with solely H$_2$O$_2$ exhibited no catalytic activity.

3.3 Results and Discussion

**Figure 3.1** shows the products generated from DACSL during a 5 hour reaction at 60 °C with chalcopyrite under the presence of H$_2$O$_2$ at pH 4 (acetic acid buffer). DACSL is a GSH type of lignin which contains all three phenylpropane units, guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H). The G:S:H ratio for DACSL is 57:19:24 determined by $^{13}$C NMR analysis. (See details in Chapter Five). Significant amounts of LMWAC including $p$-coumaric acid, $p$-hydroquinone, $p$-hydroxybenzoic acid and vanillic acid were produced after the first hour of reaction. After 2 hours of oxidation, ring cleavage of LMWAC became the predominant reaction, leading to the formation of the following DCA: malonic acid (35%), succinic acid (50%), malic acid (6%), and maleic acid (3%). The selectivity (**Figure 3.1**) and yield towards these acids continued to rise as the oxidation time increased. DCA represented more than 94% of the total soluble products at the 5 hour mark.
Figure 3.1. Products distribution of DACSL after 5 hours oxidation.

SESPL contains predominantly guaiacyl units, approximately 95% by $^{13}$C NMR analysis (See details in Chapter Five). Similar to DACSL oxidation, conversion of steam exploded spruce lignin (SESPL) by chalcopyrite/H$_2$O$_2$ led to the initial production of the LMWAC, such as benzoic acid, vanillin, and vanillic acid, and followed up with ring cleavage of these compounds to DCA. After 5 hours of reaction; malonic acid (36%), succinic acid (48%), maleic acid (2%), and malic acid (3%) combined to represent about 89% of the soluble products. Though the intermediate LMWAC products differ, the selectivity towards final DCA was very similar to that of the DACSL. The mass conversion yields from DACSL and SESPL to DCA under the tested conditions were 14% and 11% respectively. From the change in product distribution over time (LMWAC to DCA), it can be seen that chalcopyrite catalysed lignin oxidation occurs in a two-stage reaction: initial lignin depolymerisation during short reaction times followed by aromatic ring cleavage at longer...
reaction times. A variety of multi-hydroxylated/ methoxylated phenols and phenolic acids were the main products from depolymerisation, while the reaction converged to two primary DCA products during the ring cleavage.

It was surprisingly noticed the products profile keep changing over a time span to 5 hours, which has exceeded the expectation of hydrogen peroxide stability with the presence of transition-metal catalysts. The stability of hydrogen peroxide in reaction solutions is a key factor affecting reaction efficiency. Many previous studies have demonstrated that transition metals (e.g. Fe, Cu, Mn) accelerate hydrogen peroxide decomposition into molecular oxygen. (Gellerstedt and Pettersson 1982, Gierer, Jansbo et al. 1991, Brown and Abbot 1995, Salem, El - Maazawi et al. 2000) On the other hand, compounds containing earth metals (e.g. magnesium salts, sodium silicate) can function as stabilizers, reducing hydrogen peroxide decomposition even in the presence of transition metals. (Christiansen 1986, Siminoski and Macas 1990, Li, Court et al. 2004) Although an ideal metal profile is not yet known, the addition of chelating agents such as EDTA prior to reaction can improve hydrogen peroxide efficiency for delignification. Beside the chelating agents, other organic compounds such as organophosphonates (Taylor and Morrison 1999) and polylactones (Nishino, Kayama et al. 2000) can be used as stabilizers to alleviate the decomposition of hydrogen peroxide and improve reaction efficiency. (Dence and Reeve 1996) Thus, we proposed the presence of acetic acid will assist the stabilization of hydrogen peroxide.

To investigate the acetic acid effect on Iron-peroxide interactions, hydrogen peroxide decomposition was monitored employing a potassium permanganate titration method (Solvay) with a gradient of acetic acid concentration from 0.01 to 60 v/v %. The peroxide concentration was diluted as 21.5 wt%. Catalyst loading were controlled at a same level corresponding to 2% catalyst/substrate loading. Hydrogen peroxide balanced with Milled-Q water was used as the
control. Figure X plots the 24 hours monitored peroxide concentration change at 8 different acetic acid concentration. Without the presence of acetic acid, the concentration of hydrogen peroxide in the solutions drop rapidly from 21.5 wt% to 1.6 wt% after 5 hours stirring at room temperature. However, presence of 0.01 wt% of acetic acids, which means stoichiometric ratio of iron/acetic acid, would significantly improve the hydrogen peroxide stability which lead to a 5.4 wt% hydrogen peroxide concentration at 5 hour time mark. At the reaction conditions in this study where the acetic acid concentration is set close to 4%, the hydrogen peroxide stability are significantly improved.
previously reported application of copper based catalysts in selective removal of lignin side groups contributing to the formation of phenols and phenolic acids. This corresponds with the reaction conditions. To better understand the mechanism of the initial lignin depolymerisation, the cleavage of β-aryl ether bonds during mild acid hydrolysis was well understood, (Lundquist and Kirk 1971, Lundquist 1972, Lundquist 1973, Ito, Terashima et al. 1981, Yasuda, Terashima et al. 1981, Yasuda, Terashima et al. 1982) however, little is known about the kinetics and mechanism of carbon-carbon bond cleavage between phenylpropane units during mild reaction conditions. To better understand the mechanism of the initial lignin depolymerisation, hydroxymatairesinol (HMR), representing a phenylpropanyl dimer with β-β linkages commonly observed in lignin, was reacted with chalcopyrite/ H₂O₂. Approximately 66 w% of HMR was converted to vanillin, vanillic acid, and vanillyl alcohol after 1 hour of reaction (Scheme 3.1). These HMR degradation results confirmed the occurrence of depolymerisation in the initial stages of the biorefinery lignin reactions, with hydroxylation and oxidative cleavage of side chains of the lignin contributing to the formation of phenols and phenolic acids. This corresponds with the previously reported application of copper based catalysts in selective removal of lignin side groups.
to reveal the aromatic nuclei structure. (Goni and Montgomery 2000, Kaiser and Benner 2012) This reaction is likely caused by a transition metal ion catalysed Fenton-like reaction that generates hydroxyl radicals (HO•) which disrupt lignin linkages and produce monolignols. (Crestini, Crucianelli et al. 2010, Bugg, Ahmad et al. 2011) These HO• are formed as a reactive species catalysed by the Cu+/Cu2+ and Fe2+/Fe3+ redox pairs on the surface of the chalcopyrite catalyst under mildly acidic conditions (acetate buffer, pH range 3-5). Based on XRD diagram, there is little structural change occurred on chalcopyrite during the 5-hour reaction. The appearance of a peak at 2θ=47.5 is probably due to a small leaching of the transition metal ion from the catalyst surface.

<table>
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<tr>
<th>Table 3.1. Primary DCA Distribution after 5 Hours Oxidation of Lignin and 3 Hours Oxidation of Monolignols</th>
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<tbody>
<tr>
<td>DCA Distribution (%)</td>
</tr>
<tr>
<td>Malonic Acid</td>
</tr>
<tr>
<td>DACSL</td>
</tr>
<tr>
<td>SESPL</td>
</tr>
<tr>
<td>Guaiacol</td>
</tr>
<tr>
<td>Catechol</td>
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<td>Vanilllin</td>
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*Yield calculations of DACSL and SESPL are based on mass yield. Yield calculations of monolignols are based on carbon yield.*
To verify and understand the subsequent ring cleavage reaction, several model compounds guaiacol, catechol, and vanillin were used to represent different LMWAC. Chalcopyrite/H$_2$O$_2$ oxidation resulted in the selective formation of DCA products identical to those observed in both biorefinery lignin reactions. All reactions achieved high selectivity, 83% to 96%, toward DCA, with conversion yields between 18% and 33%. (Table 3.1) The high products selectivity would help facilitate the DCA separation by either recrystallization or distillation typically employed for diacids separation. (Campbell 1965, Orjuela, Orjuela et al. 2013) These product distributions not only confirms that DCA do originate from the aromatic rings in the biorefinery lignin, but also reaffirms the convergence of intermediate products to DCA as seen in the lignin reactions. This observation is the key to understanding the reaction mechanisms leading to this high selectivity.

Detail examining guaiacol reaction pathway revealed that two consecutive events occurred during ring cleavage. First, after 30 minutes of reaction, 54% of the initial guaiacol was converted to a mixture of catechol (79%) and hydroquinone (12%) with small amounts of 2-methoxyresorcinol and 3-methoxy-1,2-benzenediol. Second, after 3 hours of reaction, all aromatic rings were transformed into a mixture of malonic acid (51%), succinic acid (31%), maleic acid (4%), and malic acid (14%). These results suggest that an initial ring hydroxylation of monolignol occurred prior to ring cleavage to DCA. The ring hydroxylation reactions are postulated to be the result of a combination of two reactive species: hydroxyl radicals and hydroxonium ions (HO$^+$). The production and role of HO• has already been well explored, (Gierer 1986, Buswell, Odier et al. 1987) while HO$^+$ is known to be formed by the heterolytic cleavage of the peroxy bond (O-O) in H$_2$O$_2$. (Johnson 1980, Gierer 1986, Nam, Han et al. 2000, Tan, Yang et al. 2009) The strong reaction preference for catechol and hydroquinone as the predominant ring hydroxylation intermediates suggests the presence of HO$^+$ and its subsequent electrophilic attack on the para- or
ortho- position methoxy- group of guaiacol. To further understand the role of HO• and HO+ on ring hydroxylation and cleavage, α-tocopherol was added during guaiacol oxidation by chalcopyrite/H₂O₂ at a dosage of 2.0 mole per mole of H₂O₂ in order to inhibit radical formation. Alpha-tocopherol addition led to the formation of hydroxylation and demethoxylation products preferentially, leaving the aromatic ring intact (Scheme 3.2). Little DCA were observed even after 3 hours of reaction. This result substantiates the essential role of radicals towards ring opening reactions, and confirms that HO+ contributed to ring hydroxylation and demethoxylation.

Scheme 3.2. Proposed ring-opening pathways using guaiacol as model compounds and the hindering effect with α-tocopherol.

As reported previously on phenol oxidation (especially on cuprous catalysts), ortho-quinone structures are typically cleaved into muconic acid, while para-quinone structures are transformed into maleic/fumaric acid and oxalic acid. (Speier and Tyeklar 1980, Walsh, Furlong et al. 1999) Maleic acid was detected only in small amounts during guaiacol oxidation (4%) as well as during biorefinery lignin oxidation, indicating its presence as a brief, unstable intermediate state before ring cleavage. However, no muconic acid was detected in the reaction products by GC-MS, likely due to its low solubility in aqueous solutions. Oxidation of a pure muconic acid suspension by chalcopyrite/H₂O₂ was conducted as a control; despite low conversions, malonic acid, succinic acid, and maleic acid/fumaric acid were detected as products. Thus, it is possible that muconic acid contributed to the formation of the final end products in all reactions.
Scheme 3.3. Proposed mechanisms for (A) lignin depolymerisation and aromatic nuclei oxidation; (B) aromatic ring cleavage; and (C) formation of final products.

The results from model compounds oxidation show that the chalcopyrite/H₂O₂ system can promote a mild Fenton reaction, contributing to the selective conversion of biorefinery lignin to DCA. Both HO• and HO⁺ were present as the primary reactive species. The main reaction pathways involved in chalcopyrite/ H₂O₂ oxidation of lignin are illustrated in Scheme 3.3. The first reaction step is the depolymerisation of lignin by side-chain substitution (ring hydroxylation by HO⁺) and/or oxidative side chain cleavage to produce LMWAC which were further oxidized to para- and ortho-quinone derivatives (Scheme 3.3A). Aromatic ring cleavage of quinone to maleic acid/fumaric acid and muconic acid derivatives is the second reaction step (Scheme 3.3B). It is likely that
muconic acid then quickly converts to maleic/fumaric acid. The third reaction step is the formation of malonic and succinic acids from the hydrolysis of maleic/fumaric acid, as observed in all reaction results (Scheme 3.3C). To verify this last step, control experiments beginning with only maleic acid produced both malonic and succinic acid. Small amounts of malic acid seen in all reaction results suggest that further oxidation of malic acid accompanied by decarboxylation is likely the mechanism leading to the formation of malonic acid. The exact reaction mechanisms describing succinic acid formation are still under investigation. The observation of small amounts of formic acid during the reaction suggests a mechanism of intermolecular electron transfer between maleic/fumaric acid and formic acid to form succinic acid. Similar reaction mechanisms using formic acid derivatives as electron donors have been reported (Brunel 2007, Shen, Chen et al. 2011). It is speculated that the saturated nature of succinic and malonic acid makes them stable endpoints in the oxidation process, contributing to their high selectivity in every reaction. According to the reaction mechanism, a theoretical carbon yield of 40% can be achieved from biorefinery lignin conversion to DCA based on a guaiacyl type (C10) of monolignol unit. This yield would be significant considering lignin as a renewable-carbon-neutral feedstock. However, relatively low lignin-to-DCA conversion yields were obtained from the testing condition employed in this study. There is a significant potential to improve the DCA yield by optimizing the reaction process condition.

3.4 Conclusion

In conclusion, chalcopyrite catalysed H₂O₂ oxidation of biorefinery lignin demonstrated a new, highly selective conversion route to producing valuable dicarboxylic acid products from biorefinery lignin. In addition, the mild reaction conditions offer competitive advantages over reductive aromatic conversions to linear chain hydrocarbons. The findings from this study will
pave a new avenue to biorefinery lignin conversions and applications. However, the overall DCA yields are low. The products profile changes along with the increasing of reaction time which suggestes a two stage reaction mechanism: 1) lignin depolymerisation to monomeric phenolic compounds, and 2) aromatic ring cleavage of lignin. It is very likely these two steps reach their optimized yields at different conditions. Lack of practical strategies for lignin depolymerisation to monomeric phenolics is one of the key question need to answer. Thus in next Chapter (Chapter Four) we will explore to identify a pathway can effectively depolymerize lignin to monomeric phenolics suitable for further conversion to DCA.

3.5 Experimental Summary

**Materials:** All chemical reagents were purchased from commercial sources and used without further purification. The following reagents were obtained from Thermo Fisher Scientific Inc.: hydrogen peroxide (35 w%, stabilized), vanillin, catechol, maleic acid, fumaric acid, *trans*, *trans*-muconic acid, α-tocopherol, ethyl acetate (HPLC grade) for extraction and GC-MS, and 10% TMCS (in BSFTA) for sample silylation. Succinic acid was obtained from Sigma Aldrich Co. LLC. HMRlignan™ was purchased from Linnea SA, Switzerland. It was extracted and purified from knots wood in *Picea Abies*. DACSL and SESPL were prepared as described in Chapter Two.

**General Oxidation Procedure:** Generally, all oxidation procedures mentioned in this communication are carried out in 20 ml batch reactors (clear glass headspace vials, THERMO SCI SUN-SRI). 0.1000-0.2000 g organic substrates in ESI 2.1 were dissolved or dispersed in a mixture of 2 ml acetic acid/sodium acetate buffer, 6-7ml Mill-Q water, and 0.5 ml hydrogen peroxide. A 10% (weight percentage of substrates) loading of chalcopryte catalyst was added in to the mixture. The mixture was shaken and mixed well using mixer for 1-2 minutes (THERMO SCI TYPE 16700 MIXER). The batch reactors were kept in pre-heated incubator (60 °C) for 0-5 hours. Headspace
vials were necessary for retaining the gases generated during the reactions, which were also tested and clarified as supporting information for the proposed mechanisms.

