

# Chemistry of Bond Cleavage in Lignin Degradation during Pretreatment of Lignocellulosic Biomass



Sujala Bhattarai and Shulin Chen\*

Department of Biological Systems Engineering, Washington State University, USA

Submission: May 10, 2017; Published: July 26, 2017

\*Corresponding author: Shulin Chen, Department of Biological Systems Engineering, Washington State University, WA, USA,  
Email: chens@wsu.edu

## Abstract

Deconstructing lignin structure is a critical step in utilizing cellulosic sugar for the production of biofuels and bioproduct. This is accomplished typically in a biomass pretreatment process. Various options have been investigated for this application but comparisons of these processes have been difficult. This review summarizes the chemical nature of these processes with focus on bond cleavage. The commonly accepted technologies such as alkane, acidic, ionic, and biological processes are assessed. The chemical nature of these processes provides a scientific base for understanding and evaluating these different processes. The information presented in this paper can be used for selecting the proper pretreatment technologies for processing lignocellulosic biomass.

## Lignocellulosic Biomass

Lignocellulosic biomass is abundantly available feedstock for bioenergy production. This biomass includes trees, bushes, grass, agricultural residues, energy crops, and agricultural industrial wastes. Lignocellulosic biomass is a sustainable and cheap feedstock for the production of chemicals, fermentable sugars, and biopolymers. Biofuel production from the lignocellulosic feed stocks consists of five major unit operations: feedstock preparation, pretreatment, enzymatic hydrolysis, and fermentation. Therein, the major bottleneck is low enzymatic hydrolysis efficiency and the high expense of pretreatment, both of which can be attributed to the complex cell wall structure of lignocellulosic biomass.

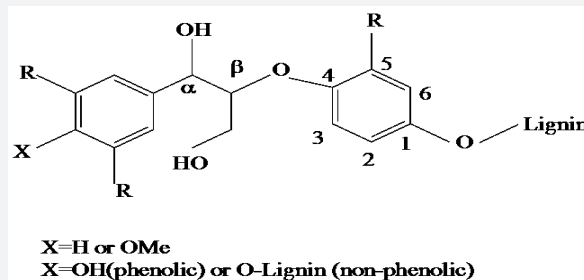
## Lignin Structure

**Table 1:** BDE for the common linkages calculated at M06-2X/6-311++G(d,p)<sup>2</sup>.

Bonds	Bond Dissociation Energy, Kcal/Mol
$\alpha$ -O-4	60.0-70.9
$\beta$ -O-4	69.5 -71.8
$\beta$ -5	103.9-106.4
$\beta$ - $\beta$	112.2-118.5

Lignocellulosic biomass is composed of lignin, hemicellulose, and cellulose. Lignin is a complex phenolic polymer formed

by radical coupling reactions of primarily three different monolignols: p-coumaryl, coniferyl, and sinapyl alcohol. The lignins formed from these monolignols are syringyl lignin (s-lignin), guaiacyl lignin (G-lignin), and hydroxyl-phenyl lignin (H-lignin). The common lignin linkages in the lignin are formed by the connection of C-O bonds ( $\beta$ -O-4,  $\alpha$ -O-4) and C-C bonds ( $\beta$ -5,  $\beta$ -1 and  $\beta$ - $\beta$ ). The amounts of these linkages are 50, 2-8, 9-12, 7 and 2%, respectively [1]. Degradation and dissolution of lignin loosen the plant cell wall structure and improve enzymatic hydrolysis. The bond dissociation energies of all common lignin linkages are shown in Table 1. The most common native  $\beta$ -O-4 lignin linkage has the BDE of 69.5-71.8 kcal/mol as calculated by density functional theory [2].



**Figure 1:** Phenolic lignin (X=OH) and nonphenolic lignin (X=H, or O-Lignin).

Lignin polymers are further classified as phenolic or nonphenolic. The former has an -OH moiety in the X position as shown in Figure 1 and nonphenolic lignin has H or O-lignin at the X position.

### Pretreatment Techniques

The goal of the pretreatment technique is to break the natural recalcitrant (lignin) of the lignocellulose to produce a high amount of fermentable sugar during the enzymatic saccharification. An ideal pretreatment technique should have the following characteristics.

