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Photonanocatalyst Aided Alkaline Pretreatment and Raman Spectroscopic Characterization of Corn Stover Biomass

Chao Wang
Iowa State University

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Photonanocatalyst aided alkaline pretreatment and Raman spectroscopic

characterization of corn stover biomass

by

Chao Wang

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Program of Study Committee:

Chenxu Yu, Co-Major Professor

Tae Hyun Kim, Co-major Professor

D. Raj Raman

Kurt Rosentrater

Iowa State University

Ames, Iowa

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Table of Contents

Chapter 1 Introduction

Objectives

Cellulosic ethanol, as the second generation of biofuels, attracts increasing attentions owing

to the competitive economic and renewable factors, compared to ethanol produced from food

and oil crops. Most lignocellulosic materials used in cellulosic ethanol production are mainly

composed of cellulose, hemicellulose and lignin. The presence of lignin causes a protective

barrier that prevents the raw materials from destruction and enormously hinders the

digestibility of cellulose to fermentable monosaccharides (Kumar et al., 2009; Alvira et al.,

2010). As a result, an effective pretreatment is an essential step in the cellulose hydrolysis

process. Currently, although quite a few pretreatment approaches have been investigated; the

huge water, chemicals and energy consumption for delignification still renders the

pretreatment step the most costly one within the ethanol production process from

lignocellulosic biomass. Meanwhile, the residual chemicals, such as sulfuric acid, sodium

hydroxide, may give rise to serious pollution problems if not handled properly. In order to

decrease the loading/dosage of chemicals and the overall energy consumption to achieve a

more environmentally friendly de-lignification route for lignocellulosic biomass

pretreatment, an advanced progressive photocatalyst assisted alkaline pretreatment method is

developed in this project.

Another challenge facing the study of biomass pretreatment is to characterize the chemical

changes occurring during the pretreatment process at the molecular level in real time, so that

optimization of the processing parameters can be better achieved to further reduce the

processing cost. In this study, we investigated the potential of utilizing Raman spectroscopy

to monitor the molecular changes associated with the photo-assisted chemical de-lignification

process. IR and Raman spectra, which are complementary to each other, could provide the complete molecular vibrational picture in any given chemical compound. Therefore, both techniques could be used to characterize molecular structures and their vibrational patterns (Agarwal and Atalla, 2000; Wartewig and Neubert, 2005). However, compared to IR, Raman spectroscopy has some unique advantages in analyzing cell wall materials. In Raman, spectra can be recorded from domains with 1 μm spatial resolution, so the problem of morphological heterogeneity on a large scale can be overcome (Agarwal and Atalla, 1986). Additionally, only very small amounts of both dry and wet samples are required, and measurement process is nondestructive (Sivam et al., 2012). Although tremendous progress has been achieved in understanding the specific Raman peaks/bands associated with lignin substituent and functional groups, sufficient reference data for various lignin materials are still lacking, especially for lignocellulosic biomass. One of our objectives in this project is to further complete the lignocellulosic biomass (corn stover in our current case) Raman spectroscopic band assignments database and to understand the dynamic mechanism for delignification of biomass in alkaline pretreatment process through periodic monitoring the change of materials' molecular structure.

1.1. Literature review

1.1.1. Background

Since the First Industrial Revolution, human beings have consumed all kinds of fossil fuels (coal, oil, natural gas) at an astonishing speed, which has caused the two giant crises we have to face nowadays: energy shortage and global warming. Owing to the rapid growth of the world population and the improving living standard in developing countries, the global energy consumption will continue to rise. Today fossil fuels are still the first choice when it

comes to energy. Huge consumption of fossil fuels has generated and will continue to contribute to the issue of global climate change. As a result, unless people could find alternatives to fossil fuels, or develop more efficient ways to utilize fuels, the world will eventually run out of energy, and face the consequences of the changing climate. Use of biomass-rich resources for bioenergy and biorenewable chemical production could contribute to the displacement of fossil fuels as our primary energy source and to the mitigation of the emission of greenhouse gases.

Biorenewable energy offers an alternative to fossil fuels, and brings ecological and environmental benefits. However, current bioenergy production has its own problems. The first generation biofuel technology uses food and oil crops, like corn and soybean, as feedstock for biofuel production (i.e., ethanol, biodiesel). Due to the low conversion rate from feedstock to biofuel (Wu et al., 2010), a great deal of foodstuffs such as corn need to be consumed to produce energy, which obviously would lead to a series of problems, such as food shortage, high water and chemical fertilizers usage as well as occupancy of arable land (Damartzis and Zabaniotou, 2011). The second generation biofuel technology will produce fuels from the whole plant matter of dedicated energy crops (i.e., switchgrass) or agricultural residues, forest harvesting residues or municipal and industrial waste (Naik, Goud, Rout, & Dalai, 2010). However, the strategies of second generation biofuels are simultaneous saccharification and fermentation (SSF), and/or simultaneous saccharification and co-fermentation (SSCF), both of which require extensive pretreatment of the lignocellulosic feedstock to facilitate the subsequent release of glucose monomers for fermentation (Demain, Newcomb, & Wu, 2005). Processing of lignocellulose to biofuels such as ethanol consists of 4 major steps: pretreatment, hydrolysis, fermentation, and product purification/fuel recovery.

With an excellent pretreatment process altering the biomass macroscopic and microscopic size and structure, a significant increase of sugar yield would be obtained in the next hydrolysis step where carbohydrate polymers (cellulose and hemicellulose) are being fractioned to monomeric sugars (Wyman, 1999) by enzymatic or chemical treatments. And then, the sugars are converted to ethanol by fermentation. The rate limiting step in the conversion of cellulose to fuel is its hydrolysis, especially the initial attack on the highly insoluble structure of crystalline cellulose. Lignocellulose is difficult to hydrolyze because it is associated with hemicellulose and enfolded by a lignin seal which has a limited covalent association with hemicellulose. Furthermore, the crystalline structure of cellulose with a potential formation of six hydrogen bonds also makes it hard to be hydrolyzed during processes (Pérez and Samain, 2010).

1.1.2. Lignocellulosic feedstock

Cellulosic ethanol is generally produced from lignocelluloses of non-edible parts of plants, such as corn stover, switchgrass, miscanthus, woodchips and the byproducts of lawn and tree maintenance. Lignocelluloses are composed mainly of cellulose, hemicelluloses and lignin. Generally, cellulosic biomass contains 40-50 % cellulose; 25-35% hemicelluloses; and 15-20% lignin. The other 5% is composed of minerals, oils, proteins, soluble sugars and other components (Wyman et al., 2005).

Cellulose is a linear polymer consisted of hundreds β- (1, 4)-glycosidic linked D-glucose units. Through hydrogen and van der Waals bonds, the long chain cellulose polysaccharides are linked and packed into microfibrils (Mosier et al., 2005). A polymer in cellulose contains about 7,000- 15,000 glucose molecules. There are two forms of cellulose presenting in biomass: crystalline and amorphous. Under a heat treatment at 320℃ and pressure of 25 MPa,

crystalline form, the major part of cellulose, can be transformed into amorphous form in water (Deguchi, Tsujii, & Horikoshi, 2006). Compared to amorphous cellulose, crystalline cellulose is stronger and more resistant to hydrolysis and enzymatic degradation. Hemicellulose has branches linked with β- (1, 4)-glycosidic bonds with short lateral chains consisting of different sugar monomers, including glucose, xylose, mannose, galactose, thamnose, arabinose, and uronic acids. Compared to cellulose, hemicellulose molecules are shorter chains with 500-3,000 sugar molecules and are more susceptible to hydrolysis and degradation (Mosier et al., 2005). In biomass, cellulose is encrusted with lignin and hemicellulose for protection. Lignin is a complex and large molecular structure containing cross-linked polymers of phenolic monomers. Because it is heterogeneous and lacks of a defined primary structure, lignin has some special properties, such as impermeability, and resistance against microbial attack (Kuhad and Singh, 2007). Guaiacyl propanol, *p*-hydroxyphenyl propanol, and syringyl alcohol can be regarded as monomers of lignin and they are linked by alkyl-aryl, alkyl-alkyl, and aryl-aryl ether bonds. Figure 1 shows how cellulose, hemicellulose, and lignin are inter-connected and bundled together in plant biomass.

The heterogeneity of lignin includes inter-phenylpropane-unit linkages between C-C and C-O, as well as side chains with various substituent and functional groups. Based on comprehensive spectroscopic analysis (near IR, FT-Raman and FT-IR) of the lignin and lignin containing materials, the main substituent and functional groups have been identified, including C=O, CHO, COOH, CH_3, OCH_3, α, β C=C, furan, and inter-unit COC and CC linkages (Agarwal et al., 2005). However, to better understand the ways that lignin molecules are bundled to cellulose and hemicellulose molecules in biomass and how these linkages can

be cleaved more effectively during pretreatment processes, more detailed spectroscopic characterization is needed. In this work we utilized Raman spectroscopy to characterize biomass during pretreatments. It has been discovered that aromatic ring stretch band on 1600 cm^{-1} wavenumber is only minimally sensitive to substituents due to the fact that it could be found in 70% lignin models. The phenyl vibrational frequency was discovered intensively between 1594 to 1604 cm^{-1} (Agarwal et al., 2005).
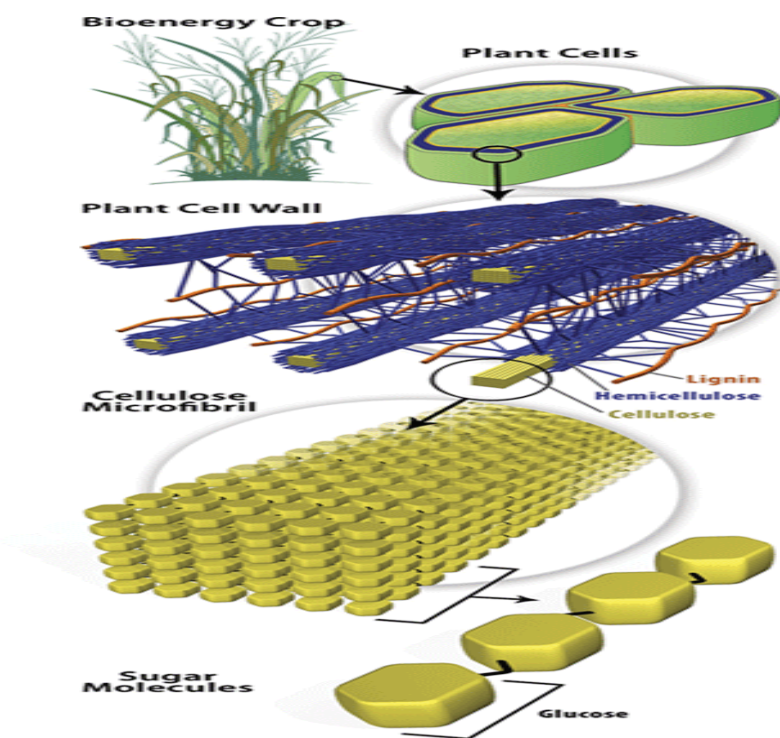
Figure 1 Cellulose, hemicellulose and lignin structure in lignocellulosic feedstock
(http://bioenergy.msu.edu/fuels/ethanol/cellulosic_ethanol.shtml)

1.1.3. Biomass Pretreatment

Although the utilization of lignocellulosic feedstock has the potential to decrease the cost of bio-ethanol due to its abundance, renewable nature and less competition with food sources (Silverstein et al., 2007), further processing advances are still needed to improve the returns and lower the cost, especially in pretreatment processing, one of the most costly steps. The solubilizing hemicellulose and crystalline cellulose structure are the main raw materials for

hydrolysis in native biomass tissues. However, due to the existing of lignin seal interpenetrating those structures, enzymes attack is hindered and then high sugar yield cannot be achieved. Therefore, a promising and inexpensive pretreatment step for removing lignin structure is needed to significantly increase the efficiency of fermentation and reduce the amount of expensive enzymes needed as well as the downstream costs (Wyman et al., 2005). The primary goal of pretreatment is to break down the shield forms of hemicellulose and lignin, prior to subsequent enzymatic hydrolysis and microbial fermentation of the exposed cellulose. Basically, the pretreatment methods can be physical (e.g. mechanical comminution and high energy irradiation), chemical (e.g. acids and bases), and biological depending on the application. Because of the large energy consumption needs in physical approaches and the relatively low efficiency in biological approaches, chemical pretreatment with higher removal efficiency for hemicellulose and lignin is becoming the most attractive method and has been extensively studied.

- Conventional chemical pretreatment methods

Conventionally, chemical pretreatment methods can be categorized into acidic methods, alkaline methods, and water-based methods.

According to Knappert et al., at 140-190°C temperature and 3-15 atm pressure, dilute sulfuric acid (0.5-1.0%) was added into biomass (10-40 wt.%), resulting in effective removal and complete hydrolysis of hemicellulose, and increased enzymatic hydrolysis yield of glucose from the cellulose. It was reported that 2% (w/v) sulfuric acid pretreatment of cotton stalks under the condition of 121°C and 15 psi for 90 min resulted in a high xylan reduction rate (95.23%) but a low cellulose to glucose conversion during hydrolysis (Silverstein et al., 2007). Except for sulfuric acid, nitric acid, hydrochloric acid, and phosphoric acid have also

been examined as pretreat agents for biomass materials (Brink, 1994; Herrera et al., 2003; Israilides, Grant, & Han, 1978; Zheng et al., 2008). The main advantage of dilute acid methods is the complete hydrolysis of hemicellulose into monosaccharides. After dilute sulfuric acid pretreatment, Schell et al. (Schell et al., 2003) measured the cellulose reactivity of corn stover with a simultaneous saccharification and fermentation (SSF) process. The results showed a 71% monomeric xylose yield and an 87% cellulose conversion rate. However, dilute acid pretreatment also has some negative influences on both efficiencies of the subsequent operations and costs of energy as well as materials. High pressure and temperature are needed in the pretreatment process; the corrosion of equipment caused by the acid increases the cost of maintenance; large quantity of water is needed for product recovery and neutralization (Hsu et al., 1996; Wyman, 1999).

H_2SO_4 and HCl are the major acid used in concentrated acid-based pretreatment to hydrolyze lignocellulosic biomass for fermentable sugar production (Goldstein et al., 1983). Even though concentrated acids are very effective to remove hemicellulose and enhance cellulose hydrolysis, the danger of handling concentrated acids brings major safety concerns. In addition, expensive equipment and complex reaction conditions are required to implement this approach (Zheng et al., 2009), which diminish its appeal to commercial users.

Liquid hot water pretreatment, strictly speaking, is a physical method in which no chemical reagent is used. Weil and coworkers (Weil et al., 1998) examined that super critical liquid water at high temperature (220-260℃) and high pressure could improve the cellulose digestibility and pentose recovery at 4.4% solid/liquid slurry corn fiber. Keeping the pH within the range of 5-7, they obtained an effective hydrolysis yields up to 84%. The mechanisms of hot water pretreatment to biomass include two aspects. Water can penetrate

cell structure of biomass and remove hemicellulose under a proper pressure. Furthermore, with a high dielectric constant, water can dissociate hemicellulose as well as part of lignin. The composition of protein and ash in biomass might lead to the stability of pH because of their buffering ability. Without other reagents added in the system, hot water pretreatment could reduce costs (e.g. energy and materials use compared to acid pretreatment) (Wyman et al., 2005).