**General Sampling Procedure:** All selectivity and yield data in this communication were based on the results from Gas Chromatography–Mass Spectrometry (GC-MS). Aqueous samples (0.5-1ml based on the concentration) were extracted with ethyl acetate (HPLC grade, 2 ml) three times. Extracted solutions were dried under nitrogen flow to evaporate all ethyl acetate and volatile compounds. Dried samples were silylated with 100 ul 10% TMCS (in BSTFA) at 60 °C for over 20 minutes, and then diluted with ethyl acetate into 1 ml solutions. The chemical composition of products was determined with a GC-MS (Agilent 7890A/5975C) fitted with a DB-5 capillary column. Structural information of lignin samples and HMR were characterized using $^{13}$C 600-MHZ NB VARIAN NMR SYSTEM supported by the Environmental Molecular Sciences Laboratory (EMSL, Richland, WA). All procedures for sample treatments and analysis followed previous published papers. (Seca, Cavaleiro et al. 2000, Jiang, Pu et al. 2009)
CHAPTER FOUR: PERACETIC ACID DEPOLYMERIZATION OF BIOREFINERY LIGNIN FOR PRODUCTION OF SELECTIVE MONOMERIC PHENOLIC COMPOUNDS

Chapter Three introduced a new catalytic pathway to selectively convert biorefinery lignin to dicarboxylic acids. The mechanisms study demonstrated a two stage reaction including lignin oxidative depolymerization and aromatic ring cleavage. However, the overall conversion yield to DCA was not satisfied, which was likely limited by the efficiency of first step lignin depolymerization. There is an urgent need to develop a practical lignin depolymerization pathway and thusly is the focus of this chapter.

3.1 Abstract

Lignin is the largest source of renewable material with an aromatic skeleton. However, due to the recalcitrant and heterogeneous nature of the lignin polymer as well as its complex side chain structures, it has been a challenge to effectively depolymerize lignin and produce high value chemicals with high selectivity. In this study, a highly efficient lignin-to-monomeric phenolic compounds (MPC) conversion method based on peracetic acid (PAA) treatment was reported. PAA treatment of two biorefinery lignin samples, diluted acid pretreated corn stover lignin (DACSL) and steam exploded spruce lignin (SESPL), led to complete solubilization and production of selective hydroxylated monomeric phenolic compounds (MPC-H) and monomeric phenolic acid compounds (MPC-A) including 4-hydroxy-2-methoxyphenol, p-hydroxybenzoic acid, vanillic acid, syringic acid, and 3,4-dihydroxybenzoic acid. The maximized MPCs yields obtained were 18% and 22% based on the initial weight of the lignin in SESPL and DACSL respectively. However, we found that the addition of niobium pentoxide catalyst to PAA treatment
of lignin can significantly improve the MPC yields up to 47%. The key reaction steps and main mechanisms involved in this new lignin-to-MPC valorization pathway were investigated and elucidated.

3.2 Introduction

Lignin is the largest source of renewable material with an aromatic skeleton. Depolymerizing lignin to low molecular weight aromatic and phenolic compounds presents an attractive pathway to produce bio-based renewable chemicals.(Whiting 2001, Holladay, Bozell et al. 2007, Zakzeski, Bruijnincx et al. 2010, Zhang, Tu et al. 2011, Song, Wang et al. 2013, Bruijnincx and Weckhuysen 2014, Ragauskas, Beckham et al. 2014, Rahimi, Ulbrich et al. 2014, Deng, Zhang et al. 2015, Van den Bosch, Schutyser et al. 2015) However, despite decades of effort, there are still very few commercially viable processes available to convert lignin to phenolic compounds. Lignin is a heterogeneous polymer consisting of phenylpropane units intricately connected by carbon-carbon bonds and ether linkages; selectively cleaving these bonds to release monomeric phenolic compounds (MPC) from lignin is a well-recognized challenge.(Whiting 2001, Zhang, Tu et al. 2011, Bruijnincx and Weckhuysen 2014, Lancefield, Ojo et al. 2014, Rahimi, Ulbrich et al. 2014) To date, an effective and complete lignin depolymerization chemistry, specifically optimized for selective chemical(s) production has not been demonstrated.(Ma, Xu et al. 2015) In addition, the configurations of both the propane side chain and aromatic nuclei differ significantly among the phenylpropane units that make up lignin macromolecules.(Lancefield, Ojo et al. 2014) Thus, the fragments obtained from existing lignin depolymerization methods typically exhibit large variations in structure, hindering the development of selective chemical production processes. Identifying a chemistry that has high efficiency in depolymerization of lignin and at the
same time can eliminate or minimize side chain variations is a key to realizing a selective lignin valorization method.

Peroxy acids are a group of acids with a perhydroxyl group (-OOH) substituting the hydroxyl group of their parent acids. It has been postulated that heterolytic cleavage of the peroxidic bond (COO-OH) produces hydroxonium (HO\(^+\)) cations, which are a strong electrophilic species that readily reacts with a number of electron-rich sites in lignin, including both aromatic ring and olefinic side chain structures.(Derbyshire and Waters 1950, Kadla and Chang 2001) A considerable amount of research has been done on the application of peroxy acids for pulping and bleaching.(Barthelemy 1934, Haney, Martin et al. 1948, Johnson 1975, Lawrence, Mckelvey et al. 1980, Gierer 1986, Thomasfolk, Myhrman et al. 1996, Sundquist and Poppius-Levlin 1997, Jaaskelainen and Poppius-Levlin 1999, Poppius-Levlin, Jaaskelainen et al. 2000, Sun, Tomkinson et al. 2000, Suchy and Argyropoulos 2001, Barros, Silva et al. 2010) Among all of the peroxy acids, peracetic aid (PAA) has attracted the most interest, and oxidation reactions using PAA typically proceed rapidly under mild conditions with minimal side reactions and by-product formation.(Swern 1949)

The results from previous studies appear to suggest that aromatic ring hydroxylation by HO\(^+\) substitution/addition is a predominant reaction in peroxyacid delignification. Therefore, this chemistry has a potential to selectively depolymerize lignin polymers to phenolic compounds. However, a complete mechanistic understanding of PAA reactions with macromolecular lignin is lacking. It is well understood that lignin consists of phenylpropane units linked by carbon-carbon (C-C) and ether bonds (C-O-C). Previous studies have focused primarily on PAA reactions with monolignols and β-aryl ether linked lignin dimeric model compounds.(Johnson and Farrand 1971, Lawrence, Mckelvey et al. 1980, Ruohonimi, Heiko et al. 1998) There is a gap in knowledge
concerning the capability and efficiency of PAA reactions with phenylpropane units linked by C-C bonds. Prior understanding of PAA delignification chemistry was largely based on treatments of wood/grass and pulp samples which consist of small percentages of lignin. The efficacy of PAA reactions with macromolecular lignin alone has not been determined.

![Figure 4.1. Reaction solution 1) DACSL before (1a) and after (1b) and SESPL before (1c) and after (1d) 60 minutes of reaction with PAA at 333 K at 0.2 g lignin/g PAA.](image)

The main objectives of this study are to demonstrate the potential of PAA for efficient depolymerization of biorefinery lignin to selective MPC and to gain a more complete understanding of the mechanism of PAA reactions with macromolecular lignin.

### 3.3 Result and Discussion

**Peracetic Acid Depolymerization of Biorefinery Lignin**

Two representative biorefinery lignin samples: diluted acid corn stover lignin (DACSL) and steam explosion spruce lignin (SESPL) were investigated in this study. Treatments of DACSL and SESPL by PAA were conducted in aqueous solution at different temperatures (303, 318, 333 and 363 K), reaction times (30-720 minutes) and PAA dosages (0.2-1 g lignin/g PAA). Both DACSL and SESPL were initially insoluble in the reaction media (Figure 4.1a and 4.1c).
Following the addition of PAA, all lignin samples were quickly dissolved within an hour of reaction at 333 K (Figure 4.1b and 4.1d) and 363 K. The amount of time required to completely dissolved lignin depended on temperature and PAA dosage. Increasing reaction temperature and PAA dosage further facilitated solubilization. A considerable amount of phenolic compounds were detected in the reaction solution through the use of Folin-Ciocalteu reagent to estimate the phenolic yields. (Singleton, Orthofer et al. 1999) Ethyl acetate, an effective solvent for extracting plant phenols from aqueous media, (Örsa and Holmbom 1994, Mitchell, Taylor et al. 2014) was used to extract the phenolic compounds from the reaction solution. (Haney, Martin et al. 1948, Singleton, Orthofer et al. 1999) The ethyl acetate extract (EAE) was silylated and then analyzed by GC-MS to identify specific chemical products. The dry weight of the total organic matter in the EAE was also measured. The EAE fraction produced after 90 minutes of PAA treatment at 333 K represented 54% and 58% of the initial weight of SESPL and DACSL respectively.
The composition of MPC in the EAE produced at different reaction conditions were determined by GC/MS analysis (Figure 4.2). It appears that the highest MPC yields were obtained mostly at 60 (DACSL) and 90 (SESPL) minutes of reaction. Higher reaction temperature caused a decrease in MPC concentrations at prolonged reaction times, a trend more significant for DACSL than SESPL. Peak MPC concentrations for SESPL were reached after 90 minutes of PAA treatment at 333 K, with 18% conversion yield based on the initial dry weight of the lignin. The highest MPC yield from DACSL (22%) was obtained after 60 minutes of PAA treatment at 333 K. This suggests grass lignin (e.g. DACSL) is more susceptible to PAA depolymerization than softwood lignin.
Figure 4.3. GC-MS spectrum of PAA treatment of SESPL at 90 min and 333 K; the major compounds structures are identified.

The detailed MPC compositions in the reaction media after PAA treatment of SESPL at 333 K after 90 minutes is shown in the total ion chromatograph (TIC) presented in Figure 4.3. Three major phenolic compounds were identified: 4-hydroxy-2-methoxyphenol (1), vanillic acid (2), and 3,4-dihydroxybenzoic acid (3). These three MPCs represented approximately 80% of the total ions detected by GC-MS. All are clearly derived from guaiacyl (G) subunits that are predominant in steam explosion spruce lignin (SESPL). This suggests that a prevailing reaction of PAA depolymerization of lignin is the cleavage of the propane side chains by either replacement with a hydroxyl group to (1) or oxidation to (2). (3) is likely a subsequent product formed after the
demethylation of the methoxy group of (2). 2-hydroxypropanoic acid (i1) is likely derived from side chain fragments. The PAA conversion of spruce lignin to a selective range of products is a significant finding, given the typically broad spectrums of products generated by most existing lignin depolymerization methods. (Vigneault, Johnson et al. 2007)

![GC-MS results for DACSL (EAE) after 90 min oxidation by PAA at 333 K without catalyst.](image)

Figure 4.4. GC-MS results for DACSL (EAE) after 90 min oxidation by PAA at 333 K without catalyst.

The products composition obtained from PAA treatment of DACSL was also analyzed (Figure 4.4). Again a selective group of MPC, including $p$-hydroquinone (4), $p$-hydroxybenzoic acid (5), vanillic acid (2), 3,4-dihydroxybenzoic acid (3), and syringic acid (6), represented approximately 85% of the total ions detected by GC-MS. DACSL contains all three types of phenyl nuclei: guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) in significant quantities, unlike the
guaiacyl dominant SESPL (G≈95%). The GSH ratios of SESPL and DACSL were estimated by both $^{13}$C NMR and thioacidolysis analyses (See details in Chapter Five). The detected products were clearly derived from all three GSH structures through the same propane side chain replacement and oxidation reactions as observed in SESPL depolymerization.

The narrow products distribution of MPC obtained from PAA depolymerization of lignin suggests a distinctive pathway that differs from most existing lignin depolymerization methods, which typically yield a mixture of phenolic monomers incorporating different C$_1$-C$_3$ side chain functionalities inherited from the lignin macromolecule. (Holladay, Bozell et al. 2007, Vigneault, Johnson et al. 2007, Lange, Decina et al. 2013, Ragauskas, Beckham et al. 2014)

Although peroxy acid reactions with β-aryl ether linked lignin dimer model compounds have been elucidated, a clear understanding of peroxyacid reaction mechanism toward propane side chain removal to produce hydroxylated MPC (MPC-H) and monomeric phenolic acids (MPC-A) is lacking. Furthermore, no prior studies have conducted a detailed investigation of peroxyacid reactions with either lignin or lignin model compounds with C-C linkages.

**Mechanism of PAA Depolymerization of Biorefinery Lignin**

To fill in the knowledge gaps and to gather an aggregated mechanistic understanding of peroxyacid reaction with biorefinery lignin, we started testing the reaction of hydroxymatairesinol (HMR), a dimeric phenylpropane compound connected by a C-C linkage, with PAA. Similar to the biorefinery lignin samples, the initially insoluble HMR was found to dissolve completely after reacting with PAA for 15 minutes at 333 K. After 90 minutes of reaction, several predominant compounds including 4-hydroxy-2-methoxyphenol (1), (7) and (8) were detected in a ratio of 4:1:1.5 respectively. These results suggest that an initial side chain replacement (SCR) reaction
occurred through \( \text{HO}^+ \) attack on \( C_1 \) to produce (1) and (7). SCR can occur on the \( C_1 \) of either phenyl nuclei, and the intermediate (8) generated can undergo a second SCR reaction to produce another (1) and side chain fragment (7) (Scheme 4.1). SCR reaction through electrophilic attack by \( \text{HO}^+ \) has been previously observed in reactions with lignin. The results from this HMR study provide the first direct evidence supporting SCR as the predominant mechanism for dissociating phenylpropane units linked by C-C bonds in lignin. The observed formation of MPC-H in both SESPL and DASCL reactions likely resulted from SCR.

**Scheme 4.1. Mechanism of step-wise PAA depolymerization of HMR.**

Previous work has shown that PAA cleavage of the \( \beta \)-aryl ether linked lignin dimer model compound, 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy) propanol, produced a significant amount of phenolic products with a propane-1,2-diol side chain attachment (pinacol structure).(Lawrence, McKelvey et al. 1980) However, compounds with pinacol structures were not detected in the present study. Instead, MPC-A such as vanillic acid (2), 4-hydroxybenzoic acid (5), and syringic acid (6) were the most abundant products. Therefore, the precise mechanism of
PAA depolymerization of ether bond linked phenylpropane units to MPC required further detailed investigation.