- Cost effectiveness
- A high sugar yield
- Low chemical consumption
- A low energy requirement
- Be selective towards lignin
- Be environmental friendly

Pretreatment methods are broadly classified as physical, chemical, and biological. All existing pretreatment techniques have their own unique way to deconstruct the recalcitrant plant cell wall structure. For instance, physical pretreatment increases the specific surface area of the feedstock to enhance the digestibility of cellulose. Fungal pretreatment is a selective and efficient technique, while chemical pretreatments are the most rapid. The key issues associated with existing pretreatment techniques are that physical methods are energy intensive and the biological pretreatment like fungi are slow and require a large physical area. Further, a chemical pretreatment technique such as dilute acid produces high concentration of inhibitors and an alkali pretreatment consumes a lot of chemicals and

is not environmentally friendly. Thus, the development of a cost effective, environmentally benign and industrially viable pretreatment technique remains a challenge. Toward that end, understanding the fundamental chemistry of how a pretreatment techniques act to deconstruct the lignin molecule is important. Herein I present a brief review regarding the state of knowledge on how alkali, acid and ionic liquid conditions decompose the most common  $\beta$ -O-4 lignin linkages in chemical pretreatment methods.

### Lignin deconstruction in alkali based pretreatment

Sodium hydroxide, lime, sodium carbonate, and aqueous ammonia are common existing alkali based pretreatments techniques. The lignocellulose degradation reactions in the alkali based pretreatment is diffusion controlled. This means the rate of reaction is controlled by the ability of the alkali solution to be transported through the biomass structure. As an example, the aqueous ammonia pretreatment of an oil palm empty fruit bunch with 35.4% acid insoluble lignin took 10 to 12hrs of pretreatment time at a solid loading of 21% (w/w) at 60 oC to obtain 36.4% lignin removal [3]. One improvement that can be made is to remove the diffusion barrier. The diffusion of an alkali solution can be enhanced by increasing the surface area of the biomass and opening up the pores to chemical degradation. For instance, the sodium hydroxide pretreatment time of wheat straw was reduced to 1hr from 12hrs when integrated with 10mins of an ozone pretreatment [4]. The ozone pretreatment alters the lignin structure in biomass. As a result, the pore size increases and diffusion barrier is removed to alkali solution. The integrated process had the sugar yield of 98% which was significantly larger compared to 50% untreated, 60% with only ozone and 80% with only alkali. The review of the existing alkali pretreatment techniques and their pros and cons are discussed in detailed in a review article [5].

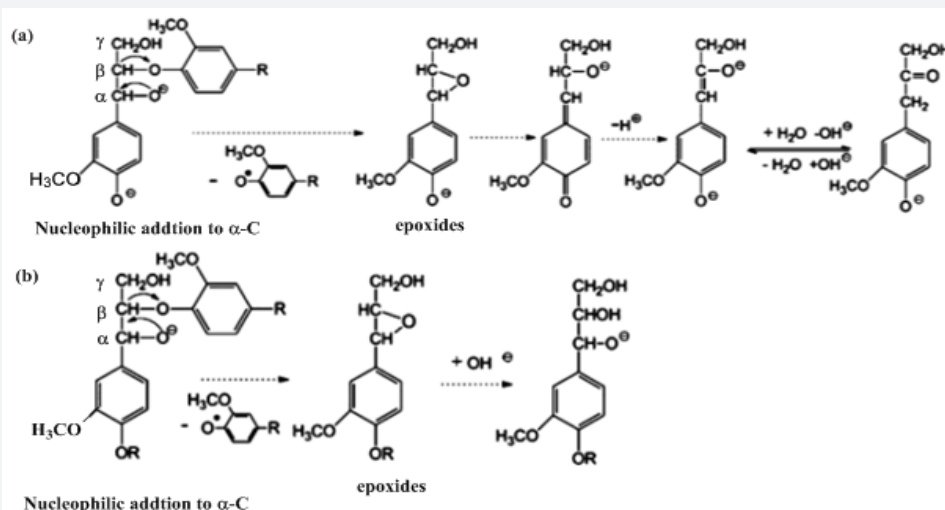


Figure 2: Lignin reactions in alkaline conditions a) cleavage of phenol type  $\beta$ -aryl ether b) fracture of nonphenol type  $\beta$ -aryl ether [6].