Another physical pretreatment approach, uncatalyzed steam-explosion, also named autohydrolysis, is considered as one of the most cost-effective pretreatment methods and can be implemented in commercial scale. Generally speaking, biomass is heated under high pressure saturated steam to augment the efficiency of hemicellulose hydrolysis. Water displays acid-like properties at high temperature; together with other organic acids formed from acetyl or other functional groups released from biomass, they are able to hydrolyze biomass. Uncatalyzed steam-explosion pretreatment can yield higher rates of removal and hydrolysis of hemicellulose (Zheng et al., 2009). Similar to uncatalyzed steam-explosion, catalyzed steam-explosion also is a cost effective pretreatment process and has even better performance in removing hemicellulose and improving subsequent enzymatic hydrolysis. Generally, SO_2 is utilized as catalyst in this pretreatment which facilitates the production of more xylose and digestible components for fermentation. Moreover, simpler reactors were needed in SO_2-catalyzed steam explosion with less costly conditions (Tengborg et al., 1998). In the process of Steam-Explosion pretreatment, high-pressure, high-temperature steam is introduced into a sealed chamber containing lignocellulosic material. Meanwhile, SO_2 is added into the steam as the catalyst because it is less corrosive than other catalysts, such as H_2SO_4. After certain retaining time, the pressure is released, causing the steam to expand

within the materials. After cooling, the lignocellulosic materials are further processed to ethanol (Mabee et al., 2006).

The mechanism of alkaline pretreatment is considered to be saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components. Additionally, acetyl and uronic acid in biomass structure which limit the accessibility of enzymes to cellulose are disrupted by bases (Chang and Holtzapple, 2000). Compared to acidic pretreatment which mainly removes hemicellulose, alkaline processes are more powerful to disrupt lignin structure and effectively cleave the covalent linkages between lignin and cellulose, such as ester bonds (Jin et al., 2006), and a significant amount of hemicellulose is solubilized through saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components(Zheng et al., 2009). Furthermore, after alkaline pretreatment, the swelling of biomass materials results in decrystallization of cellulose, which leads to more effective enzymatic hydrolysis. In alkaline pretreatments, a variety of bases have been used, including sodium hydroxide, calcium hydroxide (lime), potassium hydroxide, aqueous ammonia, ammonia hydroxide, and sodium hydroxide in combination with hydrogen peroxide (Zheng et al., 2009). Sodium hydroxide pretreatment resulted in the highest level of delignification (65.63% for 2% concentration (w/v) NaOH, 90 min, 121 ℃/15 psi) and cellulose conversion (60.8%) (Silverstein et al., 2007). Kim and Holtzapple (Kim and Holtzapple, 2005) reported that the optimal condition of calcium hydroxide pretreatment of corn stover was 55℃ for 4 weeks with aeration, 87.5% lignin was removed, and the subsequent saccharification yield of glucose and xylose were 91.3% and 51.8%, respectively. Peroxide pretreatment can improve the lignin solubilization and reduction of cellulose crystallinity, which leads to the enhancement of enzymatic conversion efficiency. Silverstein et al. (Silverstein et al., 2007)

reported that 10 g cotton stalks at a solid loading of 10% (w/v) were pretreated by 0.5%, 1% and 2% hydrogen peroxide under the experiment condition of 90 and 121℃ temperature with 15 psi pressure for 30, 60 and 90 min respectively. The results showed 6.22% (0.5%, 90 min, 90℃) to 32.01% (2%, 60 min, 121 ℃/15 psi) delignification and averaged between 8.18% (0.5%, 60 min, 90℃) and 30.56% (2%, 30 min, 121℃/15 psi) of xylan solubilization. Moreover, the percentage of glucan in the pretreated solids remaining as a result of H_2O_2 pretreatment ranged from 28.4% (1%, 30 min, 90℃) to 34.1% (2%, 90 min, 90℃).

- Soaking in Aqueous Ammonia (SAA) pretreatment method

Among all kinds of alkaline pretreatments, ammonia hydroxide pretreatment shows excellence performance in ammonia recycling through adding a recycling step at the end of the whole procedure because of the high volatility of ammonia and the low cost for evaporation (Kim and Lee, 2005). It is reported that SAA at low temperature can reserve the hemicellulose in solids through minimizing the interaction with hemicellulose. Meanwhile, an increase of surface area is achieved due to swelling in SAA pretreatment. More retained hemicellulose and cellulose, as a result, increase the amount of fermentable components and eventually the ethanol production is improved (Kim, Taylor, & Hicks, 2008). Aqueous ammonia, as a pretreatment reagent for agricultural residues, is considered environmentally friendly and inexpensive because it is easily recovered and would not lead to hazard residual problems. Furthermore, the delignification ability of SAA is relatively high compared with other conventional pretreatment approaches so that the yield of saccharification in the downstream process could be drastically increased. In particular, pretreatment of lignocellulosic resources by soaking with aqueous ammonia has been studied by some

research groups with corn stover, barley hull, and rice straw. Most research on SAA has

focused on pretreatment of corn stover (Kang et al., 2012).

Based on Kim and Lee's work, lignocellulosic feedstock (corn stover) was treated by SAA

pretreatment method over an extended time at room temperature. The results showed 74%

lignin removing, 100% glucan and 85% xylan retaining (Kim and Lee, 2007). Then

simultaneous saccharification and co-fermentation (SSCF) was used with *E.Coli* (KO11) and

a 77% ethanol yield was obtained based on both glucan and xylan conversion. Additionally,

Kim and Lee (2007) reported that modestly increase in temperature could effectively reduce

the treated time while retaining the corn stover biomass delignification level. The optimal

pretreatment conditions were verified after different combination tests: 15% aqueous

ammonia (wt.), 60℃, 1:6 solid and liquid ratio, and 12 hours. The pretreatment and SSCF

results showed modified method removed 62% lignin and retained 85% xylan and overall 77%

ethanol yield based on both glucan and xylan. In the study for barley hull SAA pretreatment,

the best experimental conditions were tested. Under 75℃ temperature, 15wt.% aqueous

ammonia and lignocellulosic biomass (1:12 of solid and liquid ratio) were treated for 48h, a

relatively high saccharification yield (83% for glucan and 63% for xylan) was obtained with

proper enzyme fermentation (Kim et al., 2008). The pretreatment of rapeseed straw by

soaking in aqueous ammonia was studied and the optimal condition for this specific biomass

SAA pretreatment was reported: 19.8% concentration ammonia water, 14.2 h of pretreatment

time, and a temperature of 69.0℃. With the above conditions, 60.7% glucose yield was
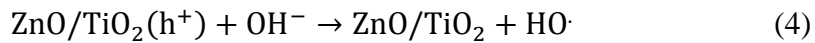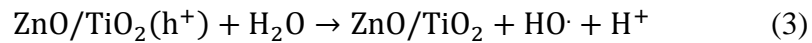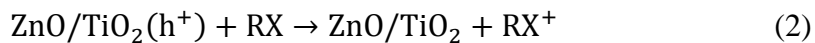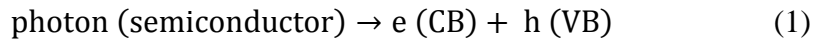
achieved (Kang et al., 2012).

1.1.4. Photocatalytic process for delignification

Although alkaline, especially soaking in aqueous ammonia, pretreatments display excellent hydrolytic ability; relatively high concentration of ammonia (15-30%) might still cause high water consumption for neutralization. In addition, ideal ammonia recovery shows remarkable environmental performance, however, more capital investment is needed for the ammonia recycling system, which would increase the total pretreatment cost. As a result, an enhanced approach is still needed to further reduce resource and capital consumption while retaining the environmentally friendly nature.

It was reported that hydroxyl radicals produced by the reaction of hydrogen peroxide with UV light or ferric ions (Fenton's reagent) can decompose lignin into carbon dioxide and hydrocarbon gases such as methane and ethane (Kobayakawa et al., 1989). Recent studies show the combination of semiconductor photocatalysts and UV light is effective in lignin degradation process based on the same reaction mechanism. Moreover, photocatalytic process is also effective in decoloration and bleaching Kraft mill effluent. Photocatalytic process has been developed for the treatment of waste water and polluted air mainly focused on removing lignin at first (Tanaka, Calanag, & Hisanaga, 1999).

The photocatalytic reactions are initiated by electrons and holes photogenerated in semiconductor particles (Serpone et al., 1986). The photon energy should be equal or higher than the band gap of the specified semiconductors. For TiO_2 and ZnO (the photocatalysts used in this study), the band gaps are 3.23eV and 3.17eV, respectively. The suitable wavelength regions are both wider than other photochemical reactions. Generally, photocatalysts having large band gaps have strong photocatalytic activities. Therefore, it is possible to enhance the production efficiency with those two semiconductors and to use solar

energy in the future after optimizing the properties of the photocatalysts. The irradiation

promotes the electrons from valence band (VB) to the conduction band (CB) of the

semiconductor. Then electrons (-) in the CB can reduce oxygen and the subsequent

superoxide anion can further improve the oxidative delignification process. While holes (+)

in the VB oxidize electron-donating substances directly or react with surface hydroxyl groups

(OH−) and adsorbed H_2O to form hydroxyl radicals. It was reported that high rate of

hydroxyl radicals have high delignification ability (Kobayakawa et al., 1989). Meanwhile,

the electrons and holes can also recombine, producing either heat or luminescence (Ohnishi

et al., 1989). The equations for each step are shown in equations (1)-(7).

$$\text{photon (semiconductor)} \rightarrow \text{e (CB)} + \text{h (VB)} \qquad (1)$$

$$ZnO/TiO_2(h^+) + RX \rightarrow ZnO/TiO_2 + RX^+ \qquad (2)$$

$$ZnO/TiO_2(h^+) + H_2O \rightarrow ZnO/TiO_2 + HO^{\cdot} + H^+ \qquad (3)$$

$$ZnO/TiO_2(h^+) + OH^- \rightarrow ZnO/TiO_2 + HO^{\cdot} \qquad (4)$$

$$ZnO/TiO_2(e^-) + O_2 \rightarrow ZnO/TiO_2 + O_2^{\cdot -} \qquad (5)$$

$$O_2^{\cdot -} \rightarrow H_2O_2 \qquad (6)$$

$$H_2O_2 + e^- \rightarrow OH^- + OH^{\cdot} \qquad (7)$$

Plenty of evidences confirm that semiconductor photocatalysts have high delignification

ability (Tanaka et al., 1999). However, the efficiency of photocatalysts along is not sufficient

for effective biomass pretreatment. Recent studies showed various supplementary materials

and modified conditions are needed to obtain a better delignification result: (1) Alkaline

solution effect: when the pH of the solution is high, the oxidation efficiency by the

photocatalytic reaction is enhanced. It is well-known that the energy levels of oxide

semiconductors move upward to the negative, by -59 mV/pH, which means that the reducing capability of electrons in the CB becomes stronger in alkaline solutions than that in neutral solutions (Ohnishi et al., 1989). (2) Noble metals effect*:* the photocatalytic activity of TiO_2 can be markedly increased by loading with noble metals, such as Pt, Au and Ag because the electrocatalytic effect of metals is able to accelerate the oxygen reduction. In this case, noble metal ions play the role as well as oxygen for removing electron (CB) and maintaining the photovatalyst band bending in the working stage. Without these electron acceptors, the bands will be soon flattened and most of the photogenerated electrons and holes will be lost by their recombination, and therefore the delignification efficiency will be decreased (Uchihara et al., 1989). (3) Heat effect: Heat-treating TiO_2 powder in a hydrogen stream could improve the oxidation ability of the photocatalyst, either. It is indicated that donor levels are formed in high density by the partial reduction of TiO_2 with such a treatment. As a result, the electron density in the conduction band is increased and the rate of electron transfer across the interface is improved (Ohnishi et al., 1989).

More and more researchers have paid attention to utilizing photocatalysis mechanism for improving the ability of delignification in processing lignocellulosic feedstock. There is no doubt that the photocatalytic pretreatment for biomass would reduce a large amount of chemical use compared to conventional pretreatment methods. Furthermore, with the help of photocatalysts, only a relatively mild condition is needed to obtain the same delignification efficiency, which could significantly decrease the energy consumption. Niu et al (Niu et al., 2009) tested the alkali assisted photocatalyst pretreatment effect for rice straw biomass. 2g/L nano-TiO_2 and 3% NaOH were added into grounded rice straw under a UV light source. 22h total reaction time and 1 h irradiation time were operated during the pretreatment process.

Cellulose composition was increased up to 71.5% and lignin was removed up to 18.5%. From Niu's result, it can be found that alkali treatment played an important role in assisting photocatalyst for delignification of rice straw. However, sodium hydroxide pretreatment would lead to environmental problems because sodium hydroxide is relatively difficult to be recovered or removed in the downstream process of biomass-ethanol fermentation. In this study, a combination of photocatalysis reaction and aqueous ammonia soaking pretreatment is investigated for obtaining better delignification efficiency and reducing chemicals loading. Most current studies have focused on Degussa P25 TiO_2 based photocatalysts in deforming organic compounds. Degussa P25, a commercial TiO_2 produced by Evonik Company, is composed by two different structures titanium dioxide: rutile and anatase, and the ratio between the two structures is 4:1 (Janus and Morawski, 2007). The average sized of the tow particles are 85 and 25 nm, respectively. It was discovered that the photocatalytic oxidation is not so efficient as the P25 when used pure rutile and anatase powders in treatment of naphthalene (Ohno et al., 2001). Therefore, most researchers preferred mixed type titanium dioxide P25, rather than pure ones during photocatalysis tests.

1.1.5. Raman Spectroscopy

Raman scattering and infrared absorption are the basic mechanisms for modern vibrational spectroscopies technologies. They are widely utilized to study chemical structures and molecular motions of different compounds, such as vibrational, rotational, and other low-frequency modes. Additionally, they are also widely used to identify substances with the characteristic spectral patterns.

Infrared absorption is usually aroused by a change in dipole, namely asymmetric vibrations. In contrast, Raman scattering is led by a change of the polarizability of the electron cloud

around a molecule. Symmetric vibrations could cause the biggest changes of polarizability and therefore give the greatest intense Raman scattering. Actually, infrared absorption and Raman scattering are complementary in understanding the vibrational structure of a molecule (Smith and Dent., 2005). In identification functional structures in lignocellulosic biomass, Raman spectroscopy is superior to IR spectroscopy (Bogdan, 2011). The optical heterogeneities of lignocellulosic materials generally have dimensions in the same range as the wavelengths of the infrared absorption in the region of the fundamental vibrations. Therefore, the Rayleigh scattering of the incident infrared beam can be significant and difficult to discriminate. Additionally, OH structure and absorbed water, which have high permanent dipole moment, in treated biomass are dominant and probably overshadow most functional bands in the infrared spectra, While H_2O is a very weak Raman scatter. Compared to traditional crystallographic methods, spectroscopic methods, such as solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy and Raman spectroscopy, can give a new insight and sometimes even better interpretation of the characterization of the aggregated states of cellulose. The key advantage of Raman spectroscopy is its sensitivity to the skeletal vibrations of the cellulose molecule, with the mode of packing in the lattice having only secondary effects. This sensitivity is a consequence of the fact that most of the skeletal bonds are C-C bonds and C-O bonds, both of which have relatively high polarizabilities and hence high Raman scattering coefficients (Bogdan, 2011).