As shown by the C₉ formulas of SESPL (C₉H₁₀.₄O₃.₂(OCH₃)₀.₉₆) and DACSL (C₉H₁₀O₂(OCH₃)) obtained from elemental analysis, there is a significant amount of oxygen present either as ether bonds or hydroxyl groups on the side chains. This provides a likely source for the production of pinacol structures after PAA treatment. In addition, PAA is known to instigate epoxidation of the olefinic side chain structures in lignin, also giving rise to pinacol structures. (Zhang, Tu et al. 2011, Ma, Xu et al. 2015)

To understand the production of pinacol structures and their fate in PAA depolymerization of lignin, isoeugenol (9) was tested as a model compound. The products from isoeugenol reaction with PAA at 333 K were analyzed by GC-MS. It was seen that isoeugenol was quickly consumed within 30 minutes of reaction with four major reaction products identified. Vanillic acid (2) is indeed produced from PAA treatment of isoeugenol. In addition, a vanillic acid ester (12) as well as two 1-(3-methoxy-4-hydroxyphenyl)-propane-1,2-diol (10a, 10b) isomers were detected; the weight ratio of (2):(12):(10) was 1:6:10 after 30 minutes of reaction. It was also observed that the concentration of (10) and (12) decreased as the reaction progressed from 30 minutes to 90 minutes while the concentration of (2) kept increasing over time. The ratio of (2):(12):(10) changed to 1:1:1.5 at the 90 minute mark.
Scheme 4.2. Mechanism of a) PAA treatment of isoeugenol through epoxidation to form pinacol side chain and further oxidation to MPC-A; b) proposed mechanism of rearrangement of the pinacol side chain followed by the Baeyer-Villiger oxidation and hydrolysis to form MPC-A.

These results provided new understanding and insights regarding the transformation of pinacol structured phenolic compounds to MPC-A (Scheme 4.2a). Pinacol rearrangement to form ketone or aldehyde is a well-known reaction (Fittig 1860, Gutierrez, Loupy et al. 1989) As shown in Scheme 4.2b, under the experimental conditions employed in this study, 10a and 10b were likely first rearranged to a phenyl ketone (11), and then converted to a vanillic acid ester (12) through Baeyer–Villiger oxidation, a common organic reaction known to occur with ketones in the
presence of peroxyacids. (12) was subsequently hydrolyzed to yield MPC-A in the acidic media. This mechanism provides a convincing explanation of MPC-A formation from PAA depolymerization of biorefinery lignin.

Based on these new findings from both the C-C cleavage and pinacol structure rearrangement reactions, an aggregated mechanistic understanding of PAA depolymerization of lignin to MPC is delineated. Scheme 3.3 summarizes the pathways for three common phenylpropane unit side chain configurations: non-conjugated structure with C-C bond (S1); β-aryl ether linked structure (S2); and olefin structure (S3). For S1, direct replacement of saturated propane side chains initiated by electrophilic HO\(^{+}\) attack on partially negative charged C\(_1\) is the prevailing reaction to produce MPC-H (Scheme 4.3a). For S2, the reaction proceeds by cleaving hydroxylated ether linked phenylpropane units to form pinacol derivatives, which then undergo sequential pinacol rearrangement, Baeyer–Villiger oxidation and hydrolysis to generate MPC-A as final products (Scheme 4.3b). This pinacol rearrangement sequence is also applicable to S3 reactions, which generate the pinacol derivatives from an initial epoxidation step (Scheme 4.3c). These three pathways provide a unique mechanism for PAA depolymerization of lignin to yield product structures that are independent of their inherited lignin side chains. A high MPC-A to MPC-H ratio yielded from both lignin samples suggests that S\(_2\) and S\(_3\) are likely the predominant structures in SESPL and DACSL.
Scheme 4.3. Mechanisms involved in PAA treatment of lignin phenylpropane units with different side chain configurations. a) non-conjugated structure with C-C bond \((S1)\); b) \(\beta\)-aryl ether linked structure \((S2)\); c) olefin structure \((S3)\).

Overall product distributions after PAA depolymerization of lignin and the potential of niobium pentoxide to improve MPC yield

The highest MPCs yields obtained after PAA treatment of SESPL and DACSL at 333 K were 18% (90min) to 22% (60min) based on the initial weight of respective lignin sample. As mentioned earlier, the EAE at these treatments represented 54% and 58% of the initial weight of SESPL and DACSL respectively. The detailed compositions of the non-EAE fraction, consisting of organic matter remaining in the reaction solution after ethyl acetate extraction, were difficult to
quantify precisely as most of these compounds are volatile. Propanoic acid derivatives were detected by GC/MS analysis as major constituents of the organics in the residual aqueous phase. Based on the mechanism understanding, these compounds are likely derived from the side chain cleavage of lignin.

It is apparent that the mass of the EAE fraction does not match the mass of the detected MPC. One main reason is due to presence of oligomeric phenolic compounds which are dissolved in the reaction media, but not quantified by GC analysis. However the fact that amount of MPCs did not increased along with the increase in either treatment temperature or time appear to suggest that MPCs may be further degraded. It is known phenol can be converted to quinones during PAA reaction. Quinones are not detected by GC/MS. To capture and determine quinones, ortho-phenylenediamine was added to the reaction solution to form phenazine complex with quinone intermediates. The ensuing phenazine complex was subsequently determined by UV/Vis spectroscopy. (Zhu, Olmstead et al. 1995) As shown in Figure 4.5, this phenazine complex has a distinct absorption peak at 398 nm. As shown in Figure 3.4, $A_{398}$ increased with reaction time, suggesting the accumulation of quinones. (Zhu, Olmstead et al. 1995, Vân Anh and Williams 2012) The conversion of phenols to quinones appeared to be significant after 90 minutes of reaction and became more pronounced after 180 minutes of reaction. This trend is correlated to the corresponding MPC yield profile, where yield peaked at 90 minutes and then leveled off. Transformation of phenols to $o$-benzoquinone can consequently lead to aromatic ring cleavage reactions resulting in lower MPC yield; the MPC yield from DACSL decreased to 8% after 300 minutes of reaction at 333 K. It is, however, interesting to observe that lower reaction temperatures can maintain slightly higher MPC yields after 300 minutes, although the maximum MPC yield was lower than that of the reactions at 333 K. These results suggested that a series of reactions can
occurred during PAA treatment of lignin. PAA treatment can first depolymerize macromolecular lignin to oligomers and subsequently produces selective MPCs. MPCs can be further converted to quinones and subsequent ring opening products at higher temperature and longer reaction time. Therefore it is apparent that improving PAA depolymerization efficiency and preventing MPC from further degradation are the two keys to optimizing MPC production from lignin.

![Graph showing UV-vis spectrum quantification of quinone formation in PAA treatment of DACSL from 0-300 minutes of reaction time without catalyst.](image)

**Figure 4.5.** UV-vis spectrum quantification of quinone formation in PAA treatment of DACSL from 0-300 minutes of reaction time without catalyst.

Previous literature has mainly attributed HO\(^+\) as the key reactive species from peroxycacid involved in lignin depolymerization. However, the information related to the characteristics of the HO\(^+\) species and the methodology to detect this species is scarce in literature. Therefore identifying
a targeted approach to enhance the PAA reactivity toward lignin is a challenging task. We have evaluated several metal oxide catalysts for their potentials to facilitate PAA conversion of lignin. (Ma, Xu et al. 2015) Niobium pentoxide (Nb$_2$O$_5$) showed a surprising effect on promoting PAA conversion of lignin to MPC. Several studies have shown that niobium oxides can function as selective and effective catalysts for a range of oxidation reactions. (Iizuka, Ogasawara et al. 1983, Paulis, Martin et al. 1999, Wachs, Briand et al. 2000, Rooke, Barakat et al. 2012) Besides acting as a strong Lewis acid, (Nakajima, Baba et al. 2011) niobium oxides also have a unique capability of stabilizing oxidative cation species, (Zemski, Justes et al. 2001, Fielicke, Meijer et al. 2003, Carniato, Bisio et al. 2014, Wu, Tang et al. 2014) which may assist the electrophilic attack of PAA on lignin.
Figure 4.6. Comparison of MPC yield from PAA treatment of SESPL and DACSL without (SESPL-NC-60, DACSL-NC-60) and with (SESPL-NB-60, DACSL-NB-60) Nb\textsubscript{2}O\textsubscript{5} catalyst at 333 K from 0-300 minutes.

Nb\textsubscript{2}O\textsubscript{5} was added as a catalyst to the PAA reactions of DACSL under identical reaction conditions and lignin-to-MPC conversion yields over 300 minute reactions at 333 K were determined. It was found that MPC yields from the catalyzed reactions (10 wt\% Nb\textsubscript{2}O\textsubscript{5}) were significantly higher than those from control (without Nb\textsubscript{2}O\textsubscript{5}) reactions throughout the course of reaction. The highest MPC yield was obtained after 90 minutes at 47\% (Figure 4.6). Applying Nb\textsubscript{2}O\textsubscript{5} catalyzed PAA treatment of SESPL also led to increased MPC yields. It is interesting to
see that the highest yields were obtained at 300 minutes. The role of Nb$_2$O$_5$ in the improvement of MPC product selectivity is intriguing. For both lignin, the maximum yields were all observed at longer reaction times: 90 minutes vs 60 minutes for DACSL and 300 minutes vs 90 minutes for SESPL. This evidence suggests that in addition to enhancing PAA efficiency, Nb$_2$O$_5$ also plays a significant role toward improving MPC stability. Another important finding is that the dry weight of the EAE fractions obtained from the Nb$_2$O$_5$ catalyzed reactions with either lignin did not change significantly. The maximum MPC yields thus account for 81% and 88% of the total EAE of SESPL and DACSL respectively. Combining the additional results shown in Figure 4.3 and S4 to S6, it is reasonable to conclude that Nb$_2$O$_5$ likely acted as a heterogeneous catalyst to react with the soluble fraction instead of directly catalyzing insoluble lignin depolymerization in this reaction. At the same time, Nb$_2$O$_5$ has clearly shown an effect on improving the stability of MPC produced from lignin depolymerization. Although Nb$_2$O$_5$ is known to catalyze oxidation reactions in the presence of peroxygen chemicals,(Zemski, Justes et al. 2001, Fielicke, Meijer et al. 2003) detailed catalytic chemistry of Nb$_2$O$_5$ is lacking.(Carniato, Bisio et al. 2014, Tarselli 2015) In addition, the direct characterization of HO$^+$ during the reaction is challenging.(Derbyshire and Waters 1950) Future research is needed to understand Nb$_2$O$_5$ interactions with dissociated and non-dissociated peroxacid species as well as soluble phenolic oligomers.

**Improved conversion of monomeric phenolic compounds to DCAs**

The depolymerization of lignin has resulted in a selective profile of hydroxylated phenolic compounds and phenolic acids compounds that can potentially be converted to DCAs by aromatic ring cleavage conditions. To further evaluate whether the efficiency can be improved, we used a group of monophenolics with similar structures as lignin derived monophenols, including catechol, guaiacol, veratrol, vanillin, vanillic acid, methylsyringol, tert-butyl catechol, eugenol,
and iso-eugenol, which covered a wide possibility of lignin subunits configuration as well lignin valorization products. To mimic the multi-hydroxylated structure, we are treating lignin model compounds under high concentration of hydroiodic acid (47 wt%) which has been employed as effective characterization method determining the methoxy contents by measuring the CH$_3$I during the process. Since the depolymerization is operated under peractic acid treatment conditions, we explored a second step using iron catalyzed PAA aromatic ring opening of phenolic rings. Table 4.1 summarized the yield of DCA from representative phenolics. The weight yield of the DCA from the phenolics structures can achieve a high yield up to 86 wt%, which is derived from G units (guaiacol). Also, the hydroxylated structure such as catechol and methylsyringol which derived from H and S units can also be converted to DCA in comparable yields. This outcomes confirms that optimizing the depolymerization of lignin to hydroxylated phenolic structures would be a promising route to improve the conversion to DCA. What should be noticed is the vanillin and vanillic acids, which is benzaldehyde and benzoic acid, still have a low conversion reactivity. The phenomena reminds to explore the depolymerization chemistry to achieve a optimized phenolic profile is the key to improve the overall lignin-to-DCA in the future work.

<table>
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<tr>
<th>Phenolic</th>
<th>Conversion</th>
<th>DCA Yield wt%</th>
<th>Meth</th>
<th>DM Conversion%</th>
<th>DM Yield wt%</th>
<th>DM-DCA Yield %</th>
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</tr>
<tr>
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<td>n.d.</td>
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</tr>
<tr>
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<td>100%</td>
<td>92</td>
<td>0%</td>
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<tr>
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<td>~99</td>
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</table>
4.4 Conclusions

This study demonstrated a novel and efficient biorefinery lignin depolymerization chemistry. PAA can completely depolymerize biorefinery lignin at mild conditions and produce a selective group of monomeric phenolic compounds (MPC). We also discovered that supplementing Nb$_2$O$_5$ catalyst to this reaction can significantly increase the MPC yield up to 47% based on the initial lignin weight. The mechanisms involved in lignin conversion to selective MPC were investigated and elucidated. The results have shown that PAA depolymerizes lignin to MPC through a unique mechanism that converts the propane side chains to either a hydroxyl or carboxyl group, contributing to products selectivity. The results obtained from this study offer a new pathway toward lignin valorization.

4.5 Experimental Summary

**Materials:** All chemical reagents were purchased from commercial sources and used without further purification. The following reagents were obtained from Thermo Fisher Scientific Inc.: peracetic acid (39 w%), glacial acetic acid, ethyl acetate (HPLC grade) for extraction and GC-MS, and 10% Trimethylchlorosilane with N,O-bis(Trimethylsilyl) trifluoroacetamide for sample silylation. Isoeugenol (98+%) and niobium (V) pentoxide (powder) were obtained from Sigma Aldrich Co. LLC. Diluted acid corn stover lignin (DACSL) was collected from the corn stover-to-bioethanol conversion process via diluted acid pretreatment. Steam exploded spruce lignin (SESPL) was collected from steam explosion pretreatment of spruce.

**Oxidation Procedure:** Generally, all oxidation procedures mentioned in this article were carried out in 50 mL Erlenmeyer flasks. Treatments of DACSL and SESPL by PAA were conducted in aqueous...
solution at different temperatures (303, 318, and 333), reaction times (30-720 minutes) and PAA dosages (0.2-1 g lignin/g PAA). Lignin and peracetic acid were added into aqueous solution (DI water) and mixed (THERMO SCI TYPE 16700 MIXER, Fremont, CA) for 1 minute prior to reaction at the selected temperature. The batch reactors were kept in a pre-heated incubator (303-333 K). During the catalyzed peracetic acid treatment, a 10% (weight percentage of substrates) loading of Nb₂O₅ catalyst was added into the mixture prior to the reaction.

Detection of MPC products and DCA products: The total amount of phenolic compounds was estimated in the reaction solution via microtiter-plated Folin-Ciocalteu assay using isoeugenol as a standard. (Zhang, Zhang et al. 2007, Magalhães, Santos et al. 2010) The products profile is determined using GC-MS methods described in Chapter Two. The DCA products are detected and quantified using GC-MS with trans,trans-,muconic acid as reference.