The glucose yield of the alkali based pretreatment ranges from 80 to 95% [5]. The rate of delignification in the alkali pretreatment depends upon the cleavage of phenol type  $\beta$ -aryl

ethers ( $\beta$ -O-4) as the C-C linkages are relatively stable [1]. Figure 2 shows the reaction mechanism by which phenolic and non phenolic  $\beta$ -aryl ethers dissociated during the alkali pretreatment.

The main reaction during alkaline treatment of phenolic  $\beta$ -aryl ether linkages is the elimination reaction of the  $\beta$ -proton as shown in Figure 2. The OH<sup>-</sup> will add to  $\alpha$ -C atoms, initiating a fracture of the linkages by the formation of the epoxides (Figure 2). The cleavage of phenol type  $\beta$ -aryl ethers can be significantly enhanced in the presence of stronger nucleophiles in pretreatment liquor [6]. For instance sulfate digestion in pulping industries is enhanced by the nucleophilic attack of HS<sup>-</sup> or S<sup>2-</sup> ions to dissociate the  $\beta$ -O-4 linkages [1].

### Lignin deconstruction in acid based pretreatment

Dilute acids are commonly used in acid pretreatment and includes sulfuric acid, nitric acid, hydrochloric acid, phosphoric acid, peracetic acid and oxalic acid. Among these, dilute sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) is the most widely used for experiments because it is cheaper, readily available and has low safety concerns. The glucose yield of the dilute acid pretreatment at an optimum temperature of 170 °C ranges from 75% to 97% [7].

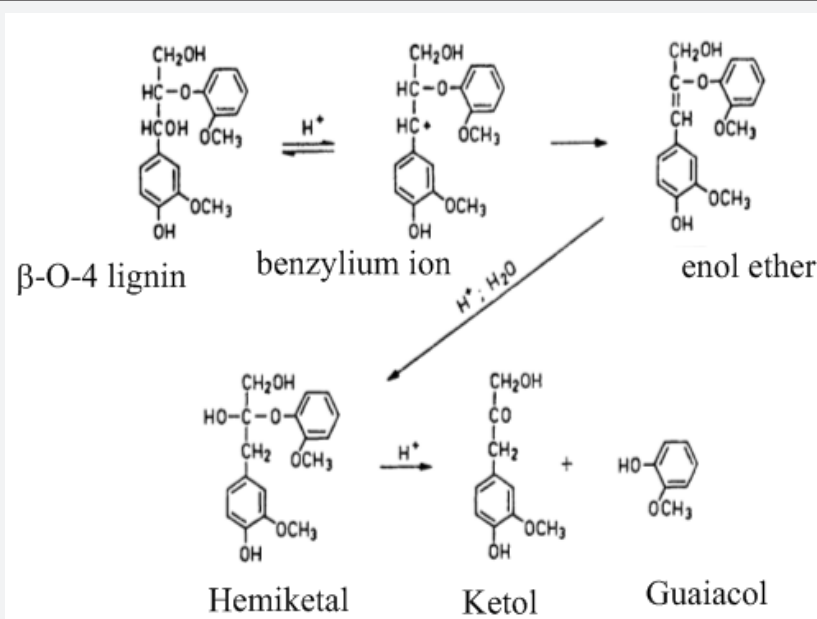


Figure 3: Reaction route for the formation of ketol and guaiacol on acidolysis [8].