However, until recently, Raman spectroscopy has not been widely utilized to study lignin and lignincellulosics materials because the high interruption of laser-induced fluorescence (LIF) always leads to relatively poor quality of the spectra. Moreover, high cost of instrumentation and lack of understanding of the molecular mechanism of Raman spectroscopic

characteristics of biomass also obstruct users pay more attention to this technique (Agarwal and Atalla, 1986; Atalla and Agarwal, 1985). Even though several methods were used to resolve the problem, the S/N ratios obtained were not good enough to detect the functional groups in both lignin and carbohydrate except for a few strong Raman bands. In 1986, a FT-Raman instrument based on NIR excitation (1064 nm) and a Michaelson interferometer was developed and this instrument significantly decreased the obstacle of fluorescence by excitation at a near IR wavelength. Optical microscope can be selectively installed in the conventional Raman spectrometer so that a spatially resolved manner for samples investigation could be realized. For lignincellulosics materials, which have a high degree of heterogeneity, this setup is particularly advantageous to exhibit both lateral and axial resolution as well as the ability to reveal the ultrastructure of the studied materials (Hirschfeld and Chase, 1986).

1.1.6.   Raman band assignment for cellulose, hemicellulose and lignin

Raman intensity can be affected by several factors. In addition to debugging the functions of the Raman instrument and the concentration of a component in a sample, effects and mechanisms like π-conjugation, resonance/pre-resonance Raman, and surface-enhanced Raman are worth of noticing, and can be applied in analyzing different Raman spectra for all kinds of materials (Agarwal and Atalla, 2000). For instance, when the Raman spectrum of black spruce was obtained using the 514.5 nm excitation, as much as 86% of the intensity of the 1595 cm^{-1} band of lignin could be assigned to conjugation and pre-resonance Raman effects (Bond, 1991). In consequence, a relatively complete bands assignment for lignocellulosic biomass based on multiple Raman techniques (i.e., NIR-Raman, FT-Raman) has become available. It should be noticed that different techniques might show some slight

differences for different functional peaks, in both wavenumbers and intensities. A summary of Raman bands that are of importance in lignocellulosic biomass analysis is listed below based on a comprehensive review of previous literatures.

Cellulose:

Cellulose bands are sharp and intense because cellulose is a highly ordered and crystalline polymer. There are several coupled modes below 925 cm^{-1} involving deformation and torsional vibrations are present (vibrations of C-C-C, O-C-O, C-O-C, C-O-H, and aromatic ring deformation modes).

300-550 cm^{-1}

- The ring torsions make significant contributions in the range;

- Skeletal-bending modes, methane bending and skeletal stretching can be associated in the range.

400-700 cm^{-1}

- Heavy atom bending involving both C-O (i.e., CCC, COC, OCC, OCO) and ring bending modes are the major vibration types;

- Some ring stretching coordinates still make minor contributions (O-H out of plane bending motions may make minor contributions);

- C-O stretches and external bending modes of the methylene group may contribute as well (Bogdan, 2011).

896 cm^{-1}

- The peak is assigned to HCC and HCO bending at C6 position of the carbohydrates.

950-1150 cm^{-1}

- There exists significant contributions from ring bond stretching;

- The skeletal structures of cellulose molecules, C-O and C-C stretching motions, also are assigned in this range showing high polarizabilities.

1098 cm^{-1}

- Cellulose orientation is mostly perpendicular to the transverse plane and the 1098 cm^{-1} band intensities are similar (or a little decrease in perpendicular) in both orientations (Agarwal, 2006);

- The band at 1098 cm^{-1} has been identified with one of the polarization-and orientation-sensitive skeletal modes of the chain (Agarwal and Atalla, 1986).

1200-1540 cm^{-1}

- The peaks are assigned to modes involving considerable coupling between methane bending, methylene rocking and wagging, and C-O-H in plane bending motions;

- The structures could show the property of angle bending coordinates involving one bond to a hydrogen atom and the other to a heavy atom;

- Some small contributions from the skeletal stretching modes were also present in this range (Bogdan, 2011).

1450 -1462 cm^{-1}

- The H-C-H bending vibration is assigned in the range;

- The CH_2 vibration can also be considered as the scissors vibration (Bogdan, 2011).

2886-2940 cm^{-1}

- The peak at 2890 cm^{-1} is assigned as the aliphatic C-H stretch residual carbohydrates in R_3C-H structures (The methane C-H stretches of cellulose molecules), which is a strong band;

- When the electric vector direction of the incident beam is changed from parallel to perpendicular with respect to the cell wall, the 2900 cm^{-1} band shows an increasing trend, which may be the result of some contribution to the 2900 cm^{-1} region from aliphatic C-H's in lignin, especially from –OCH$_3$ and –CH$_2$ groups (Agarwal and Atalla, 1986).

3100 and 3600 cm^{-1}

- H-bonding is shown in this range;

- The O-H stretching modes here reflect alignment of crystalline cellulosic domains with the chain axis in the plane of the cell-wall surface;

- These superimposed bands arise from intramolecularly hydrogen-bonded –OH groups which are parallel to the chain;

- Inherently broad in a substance as highly hydrogen bonded as cellulose;

- OH stretching is not detected because their Raman intensity is weak. Polar bonds are not easily detected in Raman Spectroscopy because they have poor scattering cross-sections.

Hemicelluloses:

The main hemicellulose components analyzed in Raman measurement are glucomannan and xylan. Compared with those of cellulose, glucomannan bands are broader and weaker because in the natural state, it is not amenable to crystallization, while xylan bands are significantly narrower than those of glucomannan. Sharper and better resoved peaks in a spectrum indicate that a polymer is crystalline and/or ordered. Therefore, xylan is likely to be more crystalline and ordered (Agarwal and Ralph, 1997).

2918 cm^{-1}

- The hemicellulose peak shown here is not overlap with any of the cellulose's Raman features and is quite intense.

494 cm^{-1}

- Sharp Raman band;

- Hardly detected in kraft pulp and other samples' spectra because of two reasons: lack of significant contribution could be xylan's low concentration and it existed in the noncrystalline state (Agarwal and Ralph, 1997).

Raman spectral sigatures of lignin

384 and 491 cm^{-1}

- Very weak peak;

- The lignin peak here possibly arises as a result of either the artifacts of the subtraction process or the intermolecular interactions between lignin and carbohydrates (Agarwal and Ralph, 1997).

785 and 986 cm^{-1}

- The peaks at 785 and 986 cm^{-1} are due to the skeletal deformation of aromatic rings.

Bands in 1000-1500 cm^{-1} region are usually hard to be assigned to one specific mode due to the serried coupled vibrations of many similar modes.

1163-1181 cm^{-1}

- the peaks here are assigned to C-O structure, typical for *p*-hydroxyphenyl, guaiacyl and syringal lignins (Iskalieva et al., 2012).

1205 cm^{-1}

- Weak peak;

- It is likely to be associated with phenolic units.

1265 cm^{-1}

- The band at 1265 cm^{-1} is contributed from aryl-O (aryl-OH and aryl-CH$_3$);

- Its intensity is related to guaiacyl/syringyl ring breathing.

1334/1331 cm^{-1}

- The peak here is contributed to aliphatic O-H bending;

- This feature is stronger in syringyl-rich ring breathing with C-O stretching groups in aspen lignin.

1363/1367 cm^{-1}

- C-H bending in R$_3$C-H structures is assigned to this peak.

1392/1395 cm^{-1}

- Phenolic O-H bending shoulder peak is located here.

1453/1455 cm^{-1}

- The peak is contributed to CH$_3$ deformation and CH, scissoring modes and guaiacyl/syringyl-ring vibration.

1508/1501 cm^{-1}

- The peak indicates the aromatic ring stretch modes;

- It is strong in IR spectrum but not in Raman.

1598-1600 cm^{-1}

- The peak here is attributed to aromatic ring symmetric stretch modes of lignin;

- Usually it is strong band;

- Lignin orientation in the S2 transverse section produced less than a 20% change in Raman band intensity when the cell wall was parallel to the laser electric vector as opposed to perpendicular (Agarwal, 2006).

1620 cm^{-1}

- The should peak at 1620 cm^{-1} band is assigned to aldehydrade α,or γ carbonyl group(Agarwal and Atalla, 2000).

1660-1690 cm^{-1}

- The peak is assigned to the ethylenic C=C and γ-C=O bond stretches in coniferyl alcohol/ sinapyl alcohol and coniferaldehyde/sinapaldehyde units of lignin;

- Coupling of C=C and C=O stretch models sometimes makes the assignment of the Raman bands difficult;

- It is also attributed to the p-quinones, which play a major role in determining pulp brightness (Adapa et al., 2009);

- The p-quinones entities in different biomass are likely to have different molecular structures and different intermolecular interactions. So the broadness of Raman feature can be explained.

2800-3100 cm^{-1}

- Aromatic and aliphatic C-H stretches are assigned here;

- Medium feature of aromatic C-H stretches are shown at 3075 cm^{-1};

- The peak at 2845/2947 cm^{-1} arises from the symmetric C-H stretch in the O-CH$_3$ group;

- 2940/2939 cm^{-1}, and 2008/2003 cm^{-1} are due to the asymmetric C-H stretches in O-CH$_3$ groups;

- 2890/2893 cm^{-1} is due to the C-H stretch in R$_3$C-H structures.

Conclusion

Lignocellulosic biomass has become an interesting alternative in biofuels production processing due to its renewable nature. However, the strong and chemically-resistant - structures of the lignocellulosic biomass make it more difficult to be hydrolyzed into monosaccharides, before eventually being fermented to ethanol and other variety of biofuels. As a result, an effective pretreatment is truly important for the biological conversion of lignocellulosic biomass. SAA pretreatment has shown excellent performance recently because of the possibility to recycle ammonia, which leads to the reduction of the chemical loading. However, the relatively high ammonia concentration still requires a large amount of water and energy for neutralization and downstream steps. Semiconductor photocatalyst is promising in delignification and has been widely used in paper industrial processing for lignin degradation. Therefore, it is considered that combining the SAA and photocatalyst as the lignocellulosic biomass pretreatment would further enhance the delignification efficiency and then increase the final ethanol production. Due to the heterogeneous structure of lignocellulosic biomass, it is difficult to reveal the lignin decomposition mechanism in the molecular scale by the conventional composition analysis methods. In this study, Raman spectroscopic technique is being explored to monitor structural and compositional change of biomass during pretreatment processes in real time. By monitoring the changes in Raman bands associated with various functional groups in lignocellulosic biomass that are being altered during the pretreatment, the cleavage and alteration of lignin bonds that underline the delignification process are characterized, and the molecular mechanism for biomass pretreatment is rectified.

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Chapter 2 Enhancement of enzymatic hydrolysis and lignin removal of corn stover

using photocatalyst-assisted alkaline pretreatment

Abstract

A novel photocatalyst-assisted alkaline pretreatment was investigated to reduce the lignocellulosic materials biological resistance for enzymatic hydrolysis and fermentation. Photocatalyst (TiO_2 and ZnO were chosen) assisted ammonia method was used for corn stover treatment under such experimental condition: 60 °C batch reactor temperature; 1:20-1:50 solid and liquid ratios and periodic oxygen supplement. Ammonia pretreatment assisted by TiO_2-catalyzed photo-degradation removed 70 % of lignin under the optimum condition (12.5 % ammonium hydroxide, 60 °C, 24 h, solid:liquid=1:20, photocatalyst:biomass=1:10 with oxygen supply). The enzymatic digestibilities of the ammonia pretreated corn stover were 85 % for glucan and 75 % for xylan at 15 filter paper unit (FPU)/g-glucan of cellulase and 30 cellobiase unit (CBU)/g-glucan of β-glucosidase loadings, a 6.25% and 17.3% improvement over ammonia pretreatment alone for glucan and xylan, respectively. Photocatalysts-assisted sodium hydroxide method was used to investigate the effect of optimized mixing and UV penetration. UV/Vis measurement confirmed significant improvement in delignification, demonstrating the effect of UV penetration on photocatalysis reaction.

2.1. Introduction

Facing the global energy shortage, the efforts to find a feasible alternative from biomass based renewable energy (e.g., biofuels) have intensified over the past decade. According to the US Energy Independence and Security Act (EISA), the consumption of biofuels, such as biomass-based diesel, ethanol and advanced biofuels, needs to be increased by 100 times

within the next 10 years (Fishman et al., 2010). Animal and human waste, trees, shrubs, yard waste, wood products and agricultural residues are all potential feedstock for production of biofuels. Among various biomass sources, lignocellulosic materials, in particular agricultural residues, are among the most promising feedstock because of their abundance and relatively low cost (Zheng et al., 2009). Lignocellulosic biomass is mainly composed of cellulose, hemicellulose and lignin. Cellulose is encrusted with lignin and hemicellulose for protection. The molecular structure of Lignin is complex; it contains cross-linked polymers of phenolic monomers which are impermeable and chemically-resistant. Therefore, lignin is considered as one of the primary reasons of recalcitrance in biomass structure (Kuhad and Singh, 2007; Sun, Simmons, & Singh, 2011). In order to convert lignocellulosic biomass into ethanol or other biofuels with high efficiency through biological/chemical processes, it is necessary to pretreat the biomass for lignin removal or alteration before the enzymatic hydrolysis and fermentation are conducted (Kumar et al., 2009).