Detection of Benzoquinone: Separate ethyl acetate extractions were conducted to obtain samples for quinone analysis. Then O-phenylenediamine and glacial acetic acid were added into the extracts. The mixture was reacted for 4 hours at 60 °C. The reaction solution was dried under nitrogen atmosphere and was diluted with ethyl acetate to desired concentration prior to UV-vis measurement. The UV-Vis measurements were carried out using a UV-Vis spectrophotometer (SpectraMax M5) with a scanning range from 200 to 800 nm of wavelength. As o-benzoquinone derivatives have a known absorption in the range of 350-450 nm according to their structural variation (Zhu, Olmstead et al. 1995), the quinone concentration in this study was determined by its peak absorption wavelength at 398 nm.
CHAPTER FIVE: QUALITATIVE AND QUANTITATIVE RELATIONSHIPS BETWEEN LIGNIN DEPOLYMERIZATION/AROMATIC-RING OPENING REACTIVITY AND THEIR STRUCTURAL CHARACTERISTICS: FUNCTIONAL GROUP, INTER-UNIT LINKAGE AND MACROMOLECULAR

Lignin is a three dimensional complex with a variable structural features determined by biomass source and isolation methods. The structural complexity of lignin is expected to have a significant impact on its reactivity, but not yet been explored. Chapter Three and Four developed a systematic mechanistic understanding towards oxidative sequential depolymerization and aromatic ring cleavage of lignin. This chapter aims to explore the qualitative and quantitative relations between lignin depolymerization/aromatic ring opening reactivity and their structural characteristics.

5.1 Introduction

Lignin, together with cellulose and hemicellulose, is one of the three major components representing 15 to 40% of the dry weight of plants in lignocellulosic biomass.(Fengel and Wegener 1984, Sjostrom 1993) Because polysaccharides have been the primary component used for biofuel and chemical production, large quantities of industrial lignin are already produced annually as a waste product of the pulp and paper industry,(Zhang, Tu et al. 2011) while the emerging biomass refinery industry will further introduce an enormous amount of lignin.(Holladay, Bozell et al. 2007) Therefore, lignin is attracting much attention due to its potential as a renewable resource for the production of bio-based materials, fuels, and chemicals, particularly aromatics. Despite considerable efforts in this direction, the major industrial uses of lignin are still limited and are
primarily associated with the macromolecular industrial application of lignosulphonates. Recent studies have shown lignin as a potential source for phenolic and dicarboxylic acid production. However, lignin derived from different isolation processes have diverse structural characteristics and specific structural features that would affect product selectivity and yields significantly. The lignin structural complexities are primarily exhibited at three different levels: functional group, inter-unit linkage and macromolecular.

Lignin is the result of copolymerization of three major groups of phenylpropanyl subunits, p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S), which are crosslinked by a variety of inter-unit linkages that fit into two major categories: ether bond linkage (e.g. α-O-4, β-O-4, 4-O-5) and carbon-carbon linkages (e.g. β–β, β-5, 5–5). The functional groups on lignin structures consists those on aromatic rings and propanyl side-chains. The GSH ratio of the lignins are predominantly controlled by the source of the feedstock, and is the primary aspect determining functional group on lignin aromatic rings. For example, lignin derived from gymnosperm biomass primarily consists of G units (~95%), lignin derived from angiosperm is rich in both G and S, and lignin from grass (corn stover, wheat straw) consists of all three types of subunits. During the lignin isolation/pretreatment processes, the side-chain functionalities were also significantly modified. The side-chains typically contain different quantities of functional groups, such as olefin, hydroxyl, carbonyl, and carboxylic groups. The nature of these GSH units to radically polymerize leads to the complexity of inter-linkages existing in lignin. The relative abundance of these linkages in native lignin varies from plant to plant, but the β-O-4 linkage is typically the most abundant. Although ether linkages are relatively more abundant, they are believed to readily cleave or partially cleave under most pretreatment/lignin isolation conditions. The cleavage of the β-O-4 is also commonly observed during acid/basic treatment, and the variation of the β-O-4 linkages left
in the isolated lignins are highly depending on the chemistry and severity of the treatment. In contrast, carbon-carbon linkages tend to be inert during most of the treating conditions, but re-condensation during the isolation processes could lead to the formation of a new type of C-C bond. Although there have been appreciable efforts on these two levels, there is still a lack of awareness that these structural features are highly correlated and also have impact on lignin conversion reactivity. Thus, a systematic comparison of all these structural features is needed. These two levels of structural complexity are usually the primary concern in current lignin research.

The last level of complexity, which will be referred as the macromolecular level, has not been focused on as a major complexity in previous studies. Existing studies tend to use molecular weight as the primary scale or the only approach to characterize lignin macromolecule. However, when lignin subunits assemble to the macromolecular level, inter-linkage bond formation is not the only force that shapes the macromolecule. The intra-molecular force is very complicated. In other words, the physical configuration of the lignin macromolecule, not just the chemical structural features, needs more attention. For example, during the lignin isolation process the oxygenate rich side chain structure makes it possible to form a variety of hydrogen bond inter and intra molecularly, which will champion the force determining the side chain interactions. Moreover, considering the lignin is the most abundant source of aromatic rings in nature, the potential π-π* interaction between subunit rings are also likely to contribute to the force determining the morphology and reactivity of lignin molecules. Despite this, current studies have focused primarily on the complexity, while the macromolecular level is largely being ignored.

The structural complexities are often observed to affect lignin’s reactivity. However, current research primarily focused on one or only a few of these structural characteristics to interpret their impact on lignin reactivity towards conversion. Lack of an assembled analysis of
lignin structure can hardly give fair evaluation. Moreover, most of current researches remain to give qualitative explanation of structure-reactivity correlation. Establish a quantitative tool is in urgent need to evaluate and predict a lignin source’s performance for valorization. In this study, we provide an assembled analysis of functional groups and inter-units linkage features on biorefinery lignin. Seven representative biorefinery lignin were selected including diluted acid pretreated corn stover lignin (provided by NREL), steam exploded spruce lignin (provided by FPInnovation), SPORL of D. Fir (provided by FPL), Milled wood lignin of D. Fir. (Provided by NARA), soda pretreated wheat straw lignin (isolated based on ref), commercial lignin (alkaline lignin purchased from Sigma-Aldrich), and deep eutectic solvent D. Fir lignin. Functional groups on lignin aromatic rings are acquired using conventional wet chemistry methods: thioacidolysis and nitrobenzene oxidation. The side-chain functional groups are estimated through a combination of the C9 formula (calculated according to CHNO elemental analysis) and FT-IR. The inter-linkage structures are compared based on quantitative 13C NMR analysis, 13C/1H HSQC NMR and GPC. The macromolecular level of complexity is presented by combining the analysis of molecular weight distribution (GPC), BET, and scanning electron microscopy (SEM). At the end, we introduce a statistical tool using R Statistics® to build quantitative correlation between lignin structural characteristics and their reactivity towards oxidative depolymerization and aromatic ring cleavage.

5.2 Results and Discussion

5.2.1 Lignin preparation strategy and Composition Analysis

In this study, we investigated the seven representative lignins isolated from both woody biomass (Spruce, D. Fir) and agricultural residue (corn stover, wheat straw) by a variety of pretreatment methods (acidic, alkaline, sulfite, mechanic milling and DES pretreatment). The
purity of the representative lignin is measured by Klason lignin content and presented in Table 1. A description of these pretreatment methods is provided below.

Acidic pretreatment (e.g. diluted acid, steam explosion) has been applied to a variety of lignocellulosic materials. Diluted acid pretreatment is typically carried out at temperatures above 160°C with mineral acids as catalyst. During this acid treatment process, the majority of lignin remains as a solid residue after enzymatic hydrolysis. Previous work has shown that diluted acid lignin contains low carbohydrate content. The ether and ester linkages are partially cleaved during diluted acid pretreatment, which may also destroy the matrix structure and generate low molecular-weight lignin fragments with increased hydroxyl group content. However, the detailed structural information of the lignin samples treated with diluted acid still needs further investigation.

Alkaline pretreatment is another process commonly applied in paper making and biorefineries. The alkaline conditions likely caused the breakdown of the protolignin and thus extracted lignin by dissolution of lignin fragments. The crude lignin was extracted from the remaining extraction solution through precipitation, which was done by neutralizing the solution to a pH of 2-4. The labile cleavage of lignin-carbohydrate complex resulted in a significant amount of residual saccharide in the isolated alkaline lignins. In addition, the residue mineral is higher compared to other isolation processes due to the neutralization step for lignin collection.

Mechanical milling is an industrial process used to produce fibrous pulp from biomass, predominantly wood, by a combination of heat and mechanical force. This is the existing commercial process to make either paper products such as newsprint or fiber board products such as medium density fiber board. With minimized structural modification by chemical treating, the milled wood lignin is considered the most resemble to native lignins existing in biomass.
SPORL (sulfite pretreatment to overcome recalcitrance of lignocellulose) is a biomass pretreatment modified from sulfite pulping. SPORL yields sulfonated lignin (lignosulfonates) both dissolved in spent pretreatment liquor and retained in the solid substrate. The existing commercial products from lignin are predominantly derived from lignosulfonates. Compared to other types of biorefinery lignin, lignosulfonates have higher average molecular weight and hydrophilicity. SPORL lignin are also likely to contain a high sulfur content compared to other biorefinery lignins.

DES lignin is a novel type of lignin developed in our lab that applies deep eutectic solvent as an alternative to ionic liquid in order to extract high purity lignin with minimum loss of saccharides. By washing the collected lignin with a water and ethanol mixture multiple times, high purity lignin can be obtained (>95% klason lignin purity) with little ash or sugar residue. Moreover, this process not only extracts lignin in high purity, but in a high recovery yield as well (~60% of original lignin determined by comparing lignin content of residue before and after treating). Our previous study indicated that a high degree of ether linkage cleavage occurred during DES treatment, resulting in a low average molecular weight.

<table>
<thead>
<tr>
<th>Lignin</th>
<th>Lignin Content</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>AIL</td>
<td>ASL</td>
<td>Sum</td>
</tr>
<tr>
<td>AKL</td>
<td>45</td>
<td>2</td>
<td>47</td>
</tr>
<tr>
<td>DACSL</td>
<td>88</td>
<td>3</td>
<td>91</td>
</tr>
<tr>
<td>SESPL</td>
<td>75</td>
<td>2</td>
<td>77</td>
</tr>
<tr>
<td>Soda-WSL</td>
<td>58</td>
<td>4</td>
<td>62</td>
</tr>
<tr>
<td>MWL</td>
<td>55</td>
<td>2</td>
<td>57</td>
</tr>
<tr>
<td>DESL</td>
<td>91</td>
<td>4</td>
<td>95</td>
</tr>
<tr>
<td>SPORL</td>
<td>23</td>
<td>4</td>
<td>27</td>
</tr>
</tbody>
</table>

Table 5.1. Analysis of lignin purity by Klason lignin content.
5.2.2 Characterization of Lignin Functional Groups and Inter-unit Linkage

GSH variation is the most basic structural features reflecting functional groups on lignin aromatic rings. The GSH ratios are varied in biorefinery lignins and are primarily determined by the mother source of the biomass. To date, many chemical degradation or analytical methods have been developed attempting to determine the GSH ratio in a lignin samples, such as acidolysis, thioacidolysis, alkaline nitrobenzene oxidation, cubric oxide oxidation, permanganate oxidation, and pyrolytic GC-MS. However, there has not been an agreed method that could fully represent the real GSH ratio within a lignin sample. This paper chooses to compare the results from alkaline nitrobenzene and thioacidolysis to determine the GSH ratio of representative lignin. Nitrobenzene oxidation of lignins in alkaline solution is one of the most significant lignin subunit analysis methods, converting GHS units to the benzaldehyde structures respectively. A typical recovery yields of less than 30% total of benzaldehyde of original lignin weight can be achieved. The method is relatively simple compared with the potassium permanganate oxidation of lignins. However, the reaction mechanisms of nitrobenzene oxidation remained unknown though there is some preliminary work on the model compounds. Thus, it is hard to speculate the possible side-chain structures based on nitrobenzene oxidation results. In contrast, thioacidolysis is an acid-catalyzed reaction which results in the depolymerization of lignins with certain modification of lignin side-chain. The method may be used to estimate the amount and composition of uncondensed alkyl aryl ether structures without affecting methoxyl groups on the aromatic ring. Additionally, it can be used to analyze several minor structures in both isolated and in situ lignins. The selective modification of propanyl side chain by the thioacidolysis provides unambiguous evidence for the occurrence of arylglycerol aryl ether structures (β-O-4 ether linkage in other words) and allows for a precise quantitative analysis of each unit based on the specific mass
spectroscopy. Thus, integrating this two acid-alkaline based analytical method is likely to give a balanced interpretation of the GSH ratio in lignin.

<table>
<thead>
<tr>
<th></th>
<th>Alkaline Nitrobenzene Oxidation</th>
<th>Thioacidolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>G</td>
</tr>
<tr>
<td>AKL</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>DACSL</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>SESPL</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>MWL</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>SPORL</td>
<td>-</td>
<td>96</td>
</tr>
<tr>
<td>DESL</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>Soda-WSL</td>
<td>7</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 5.2. Comparative analysis of GSH ratio of representative biorefinery lignins determined by alkaline nitrobenzene oxidation and thioacidolysis.

Table 5.2 summarized the GHS ratio of the seven biorefinery lignins obtained using both alkaline nitrobenzene oxidation and thioacidolysis. In general, the GSH ratios determined by both reactions are highly aligned. AKL, SESPL, MWL, SPORL and DESL are lignins extracted from gynompsperm wood, in which guaiacyl units are usually predominant (>95%). The total percentage of detected G units in these lignins can vary from 85% to 99%. In particular, MWL and DESL are derived from D. Fir lignin from same species and area. However, it is apparent that the MWL and DES lignin have a very different pattern towards alkaline nitrobenzene oxidation and thioacidolysis. MWL has shown a much higher reactivity towards both nitrobenzene oxidation (17.3%) and thioacidolysis (12.1%) compared with the respective 6.4% and 0.9% of the yield from
DESL. The nitrobenzene oxidation indicated 15% H units together with 85% G units in DES lignin with a total recovery yield of approximately 6.4% of the original weight. The thioacidolysis only detected the G units with a very low recover yield of 0.9% of the original weight of DES lignin. Similarly, the lignins isolated by sulfite treating processes (AKL, SPORL) have relatively low recover yields (~5-6%) in the thioacidolysis reaction but relatively higher yield in nitrobenzene oxidation. This is likely due to the fact that, in DES or sulfite treating lignin isolation processes, a majority of the ether linkages were cleaved so that only a few units were left to be subjected to the thioacidolysis reaction. The mechanisms of nitrobenzene oxidation allow more accessibility to the oxidization of the side-chain, which yields the detectable monomers. DACSL and Soda-WSL are extracted from grass type biomass, thus containing all three of the GHS type of subunits. Besides the regular structures detected in nitrobenzene oxidation and thioacidolysis, both samples also contain a considerable amount of cinnamic acid and ferulic acid which make up to approximately 10% of the total recover yield. The GSH ratio obtained from alkaline nitrobenzene oxidation and thioacidolysis are overall consistent, but they only represent the GSH ratio of less than 30% of the lignin samples due to their low reaction yield. Advanced instrumentation, such as NMR would provide more fair quantification of the lignin GSH ratio and will be introduced in later discussion. These wet chemistry approaches still provide important structural information such as side-chain configurations, and will be more useful if reaction mechanisms is well investigated.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>H</th>
<th>O</th>
<th>Chemical Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKL</td>
<td>57.2</td>
<td>7.4</td>
<td>35.4</td>
<td>C₉H₁₃₂O₃.₈⁹(OCH₃)₀.₅₄</td>
</tr>
<tr>
<td>SESPL</td>
<td>62.7</td>
<td>5.7</td>
<td>31.6</td>
<td>C₉H₁₀₁O₂.₀₀(OCH₃)₁.₀₁</td>
</tr>
<tr>
<td>DACSL</td>
<td>57.4</td>
<td>6.4</td>
<td>36.2</td>
<td>C₉H₁₀₄O₃.₂₀(OCH₃)₀.₉₆</td>
</tr>
<tr>
<td>MWL</td>
<td>51.3</td>
<td>5.6</td>
<td>43.0</td>
<td>C₉H₁₂₆O₅.₈₃(OCH₃)₀.₄₅</td>
</tr>
<tr>
<td>DESL</td>
<td>65.8</td>
<td>5.2</td>
<td>29.0</td>
<td>C₉H₄₅O₁.₆₆(OCH₃)₁.₉₇</td>
</tr>
</tbody>
</table>
In addition to GSH ratio, another key aspect that controls the lignin monomeric structures is the side-chain variations. Functional groups like olefin, hydroxyl, carbonyl, and carboxylic commonly exist in the protolignins, while the isolation processes would bring in significant modifications. In this study, we integrated the CHO elemental analysis and FT-IR to provide qualitative and quantitative characterization of the functionalities on the side-chain structures. Elemental analysis data can help speculate the C9 formula of the lignins, which can be used to estimate the average quantities of methoxy content, side-chain oxygen content and unsaturation based on per phenolic structure. Table 5.3 presents the CHO elemental analysis results of these biorefinery lignin. We estimate their structure and present in C9 formula. The oxygen contents, which represents the overall functionality on aromatic ring and side-chains, are varied significantly lignin by lignin in a range from 29.0% for DESl to 43.0 for MWL. The high oxygen content in MWL does not necessarily mean the high functionalities, but could be a results of high sugar residue content left after isolation. Most of the oxygen contents of other lignin are around 30 to 38%, which also suggests an oxygen-rich side-chain structures. However, elemental analysis can only provide a quantitative estimation of the combining abundance of oxygen containing functionalities, such as hydroxyl, methoxy, carbonyl, and carboxylic group, but not providing the evaluation of each functional group.