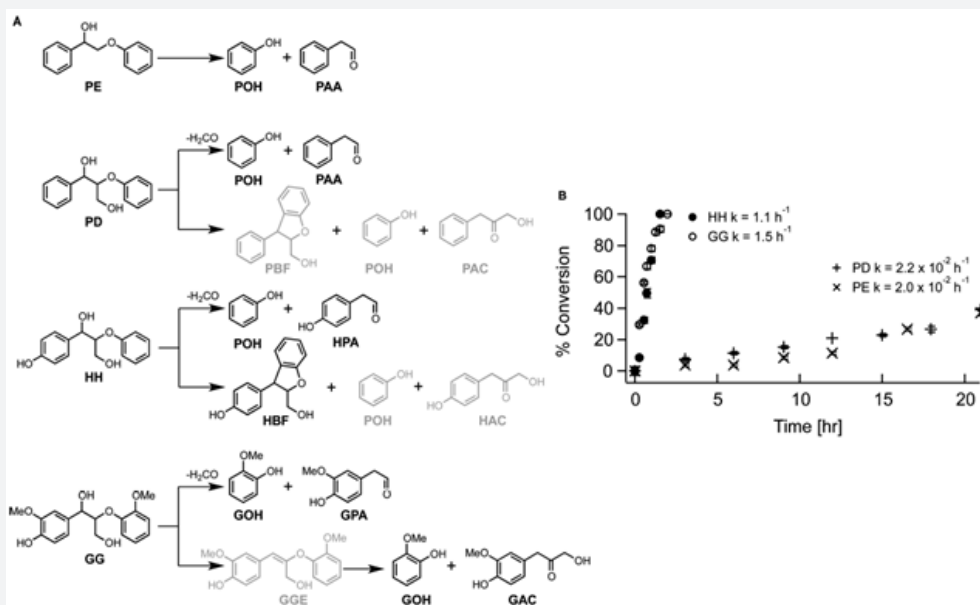


Figure 4: Dissociation of  $\beta$ -O-4 type HH, GG, PE and PD types of lignin in the dilute acid pretreatment (POH=phenol; GOH=guaiacol).

The proposed reaction pathway for the cleavage of  $\beta$ -O-4 linkage in acid solution is shown in Figure 3. The acidolysis of  $\beta$ -O-4 type lignin model compound with 0.2M HCl in dioxane

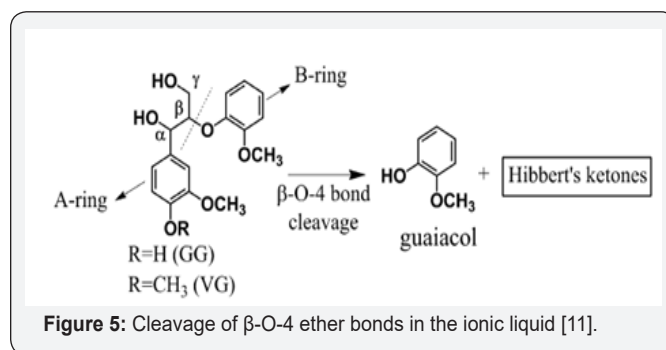
water (9:1) reveals the guaiacol and ketol as the primary products [8]. The presence of a benzyl alcohol group on the carbon adjacent to the  $\beta$ -O-4 bond, results in a benzylium ion

and that converts to an enol ether on acidolysis. The Enol ether then undergoes hydrolysis (breaking of a molecular bond in the presence of water) via hemiketal forming ketol and guaiacol as shown in Figure 3.