It is well known that lignin, as a major chemical oxygen demand (COD) component in pulp mill effluent, can be oxidatively decomposed by catalyzed photo-degradation. Photocatalytic degradation process is also effective in decoloration and bleaching of Kraft mill effluent (Tanaka, Calanag, & Hisanaga, 1999). Semiconductor-photocatalyzed degradation processes have also been developed for the treatment of waste water and polluted air since 1980s (Tantemsapya et al., 2004). In recent years, increasing efforts have been made to develop photocatalytic systems with the combination of photocatalysts and ultraviolet light for highly effective oxidation of aromatic compounds (Kansal, Singh, & Sud, 2008) and TiO_2 and ZnO were shown to be among the most effective photocatalysts in various photo-degradation processes (Chakrabarti et al., 2008; Peralta-Zamora et al., 1998).
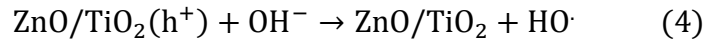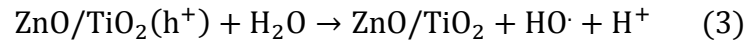
The photocatalytic reactions are initiated when holes (in valence band, VB) and electrons (in conducting band, CB) are photogenerated in the semiconductor particles such as TiO_2 and ZnO (1) (Ohnishi et al., 1989; Serpone et al., 1986) by photons with equal or higher energy than the band gap of the semiconductors. For TiO_2 and ZnO, the band gaps are 3.23 eV and 3.17 eV, respectively
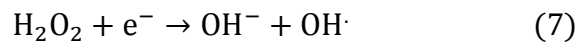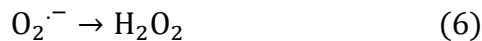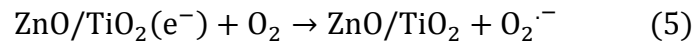
$$photon + \ (semiconductor) \rightarrow e \ (CB) + \ h \ (VB) \qquad (1)$$
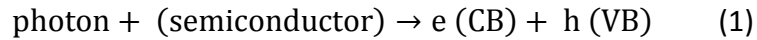
The holes (+) either oxidize the electron-donating substances (i.e., lignin) directly (2),

$$ZnO/TiO_2(h^+) + RX \rightarrow ZnO/TiO_2 + RX^+ \qquad (2)$$
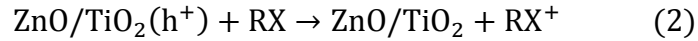
or react with surface hydroxyl groups (OH-) and/or adsorbed H_2O to form hydroxyl radicals (3), (4).

$$ZnO/TiO_2(h^+) + H_2O \rightarrow ZnO/TiO_2 + HO^\cdot + H^+ \qquad (3)$$

$$ZnO/TiO_2(h^+) + OH^- \rightarrow ZnO/TiO_2 + HO^\cdot \qquad (4)$$

The hydroxyl radicals then decompose lignin into carbon dioxide and hydrocarbon gases (Kobayakawa et al., 1989). The electrons (-) in the CB (1) can reduce oxygen and the superoxide anions formed are highly effective in various oxidative degradation processes (5)-(7) (Ohnishi et al., 1989).

$$ZnO/TiO_2(e^-) + O_2 \rightarrow ZnO/TiO_2 + O_2^{\cdot -} \qquad (5)$$

$$O_2^{\cdot -} \rightarrow H_2O_2 \qquad (6)$$

$$H_2O_2 + e^- \rightarrow OH^- + OH^\cdot \qquad (7)$$

However, it has been shown that the photocatalyzed degradation methods alone were not capable of reducing lignin incorporated in biomass effectively (Niu et al., 2009). Conventionally, lignin degradation is achieved through chemical pretreatment methods. Among these pretreatment methods, ammonia pretreatment shows excellence performance in sugar conversion with high selectivity for reaction with lignin over those with carbohydrates (Agbor et al., 2011). Ammonia is also easy to handle and recycle because of its high volatility and non-corrosive nature (Kim et al., 2003). Although ammonia pretreatment is considered as a promising method, excessive energy consumption and environmental pollution with ammonia use are still concerns need to be dealt with. It is desirable to find a way to improve the effectiveness of pretreatment without relying on increasing ammonia loading or reaction temperature. In this study, photocatalyst-assisted ammonia pretreatment was investigated to improve bioconversion of corn stover.

Herein, degradation or alteration of lignin using photocatalyst-assisted ammonia pretreatment to lower their affinity towards enzymes was the goal of this study. Two different photocatalysts, titanium dioxide (TiO_2) and zinc oxide (ZnO), were added into ammonia solution to pretreat corn stover. Ammonia concentration and solid-to-liquid loading were investigated as important control factors to optimize the subsequent sugar conversion yield by enzymatic hydrolysis of the pretreated biomass. The overall lignin mass balance throughout the pretreatment process was monitored by analyzing hydrolyzed lignin in the liquid pretreatment hydrolysate, and Raman spectroscopy was utilized to explore the structural changes in the biomass before/after the pretreatment.

2.2. Materials and Methods

2.2.1. Materials

Corn stover was collected from central Iowa in 2010, washed with deionized water and air-dried at ambient temperature. The corn stover was ground and screened to a nominal size of 9-35 mesh. The initial composition of the corn stover was 37.3 wt.% glucan, 22.5 wt.% xylan, 2.5 wt.% galactan, 3.3 wt.% arabinan, 17.2 wt.% lignin (acid insoluble + acid soluble), 4.8 wt.% protein, 1.5 wt.% sucrose, 4.6 wt.% ash, and 6.3 wt.% other extractives. Zinc oxide (Sigma-Aldrich, Cas #1314-13-2) and Titanium dioxide P25 (Evonik Degussa, Cas #13463-67-7) were used as photocatalysts.

Cellulase GC 220 (Lot #301-04232-162) was provided by Genencor, a Danisco Division. The average activity of cellulase (GC-220) was 45 filter paper unit (FPU)/ml. The β-glucosidase enzyme, Novozyme 188 (Novo Inc., Lot #11K1088), was purchased from Sigma-Aldrich (St. Louis, MO). Activity of Novozyme 188 was 750 cellobiase units (CBU)/ml.

2.2.2. Photocatalyst-assisted ammonia pretreatment

Corn stover was treated by aqueous ammonia solution with photodegradation under UV catalyzed by the photocatalysts. Different ratios of biomass to solution were applied from 1:20 to 1:50 (g/ml). The amount of photocatalyst (TiO_2 and ZnO) was 10 wt.% of biomass loading. Biomass and photocatlysts were loaded in screw-capped 250 ml bottles with 5% and 12.5 % of ammonium hydroxide. The reaction temperature was maintained at 60 °C by isotemp basic ceramic stirring hotplate (Fisher, Cat. #11-100-100SH) and reaction time was 24 hours. The UV light source was 100 W UV lamps (American ultraviolet company,

Lebanon, IN) with irradiation wavelength 380 nm. During the first 6 hours, oxygen was supplied to enhance the photocatalytic reactions.

In order to evaluate the effect of UV light penetration for the photocatalytic reaction, another experiment set up was investigated. All reactants were kept in 150 dia×20mmH corning PYREX Brand glass petri dishes; then the UV lamp and petri dishes were simultaneously put into incubator shaker for heating and mixing. Due to the evaporation limitation, 0.1% sodium hydroxide was used to substitute 5% ammonia in this experimental design. Other conditions and ratios were identical to the previous tests. Moreover, in order to test the effect of the duration of UV illumination during the pretreatment processes, 2, 6, 24 hours UV treatments were used. All samples were kept in incubator shaker for 24 hours for optimal alkaline delignification efficiency.

2.2.3. Enzymatic hydrolysis of pretreated biomass

The enzymatic digestibility of the corn stover samples was determined in duplicates according to the NREL Chemical Analysis and Testing Standard Procedure (NREL, 2008). For the enzymatic digestibility tests of pretreated solid residue, 15 FPU of GC 220 and 30 CBU of Novozyme 188 per gram of glucan supplemented were loaded into 250 ml screw-capped Erlenmeyer flasks. The initial glucan concentration was 1 % (w/v) based on 100 ml of total liquid and solid, and pH was adjusted to 4.8 by 0.05 M sodium citrate buffer solution. Prepared flasks were placed in the incubator shaker (New Brunswick Scientific, Edison, NJ) and agitated at 150 rpm at 50 °C. Samples were taken periodically (6, 12, 24, 48, 72, 96, and 120 h) and analyzed by HPLC to calculate the glucose and xylose contents.

The glucan and xylan digestibilities were calculated as follows:

$$\text{Glucan digestibility} = \frac{\text{Total released glucose (g)} \times 0.9}{\text{Initial glucan loading (g)}} \times 100$$

0.9 is the conversion factors of glucose to equivalent glucan.

$$\text{Xylan digestibility} = \frac{\text{Total released xylose (g)} \times 0.88}{\text{Initial xylan loading (g)}} \times 100$$

0.88 is the conversion factors of xylose to equivalent xylan.

2.2.4. Compositional analytical methods

Carbohydrates and lignin contents in the untreated and pretreated corn stover were analyzed

following the NREL LAP (NREL, 2008). Each sample was analyzed in duplicates.

Carbohydrates were determined by HPLC with a Bio-Rad Aminex HPX-87P column and a

refractive index detector (Varian 356-LC, Varian, Inc., CA).

Dissolved lignin in the pretreated liquid hydrolysate was monitored using LAMBDA™ 750

UV/Vis/NIR spectrophotometer (PerkinElmer, Shelton, CT). The absorption at 290 nm was

used to characterize the soluble lignin (Tanaka et al., 1999). Alkali lignin (Sigma-Aldrich, St.

Louis, MO) was dissolved in the same concentration of ammonia (as used in the pretreatment)

and used as standard for the generation of the calibration curve. During the pretreatment

experiments, 1 ml of the reactant was periodically withdrawn from the bulk to monitor its

soluble lignin content: after centrifugation, the supernatant was subjected to the UV-Vis

measurement.

2.3. Results and discussion

2.3.1. Composition analysis of photocatalyst-assisted ammonia pretreatment

The composition analysis results for the corn stover photocatalytic pretreatment were shown

in table 1, 2 and 3 with different ammonia concentration and solid & liquid ratios. The

standard deviation calculation results of all the measurement are less than 0.2 (wt. %). For table 1, the ammonia concentration was 5% and the solid & liquid ratio was 1:50. Aqueous ammonia alone method has already shown the excellent performance in lignocellulosic materials pretreatment (Kim et al., 2011). The individual ammonia treatment removed 57% lignin and preserved 94% glucan as well as 80 % xylan in this study. The delignification ability of photocatalysts treatment without alkaline promotion was relatively low: 5% and 10% for titanium dioxide and zinc oxide, respectively. However, a certain improvement for delignification was shown in the combination of photocatalysts (TiO_2 and ZnO) and alkaline compared to ammonia alone (see table 1). Furthermore, although the effect of each photocatalyst on lignin removal was similar, more carbohydrates were preserved in the titanium dioxide treatment.

Table 1 Composition analysis in 5% ammonia photocatalyst treatments

Components	Untreated	TiO_2	ZnO	NH_4OH	$NH_4OH+TiO_2$	$NH_4OH+ZnO$	Standard Deviation
	Comp. Wt.%	Comp. Wt.%	Comp. Wt.%	Comp. Wt.%	Comp. Wt.%	Comp. Wt.%	Comp. Wt.%
Glucan	37.3	35.7	35.4	35.1	34.7	34.3	<0.2
Xylan	28.3	26	25.2	22.8	21.9	20.8	<0.2
Lignin	17.2	16.4	15.5	7.3	6.9	6.8	<0.2

Table 2 revealed the composition analysis results focused on TiO_2 assisted 5% ammonia treatments with different solid and liquid ratios (1:20, 1:30, 1:50). An assumption was considered that lower liquid loading might result in higher concentration of free hydroxyl radicals generated from the photoreaction and therefore more lignin component would be removed from the corn stover biomass. In the study, three different solid and liquid ratios were tested. However, the lignin composition results did not support this assumption. Therefore, other consideration for improving the photocatalytic pretreatment efficiency should be explored.

Table 2 Composition analysis in different solid & liquid ratios photocatalyst treatments

Components	Untreated	1:50	1:30	1:20	Standard Deviation
	Comp. Wt.%	Comp. Wt.%	Comp. Wt.%	Comp. Wt.%	Comp. Wt.%
Glucan	37.3	34.7	35.6	35	<0.2
Xylan	28.3	21.9	20.8	19.3	<0.2
Lignin	17.2	6.9	6.5	6.5	<0.2

For table 3, the ammonia concentration was increased to 12.5 % and the solid & liquid ratio was 1:20 in order to obtain the higher hydroxyl groups concentration and thus the better delignification efficiency. The delignification abilities were 65% and 70% for ammonia alone and photocatalyst assisted ammonia treatments, respectively. So that improvement of lignin removal by enhancing alkaline concentration could be realized in practice. Additionally, similar to the above composition results, the treatment of TiO_2 photocatalyst displayed the excellent performance in preserving more glucan and xylan in the solid part compared to the result of ZnO treatment.

Table 3 Composition analysis in 12.5% ammonia photocatalyst retreatment

Components	Untreated	NH_4OH	$NH_4OH+TiO_2$	$NH_4OH+ZnO$	Standard Deviation
	Comp. Wt.%	Comp. Wt.%	Comp. Wt.%	Comp. Wt.%	Comp. Wt.%
Glucan	37.3	35.3	35.7	32.9	<0.2
Xylan	28.3	22	19.1	17.8	<0.2
Lignin	17.2	5.9	5.2	5.2	<0.2

Figure 1 showed lignin concentration profile in the liquid hydrolysates during photocatalyst-assisted ammonia pretreatment by the measurement of UV/Vis spectrometer. It clearly showed that the delignification performance was enhanced through the addition of photocatalyst into alkaline treatments. Furthermore, Compared to TiO_2 in ammonia, ZnO and ammonia combination generated more soluble lignin from biomass from 6 hours to 24 hours; however, soluble lignin recovery by the photocatalyst-assisted process was almost identical

after 24 hours; It should be noticed that photocatalyst-assisted processes showed higher hydrolysis rates within the first 12 hours than that of ammonia-alone process, which could have major implication from energy consumption standpoint: an acceptable "pretreatment level" could be reached in shorter processing time with less energy needed.
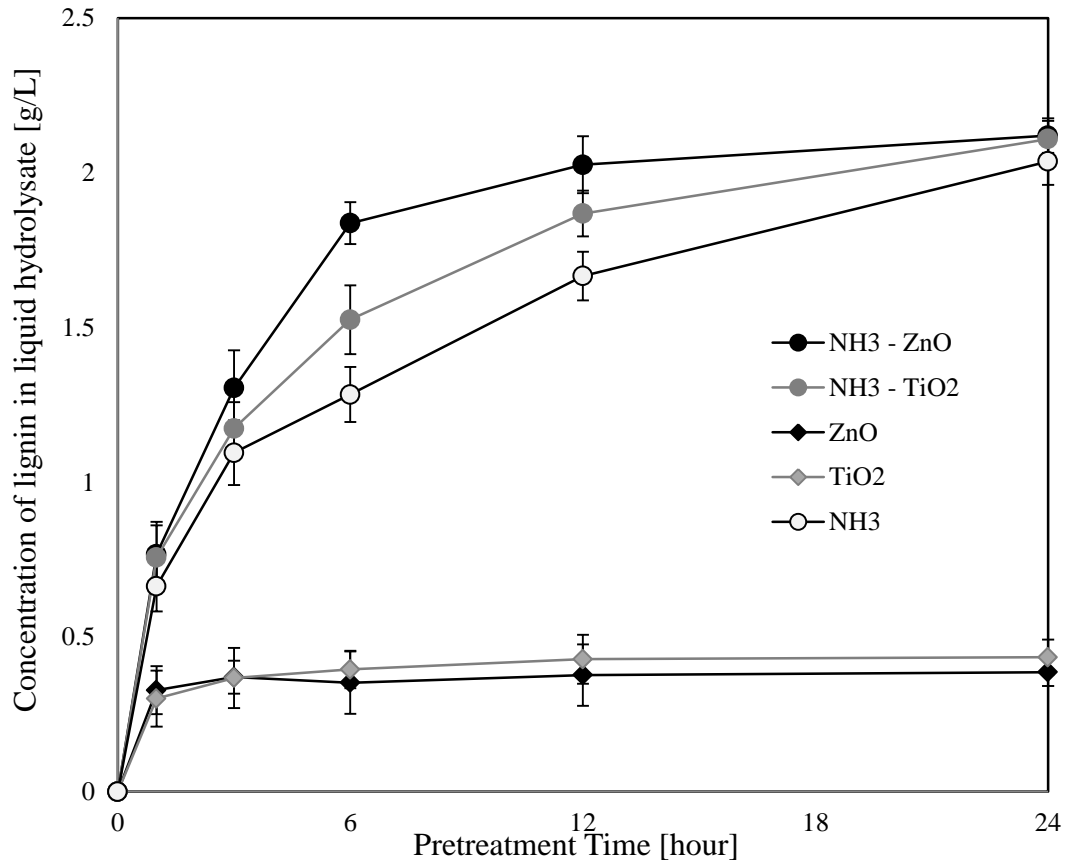

Figure 1 Dissolved lignin from corn stover in the liquid product during ammonia-photocatalyst pretreatment

2.3.2. Effect of photocatalyst-assisted ammonia pretreatment on enzymatic digestibility

Each sample in the above composition analysis was investigated in enzymatic digestion, however, only the highest digestibility results were shown in this discussion. Subsequent enzymatic digestibility tests were conducted with ammonia-TiO_2 treated corn stover using 15

FPU/g-glucan of cellulase (GC-220) and 30 CBU/g-glucan of β-glucosidase enzymes (Novozyme 188). The pretreatment conditions were as follows: 12.5 % ammonia loading, solid:liquid=1:20, photocatalyst (TiO$_2$):biomass (corn stover)=1:10 (w.t.), temperature: 60 °C, 24 h treatment with oxygen supply and UV light irradiation. Glucan and xylan digestibilities of aforementioned Titanium Dioxide assisted ammonia treated biomass are summarized in Figure 2.