FT-IR provides a qualitative comparison of the functional groups of the side-chains. Integrating both results are meant to provide mechanistic information on the isolation chemistry.
and potential reactivity evaluation of biorefinery lignins for specific type of depolymerization chemistry. Lignin-KBr pellets were prepared to get the high resolution of lignin FT-IR spectroscopy. The comparative FT-IR were presented in four wavenumber segment in as shown in Figure 5.1, including 3050-2750 cm\(^{-1}\), 1830-1550 cm\(^{-1}\), 1550-1175 cm\(^{-1}\), and 1175-800 cm\(^{-1}\). These four segments of wavenumbers represent the regions of signals reflecting the functionality of C-H stretch in methyl and methylene groups, C=O stretch, aromatic skeletal vibration, and C-H deformation, respectively. Interpretation of band below 1175 cm\(^{-1}\) is usually difficult. To provide comparative analysis of FT-IR spectroscopies of these biorefinery lignins, the absorbance at 3050-2750 cm\(^{-1}\) was matched to a similar level since the relatively high consistency of methyl/methylene functionality in most treating conditions. Remarkable difference in the band range 1800-1650 cm\(^{-1}\) were observed, which indicate the unconjugated (centered >1700 cm\(^{-1}\)) and conjugated (centered 1650-1700 cm\(^{-1}\)) C=O stretch. The existence of both conjugated and unconjugated C=O stretch was observed in almost all biorefinery lignins samples, primarily in the form of ketone. In DACSL, DESL, and soda-WSL, unconjugated C=O structures seems to be more predominant, while in MWL, SESPL, and SPORL (all three derived from softwood) there are more C=O conjugated to phenolic rings. The DES lignin has a broad response in the range of 1700-1750 cm\(^{-1}\), which can be deconvoluted to two peaks centered at 1740 cm\(^{-1}\) and 1720 cm\(^{-1}\) respectively indicating the presence of unconjugated ketone and aldehyde. In addition, DESL has a comparable high free phenolic OH content since its high absorbance at 1375 cm\(^{-1}\). These results correspond to the proposed mechanisms for DES cleavage of β-aryl ether bond and formation of H-K ketone during lignin extraction processes. Similar degrees of adsorption at 1375 cm\(^{-1}\) are observed in other biorefinery lignins beside DESL, indicating similar content of free phenolic groups. Increased vibration at 1654 cm\(^{-1}\) is assigned to lignin condensation reactions at the expense of C=C double
bonds in conjugated carbonyl groups, which were observed in DACSL and SESPL. Most of the time, aromatic rings exhibit a characteristic band at approximately 1500 cm\(^{-1}\), corresponding to benzene ring stretching vibrations. A shift of the maximum absorption from 1505 cm\(^{-1}\) to approximately 1512 cm\(^{-1}\) due to breaking of aliphatic side-chains was observed in DESL, Soda-WSL, SESPL and SPORL, which suggests that the DES treatment, soda treating, steam explosion and SPORL processes have a relatively higher efficiency at cleaving the lignin ether linkage during fractionation processes. DACSL and soda-WSL have a noticeable adsorption at 1330 cm\(^{-1}\) and 835 cm\(^{-1}\) that represents the S unit and C-H deformation on S units’ rings while not showing in other lignins extracted from softwood. This corresponds to the GSH ratio analysis results showing that DACSL and soda-WSL contain more than 30% of S units and that almost no S units are detected in softwood lignins. The band at 1140 cm\(^{-1}\) is the result of the sum of the contribution of C-H deformation in aromatic rings and C-O stretching in primary alcohols. Interestingly, it therefore shows a higher content of primary alcohol existing in MWL and DESL, while maintaining a similar level in other lignins.
Figure 5.1. FT-IR spectra of representative biorefinery lignin.

\[^{13}C\] NMR can determine minor structure not amenable to analysis by other methods. The \[^{13}C\] NMR spectrum of lignin can be divided into three main segment: the first (200 to 165 ppm) contains signals assignable to carbonyl carbons, the second (165 to 100 ppm) is ascribable to aromatic and olefinic carbons, and the third (100 to 10 ppm) is attributed to aliphatic carbon atoms. To improve lignin solubility in solvents and the spectra resolution, the acetylation derivation was performed on all lignin samples. Due to the sulfonate group on AKL and SPORL, which are lignosulfonate from sulfite pretreatment processes, these two lignins cannot be acetylated and therefore are not compared in this section. The other five types cover biorefinery lignins from both woody biomass and grass biomass and are treated by a variety of methods including acid, alkaline, and DESL. Table 5.4 summarizes the major assignment of chemical shifts of spectral region and the quantitative analysis of five types of biorefinery lignins including MWL, DESL, DACSL, SESPL, and Soda-WSL. Normally either methoxy content or aromatic carbons were normalized as internal standards. However, in this study, considering how mother biomass species or isolation process can cause potential differences in methoxy content, the integration of total aromatic carbons (160-103 ppm) were normalized to six as internal standards for the quantitative analysis.
of biorefinery lignin samples. Since G units are the predominant monomeric units in softwood, MWL and DESL have a methoxyl content very close to 1. DESL has relative low methoxyl content, which could be due to either partial conversion of methoxyl groups to free hydroxyl groups or to the solubilization of G units in DES, therefore increasing the ratio of H units. DACSL and Soda-WSL have a methoxyl content greater than 1 since S units make up over 30% of these two lignins. DACSL has a relatively low methoxyl content compared to soda-WSL, which could be due to the existence of more H units in the form of cinnamic acid/ester in DACSL. This result corresponds to those obtained from thioacidolysis and Nitrobenzene oxidation. In the aromatic region (160-103 ppm) significant differences were observed between the various lignins, which provides another way of estimating HGS ratio. The HGS ratio was estimated based on the integration of resonance intensity at 121.5 ppm for H units, at 150 ppm for G units, and 152 ppm for S units. The results are normalized to 100% and summarized in Table 4. It appears that the G and S unit estimate obtained from 13C NMR closely matches the results obtained from thioacidolysis and nitrobenzene oxidation. However, a lack of resolution with the H units is likely due to the difficulties of baseline correction near the chemical shift at 120 ppm region. Besides estimating the HGS ratio, a comparison of the $C_{Ar}-C$ bond at the 141-125 ppm range provides important information regarding the formation of new C-C bonds, in another word the lignin recondensation, during isolation processes. Higher intensity at this region assigned to higher degree of condensation. It is apparent that MWL and DESL have a lower degree of condensation compared to the other three lignins, while DACSL and SESPL have the highest degree of condensation by forming new C-C bonds on the aromatic ring during the isolation processes. DES lignin, DACSL, and SESPL have a higher phenolic hydroxyl content, which might be released from the cleavage of ether linkage. It is understandable that there is less phenolic content in MWL, considering that
its structure is similar to protolignin. However, it is interesting that soda-WSL also has a low phenolic hydroxyl content. All of these lignin samples have shown an amount of aliphatic hydroxyl group (171-168.5 ppm) on the propanyl side chains.

<table>
<thead>
<tr>
<th>Spectral Region</th>
<th>Chemical Shift (ppm)</th>
<th>Numbers of moieties per aromatic rings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MWL</td>
</tr>
<tr>
<td>Methoxyl content</td>
<td>57-54</td>
<td>0.97</td>
</tr>
<tr>
<td>Car-H</td>
<td>125-103</td>
<td>2.75</td>
</tr>
<tr>
<td>Car-C</td>
<td>141-125</td>
<td>1.66</td>
</tr>
<tr>
<td>Car-O</td>
<td>160-141</td>
<td>1.59</td>
</tr>
<tr>
<td>Phenolic hydroxyl</td>
<td>174-171</td>
<td>0.05</td>
</tr>
<tr>
<td>Aliphatic hydroxyl</td>
<td>171-168.5</td>
<td>1.62</td>
</tr>
<tr>
<td>Saturated CH2 or CH3 on aliphatic side chain</td>
<td>40-20</td>
<td>1.83</td>
</tr>
<tr>
<td>C-γ in β-5 and β-O-4 with C=O</td>
<td>64-62</td>
<td>0.65</td>
</tr>
<tr>
<td>Ca in β-O-4</td>
<td>71-73</td>
<td>0.57</td>
</tr>
<tr>
<td>Cβ in β-O-4</td>
<td>84.5-80</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Table 5.4: Quantitative 13C analysis of five representative biorefinery lignins

13C/1H HSQC NMR spectra were further applied to investigate the unique interunit linkage properties of biorefinery lignins. Again, only MWL, DESL, DACSL, SESPL, and Soda-WSL were used in this section due to difficulties of SPORL and AKL acetylation. Zero-filling, apodisation and linear prediction parameters was assessed to improve the spectral resolution without affecting signal intensities. Linkage and moiety signals are assigned according to the validation from dimeric model compounds obtained from previous literature studies. Two regions
of interests are discussed here: aliphatic oxygenated side-chain region (δC/δH 90-45/6.4-2.4) and aromatic-unsaturated region (δC/δH 148-98/8.6-5.8).

The oxygenated side-chain region of the HSQC NMR spectra is depicted in Figure 5.2. The relatively high xylan content was observed in SESPL and DACSL as signals shown in 2D NMR at δC/δH 73.1/3.1, 73.9/3.3 and 75.6/3.6. In contrast, the MWL, DESL, and Soda-WSL have little trace of xylan. The β-aryl ether (β-O-4), phenylcoumaran (β-5) and resinol (β- β) linkages were almost omnipresent in these five lignins. However, their relative abundance in the isolation processes varied significantly with the mother biomass source and the type of isolation processes. Besides DESL, all of the other lignins contained the β-O-4 linkages in the highest quantity, with only small amount of phenylcoumaran (β-5) and resinol (β- β) linkages present. DACSL and Soda-WSL, lignins derived from grass biomass, had a unique type of coumarate linkages shown at δC/δH 62.0/3.7. The signals of β-O-4 linkages were completely absent in DESL, which highly suggests that this bond is cleaved during DES treatment. Thus, β-5 and β-β became the dominant inter-units linkage for DES lignin. Moreover, it was observed that Hibbert’s Ketone structures formed as a new type of side-chain only found in DES lignin, which corresponds to the previous investigation on chemistry mechanisms during DES treatment of biomass.

The aromatic region of the HSQC NMR spectra is depicted in Figure 5.3. First, the signals in this region provide a confirmation of GSH presence in all lignins. Biorefinery lignins derived from softwoods (MWL, DESL, SESPL) consisted of primarily G units with a small amount of H units signals observed. While in grass lignin (DACSL, Soda-WSL), all signals from S, G, and H units were detected and presented an appreciable amount of coumarate/ferulate structures as marked in Figure 5.3D&E. However, the isolation processes granted certain patterns within these lignins. For instance, during the acid catalyzed lignin isolation processes, such as steam explosion...
and diluted acid treatment, a significant amount of α-carbon connected to G units (G’2: δC/δH 111/7.5; G’5+G’6: δC/δH 122-114/7.6) in ether linkages will be oxidized to ketone structure. During soda-treatment, a cross peak is observed at δC/δH 107.4/7.4 ppm which can be assigned to α-oxidized S units, while G units remain intact. The DES treatment presents a unique, but selective impact on lignin aromatic rings in which only G units with β-carbon oxidization were observed. This also aligns with the proposed formation mechanisms of HK structures observed in oxygenated side-chain regions.
Figure 5.2. 13C/1H HSQC NMR of the inter-linkage region of selected biorefinery lignin.
Figure 5.3. 13C/1H HSQC NMR of the aromatic region of selected biorefinery lignin.
5.2.3 Characterization of Lignin Macromolecular Level

The seven methods presented in the previous section provide a detailed examination of the chemical and structural features of lignin from a variety of aspects, both qualitatively and quantitatively. To further study the structural features of these biorefinery lignins at the macromolecular level, we conducted gel permeation chromatography (GPC), scanning electron microscopy (SEM) and nitrogen isothermal adsorption/desorption.

The molecular weight distributions and peak molecular weight of these biorefinery lignins were also determined by GPC analysis following a previous method. (Stewart, Akiyama et al. 2009) The lignins were acetylated in pyridine and acetic anhydride mixture prior to analysis. Polystyrene standards (Alfa Aesar) with molecular weights ranging from 1,300 to 123,000 g/mol as well as veratrol (138 g/mol) were used to calibrate the molecular weight based on retention time. Figure 5.4 shows the molecular weight distribution of representative lignin determined by GPC. Among all of the lignins analysed, milled wood lignin has the highest peak molecular weight at 2950 g/mol and broadest molecular weight distribution. Compared to MWL and other typical softwood lignin, DESL lignin shows a narrower and lower molecular weight distribution ranging from 490-2600 g/mol with a peak molecular weight at about 890 g/mol. This suggests that DES depolymerized native lignin by selective cleavage of ether bonds without a subsequent condensation reaction. DACSL has similar peak molecular weight at 1020 g/mol and molecular weight distribution to DESL, but it is apparent that the DES has relatively higher uniformity with less shoulder peaks in the spectra. Soda-WSL has a relatively lower peak molecular weight at 620 g/mol. This could be due to the fact that alkaline re-precipitation selectively extracts certain molecular lignins from crude lignins. The rest of the molecular weight data was collected from the references for
Figure 5.4. GPC chromatography of Mw distribution of representative lignins.