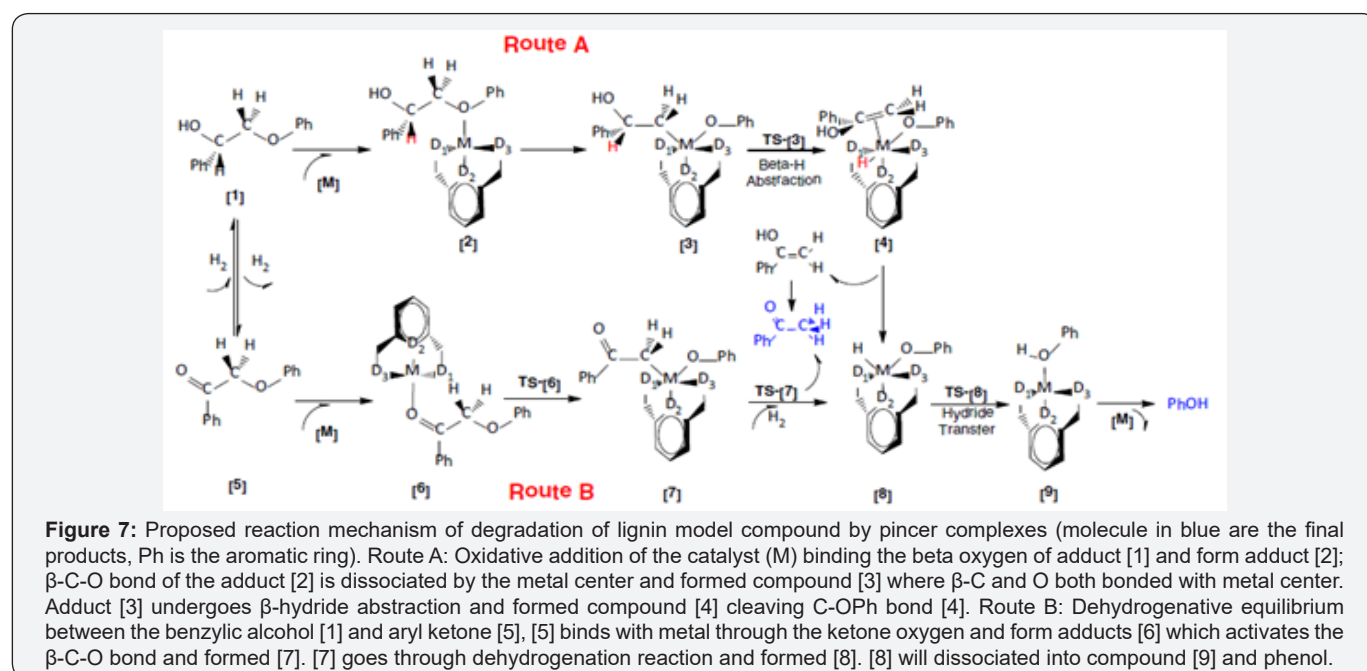
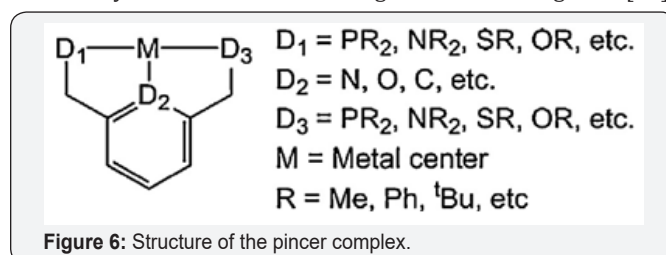
Depolymerization of phenolic and nonphenolic  $\beta$ -O-4 lignin model compounds (PE, PD, HH and GG as shown in Figure 4) in the acidic environment (0.2M  $H_2SO_4$  at 150 °C in water) was studied both experimentally and computationally. GC-MS was used to identify the products structures and HPLC was used to determine the concentration and kinetics of the products. The major products obtained from the PE and PD was guaiacol and phenol, and from HH and GG were phenol, guaiacol and acetaldehyde. Using DFT the structures of the intermediates and products were analyzed and the energetics of the reaction pathway was proposed along with a determination of the reaction rate. Their results indicate that the

presence of phenolic-hydroxyl group accelerates the reaction rate (1.1 and 1.51/h) of C-O bond cleavage in lignin model compounds with  $\beta$ -O-4 linkages [9].

Ionic liquids are salts made of from an organic cation and an organic/inorganic anion, and utilizing these for the lignin deconstruction is termed as ionic liquid pretreatment. Ionic liquid pretreatment is conducted in mild temperature (<100 °C) and pressure. It is environmentally benign compared to chemical pretreatment. However, the major bottleneck of this pretreatment is the high price and toxic effects of ionic liquids in enzymes during the enzymatic hydrolysis process for cellulose conversion to simple sugars. Moreover, ionic liquids have high viscosities that limit its dissolution ability and mass transfer. This problem is overcome by the addition of conventional solvents such as DMSO, which makes ionic liquid toxic to enzymes [10].