TiO$_2$-assisted UV treatment alone showed no improvement over enzymatic digestibility; while TiO$_2$-assisted UV treatment in conjunction with ammonia improved the digestibility of the corn stove biomass from 18% for glucan and 8% for xylan to 85% and 75%, respectively. Compared to the treatment with same ammonia loading alone, an improvement of 5 % for glucan digestibility and 11 % for xylan digestibility were observed with the TiO$_2$-assisted UV treatment.

The average standard deviation of 2.8 % in the different pretreatment methods firmly demonstrated that the enhancement of delignification was relatively effective between pretreatments with and without photocatlyst (Titanium Dioxide) for both glucan and xylan digestibility.

Previous study revealed that more retained hemicellulose could be obtained in the Soaking in Aqueous Ammonia (SAA) which effectively increased the amount of fermentable components and eventually the ethanol production (Kim, Taylor, & Hicks, 2008). However, the results for photocatlyst assisted ammonia pretreatments showed the better hemicellulose preservation and hydrolysis ability compared to ammonia alone pretreatment even though the whole digestibility improvement by photocatalyst pretreatment was not as significant as we have expected.

(a) Glucan digestibility
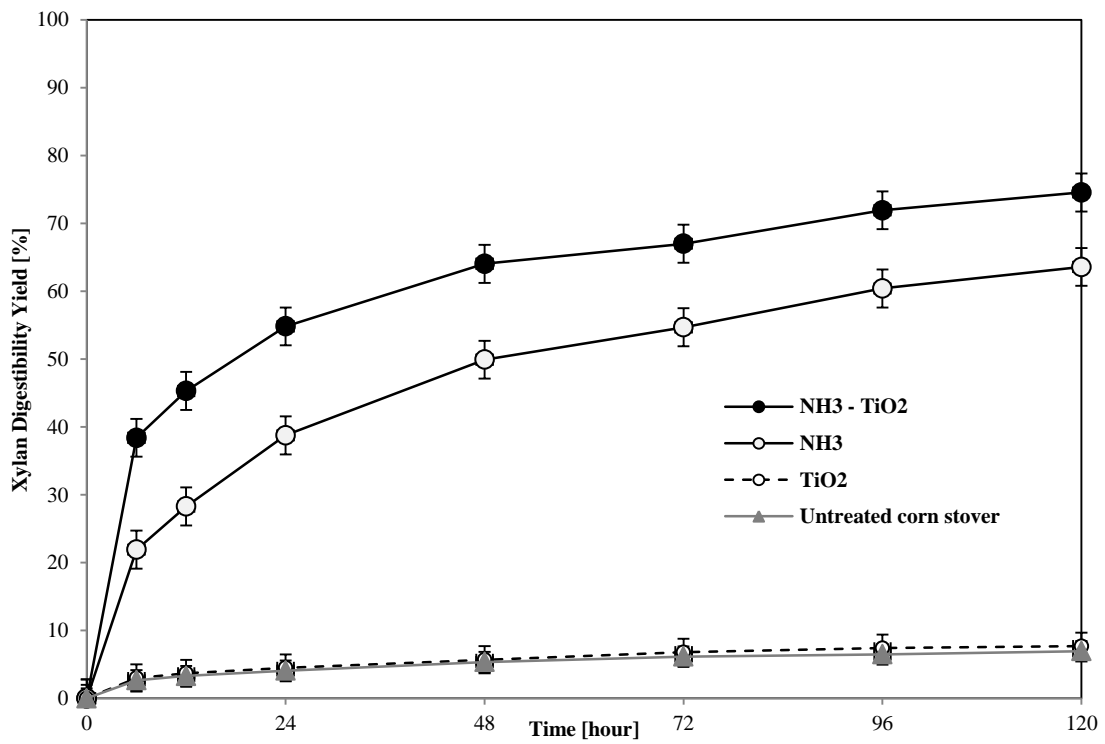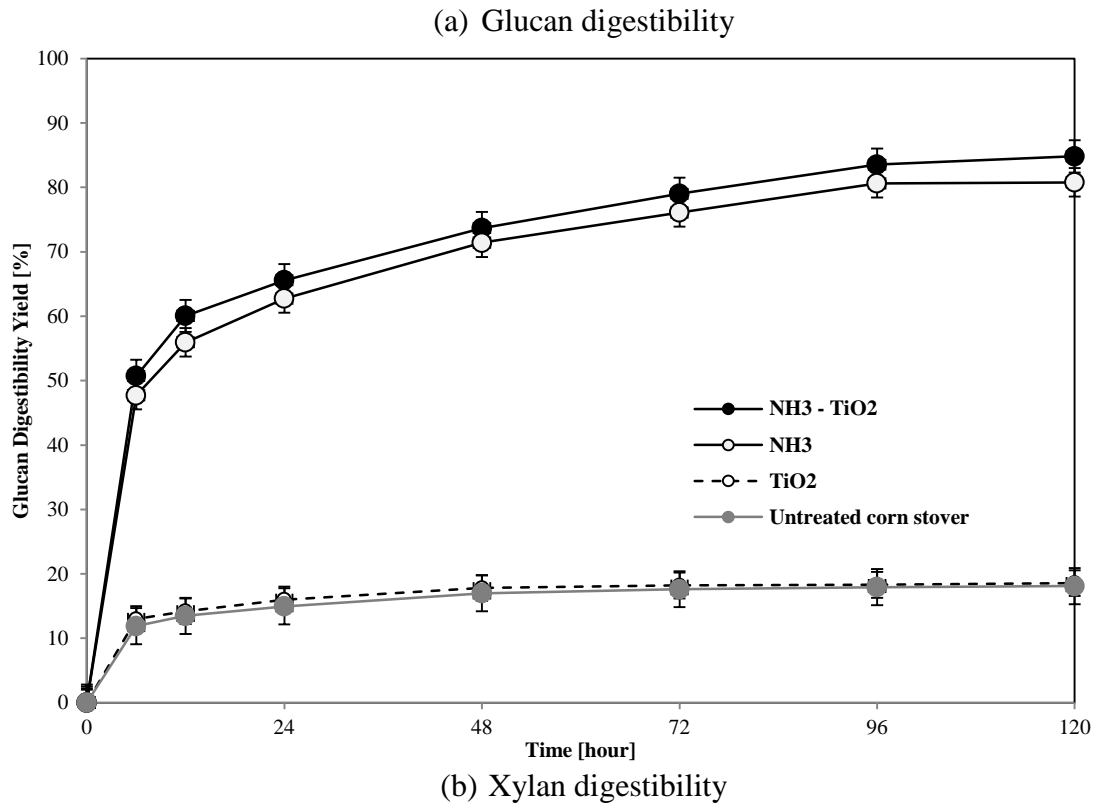

(b) Xylan digestibility

Figure 2 Enzymatic digestibility of ammonia-photocatalyst treated corn stover

2.3.3. Analysis of the effect of penetration efficiency of UV light on biomass

pretreatment

Based on the previous results in our experiment, no significant difference of delignification

effect was found between alkaline and photocatalysic-aided alkaline pretreatments for corn

stover particles. It should be noted that the activity of the photocatalysts is affected by its

own properties, such as particle size and concentration of impurities, and the experimental

conditions. In our experimental design, magnetic stirring hot plate and water bath were used

for heating and mixing of photocatalysts with the reactants. And reactants were loaded in
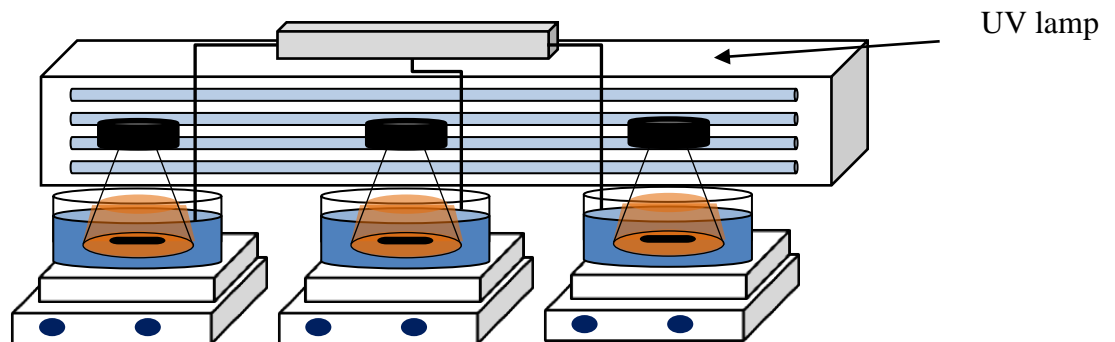
screw-capped conical flask (see figure 3).

UV lamp

Figure 3 Old experimental setup of photocatalyst-assisted ammonia pretreatment
However, the relatively thick bottle wall and the presence of surrounding water significantly

reduced the UV lamp light power delivered into the reaction system and the penetration

depth of the UV light, which might have severely reduced the efficiency and the

effectiveness of the photo-degradation pretreatment. In order to investigate the relationship

between the delignification result and the UV light penetration depth, an alternative

experimental set up was implemented (see figure 4). Additionally, in order to test the periodic

change during the photocatalysis pretreatment, UV/Vis spectra were measured in different

time points to determine the soluble lignin content in the reaction system throughout the pretreatment process.
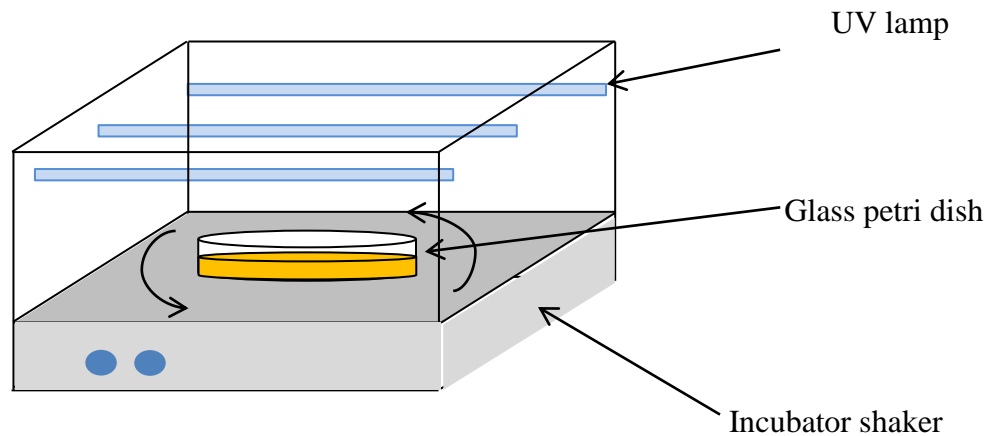

Figure 4 Alternative experimental set up for photocatalyst assisted sodium hydroxide treatments

In the previous test for photocatalyst-aided ammonium pretreatment with screw capped bottle reactor, constant increase in soluble lignin was observed in the reaction system. Figure 5 (a) showed a control test with sodium hydroxide and TiO_2 in the previous set up which also showed the increasing trend of soluble lignin. However, under the new experimental set up, it was observed that a decrease of soluble lignin appeared in the liquid lignin test. Previous study on photocatalyzed degradation of pure soluble lignin has proved that effective UV penetration and longer illumination of UV light was able to decompose lignin in liquid gradually (Tanaka et al., 1999). As a result, it was hypothesized that improving the UV penetration would not only promote the delignification process for corn stover but also lead to a degradation of soluble lignin in the reaction system, as shown in Figure 5(b). Comparing to the alkaline treatment along, the soluble lignin content in the reaction system was significantly reduced due to the photodegradation as the duration of UV treatment was increased.
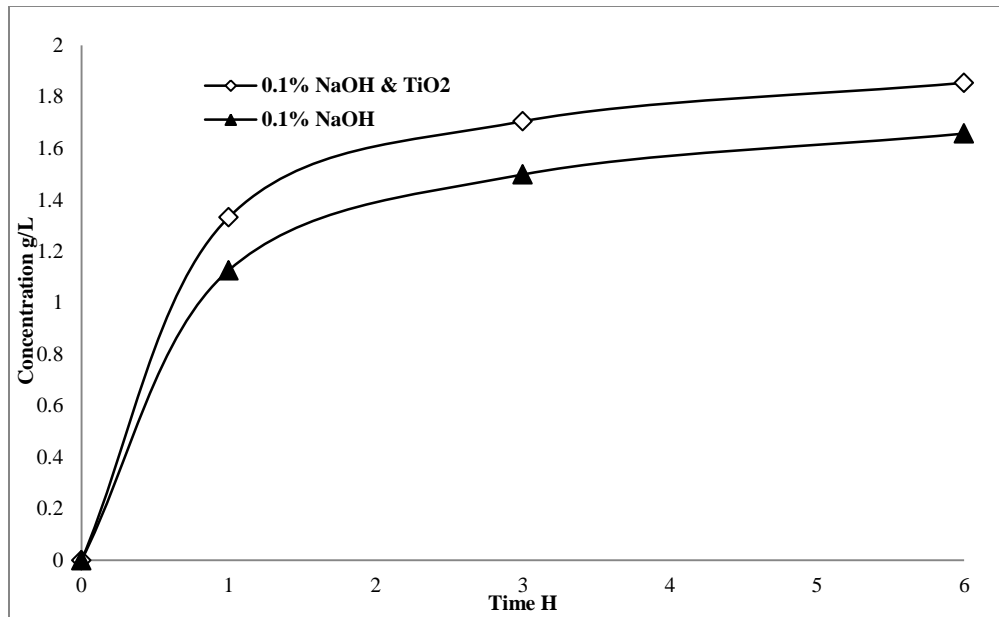
(a)
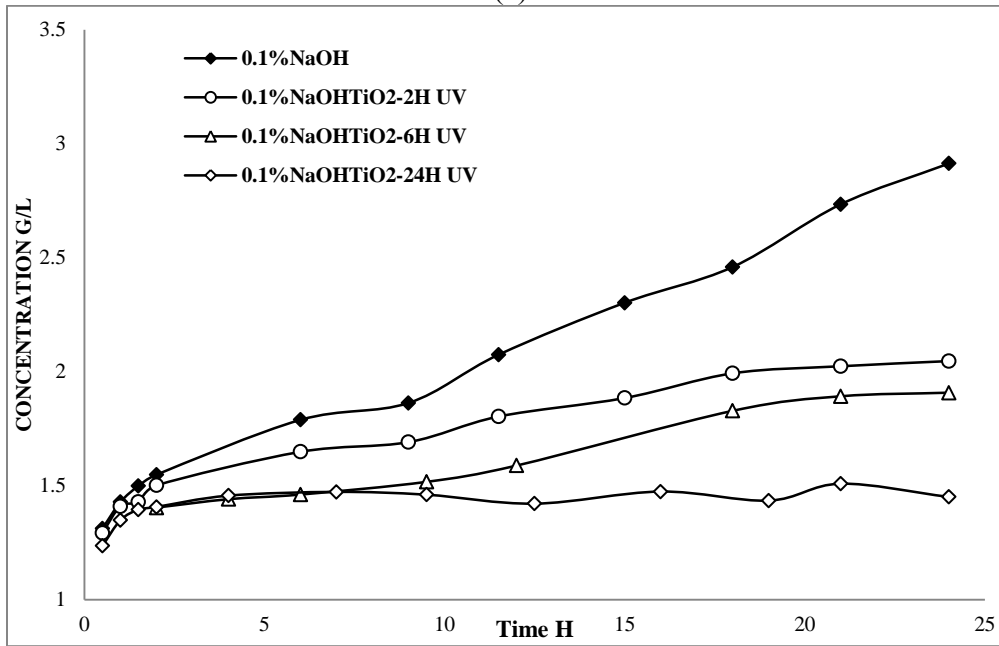
(b)

Figure 5 Soluble lignin from corn stover in the liquid product for sodium hydroxide
photocatalyst pretreatments in different experimental designs

Conclusion

Photocatalyst-assisted UV treatment could further improve the effectiveness of ammonia pretreatment to remove lignin from biomass. As a result, higher enzymatic digestibility and fermentability could be obtained.