Chemically, molecular weight indicates the degree of lignin polymerization. However, we believe that different degrees of particle aggregation also occur during lignin isolation and drying, which would change the physical morphology of lignin at the nanoscale. Thus, we further characterize the lignin particle using scanning electron microscopy (SEM) and nitrogen isothermal adsorption/desorption. Table 5.5 lists the surface area and pore distribution of each lignin characterized by isothermal nitrogen adsorption/desorption. Lignin surface area is calculated based on B.E.T. theory. Among the tested lignin, DACSL, SESPL and SPORL has a relative high B.E.T. surface area, ranging around ~5 m²/g. However, AKL and DESL have relative low surface area, below 4 m²/g. It is important to note that a smaller average molecular weight does not necessary correlate to a higher available surface area. For example, DESL and DACSL have very similar
peak molecular weights, but the DESL has a surface area around 3.05 m²/g, which is around 40% less than DACSL lignin (5.39 m²/g).

<table>
<thead>
<tr>
<th></th>
<th>AKL</th>
<th>SESPL</th>
<th>DACSL</th>
<th>SPROL</th>
<th>MWL</th>
<th>Soda-WSL</th>
<th>DESL</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.E.T (m²/g)</td>
<td>3.87</td>
<td>5.42</td>
<td>5.39</td>
<td>5.28</td>
<td>4.05</td>
<td>1.17</td>
<td>3.05</td>
</tr>
</tbody>
</table>

Table 5.5. B.E.T analysis of lignin Surface area.

We also use the SEM to provide visual evidence of lignin physical morphology at the nanometer to micrometer scale. Figure 5.5 are SEM images of the seven tested lignin at a 10,000 times magnification. It is apparent that the nanoscale morphology of lignin is highly dependent on the isolation method. Lignins derived from softwood biomass (AKL, SESPL, SPROL, DESL, and MWL) have a rougher surface compared to those from grass (DACSL and Soda-WSL). However, lignin morphology at the dry powder state can hardly represent their reactivity in solution reaction. AKL and SPROL can be completely dissolved in aqueous solution when the pH is above 4. DACSL and Soda-WSL are aggregated into lignin nanospheres (~50 nm diameter spheres) when shown in bulk in the powder state. However, these spheres are potentially dispersed in solution upon stirring or sonication. To investigate lignin structure in solution, both the chemical interaction with the solvent and the physical force of the dispersion process will be significant topics for lignin valorization in future study.
To summarize, we investigated the key structural features of the seven representative lignins, and they varied significantly according to the mother biomass source and the isolation method. Lignin derived from softwood primarily consists of guaiacyl units (G), while grass lignins contain all three GHS subunits. Lignin isolated from all tested conditions have an oxygen rich side-chain structure, presented as different amounts of aliphatic OH groups per aromatic ring (Ar) or variable partial oxidation (determined by C=O on side-chain). Additionally, lignin isolated from acid treatment have relatively higher phenolic OH content. Both acidic (diluted acid, steam explosion, and DES) and basic (alkaline and sulfite) treatments are likely to cleave a significant amount of ether linkages, resulting in a relatively lower molecular weight compared to other treatment conditions. However, mechanical milling will likely isolate a lignin with much less inter-linkage cleavage or side-chain modification, and thusly more representative of a native lignin. Grass lignin often has relatively lower molecular weight compared to lignin derived from wood, regardless of pretreatment conditions. These structural varieties will likely have an impact on...
lignin conversion capabilities, which is a definite area of further study. Finally, we adapted oxidative conversion methods from our previous research to test lignin reactivity toward phenolic and dicarboxylic acid.

5.2.4 Comparison of Biorefinery Lignin Reactivity towards Oxidative Conversion

It is apparent that lignin structure varies significantly depending on both the mother biomass resources and isolation processes. The lignin resulting from the biorefinery process, considered a waste product, are expected to perform differently towards the valorization process. However, there hasn’t been much efforts in developing a quantitative correlation between lignin conversion reactivity versus their structural features. As an example, we investigated these lignin structures’ impact on two major reactions: lignin depolymerization and aromatic ring opening.

Figure 5.6 shows lignin depolymerization efficiency to total phenolics from Nb2O5 catalyzed peracetic acid(PAA) depolymerization of biorefinery lignins versus the different structural variations (S/G ratio, H/G ratio, oxygen content, ether-linkage abundance, aliphatic OH content, phenolics OH content, and average molecular weight). The total phenolic yields are estimated by Folin-phenol protocols, which are based on the number of soluble grams of phenolic yield per gram of klason lignin. In softwood biorefinery lignins (SESPL, AKL, MWL, DESL, and SPORL), the MPC combined the products primarily derived from guaicyl units, such as 4-hydroxy-2-methoxyphenol, vanillic acid and 3,4-dihydroxybenzoic acid. However, in lignins extracted from grass biomass (DACSL, Soda-WSL), the MPC combined all possible hydroxylated phenolics and phenolic acids derived from p-hydroxyphenyl, guaiacyl, and syringyl units. Among the tested lignins, the TOTAL PHENOLIC obtained from DACSL has the highest overall yield of up to 47% based on original lignin weight, while the MWL has the lowest TOTAL PHENOLIC yields(<5%). The SESPL has a relatively high yield (up to 35%) at a temperature of 45 °C and 60
The Soda-WSL, AKL, and SPORL lignin have comparable MPC yields around 10-20%. These results confirm that the pretreatment method has a significant effect on lignin conversion to phenolic compounds through PAA treatment. The acid treated lignins (e.g. DACSL, SESPL) perform better compared to those obtained from alkaline extraction processes (e.g. Soda-WSL, AKL, and SPORL). When comparing the similar isolation methods, DACSL has a higher total phenolic yield than SESPL, while Soda-WSL has the highest yield among the lignin extracted by alkaline processes. It is suggesting that lignins extracted from grass are more reactive towards PAA treatment and phenolic production compared with those extracted from woody biomass. The MWL has the lowest total phenolic yield (<5%) during conversion, which could be due to both its high molecular weight and physical condensation during the pretreatment processes. The yield distribution versus the investigated parameters may appear scattered at first glance, but a closer look reveals that there are observable trends. For example, the S/G ratio, H/G ratio, and Phenolic OH content seem to be positively correlated to total phenol yield, while the ether linkage content, aliphatic OH content, and average molecular weight content is negatively correlated to total phenol yield. There is, however, no apparent correlation between oxygen content and total phenol yield.
Figure 5.6. Lignin structural feature versus depolymerization reactivity
We also investigated lignin reactivity towards conversion to DCA by chalcopyrite catalyzed H$_2$O$_2$ oxidation at mild acidic conditions. Figure 5.7 shows the lignin aromatic ring cleavage efficiency (DCA yield) versus same structural parameters. The DCA structures are determined by GC-MS. Regardless of the source of the lignin, the resulting structures are limited to a small group of DCA including malonic acid, succinic acid, maleic acid, fumaric acid as well as a few other C$_4$-C$_5$ DCAs. Among the tested lignins, the DCA obtained from SPORL has the
highest overall yield of up to 15% based on original lignin weight, while the MWL has the lowest DCA yields (<1%). The DACSL and SESPL have a relatively high yield of up to 14% and 11% respectively. The Soda-WSL and AKL lignin have comparable MPC yields at around 5%. DES lignin has a low DCA yield around 3%. SPORL, DESL, and MWL show a tremendous difference in their conversion to DCA, despite coming from the same biomass source. This confirms that the pretreatment method has a significant effect on lignin conversion to DCA at the tested conditions. In opposite to lignin depolymerization, it is hard to extrapolate a linear relation between lignin aromatic ring cleavage versus those structure properties characterized. Ether-linkage abundance and aliphatic OH content are negatively correlated to their conversion yield. The other structural properties seem have very weak correlation with conversion yield.

To further the study quantitatively, we use R-Statistics as a tool to explore the intrinsic relation between lignin structural features and conversion reactivity. First, we evaluated the overall linear correlation between every pair of structural variables. The resulting correlation efficiencies are listed in Table 5.6. Correlation efficiency is a factor in the range of [-1,1], which indicates the degree of correlation between two variables. When the correlation efficiency is close to 1, the variables are positively correlated, while a correlation efficiency close to -1 suggested a negative correlation. If the correlation efficiency is close to 0, there is no correlation between the two variables. Variables that are highly correlated have correlation efficiencies greater than 0.50 or less than -0.50. We have highlight these pairs either yellow if they are positively correlated or blue if they are negatively correlated. The highest correlation is between phenolic OH content and C-O-C linkage abundance, with a correlation efficiency of -0.92. This matches the mechanism that suggests that cleavage of ether linkage will increase the amount of phenolic OH group in the lignin. Less cleavage of ether linkages will likely result in a higher molecular weight (CE=+0.64 of Mw
versus C-O-C%). However, it is intriguing to discover that phenolic OH content is negatively correlated to the overall oxygen content of the lignin, which suggests that the cleavage of the ether bond is not necessary to increase the overall oxygen content. This might due to the pinacol rearrangement of the side chain during the lignin isolation process and the occurrence of aromatic ring side chain re-condensation. The modeling also suggests that H/G content could positively correlate to S/G content, as the H unit is an important intermediate in the S unit synthetic pathway. S/G ratio and H/G ratio are negatively correlated to the aliphatic OH content according to this set of data, but there is no existing theory to explain this.

<table>
<thead>
<tr>
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<th>S/G</th>
<th>H/G</th>
<th>Oxygen %</th>
<th>Aliphatic OH</th>
<th>Phenolic OH</th>
<th>C-O-C%</th>
<th>M.w.</th>
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<tbody>
<tr>
<td>S/G</td>
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<td>H/G</td>
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<td>Oxygen%</td>
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<td>Aliphatic OH</td>
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<td>Phenolic OH</td>
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<td>C-O-C</td>
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<tr>
<td>Mw</td>
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Table 5.6. Overall linear correlation analysis between structural variations.

There is some scattered information in previous studies attempting plot the product yields versus a single variable to interpret the structural information on lignin conversion. However, an analysis of the correlation between structures proves it is necessary to integrate the contribution of each potential variables in a systematic way. Thus, we explored the linear correlation between these variables and total phenol/DCA yield by conducting multiple-variable linear estimation (MVLE). Since seven lignin samples were tested in this study, the maximum degree of freedom (allowed amount of variables) should be no more than five. To reduce the degree of freedom, a
number of variables were ruled out. We also ruled out influent of S/G ratio since most of the tested lignins are derived from softwoods, which have low S unit presence. Since there are missing data in phenolic aliphatic OH, C-O-C% due to the technical difficulties in NMR test, we generated two sets of models: 1) evaluating product yield as a function of H/G, oxygen, and Mw based on the seven data points of reaction yields; and 2) evaluating product yield versus Mw, Aliphatic OH, and C-O-C% based on five data points of reaction yields. The estimated linear correlations can be expressed in the following form: \( y = ax_1 + bx_2 + cx_3 + \text{residue} \).

1) evaluating products yield versus H/G, oxygen and molecular weight (Mw)

\[
\begin{align*}
M1: \quad Y_{\text{Phenol-1}} &= 31.7 \cdot \frac{H}{G} - 0.999 \cdot \text{Oxygen}\% - 0.00180 \cdot \text{Mw} + 57.8 \quad (\text{Multiple } R^2 = 0.835) \\
M2: \quad Y_{\text{DCA-1}} &= 11.4 \cdot \frac{H}{G} - 0.271 \cdot \text{Oxygen}\% + 0.00143 \cdot \text{Mw} + 11.9 \quad (\text{Multiple } R^2 = 0.267)
\end{align*}
\]

2) evaluating products yields versus Mw, aliphatic OH and C-O-C

\[
\begin{align*}
M3: \quad Y_{\text{Phenol-2}} &= 0.00247 \cdot \text{Mw} - 0.362 \cdot \text{Aliphatic OH} - 0.608 \cdot \text{C-O-C}\% + 90.1 \quad (\text{Multiple } R^2 = 0.910) \\
M4: \quad Y_{\text{DCA-2}} &= 0.000847 \cdot \text{Mw} - 0.199 \cdot \text{Aliphatic OH} - 0.449 \cdot \text{C-O-C}\% + 32.7 \quad (\text{Multiple } R^2 = 0.993)
\end{align*}
\]

Besides \( R^2 \) in M2 (\( Y_{\text{DCA}} \) versus H/G, Oxygen, and Mw model), it seems that all of the \( R^2 \) values are in an acceptable range (\( R^2 > 0.8 \)). The results indicated that H/G content has a positive influence on TOTAL PHENOLIC yield, while oxygen content, aliphatic OH group and C-O-C% are negatively influencing the TOTAL PHENOLIC yield. Similarly, H/G content has a positive influence on total phenolic yield, while oxygen content, aliphatic OH group and C-O-C% are negatively influencing the DCA yield. The molecular weight is showing a contradictory impact in
two different models, might due to it is correlated to C-O-C in M3 as well. Thusly, it should have an overall negative impact on phenolic. The current results suggest that a higher overall oxygen content and oxygen content on the side-chain (aliphatic OH) will result in lower lignin conversion potential. More interestingly however, the C-O-C content is not necessarily positive correlated to lignin molecular weight, as a higher ether-linkage content will still have a negative impact on lignin conversion. This contradicts to some existing theories, in which higher ether linkage content is easier to cleave and degrades to low molecular weight products. The intrinsic reason of the ether linkage content and lignin conversion reactivity is an interesting aspect for future research.

To further evaluate the reliability of these models, we conducted t test to evaluate the significance of each variable in the models. Though the MVLE, statistical analysis provides a quantitative correlation between total phenolic/DCA and structural features. However, the t test indicates that not all of the variables are significant, or in other words, not all variables have a confirmed linear correlation to the product yields. For instance, when predicting depolymerization reactivity (M1&M3), H/G is the most significant in M1, while aliphatic OH and C-O-C content have a higher chance to be significant in M3. When predicting DCA yield (in M2 & M4), only Aliphatic OH is the only significant variable that can pass the test. These results do not necessarily mean that the significant variables are the only structural features that affect lignin conversion and products yield, but it does show a that the higher level of lignin structural properties seems to have more of an impact on lignin conversion reactivity. This results shows that the efficiency of converting biorefinery lignin to value added products is in fact affected by a group of structural variables, not just singular factors. The limitation of the determined significant variables is due to the limited amount of points acquired in this study. However, integral understanding of these structural properties will be critical for predicting lignin reactivity towards a certain conversion
method. Future studies to establish a database for lignin characterization and conversion to value-added products would provide key information to establish better lignin structure-conversion correlations and will be a vital tool for lignin re-engineering to obtain lignin feedstock with desired structural features.