Ionic pretreatment has also demonstrated a high amount of dissociation of the  $\beta$ -O-4 type lignin linkages. The hydrolytic cleavage of the  $\beta$ -O-4 ether bonds in lignin model compounds has been studied experimentally in 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) with metal chlorides ( $FeCl_3$ ,  $CuCl_2$  and  $AlCl_3$ ) and water. [BMIM]Cl (100g) was mixed with metal chlorides (5mol% to lignin) and water (2.25 $\mu$ L). The reaction temperature was 110 to 150 °C. HPLC was used to analyze the reactants and products structure. The cleavage of the C-O bond was determined based on the concentration of guaiacol in the products. About 75 to 80% of the  $\beta$ -O-4 ether bond was cleaved. They proposed that HCl formed *in situ* by the hydrolysis of metal chlorides might have catalyzed the C-O bond cleavage as shown in Figure 5 [11].



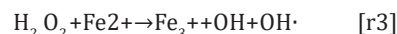
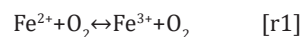
DFT (B3LYP/CEP-31G (the Stevens/Basch/Krauss ECP split valance basis set)) study indicated the activation of C-O bond during the reaction of  $\beta$ -O-4 type lignin compounds with metals solution. Metal solution was pincer complex (Fe, Ru and Os as shown in Figure 6). The C-O bond cleavage mechanism was described by two schemes as illustrated in Figure 7. Results indicated that the C-O activation energies follow the electro negativity trend i.e. Fe>Ru>Os and concluded that C-O bond dissociation depends upon the ability of the metal ions to donate electrons to the C-O bond [12].

### Termite based pretreatment

Chemical pretreatments need high temperature and pressure conditions because the C-O bonds and C-C bonds in lignin are strong (Table 1) and cannot be easily cleaved in ambient conditions. Biological pretreatments, like fungi takes a long time (15 to 60 days) to degrade lignin and needs a large space (25 ml flask per g of biomass in lab scale) to grow [13,14]. However, termite based pretreatment is promising alternative because it can be conducted in ambient conditions. Termites have been able to survive in the lignocellulosic feedstock while pretreating the lignocellulosic feed stocks in its digestive tract. Termites consume lignocellulosic biomass rapidly; 1 gram of the *C. Formosanus* termite consumes 23.4mg of wood in a day [15]. Studies show that termites can digest 99% of cellulose from wood particles within 24 hours at room temperature [16-18]. There are several hypothetical pathways postulated regarding termites'

lignin deconstruction mechanisms. Termites have a crop and gizzard that grind the wood particles in to very small sizes modifying the lignin structure [19,20] anticipated that lignin is depolymerized and mineralized by radical reactions that are triggered by oxidation with the enzymes such as lignin peroxidase (Lip), manganese peroxidase (MnP) and laccase [20]. The presence of lignolytic (laccase, Lip) and cellulolytic ( $\beta$ -glucosidase) enzymes were found in the digestive tract which indicates the possibility of enzyme induced lignin modification in termite. The termite digestive tract is composed of the foregut, midgut and hindgut. The  $\beta$ -glucosidase activity was experimentally measured as 2.33 in salivary glands, 0.2 in foregut, 0.11 in midgut, and 0.47U (enzyme unit defined as the amount of enzyme that produces certain amount of enzymatic activity) per gram per termite in hindgut [21]. The termite gut environment is oxidative. The dissolved oxygen in the termite digestive tract was experimentally measured using a microelectrode with a tip diameter <10 $\mu$ m of termite *C. formosanus*. The oxygen concentration in the foregut, midgut, and hindgut were ranged from 2-2.5, 3-4, and 2-1mg/L, respectively [17]. The redox potential of the gut environment measured using micro sensors along the central axis was 200-0 for midgut and 0-200 for the hindgut [22]. The pH was 6.7 to 7 in the midgut 5. 5-6.7 in the hindgut [22]. The hydrogen peroxide and Fe(II) concentration was also measured in the midgut [23]. The presence of an oxidative environment is favorable for the occurrence of

Fenton-like reactions in the midgut, wherein molecular oxygen reacts with hydrogen to produce hydrogen peroxide, and in the presence of hydrogen peroxide and iron, the hydroxyl radical OH. is produced (reactions r1-r3) which is responsible for the cleavage of  $\beta$ -O-4 linkages in termite [19].