With ammonia-TiO_2-treated corn stover under the optimal conditions (12.5 % ammonium hydroxide, 60 °C, 24 h, solid:liquid=1:20, photocatalyst:biomass=1:10, oxygen supply), 85 % of glucan digestibility and 75 % of xylan digestibility were obtained using 15 FPU/g-glucan of cellulase and 30 CBU/g-glucan of β-glucosidase. The Photocatalyst-assisted UV treatment in conjunction with ammonia soaking can shorten the time needed for pretreatment, and potentially it can lead to significant reduction in energy consumption of pretreatment. Besides, with new generation of photocatalysts that are more effective under visible light, sun light can eventually be harvested to improve the efficiency and effectiveness of biomass pretreatment processes.

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Chapter 3 Raman Spectroscopic characterization of Photonanocatalyst Aided Alkaline Pretreated Corn Stover biomass

Abstract

The most expensive and principal step in the process of converting cellulosic biomass into biorenewables and biofuel is the pretreatment during which lignin and cellulose crystalline structure is broken down. It is also the step that needs the most improvement in terms of efficiency and cost reduction. In this study, TiO_2 nanoparticles were utilized as photocatalyst in addition to alkaline (ammonia and sodium hydrate) treatment of corn stover biomass to improve delignification ability and reduce the energy and chemical loadings to make the process more environmentally friendly. Raman Spectroscopic imaging, as an accurate and rapid chemical characterization method, was utilized to investigate the molecular compositional and structural changes occurring during the pretreatment process in the corn stover biomass qualitatively and quantitatively to illustrate the lignin hydrolysis mechanism. Lignin characteristic peaks at 1,600, 1,620 and 1,690 cm^{-1} as well as cellulose characteristic peaks at 1,059 and 2,888 cm^{-1} were identified through Raman Spectroscopic measurement. real-time quantitative analysis of these functional peaks revealed the molecular structural changes during delignification, potentially may lead to optimization of the processing parameters to reduce the processing cost in the future.

3.1. Introduction

The decreasing fossil fuels resources and the mounting environmental deterioration due to global pollution have aroused increasingly efforts to discover alternate resources and better approaches that are more environmentally friendly and sustainable. Lignocellulosic biomaterials have drawn substantial attention in recent years as a new energy resource due to

its abundance and renewability. Crops residues and dedicated perennial grasses such as switchgrass are ideal biomass resource in biofuels production because of their low cost, abundance and proximity to existing grain-to-ethanol conversion (Lee et al., 2007). There are three major components in lignocelulosic feedstocks, including cellulose, hemicellulose (carbohydrate polymers) and lignin (phenolic polymers). Cellulose, the β-1, 4 glycosidic linked polymer of anhydroglucose, makes up the framework in the whole structure. The principle function of hemicelluloses and lignin is to fill in the blank (Bond and Atalla., 1999). Lignin is a three-dimensional network built up of dimethoxylated (syringyl, S), monomethoxylated (guaiacyl, G) and non-methoxylated (p-hydroxyphenyl, H) phenylpropanoid units, derived from the corresponding p-hydroxycinnamyl alcohols, which give rise to a variety of subunits including different ether and C-C bonds. Acetylated lignin units have been recently identified in non-woody plants using analytical pyrolysis. Lignin is considered as one of the primary reasons of recalcitrance in biomass structure (Del et al., 2004). Therefore, removing lignin is the first and most important step for utilizing cellulose and hemicellulose as feedstocks to biofuels production.

TiO_2 has been utilized in heterogeneous photocatalytic process to decompose lignin under UV illumination (Ma et al., 2008). However, most of the studies are focused on decomposing the dissolved and trace lignin in waste water or pulp industry (Tanaka, Calanag, & Hisanaga, 1999). Limited literatures showed the photocatalytic delignification of insoluble lignin in biomass. To further explore the potential of photodegradation of insoluble lignin in biomass as an energy-effective way to pretreat biomass for biorenewables and energy production, it is essential to understand the molecular mechanisms of lignin removal and alteration.

Our objective in this study is to develop a Raman spectroscopic method to investigate and quantify the effect of TiO_2-assisted photodegradation to decompose soluble and insoluble lignin in corn stover particles to gain insight into the molecular mechanism of lignin removal. Vibrational spectroscopic techniques such as IR and Raman spectroscopies have been used to study the compositional and molecular structural characteristics of plant materials (Agarwal and Atalla. 2000). In recent years, due to the utilization of monochromatic Near-IR excitation laser that effectively reduces the interfering fluorescent background caused by visible excitation laser, polarized Raman microspectroscopy has emerged as a widely adopted method for analysis of biological materials. In the area of plant structure characterization, it has been used to explore orientation and compositional variability of the cell wall components (Bond and Atalla., 1999). Compared to IR Spectroscopy, an unique advantage of Raman spectroscopy is that solid or powder samples could be measured directly without any preparation (Takayama et al., 1997). In this study, Raman Spectroscopy is used to analyze the molecular changes and the dynamic mechanism associated with the photo-assisted chemical delignification process through real-time monitoring of the change of the biomaterials under the pretreatment.

3.2. Materials and methods

3.2.1. Materials

Corn stover was collected from central Iowa in 2010, washed with deionized water and air-dried at ambient temperature. The corn stover was ground and screened to a nominal size of 9-35 mesh. Titanium dioxide P25 (Evonik Degussa, Cas #13463-67-7) was used as photocatalyst for the photodegradation of the corn stover biomass.

3.2.2. Photocatalyst-assisted ammonia pretreatment of corn stover biomass

Corn stover was treated by aqueous ammonia solution and sodium hydroxide with photodegradation under UV catalyzed by the photocatalysts with solid and liquid ratios of 1:20(g/ml). The amount of photocatalyst (TiO_2) was 10 wt. % of biomass loading. Biomass and the photocatalyst were loaded in screw-capped 250 ml bottles with 12.5 % of ammonium hydroxide. The reaction temperature was maintained at 60 °C by isotemp basic ceramic stirring hotplate (Fisher, Cat. #11-100-100SH) and reaction time was 24 hours. The UV light source was 100 W UV lamps (American ultraviolet company, Lebanon, IN) with irradiation wavelength at 380 nm. During the first 6 hours, oxygen was supplied to enhance the photocatalytic reactions.

In order to evaluate the effect of UV light penetration for the photocatalytic reaction, another experiment set up was utilized. All reactants were kept in 150 dia×20mmH corning PYREX Brand glass petri dishes; then the UV lamp and petri dishes were put into incubator shaker for heating and mixture. Due to the evaporation limitation, 0.1% sodium hydroxide was used to substitute 5% ammonia in the new experimental design. Other conditions and ratios were identical to the previous tests.

3.2.3. Raman spectroscopic characterization of the biomass under pretreatment

DXR Raman microscope (Thermo Scientific, Waltham, MA) was used for acquisition of Raman spectra with 780 nm excitation at 14 mW, 20X objective and 50 μm slit. The laser exposure time was 5 seconds and spectral resolution was 2.5 cm^{-1}. The OMNIC™ suite (Thermo Scientific, Waltham, MA) was used for data processing. The spectra were baseline-corrected and smoothed. An iterative polynomial background removal algorithm was

implemented to remove background fluorescence from the Raman spectral data (Zhang et al., 2010). 10 spectra were acquired from each sample and the average spectrum was calculated.

3.3. Results and discussion

3.3.1. Raman spectroscopic characterization of photocatalyst-assisted ammonia treated corn stover

Raman spectroscopy was used to characterize the structure constituents and chemical bonds in lignocellulosic biomass. Figure 1 shows the Raman spectra of untreated corn stover, and 12.5 % ammonia $+TiO_2$-assisted UV treated corn stover with oxygen supply. Raman bands associated with cellulose, hemicellulose, and lignin can be directly identified. In addition, semi-quantification of lignin and cellulose components in corn stover samples before/after pretreatment could also be achieved by calculating spectral intensities of relevant Raman bands (Daferera et al., 2002).

In the untreated corn stover, two major lignin bands were identified at 1,598-1620 cm^{-1} and 1,660-1,690 cm^{-1}. The most intense band at 1,598 cm^{-1} can be attributed to symmetric stretching of aromatic ring, the 1620 cm^{-1} band is assigned to aldehydrade α,or γ carbonyl group, and the 1,660-1,690 cm^{-1} band is attributed to the p-quinones (Agarwal and Atalla; 2000; Adapa et al., 2009). Additionally, peak(s) at 785 and 986 cm^{-1} due to the skeletal deformation of aromatic rings, and at 1,333 cm^{-1} for the syringyl ring breathing with C-O stretching groups in the lignin could be observed. Moreover, the peak between 1,163 to 1,181 cm^{-1} is assigned to C-O structure, typical for *p*-hydroxyphenyl, guaiacyl and syringal lignins (Iskalieva et al., 2012; Sun, Simmons, & Singh, 2011). These peaks were significantly reduced by ammonia treatment, and almost disappeared by photocatalyst-assisted ammonia pretreatment.
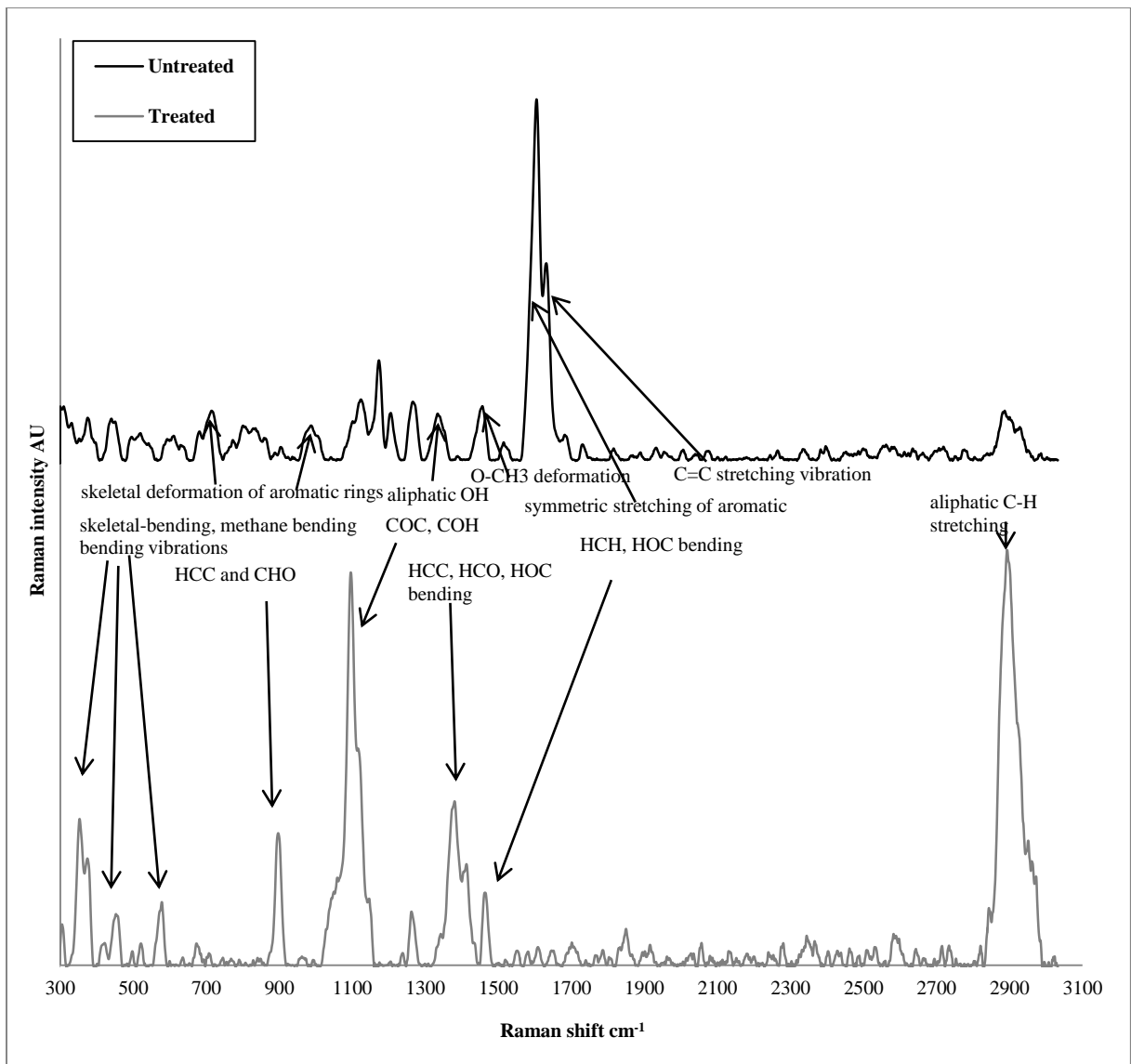
Figure 1 Part of the Raman peaks assignment for untreated and ammonia treated corn stover After pretreatments (12.5 % ammonia + TiO_2), 60-70% of the lignin components were

removed or degraded/altered to various degrees and part of the hemicellulose components

were decomposed as well. Investigation of the Raman spectra measured from the corn stover

samples before and after the TiO_2-assisted ammonia pretreatment indicated a clear switch

from lignin dominated signatures to cellulose dominated signatures. The skeletal structures of

cellulose molecules are mostly C-C bonds and C-O bonds which show relatively high

polarizabilities and high Raman scattering coefficients (Agarwal, 2006; Atalla and Agarwal,

1985; Atalla and Isogai, 2010). In particular, cellulose bands at 1,059-1,148 cm^{-1} due to the

C-O-C and C-O-H bending vibrations. Other authors considered the bands as β-(1-4)-

glycosidic linkages in cellulose, namely the COC symmetric and asymmetric stretching

modes. The band at 1,462 cm^{-1} is assigned to CH_2 vibration (Shih and Smith, 2009). At 1,377

cm^{-1} due to HCC, HCO and HOC bending, and at 2,888 cm^{-1} due to aliphatic C-H stretch

residual carbohydrates (methane C-H stretches of cellulose molecules) were remarkably

improved after the pretreatments. This is because the relative content of cellulose in the

biomass increased while the relative content of lignin decreased after the pretreatments. This

trend was further confirmed by cellulose peaks between 350 to 550 cm^{-1} of skeletal-bending

modes (CCC, COC), methane bending (CCH, OCH) and skeletal stretching (CC, CO) and

896 cm^{-1} (assigned to HCC and HCO bending at C6), and 1,456-1,469 cm^{-1} (due to HCH and

HOC bending vibration) (Agarwal, 2006; Agarwal and Ralph, 1997; Atalla and Agarwal,

1985; Tanaka et al., 1999). The specific Raman peaks assignment could be found in figure 1.