5.3 Conclusion:

This chapter provides a detailed comparison of structural features from a number of representative biorefinery lignins that have been derived from both forest and agricultural residues and isolated by representative pretreatment methods. We establish an understanding of lignin structural complexity at three different levels: functional group, inter-unit linkage and macromolecular. Ten different methodologies were conducted to characterize the lignin structural properties and to express the two levels of complexity including Klason lignin purity determination, alkaline nitrobenzene oxidation, thioacidolysis, CHNO elemental analysis, FT-IR, $^{13}$C NMR, $^{13}$C/$^1$H HSQC NMR, GPC, B.E.T., and SEM. We also explored the correlation between lignin structural properties and their conversion reactivity towards oxidative depolymerization and aromatic ring opening using a statistical method. The results from this study establish a new tool to quantitatively predict that lignin conversion reactivity based on structural properties. We believe that the outcome of this work will provide new insight and methodology in biorefinery research, especially in lignin valorization.
CHAPTER SIX: TEA ANALYSIS OF LIGNIN-TO-DCA PROCESS AND CONVERSION OF LIGNIN TO OLIGO-DCA

Chapter Three to Chapter Five in this dissertation demonstrated a new pathway producing dicarboxylic acids as new platform chemicals through a two-stage reaction mechanisms. Model compounds study helped elucidate the reaction mechanisms. New catalytic systems were developed to improve the lignin depolymerization and aromatic ring cleavage. I also explored to establish a qualitative and quantitative method to predict the lignin reactivity based on their structural characteristics. In this chapter, I will present a brief techno-economic model that is developed to evaluate the economic benefit of the lignin-to-DCA process, and to point out the key hurdles for future study.

6.1 Introduction:

Lignin is a ubiquitous component in almost all plant biomass and the largest source of renewable material with an aromatic skeleton. Large quantities of industrial lignin are already produced annually as a waste product of the pulp and paper industry, where the vast majority is burned as a low cost fuel to provide energy for the chemical pulping process. The emerging biomass refinery industry will further introduce an enormous amount of lignin. However, selectively and effectively converting lignin into valuable products is a very well recognized challenge epitomized by the myth “You can make anything out of lignin… except money”.

In nature, lignin depolymerization is accomplished primarily by powerful oxidative enzymes generated by rot fungi and some bacteria. A large body of literature on the development of practical oxidative delignification chemistries has already been created
decades ago, with a primary focus on sustaining the pulp and papermaking industry. Majority of previous in area have been focused on depolymerizing lignin to low molecular weight phenolic compounds (LMWPC). Besides LMWPC, dicarboxylic acids (DCAs) have also been commonly observed as products from the oxidative cleavage of aromatic rings in lignin or its fragments, yet this pathway has not been explored until recently. DCAs such as muconic acid, muconolactone, maleic acid, succinic acid, and malonic acid are important platform chemicals for the polymer, pharmaceutical, and food industries.

In our previous work, we reported a chalcopyrite catalyzed H$_2$O$_2$ conversion of biorefinery lignin and its model compounds to a mixture of DCAs by a mild Fenton’s reaction. The preliminary results suggested the reaction was through a two stage mechanisms including the lignin depolymerization to LMWPC and further aromatic ring cleavage. The o-benzoquinone structures and cis,cis-muconic structures were proposed to be the intermediates of the DCAs formation. Hydroxy radical was postulated as the key reactive specie which lead to the aromatic ring cleavage occurring. It was noticed the acidic acid/acetate buffer does reduce the intensity of H2O2 degradation with the catalyst and provided a mild Fenton’s environment. The finding provided a new pathway for lignin valorization for production of DCA as a new group of value-added chemicals. However, the reaction intensity still remained high and significantly limited the DCA yield (~14% from Lignin, and ~30% from Catechol) and the control of aryl chain length of the final DCA products. Thus, it is likely the reaction would be optimized if the first stage of lignin depolymerization could be improved.

After evaluating several catalysts for their potential to facilitate lignin depolymerization to LMWPC, Niobium oxide (Nb$_2$O$_5$) was found to have a pronounced effect of promoting PAA
conversion of lignin to LMWPC. Nb₂O₅ was supplemented as a catalyst to the PAA reactions of DACSL and SESPL under identical reaction conditions. By comparing the lignin-to-LMWPC conversion yield between the control (no Nb₂O₅) and catalyzed (10 wt% Nb₂O₅ addition) PAA reaction at 333 K after 60 minutes, it was seen that the production of LMWPC increased from 18% and 22% to 35% and 47%, for the SESPL and DACSL reactions respectively. The amounts of EAE determined after 60 minutes PAA treatment at 333 K represented 54% and 58% of the initial weight of SESPL and DACSL respectively. MPC thus account for 81% and 88% of the total EAE, for SESPL and DACSL respectively.

Preliminary work has demonstrated that the chemistry of lignin depolymerization and subsequent aromatic ring cleavage to produce DCAs. In order to further evaluate the economic potential of the lignin-to-DCA strategy, I also conducted a preliminary TEA exercise collaborating with our group member. We conducted techno-economic analysis using Aspen Plus® based on the results collected from previous Chapter.

6.2 Techno-economic Analysis (TEA)

The production of dicarboxylic acids from the ring opening step requires the subsequent separation and purification of the dicarboxylic acid products from the reaction mixture. This downstream processing is typically said to constitute the majority of the process costs for producing carboxylic acids. There are currently several different methods being developed specifically for the separation and purification of these products, mostly based on upstream fermentation processes. These can be potentially adapted for use in our lignin-to-DCA process. Examples include direct crystallization, precipitation, membrane separation, reactive extraction, and chromatography. These separation processes are complicated by the complexity of the initial mixture, the affinity of carboxylic acids for aqueous solutions, and the need for a high purity
product. Because of these difficulties, traditional approaches such as solvent extraction or CaOH precipitation require unreasonable amounts of solvent, acid, alkali, or waste treatment to achieve adequate separation. Additionally, because our lignin-to-DCA process also uses acetic acid as a feed/solvent, it is important to design these downstream processes in such a way that the acetic acid in the product stream can be captured and reused for the production of peracetic acid. This added requirement, combined with the even mixture of several DCA products, means that the downstream process must be carefully selected.

![Figure 6.1. Integrated Process Flow for Succinic Acid Production](image)
An example of a potential approach to an integrated upstream and downstream process flow is illustrated in Figure. With the assumption that the primary desired product is succinic acid, a reactive extraction separation is combined with crystallization to isolate a pure product while still allowing for the recycling of acetic acid for the production of peracetic acid. Reactive extraction allows for a more efficacious separation of carboxylic acids from the aqueous phase by using an extractant to form a complex with the acids that is easier to remove. The extractants commonly used are secondary and tertiary amines such as trioctylamine (TOA) as well as phosphate based salts. The binding of the extractant to the acids is dependent on the pH of the mixture beforehand and the pKa of the particular acid. In order to facilitate the separation of DCA and carboxylic acids such as acetic acid, the pH of the reaction mixture is adjusted to around 5, where DCAs such as succinic acids are not dissociated and do not bind with the extractant while single carboxylic acids are dissociated and bind readily. This is conveniently the pH at which the ring opening reaction needs to occur in order to ensure catalyst stability and thus additional pH modification is unnecessary. The separation of the extractant-acid complex from the aqueous mixture is aided by the addition of a diluent such as a long-chain alcohol like 1-octanol, yielding an aqueous phase consisting of largely pure DCA product and a solvent phase consisting mostly of the acetic acid-extractant complex. In addition, salts and other ionic compounds contaminants can be removed by this reactive extraction process. Depending on the desired fractionation of acids, the extractant, diluent, and pH can be changed to tailor this reactive extraction. Vacuum distillation and crystallization of the aqueous fraction has been shown to remove the remaining sugar and acid contaminants to yield a highly pure crystalline succinic acid product. The solvent phase of the reactive extraction, consisting of the extractant-acid complex, can be separated through a back extraction technique, including temperature swing, diluent swing through
distillation, or the addition of a strong base. The result is an aqueous fraction of free acetic acid and a solvent fraction of regenerated extractant and diluent to be recycled. The aqueous fraction of acetic acid can be used for the regeneration of peracetic acid. The regeneration of peracetic acid from hydrogen peroxide and acetic acid, catalyzed by sulfuric acid, can be predicted from literature data on the equilibrium constants and conditions of this reaction.

The advantage of performing a techno-economic analysis on this type of process model is that the separation of different compounds in the reactive extraction and back extraction processes can be modeled by either measuring the distribution coefficients ($K_d$) experimentally or finding the appropriate references in the literature. Thus, process modeling software can be used to optimize the conditions for reactive extraction based on the specific product distribution from the upstream ring opening reaction. Because the reactive extraction process is not yet fully developed, especially for the back extraction and extractant regeneration steps, the amount of makeup diluent and extractant used needs to be balanced with regards to the recovery of the DCA product and acetic acid. Some extractants/diluents have a high cost, and without efficient recovery may not prove viable economically, despite good DCA products yields. The production of peracetic acid through equilibrium of hydrogen peroxide/acetic acid may also prove to be a limiting factor in the peracetic acid concentration of the initial depolymerization reaction step. Optimization of this relationship through process modeling will be necessary.
The preliminary TEA analysis drew a conclusion that the cost of the high PAA dosage and the recovery of acetic acid takes a major portion of the total cost in the overall processes. The complete depolymerization of lignin required the consumption of high amount of PAA to remove the lignin propanoyl side chains. However, a complete depolymerization of lignin will be inevitably encountered with a considerable loss of carbon, especially from propane side cleavage as shown in Figure 6.3. The side chain cleavage does bring benefit to produce selective monolignol from lignin which is a promising strategy if phenolic compounds are the target products. To achieve a high carbon yield for DCA production which can be potentially used as fuel or polymer precursor, partial depolymerization of lignin to retain most of the propane side chains may be a more practical strategy.

Figure 6.2. Process Model of Integrated Production of Succinic Acid from Lignin
Figure 6.3. Mechanisms involved in PAA treatment of lignin phenylpropane units with different side chain configurations. a) non-conjugated structure with C-C bond (S1); b) β-aryl ether linked structure (S2); c) olefin structure (S3).

However, there haven’t been a well-known technique can selectively produce partial depolymerized lignin (PDL) in high selectivity and efficiency. Yet the aromatic ring opening on the PDL to produce oligo-DCA has been demonstrated. Identify practical technology to produce PDL will be a long term study and is a significant part proposed as my future work which will be presented in Chapter Seven. To prove the concept to produce oligo-DCA, hydromatairesinol (lignin dimeric model) was applied as a simple model compounds of PDL. As mentioned in Chapter Four, hydroxylated phenolic structures, especially branched catechol structures, are highly reactive towards a mild conversion to dicarboxylic acids with high yield. Thus, we demethylated Hydromatairesinol (HMR), a dimeric C-C linked lignin model compounds, as the substrate for
further aromatic ring cleavage reaction. HMR is mixed with 46% HI solution and refluxed for 2 hours. After the demethylation, the HMR is still not soluble in HI solution, while the color changed from ivory to light pink. The powder was centrifuged and washed with water to remove the residue HI. ~65.7 wt% of the powder was recovered from the demethylation processes. FT-IR was employed to characterize and predict products structure. The hydromatairesinol has a very typical FT-IR spectrum that highly resemble those spectra obtained from lignin in literature. After HI treatment, we consider the C=O bond on the side-chain linkages whose signal centered 1768 cm$^{-1}$ would remain similar. Reduced signal at 1450 and 1400 cm$^{-1}$ indicated the loss of -CH$_3$ bending signals. It is also noticed loss of peaks at 1250 cm$^{-1}$ and 1040 cm$^{-1}$, which are normally assigned to C-O stretch in a phenyl alkyl ether. Instead, emerging of new peak at 1215 cm$^{-1}$ suggested the new type of C-O stretch on the aromatic ring. It corresponded to the increase of H-bonded phenolic O-H group absorbance centered at 3400 cm$^{-1}$. All of these evident the success of methoxy group removal and the ensuing product should be in a structure as DM-HMR in Scheme 6.3.1.
Figure 6.4. FT-IR spectra of 1) Hydromatairesinol (HMR, Blue), 2) HI demethylated HMR (DM-HMR, Green); and 3) Fe-PAA oxidation of DM-HMR (DM-HMR-OX).
150 mg of the DM-HMR was charged to the Fe-PAA solution scaled down according to the ratio as run in previous reaction. According to the difficulties to solubilize DM-HMR in solvent, the DM-HMR was dispersed in glacial acetic acid as a slurry and pumped into the mixture of Fe catalyst and PAA. The DM-HMR was quickly solubilized in the solution and the dropped-in solution turned to a clear orange/light brown solution. While after around 5 hours of stirring, some white/ivory color powder started precipitating from the reaction solution and more precipitations was accumulated while the preceding of the reaction. The precipitations were collected as DM-HMR-OX and washed with DI water to remove the residue PAA and solubilized Fe cation on the surface. A total yield of 130 mg (87 wt%) DM-HMR-OX was collected. However, there hasn’t been a proposed analytical methodology to confirm the structure of DM-HMR-OX is an oligo-DCA products. Here, we explored to integrate FT-IR together with $^{13}$C and $^1$H/$^{13}$C 2D HSQC NMR to confirm the selective removal of aromatic ring structures and generation of oligo-DCAs.

FT-IR was firstly employed to qualitatively characterize and predict products structure. (Figure 6.4) Compared to DM-HMR, the reduction of absorbance at 1619 cm$^{-1}$ and 1512 cm$^{-1}$, which assigned to C=C stretch on aromatic rings, indicated the complete cleavage of aromatic nucleis during the oxidation. A broad signal ranging from 3650-2750 cm$^{-1}$ together with a strong band centered at 1744 cm$^{-1}$ suggested the presence of conjugation of carboxylic group with α,β unsaturated C=C. Two separated, but very closed band at 1645 cm$^{-1}$ and 1636 cm$^{-1}$ suggested at least two types of C=C stretch, in another word, corresponding to the prediction of branched muconic acid structure formation during the ring cleavage reaction.

$^1$H/$^{13}$C HSQC NMR were further utilized to confirm the deconstruction of aromatic nucleis. Figure 6. 5 showed the aromatic region ($\delta$H/$\delta$C: 8-5.5/140-110 ppm/ppm) of the 1H/13C HSQC
NMR spectra of HMR, DM-HMR, and DM-HMR-DCA. It is clear that in HMR and DM-HMR all has the very similar abundance of aromatic structures. The change of the signal chemical shift is due to the removal of the methyl group and reveal the hydroxyl on the aromatic ring. It is very clear no aromatic ring signal was presented in DM-HMR-DCA at the same region. This is another clear evidence indicate the almost complete deconstruct of the aromatic nuclei in the oligomeric lignin after Fe-PAA oxidation. This can also be referred in the later study to confirm the efficiency of aromatic ring deconstruction.