At the same time, it has been observed that termite-degraded wood retains its aromatic structure [16,24,25] which corroborates the potential reactivity of OH [26,27] with lignin, as it is known that hydroxyl radical reactions with aromatic systems do not destroy the phenyl rings [28]. Previous investigation of OH. reactivity has been observed to be 5-6 times higher with lignin linkages than for carbohydrate linkages, supporting the potential for selective OH. modification of lignin in the biomass [29]. Radicals have a very short half-life; therefore, experimentally they are difficult to trap and study their reactivity. In this circumstance, computational chemistry can provide valuable information for characterizing the potential process by which a reaction takes place between the hydroxyl radicals and lignin model compounds.

This chapter has introduced the strategies used to study pretreatment techniques from different field of study. In the remaining chapters we discuss further experimental and theoretical study using computational fluid dynamics, kinetics, quantum mechanics and molecular dynamics. The second chapter is a review of the computational methods employed in this thesis including quantum mechanics, statistical mechanics. The third chapter describes the computational fluid dynamics and kinetic study of the ozone pretreatment of wheat straw with experimental validation. The fourth chapter describes the application of quantum mechanical approaches to study the reaction between hydroxyl radical and lignin linkages leading to the ring hydroxylation reactions that are a common type of products found in termite pretreatment. The fifth chapter discusses the application of molecular dynamics methods for studying the reactive systems of hydroxyl radical, phenol and water and its application to lignin.

### References

1. Sjostrom E (2013) Wood chemistry: fundamentals and applications. Elsevier, Netherlands.
2. Kim S, Chmely SC, Nimlos MR, Bomble YJ, Foust TD, et al. (2011) Computational study of bond dissociation enthalpies for a large range of native and modified lignins. The Journal of Physical Chemistry Letters 2(22): 2846-2852.
3. Jung YH, Kim IJ, Han JI, Choi IG, Kim KH (2011) Aqueous ammonia pretreatment of oil palm empty fruit bunches for ethanol production. Bioresource technology 102(20): 9806-9809.
4. Bule M, Gao A, Chen S (2013) Advanced Methods for Sugar Production from Lignocellulosic Biomass and Fermenting Sugars to Microbial Lipids. Google Patents.