      3.3.2. Qualitative changes in Raman spectroscopic signatures associated with the

           pretreatment of biomass

Figure 2 and 3 show the typical cellulose dominant Raman spectrum and lignin dominant

Raman spectrum measured from biomass samples, respectively and the microscopic images

of the biomass structures.

From figure 3, an obviously decreasing trend can be identified correlating the duration of the

pretreatment to the lignin specific bands such as1,598 cm^{-1}, the symmetric stretching of

aromatic ring and 1,630 cm^{-1}, aldehydrade α, or γ carbonyl group. The 1,598 cm^{-1} specific

relationship between pretreatment time and intensities could be found in the figure 3, either.

However, there was no similar trend found in the cellulose dominant spectra. For 1 hour

pretreatment, relatively high cellulose specific bands have already emerged (352 cm^{-1}, 420

cm[-1], 1.095 cm^{-1} and 2.886 cm^{-1}). Meanwhile, it should be noticed that no cellulose dominant

spectrum could be observed in untreated corn stover samples, indicating the omnipresence of

lignin bundling with cellulose in the untreated biomass. Cellulose functional bands derived

from both polysaccharide and oligosaccharide could be preciously identified in Raman

spectra once lignin started to break down. As a result, no matter how long the treatment

would be, the possibilities for measuring high intensity cellulose component bands are
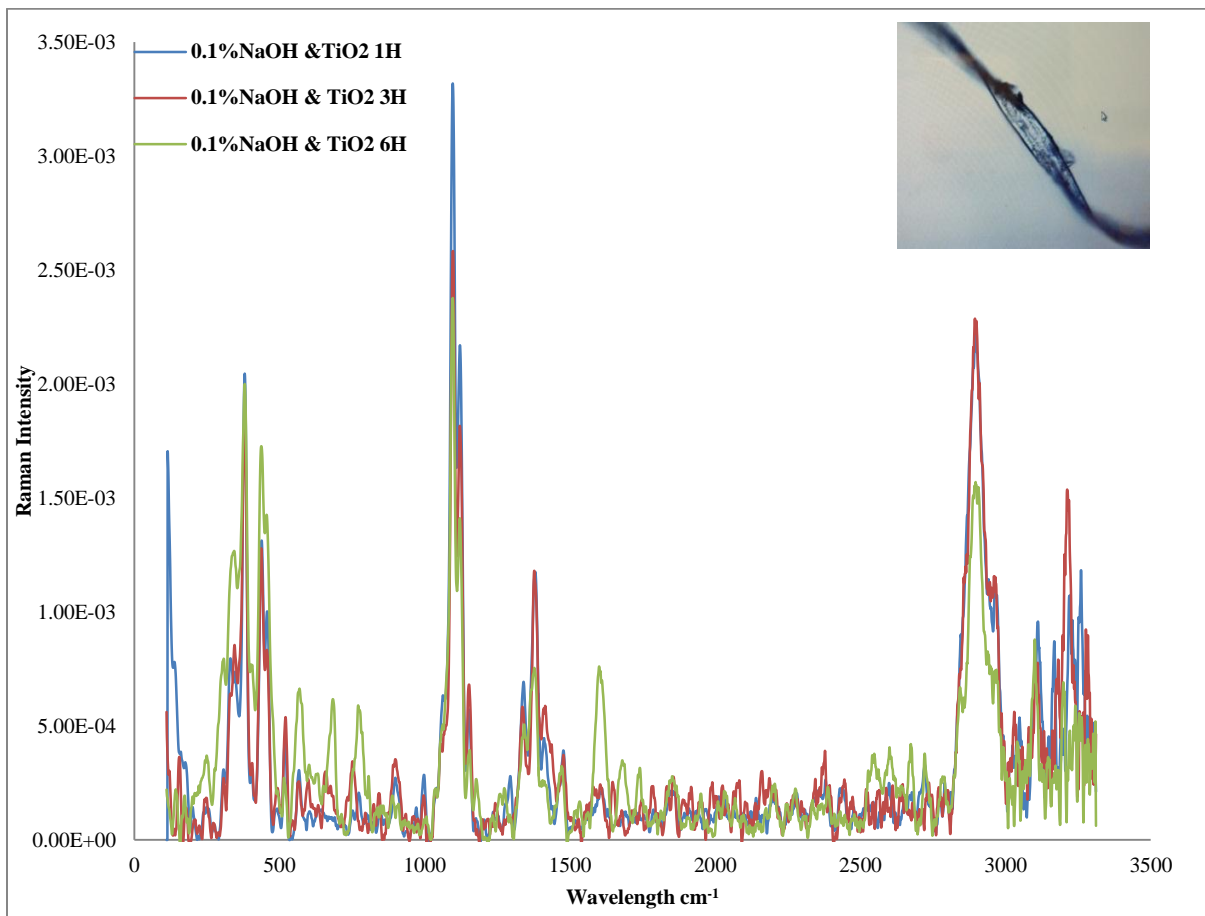
relatively constant.

Figure 2 Average cellulose style spectra of Photocatalyst assisted 0.1% Sodium Hydroxide
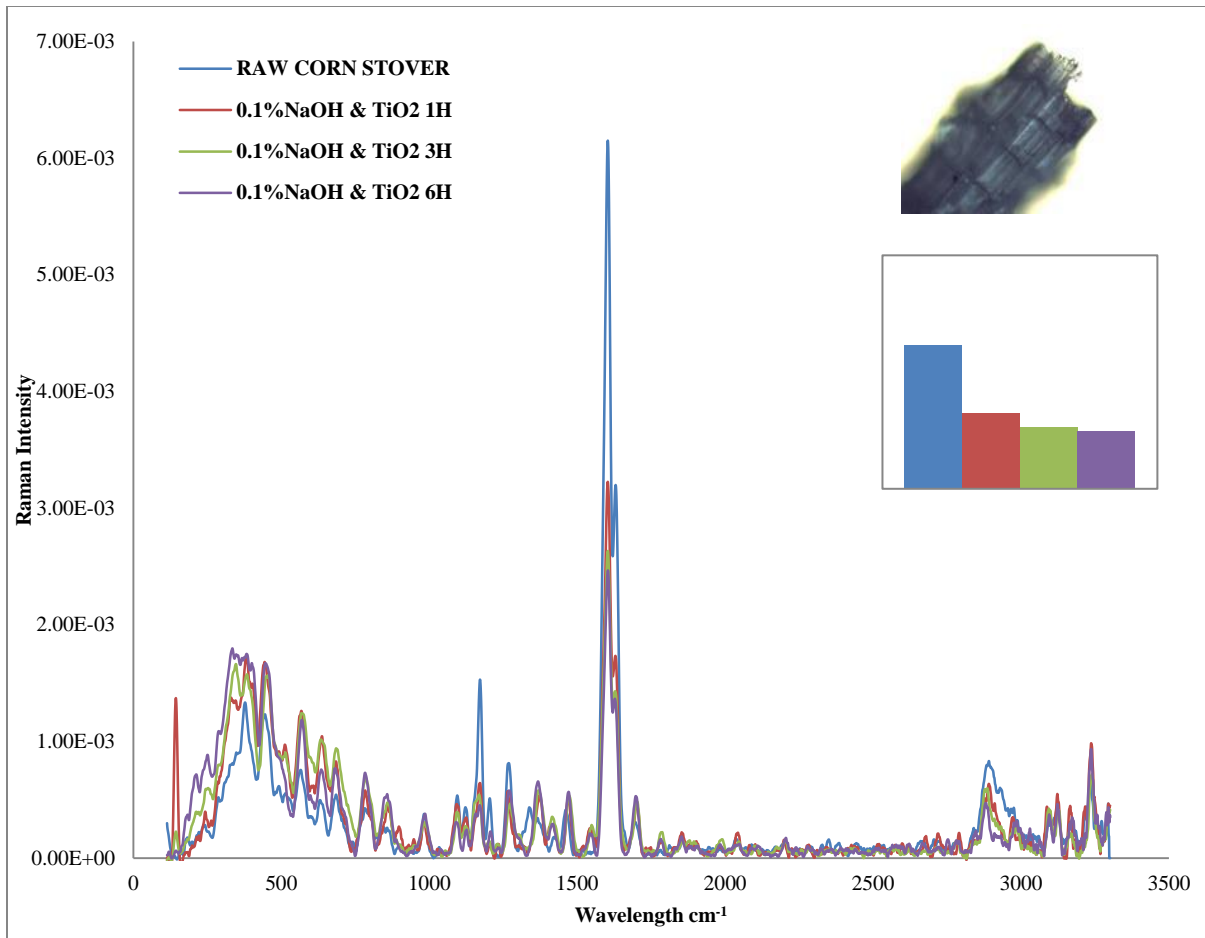pretreatment at different time points

Figure 3 Average lignin style spectra of Photocatalyst assisted 0.1% Sodium Hydroxide pretreatment at different time points and the intensities at 1600 cm^{-1} for each time point

3.3.3. Raman spectroscopic analysis of the effect of the UV penetration efficiency on the photodegradation of the biomass

Based on the previous results in our experiment, no significant difference of delignification effect was found between alkaline and photocatalysic-aided alkaline pretreatments for corn stover particles. It should be noted that the activity of the photocatalysts is affected by its own properties, such as particle size and concentration of impurities, and the experimental conditions. In our experimental design, magnetic stirring hot plate and water bath were used for heating and mixing of photocatalysts with the reactants. And reactants were loaded in screw-capped conical flask (see figure 4).
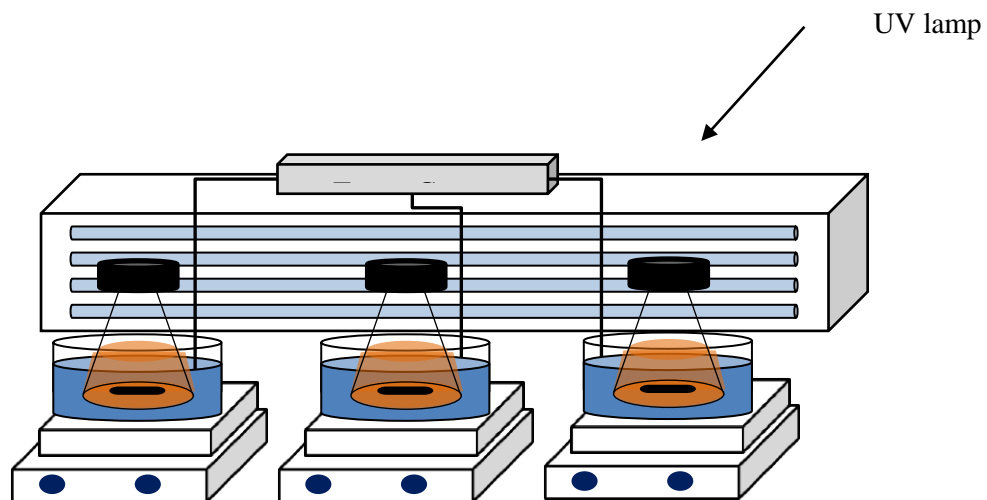
UV lamp

Figure 4 Old experimental setup of photocatalyst-assisted ammonia pretreatment

However, the relatively thick bottle wall and the presence of surrounding water significantly

reduced the UV lamp light power delivered into the reaction system and the penetration

depth of the UV light, which might have severely reduced the efficiency and the

effectiveness of the photodegradation pretreatment. In order to investigate the relationship

between the delignification result and the UV light penetration depth, an alternative

experimental set up was implemented (see figure 5). Additionally, in order to test the periodic

change during the photocatalysis pretreatment, UV/Vis and Raman spectra were measured in

different time points for the soluble lignin structure throughout the pretreatment process.
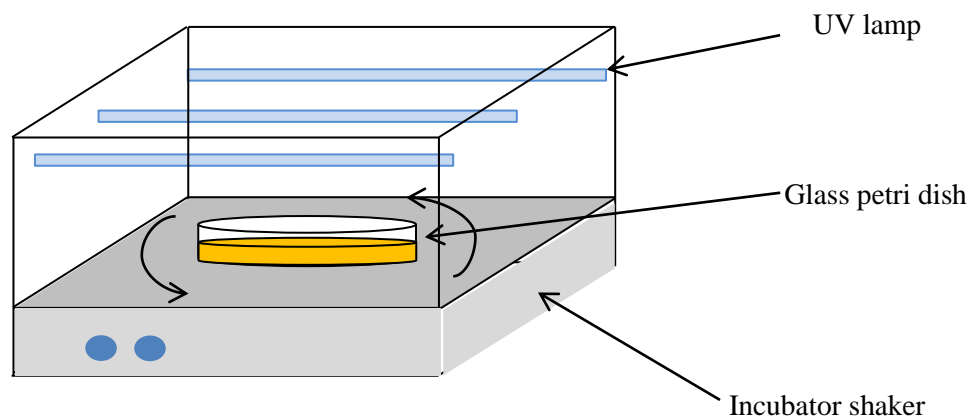
UV lamp

Glass petri dish

Incubator shaker

Figure 5 Alternative experimental set up for photocatalyst assisted sodium hydroxide
treatments

Raman spectra of the treated biomass were measured to analyze the insoluble lignin retained

in the solid part, alongside with UV/Vis analysis of the soluble lignin in the supernatant.