Figure 6.5 1H/13C HSQC NMR spectra of HMR, DM-HMR, and DM-HMR-DCA

The FT-IR and 1H/13C HSQC NMR methods presented above qualitatively demonstrated the construction of lignin aromatic nucleis and the formation of DCA structures. We also adapted 13C NMR as a quantitative approach to evaluate the process. Figure 6.6 presented the 13C NMR spectra of HMR and DM-HMR-DCA. Disappearance of peak at 54 ppm indicate the removal of
methoxy group during demethylation treatment by HI. Absence of peak from 155-120 ppm in DM-HMR-DCA is a clear evidence of nearly complete deconstruction of aromatic ring nuclei. The newly arising peak at 105-103 ppm in DM-HMR-DCA can be assigned as branched olefin carbon in -C=C-COOEt (C2,5,14,17) structure, which confirmed the formation of DCA after Fe-PAA treatment. Additional peak at 210 ppm companioned by the disappearance of peak at 75 ppm indicated the α-hydroxy group in HMR side chain is oxidized to ketone structure during the treatment. Since this α- carbon on HMR structure can be distinctive either before or after aromatic ring cleavage, we can use it as reference peak to quantify the conversion of aromatic ring or the efficiency of aromatic ring selected cleavage. In Figure 6.3.6a, integration of the peaks between δC at 155-120 ppm over integration of peak at 75 ppm is 11.9 which is very close to the total of 12 carbons presented on HMR aromatic rings. Similarly, in the 13C NMR spectra of DM-HMR-DCA, δC105-103/ δC210 is 4.5, which is corresponding to the ratio between C2,5,14,17 in DM-HMR-DCA over the C7. The ratio is ~10% larger than the theoretical number might due to the high noise-signal ratio in the test and can be further improved in future work.
To sum up, integrating the FT-IR, 1H/13C HSQC NMR and the quantitative 13C NMR, we conclude that the dimeric HMR can be selectively degraded to oligo-DCA under proper treatment condition without being depolymerized to monomeric size. Scheme 6. 1 summarizes the overall chemistry occurred from HMR to DM-HMR and finally to DM—HMR-DCA. Theoretically, for one mole of the HMR dimeric molecule, it saves at least 2 moles of PAA, which is about 50% of the PAA consumed for aromatic ring opening. This demonstrates the concept that producing PDL from lignin to convert to DCA can be a promising route to utilize lignin derived DCA in a more economic viable strategy.

**Figure 6. 6 Quantitative 13C NMR spectra of HMR and DM-HMR-DCA.**
Scheme 6. 1. Comparison of current strategy and old strategies applied on HMR.

6.3 Conclusion

Techno-economic analysis (TEA) is conducted to evaluate the proposed lignin-to-DCA process. It concludes that the reduction of the peroxo acid dosage at the lignin depolymerization stage is one of the keys to improve the economic viability of the lignin-to-DCA process. Instead of complete depolymerization of lignin to monomeric size, partial depolymerization of lignin to an oligomeric scale is proposed as a potential strategy to reduce the chemical consumption and to produce new type of partial depolymerized lignin (PDL) as the feedstock for DCA production. The model compound study using hydromatairesinol demonstrated that it is viable to convert the PDL to oligo DCA selectively. Development of practical chemistry and technologies to produce PDL in high selectivity and yield should be an emphasized area in future study.
CHAPTER SEVEN: CONCLUSIONS AND FUTURE WORK

7.1 Conclusions

The overall goal of this work was to develop a new pathway to selectively produce dicarboxylic acids (DCAs) as new platform chemicals from biorefinery lignins and to investigate the reaction mechanism. In this work, we established a systematic understanding of lignin sequential depolymerization to DCAs through lignin depolymerization and aromatic ring cleavage. We hypothesized that combining the peroxo oxidants and transition metal catalysts would generate a synergistic effect to degrade biorefinery lignin to selective products. We also evaluated the techno-economic benefits of the implementation of the proposed pathway, and pointed out the direction for future research. We anticipated the results from this work would provide new leads toward developing new practical pathways to producing DCAs as new platform chemicals through lignin oxidative depolymerization, and thus enhance the economic viability of implementing sustainable bioenergy and bioproduct processes at an industrial scale.

A number of selected biorefinery lignins were prepared representing the most common lignins from popular biomass (both forests and agriculture residues) and biorefinery pretreatment sources. Representative monomeric and dimeric model compounds were purchased to assist in the identification of reaction intermediates and the discussion of reaction mechanisms. Integration of classic wet chemistry methods and state-of-art instrumentation and analytical methods was applied to characterize the biorefinery lignin structural features and to provide qualitative and quantitative analysis of lignin degradation products (Chapter Two).

The preliminary work (Chapter Three) developed a new method for highly selective production of dicarboxylic acids (DCAs) via Chalcopyrite (CuFeS$_2$) catalyzed H$_2$O$_2$ oxidation of
lignin and model compounds. Up to 95% selectivity towards stable DCAs was obtained for several
types of biorefinery lignin and model compounds under mild, environmentally friendly reaction
conditions. The overall conversion of biorefinery lignin to DCAs involved two major steps: 1)
depolymerization of lignin to low molecular weight phenolic compounds, and 2) aromatic ring
cleavage to DCAs. The results from model compound oxidation showed that the chalcopyrite/H₂O₂
system could promote a mild Fenton reaction, contributing to the selective conversion of
biorefinery lignin to DCAs. Both HO• and HO+ were present as primary reactive species. In
addition, the mild reaction conditions offered competitive advantages over reductive aromatic
conversions to linear chain hydrocarbons. However, the reaction yield was not satisfactory,
possibly because of insufficient depolymerization in the initial step.

Thus, the subsequent research (Chapter Four) focused on developing a novel and efficient
biorefinery lignin depolymerization chemistry. Peracetic acid (PAA) can completely depolymerize
biorefinery lignin at mild conditions and produce a selective group of monomeric phenolic
compounds (MPC). The results demonstrated that PAA depolymerizes lignin to MPC through a
unique mechanism that converts the propane side chains to either a hydroxyl or carboxyl group,
contributing to product selectivity. It was discovered that supplementing Nb₂O₅ catalyst to this
reaction can significantly increase the MPC yield up to 47% by stabilizing the oxidative cation
species and acting as a strong Lewis acid. The mechanisms involved in lignin conversion to
selective MPC were investigated and elucidated. Formation of benzoquinone as a by-product was
also observed from oxidation of formed MPCs. The lignin derived MPCs structures were
demonstrated have an enhanced reactivity towards further conversion to DCAs.

To further optimize the lignin sequential degradation, the representative biorefinery lignins
prepared in Chapter Two were characterized and used to investigate the correlation between lignin
structural variation and their conversion reactivity (Chapter Five). An understanding of lignin structural complexity was established at two different levels: molecular and macromolecular. Ten different methodologies were conducted to characterize the lignin structural properties and to express the three levels of complexity including Klason lignin purity determination, alkaline nitrobenzene oxidation, thioacidolysis, CHNO elemental analysis, FT-IR, $^{13}$C NMR, $^{13}$C/$^1$H HSQC NMR, GPC, BET surface area analysis, and SEM. The results demonstrated that lignin conversion reactivity is highly dependent on their structural properties, but the impact would vary towards different conversion strategies. Quantitative modeling of lignin conversion reactivities versus lignin structural variables at the molecular and macromolecular level was also developed.

In Chapter Six, techno-economic analysis (TEA) was conducted to evaluate the proposed lignin-to-DCA process. It indicated that the reduction of peroxy acid dosage for lignin depolymerization is one of the keys to improving the economic viability of the lignin-to-DCAs process. Instead of the complete depolymerization of lignin to its monomeric components, partial depolymerization of lignin to oligomeric products is proposed as a potential strategy to reduce chemical consumption and produce new type of partially depolymerized lignin (PDL) as the feedstock for DCA production. Model compound study using hydromatairesinol demonstrated the viability of converting the PDL to oligo-DCA selectively. Developing the practical chemistry and technologies to produce PDL in high selectivity and yield should be an area of emphasis in future study.

The work described in this thesis provides an advanced understanding of the catalytic oxidation of biorefinery lignin to DCAs. Catalytic oxidation chemistry exhibits great potential to efficiently depolymerize biorefinery lignin and to convert lignin to selective products. The complexity of the lignin structure is the key hurdle impeding the progress of lignin valorization.
Integrating lignin structural characterization and statistical analysis is a powerful method for predicting lignin reactivity and to optimize the lignin isolation/conversion process. Beyond optimizing the lignin structure and oxidation conditions, developing a new platform molecule that oligomeric size can be a promising strategy to reduce the oxidant consumption to improve the overall economic viability.

7.2 Recommendation for Future Work

The work described in this thesis demonstrates the production of DCAs as new platform chemicals from biorefinery lignin. Key mechanisms of this process have been investigated. It was determined that an oxidation-based strategy can effectively depolymerize lignin to LMWPC at very mild conditions to improve the subsequent conversion to DCAs. However, TEA analysis presented in Chapter Six indicated that the over-consumption of oxidant to achieve complete lignin depolymerization is one of the key hurdles in process implementation. Preliminary results in Chapter Six suggested that developing a partial lignin depolymerization method is a promising pathway to reduce the oxidant dosage while producing partial depolymerized lignin (PDL) that can be further ring opened to oligo-DCAs. Thus, there is an urgent need to identify a practical lignin partial depolymerization pathway, and should be emphasized in future work. The following are detailed suggestions for future work and some preliminary data.

7.2.1 Exploring partial depolymerization of lignin to generate oligomeric lignin as a new platform.

Side chain cleavage is beneficial for producing selective monolignol from lignin, and is a promising strategy if phenolic compounds are the target products. However, the complete depolymerization of lignin will inevitably result in a high consumption of oxidant and a considerable loss of carbon, especially from propane side cleavage. It also leads to low carbon
yields when converting these monomeric phenolics to subsequent aromatic ring cleavage products. Mitigating the carbon loss from each conversion step will greatly improve the economic feasibility of this process when implemented at an industrial scale. Therefore, to achieve a high carbon yield, partially depolymerizing lignin in order to retain most of the propane side chains may be a more practical strategy. To date, there hasn’t been a practical method can selectively cleave the lignin inter-linkages to generate an ideal PDL. In most lignin depolymerization methods, lignin is inevitably re-condensed under severe treatment conditions.

To address this gap in knowledge, preliminary research was conducted to identify a proper lignin partial depolymerization strategy. The recent discovery and application of deep eutectic solvent (DES) provided new insight toward its potential use for lignin extraction. (Liu, Hou et al. 2012, Francisco, van den Bruinhorst et al. 2013, Radošević, Cvjetko Bubalo et al. 2015) DES is a mixture of two or more chemicals acting as either hydrogen-bond donors (HBD) or hydrogen-bond acceptors (HBA). The results from a number of recent studies suggested that DES may have the unique capacity to operate as both solvent and catalyst for solubilizing and depolymerizing lignin. We tested several HBA and HBD combinations, and identified the Choline chloride-lactic acid (ChCl-Lac) pair to be capable of effectively solubilizing and extracting PDL directly from representative woody biomass in high purity and selectivity. Treatments of woody biomass by ChCl-Lac were conducted at four different temperatures: 90°C, 120°C, 145°C and 180°C, with different treatment times. PDL can be solubilized in ChCl-Lac DES and extracted, with an optimal treatment condition of nine hours at 145 °C. A high yield of up to 78% of the original lignin can be depolymerized and recovered after proper precipitation and wash procedures. The collected PDL has a very high purity, exceeding 95% based on the Klason lignin test, even in large-scale batch reactions (600 g of biomass/batch). The preliminary work demonstrated that DES can be a
interesting system for generating PDL in high selectivity and yield directly from biomass. This part of the preliminary work conducted in cooperation with Dr. Carlos Alverez-Vasco, and has been included in part of a recent joint publication. However, the mechanism for how DES depolymerizes and extracts PDL with such high selectivity and efficiency remains unclear, and requires significant attention in future study. Mechanistic study is expected to give important clues for the further optimization and development of new lignin partial depolymerization strategies.

7.2.2 Characterization of the partial depolymerized lignin

The partial depolymerized lignin is expected to have its own distinct structural features, differing in functional group, molecular weight, interlinkages on the molecular level and molecular assembly on the macromolecular level, all of which will be highly dependent on the partial depolymerization technique and treatment severity. Investigating the structural characteristics of PDL is fundamental to developing a method for further conversion to oligo-DCAs.

To demonstrate the unique characteristics of PDL, we adapted the analytical methods integrated in Chapter Five presented in this thesis, with particular emphasis on quantitative $^{13}$C NMR, HSQC NMR, and gel permeation chromatography (GPC) analyses which provide the most important characteristics dictating the subsequent conversion to DCAs. The preliminary work from 7.2.1 exploring the use of DES treatment to obtain PDL was used as the basis for further investigation. Milled wood lignin (MWL) extracted from the same D. fir was also prepared and used as a reference for comparison with PDL. The high purity of the PDL was established by $^{13}$C NMR analysis which confirmed the absence of any carbohydrates. $^{13}$C NMR analysis yielded remarkable results for the lignin structure of PDL, revealing a negligible presence of ether linkages as well as a much higher phenolic hydroxyl group content compared to that of MWL and other typical softwood lignins. (Sen, Patil et al. 2015, Constant, Wienk et al. 2016) This confirms the
cleavage of ether linkages during DES treatment. The absence of β-O-4 ether linkage in PDL was also substantiated by HSQC NMR spectra. HSQC NMR spectra further demonstrated that carbon-carbon (C-C) bonds such as β-β and β-5 linkages remained as the predominant interunit linkages in PDL. An appreciable amount of Hibbert’s ketone (HK) was also detected in PDL, again verifying the cleavage of β-O-4 linkages by DES. No condensation structures were detected. The molecular weight distribution and peak molecular weight of this DES lignin was then determined by GPC analysis. Compared to MWL and other typical softwood lignins, PDL had a narrower and lower molecular weight distribution ranging from 490-2600 g/mol with a peak molecular weight of about 890 g/mol. This provided evidence that DES depolymerizes native lignin by selective cleavage of ether bonds without subsequent condensation reactions, a characteristic distinct from other lignin depolymerization methods.(Gierer 1985, Gierer 1986) It is conceivable that because ether bonds are more susceptible to disruption than C-C bonds, a reduction or elimination of ether bonds in lignin will likely afford PDL with greater oligomer stability. It is evident that PDL possesses unique structural properties that are distinctive from all existing prepared lignin, with great potential for high value applications. This part of the preliminary work has been included in part of our recent publication. Future study will adapt the systematic analytical methods assembled in this thesis work and focus on fully characterizing PDL, as well as identifying opportunities for conversion to oligo-DCAs as valuable platform chemicals/intermediates.

7.2.3 Demonstrate the PDL to Oligo-DCA pathway and explore the application of Oligo-DCAs

Partially depolymerized lignin (PDL) is expected to have extensive applications as a new type of feedstock. Preliminary data using hydromatairesinol as a model compound demonstrated
the viability of converting lignin oligomers to DCA structures selectively and validated the PDL-to-DCA concept. Future work will examine the efficiency of converting PDL to Oligo-DCA. Integrating FT-IR, 1H/13C HSQC NMR and Quantitative 13C NMR introduced in Chapter Six will provide a practical set of tools to qualitatively and quantitatively characterize oligo-DCA. Utilizing oligomeric lignin will also significantly alleviate concerns about product separation and purification. Characterization of the oligo-DCA would provide significant clues toward identifying new applications of oligo-DCA for high value-added fuel, chemical, and material production. The unsaturated fatty carbon chains of the oligo DCA are readily to be converted through reactions such as olefin metathesis, which might generate fuel products. The oligo-DCA can also be promising feedstock for polymer production considering their multi-functionality by carboxylic and hydroxyl group.
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