5. Xu H, Li B, Mu X (2016) Review of alkali-based pretreatment to enhance enzymatic saccharification for lignocellulosic biomass conversion. *I & EC Research* 55(32): 8691-8705.
6. Gierer J (1970) The reactions of lignin during pulping. A description and comparison of conventional pulping processes; DTIC Document.
7. Singh J, Suhag M, Dhaka A (2015) Augmented digestion of lignocellulose by steam explosion, acid and alkaline pretreatment methods: a review. *Carbohydr Polym* 117: 624-631.
8. Lundquist K, Lundgren R (1972) Acid degradation of lignin. VII. The cleavage of ether bonds. *Acta chemica scandinavica*.
9. Sturgeon MR, Kim S, Lawrence K, Paton RS, Chmely SC, et al. (2013) A mechanistic investigation of acid-catalyzed cleavage of aryl-ether linkages: Implications for lignin depolymerization in acidic environments. *ACS Sustainable Chemistry & Engineering* 2(3): 472-485.
10. van Osch DJ, Kollau LJ, van den Bruinhorst A, Asikainen S, Rocha MA (2017) Ionic liquids and deep eutectic solvents for lignocellulosic biomass fractionation. *Physical Chemistry Chemical Physics*.
11. Jia S, Cox BJ, Guo X, Zhang ZC, Ekerdt JG (2010) Hydrolytic cleavage of  $\beta$ -O-4 ether bonds of lignin model compounds in an ionic liquid with metal chlorides. *Industrial & Engineering Chemistry Research* 50(2): 849-855.
12. Liu C, Wilson AK (2015) Cleavage of the  $\beta$  O 4 linkage of lignin using group 8 pincer complexes: A DFT study. *Journal of Molecular Catalysis A: Chemical* 399: 33-41.
13. Yu H, Zhang X, Song L, Ke J, Xu C, et al. (2010) Evaluation of white-rot fungi-assisted alkaline/oxidative pretreatment of corn straw undergoing enzymatic hydrolysis by cellulase. *J Biosci Bioeng* 110(6): 660-664.
14. Millati R, Syamsiah S, Niklasson C, Cahyanto MN, Ludquist K, et al. (2011) Biological pretreatment of lignocelluloses with white-rot fungi and its applications: a review. *Bio Resources* 6(4): 5224-5259.
15. Haverty MI (1976) Termites. *Pest control* 44(5): 12-17.
16. Ke J (2012) Lignin Unlocking Process in Wood Feeding Termites For Effective Biomass Sugar Release.
17. Ke J, Sun JZ, Nguyen HD, Singh D, Lee KC, et al. (2010) In-situ oxygen profiling and lignin modification in guts of wood-feeding termites. *Insect Science* 17(3): 277-290.
18. Ke J, Laskar DD, Singh D, Chen S (2011) In situ lignocellulosic unlocking mechanism for carbohydrate hydrolysis in termites: crucial lignin modification. *Biotechnology for biofuels* 4(1): 17.
19. Brune A (2014) Symbiotic digestion of lignocellulose in termite guts. *Nature Reviews Microbiology* 12(3): 168-180.
20. Ni J, Tokuda G (2013) Lignocellulose-degrading enzymes from termites and their symbiotic microbiota. *Biotechnol Adv* 31(6): 838-850.
21. Tokuda G, Saito, H, Watanabe H (2002) A digestive  $\beta$ -glucosidase from the salivary glands of the termite, *Neotermes koshunensis* (Shiraki): distribution, characterization and isolation of its precursor cDNA by 5-and 3-RACE amplifications with degenerate primers. *Insect Biochem Mol Biol* 32(12): 1681-1689.
22. Ebert A, Brune A (1997) Hydrogen concentration profiles at the oxic-anoxic interface: a microsensor study of the hindgut of the wood-feeding lower termite *Reticulitermes flavipes* (Kollar). *Applied and Environmental Microbiology* 63(10): 4039-4046.
23. Ke J (2012) Lignin unlocking process in wood feeding termite. Washington State University, WA, USA.
24. Katsumata SK, Jin Z, Hori K, Iiyama K (2007) Structural changes in lignin of tropical woods during digestion by termite, *Cryptotermes brevis*. *Journal of Wood Science* 53: 419-426.
25. Hyodo F, Azuma JI, Abe T (1999) Estimation of effect of passage through the gut of a lower termite, *Coptotermes formosanus* Shiraki, on lignin by solid-state CP/MAS  $^{13}\text{C}$  NMR. *Holzforchung* 53(3): 244-246.
26. Gierer J (1997) Formation and Involvement of Superoxide ( $\text{O}_2^-/\text{HO}_2^\bullet$ ) and Hydroxyl ( $\text{OH}^\bullet$ ) Radicals in TCF Bleaching Processes: A Review. *Holzforchung-International Journal of the Biology, Chemistry, Physics and Technology of Wood* 51(1): 34-46.
27. Wu P, Li J, Li S, Tao FM (2012) Theoretical study of mechanism and kinetics for the addition of hydroxyl radical to phenol. *Science China Chemistry* 55(2): 270-276.
28. Curtiss LA, Redfern PC, Raghavachari K (2007) Gaussian-4 theory using reduced order perturbation theory. *J Chem Phys* 127(12): 124105.
29. Ek M, Gierer J, Jansbo K, Reitberger T (1989) Study on the selectivity of bleaching with oxygen-containing species. *Holzforchung-International Journal of the Biology, Chemistry, Physics and Technology of Wood* 43(6): 391-396.



This work is licensed under Creative Commons Attribution 4.0 License  
DOI: [10.19080/AIBM.2017.04.555643](https://doi.org/10.19080/AIBM.2017.04.555643)

### Your next submission with Juniper Publishers will reach you the below assets

- Quality Editorial service
- Swift Peer Review
- Reprints availability
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attainment for your research
- Manuscript accessibility in different formats  
( Pdf, E-pub, Full Text, Audio )
- Unceasing customer service

Track the below URL for one-step submission

<https://juniperpublishers.com/online-submission.php>