After baseline correction and area normalization, 10 lignin-dominant Raman spectra were

averaged to yield one characteristic Raman spectrum for each UV treatment time, as shown
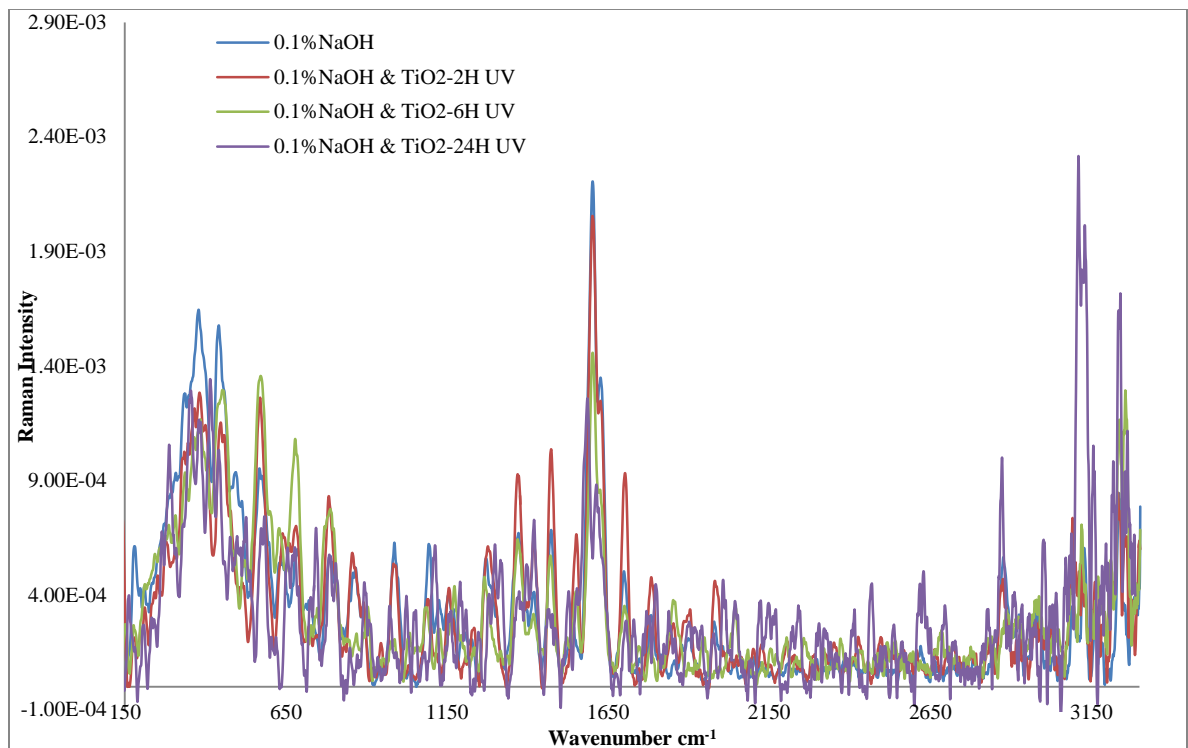
in figure 6.

Figure 6 Raman spectra of different UV treatment time lengths photocatlyst aided sodium
hydroxide methods

The peak of symmetric stretching of aromatic ring at 1,598 cm^{-1} reveals a clear decrease

tendency with the extension of UV illumination time, indicating the disappearance of

insoluble lignin in the biomass. Additionally, from the UV/Vis absorption spectra of soluble

lignin within the new experimental designs (details could be found in Chapter 2), the

significant decreasing trend of the lignin peak at 290 nm demonstrated the huge soluble

lignin decomposition in liquid. In conclusion, the UV light penetration played an important

role in photocatalyst assisted alkaline pretreatment for biomass. Moreover, the elevated

photocatalyst delignification efficiency could be found in both soluble and insoluble lignin composition in the biomass materials.

3.3.4. Raman characterization of the hydrolysis mechanism of photocatalyst aided alkaline pretreatment

By carefully examining the Raman spectroscopic changes associated with chemical functional groups of the biomass, a molecular pattern showcasing the delignification mechanism started to emerge.

Figs. 7 and 8 show the ratio of relevant intensities between Raman bands representing important chemical functional groups that are involved in delignification process. It was found that the ratios of 1,690: 1,620, 1,690: 1,598, 1,462: 1,173 demonstrated distinct increasing tendency.

For lignin, on average every 10 substructural phenyl propane units contain one phenolic group; among those phenolic groups, many are α-hydroxyl moieties in the propyl side chain (Weinstock et al., 1993). Theoretically, oxides are able to oxidize phenols and benzylic alcohols to quinones or carbonyls and aldehydrade (α, or γ-carbonyl group), respectively (Kihara et al., 2002). The specific oxidation procedures are shown in scheme 1. The Raman bands of p-quinones are in 1,665-1,690 cm^{-1} region, it is difficult to further discompose the individual Raman peaks due to the coupling of C=C and C=O stretch modes. (Agarwal and Atalla, 2000). The α, or γ-carbonyl group is detected at 1,620 cm^{-1} (Kihara et al., 2002). The peak between 1,163 and 1,181 cm^{-1} is assigned to C-O structure, typical for p-hydroxyphenyl, guaiacyl and syringal lignins (Iskalieva et al., 2012; Sun et al., 2011). Meanwhile, the CH$_2$ vibration band at 1,462 cm^{-1} is usually regard as cellulose peak in side chain (Shih and Smith, 2009).
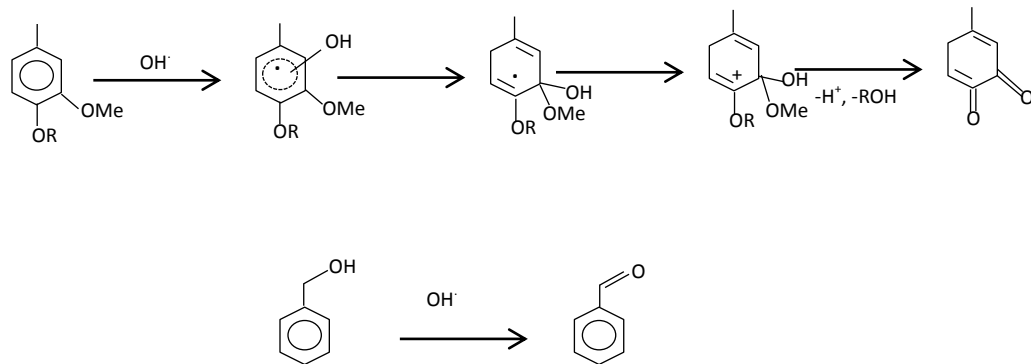
Scheme.1. Reactions of hydroxyl radicals with lignin forming quinones and carbonyls structures (Machado et al., 2000). R=H or lignin

The increase in the ratio of 1,690: 1,620 was consistent with the main oxidation mechanism discussed above that hydroxyl moieties disappeared and more carbonyls and quinones emerged in the residual biomass. For the increase of the ratio between 1,690: 1,598 pair, besides the increasing quinones amount that enhance the 1,690 cm^{-1} band, the breaking down of phenyl ring which led to the decrease of the symmetric stretching of aromatic ring at 1,598 cm^{-1} was also a contributing factor. Furthermore, the significantly improvement in pair 1,462: 1,173 ratio demonstrated that the cellulose component (a weak cellulose peak at 1,462 cm^{-1}) tended to increase while the lignin component (from p-hydroxyphenyl, guaiacyl and syringal lignins) started to decrease. Except these three obvious trend combinations with the increasing processing time, the other three ratios combinations kept stable which probably due to the consistent reaction mechanism in the pretreatment process. For example, the 1,620: 1,598 pair showed similar ratio value with the processing time because both characteristic peaks were typical lignin peaks.

The lignin hydrolysis mechanism for alkaline-based treatment and photodegradation seems to be similar. However, the higher ratios observed for photocatalyst-assisted alkaline treatment (e.g. alkaline: alkaline & TiO$_2$ =1.4: 1.5 in 1,462: 1,173 pair and 0.2: 0.3 in 1,690: 1,598 pair)

suggest that the addition of photodegradation into the pretreatment processing stream led to better delignification efficiency than that without photocatalysts.
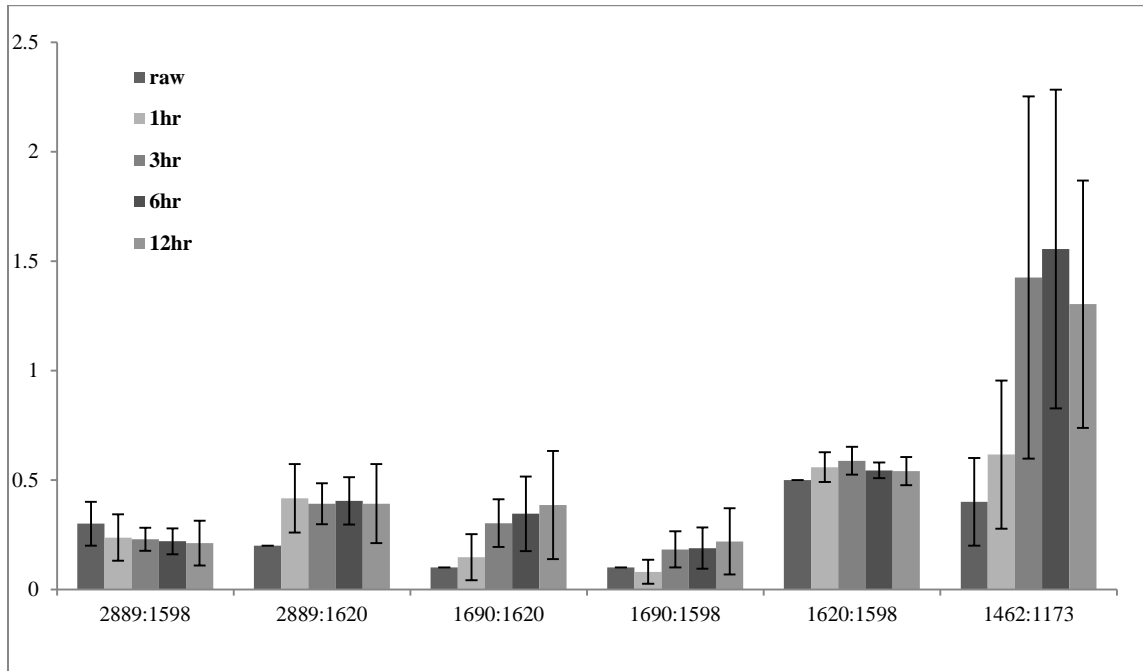

Figure 7 Ratios of different functional groups pairs in 0.1% Sodium Hydroxide treatment sampling at different time points
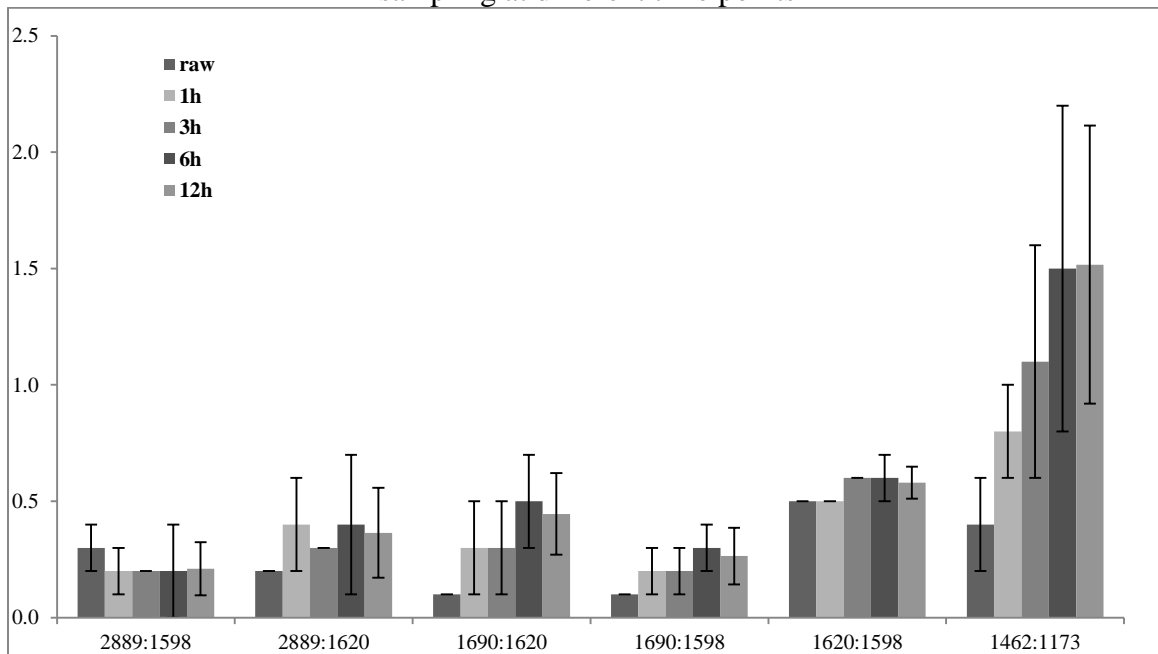

Figure 8 Ratios of different functional groups pairs in photocatalyst assisted 0.1% Sodium Hydroxide treatment sampling at different time points

Conclusion

The Raman spectroscopic investigation clearly revealed that lignin structure was significantly affected or decomposed, and the relative content of cellulose was increased by photocatalyst-assisted alkaline pretreatment. Two spectra models and relatively complete functional groups assignment were proposed in the study to further our understanding of the lignocellulosic biomass spectroscopic characteristics. Additionally, Raman spectroscopic analysis for insoluble lignin suggests that the UV light penetration is one of the most important factors affecting the photocatalyst pretreatment of biomass. Moreover, Investigation of Raman peak ratios verified that the theoretical lignin hydrolysis mechanism could be used to explain the photocatalyst pretreatment delignification processing. It should be noticed that most of Raman bands of hemicellulose overlap with those of cellulose; therefore, structure changes of hemicellulose caused by the pretreatment were not clearly shown in the Raman analysis.

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Chapter 4 Conclusion and Future Perspective

In conclusion, to improve the chemical pretreatment efficiency of bio-ethanol, a

photocatalyst assisted alkaline approach was developed to remove lignin structure and

increase the amount of fermentable component in lignocellulosic biomass (corn stover). Two

kinds of base tested in this study were aqueous ammonia and sodium hydroxide, respectively.

Due to the high volatility, a special experimental design was used in ammonia pretreatment,

which limited the UV penetration, hence only moderate improvement on enzymatic

digestibility of the treated biomass could be obtained through the photocatalyst-assisted

photodegradation. Meanwhile, testing with sodium hydroxide yielded elevated

delignification result and confirmed that the UV penetration played an essential role in

photocatalytic treatment. However, compositional analysis and enzymatic hydrolysis tests for

the new reactor design are still needed to further verify the effect of the improved UV

penetration on the saccharification and enzymatic digestibility of the treated biomass.

Moreover, more effective reactor design needs to be explored in the future to support better

UV penetration for ammonia-based pretreatment, where the volatilization of ammonia needs

to be prevented.

Spectroscopic analysis in this study revealed some notable findings. The soluble lignin peak

found in UV/Vis spectra distinctly and quickly indicated the lignin content in the treated

solution. From Raman spectroscopic investigation, two spectra types were distinguished in

real time based on abundance of different compositional components of the untreated and

treated biomass. The two types distinguish the cellulose and lignin component functional

groups, respectively. Raman spectroscopic analysis clearly revealed multiple functional

peaks in lignocellulosic biomass materials, from which the molecular delignification mechanism for the photocatalyst assisted alkaline pretreatment could be deduced. Based on the findings presented in this work, a complete spectroscopic map of lignocellulosic materials can be established in the future to help further our understanding on lignocellulosic biomaterials interact with other chemical and biological agents. Additionally, even though periodic measurement of Raman spectra throughout the pretreatment process displayed the dynamic tendency of different component peaks, an exceedingly accurate criterion for quantitative change of each functional group is still needed. As for the future study, continuous Raman spectroscopic monitoring and measurement should be implemented to yield a real-time, changing chemical map of the pretreatment process to further reveal the dynamic mechanism for delignification of biomass in different pretreatment approaches.

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