

ABSTRACT

ZHANG, XIMING. Pretreatment of Corn Stover for Sugar Production by Using the Combination of Alkaline Reagents and Switchgrass-Derived Black Liquor. (Under the direction of Jay J. Cheng.)

Corn stover pretreatment using the combination of sodium hydroxide (NaOH) and calcium oxide (CaO) was investigated in this study at room temperature for improved cost-effectiveness of biomass-to-sugar conversion. The effects of NaOH concentration, CaO loading, and residence time on enzymatic hydrolysis were studied and the total reducing sugar yield in enzymatic hydrolysis was used to evaluate the pretreatments. The results show that, compared with NaOH pretreatments, pretreatments with the combination of NaOH and CaO resulted in similarly high sugar productions but at substantially lower costs. The addition of CaO not only increased the alkalinity, which favored biomass digestibility improvement, but also contributed to better biomass preservation. Based on sugar production and cost-benefit considerations, the two recommended pretreatment conditions were 3h, 0.5% NaOH, 0.1 g CaO g⁻¹ raw biomass and 6h, 0.5% NaOH, 0.05 g CaO g⁻¹ raw biomass, at which the total reducing sugar yields were respectively 529.0 and 538.9 mg g⁻¹ raw biomass, at overall carbohydrate conversions of 75.6% and 77.0%, respectively.

Switchgrass and corn stover are two of the most intensively studied herbaceous feedstocks for sugar production. To improve the cost-effectiveness of biomass-to-sugar conversion, sodium hydroxide (NaOH) pretreatment of switchgrass was carried out at room temperature using the previously determined best conditions, and the spent alkaline liquid (black liquor) was recycled to pretreat corn stover, the feedstock exhibiting a higher susceptibility to alkaline attack, for improved enzymatic hydrolysis. The results show that,

because of the high pH and the appreciable amount of carbohydrates in the initial black liquor, the sugar productions of the corn stover pretreated with the black liquor were comparable with those of the biomass pretreated with 1% NaOH in enzymatic hydrolysis. After black liquor pretreatment at the best residence time of 24 h, the total reducing sugar, glucose, and xylose yields of corn stover reached 478.5, 287.7, and 145.3 mg/g raw biomass, respectively.

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Pretreatment of Corn Stover for Sugar Production by Using the Combination of Alkaline
Reagents and Switchgrass-Derived Black Liquor

by
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DEDICATION

*This is for my beloved parents and friends, their endless love and everlasting support,
encouraging me along the way.*

BIOGRAPHY

Ximing Zhang was born on February 23, 1987 in Benxi, Liaoning Province, P.R. China. During the first 18 years of his life, he spent his childhood and school time in Benxi, a city with heavy industry such as iron and steel production. The overdevelopment of the city brought a lot of environmental issues including air and water pollution, making it, as reported, known for one of the cities that are invisible from satellites in the 1980s. Since his childhood, he was committed to the environment protection to bring about cleaner city with industry. The first step became true when Ximing chose the major in Biosystem and Energy Engineering in Shenyang Agricultural University (SYAU). He focused on the biofuel production which is totally environmentally friendly and would greatly replace the petroleum. His research experience got started with “cellulosic ethanol” four years ago when he was a junior. As his time with research progressed, more words were successively added into the vocabulary of his lab experience, for instance, enzyme, microorganism, fermentation, pretreatment, mathematical modeling, biological conversion, bioenergy, biorefinery, and innumerable other terminologies and concepts. Ximing Zhang received his Bachelor’s degree in July 2010.

He believes that learning is a three-step process starting from minutiae, going through expansion, integration and generalization, and finally returning to a focused topic. Carrying on the green dream with biofuel, he attended North Carolina State University and started his master degree education in Department of Biological and Agricultural Engineering in August 2010.

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CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

Petroleum demand is dramatically increased during the past two decades around the world. Demand for liquid transportation fuels is projected to increase by 17 percent from 2006 to 2030 (EIA, 2008). However, it is not optimistic that the production of fossil fuels would catch up with the quickly increasing demand before world oil production peak, which would occur sometime between 2030 and 2050 (Jackson, 2007). As the upheavals of international situation and limit of fossil fuels especially crude oil production affect the petroleum price greatly, the biofuel production industry is playing an important role through the past 10 years in USA.

In 2008, the U.S. ethanol industry produced 9 billion gallons of ethanol, with an increase of 5.4 times over the past decade (RFA, 2009). Bioethanol is regarded as a promising additive and supplement to the transportation fuels. The corn based first-generation bioethanol has greatly increased the food marketing price as corn is an important food and feed source. Using of resources which are affordable and with less impact on food market to produce bioethanol is the starting point of second-generation bioethanol, namely lignocellulosic material based bioethanol.

Lignocellulosic material is a kind of renewable and abundant resource referring to biological materials derived from natural or human activities. As the lignocellulosic material is produced on a renewable basis, the growth of the biomass can absorb CO₂ emitted during the consumption of biomass-based energy source (Spatari et al., 2005). In this way, the lignocellulosic material based bioethanol can play an important role in addressing issues with both greenhouse gas emissions of transportation and the dependency on fossil fuels. The development of biomass-based energy industry will not only be beneficial to the social aspect

of sustainability by creating good jobs in local areas (Lin and Tanaka, 2006), but also provides a profitable way to dispose some biomass waste such as agricultural residues, yard wastes, forestry wastes, and municipal wastes.

Switchgrass and corn stover are the two most extensively studied lignocellulosic feedstocks for ethanol production. Switchgrass is a perennial grass native to North America. It is regarded as a promising dedicated energy crop due to its excellent yield and low agricultural inputs for cultivation (Keshwani and Cheng, 2009). Corn Stover, referring to stalks, leaves, and cobs that remain in corn fields after the grain harvest, is considered as a potential feedstock for sugar production due to its great amount and easy access in the United States. Like all other lignocellulosic feedstocks, the conversion of corn stover to ethanol is more challenging than that of corn grain because of the complex structure of lignocellulosic materials.

Cellulose, hemicellulose, and lignin are three major components of lignocellulosic materials. Cellulose and hemicellulose are polysaccharides. They include glucan and xylan which can be used for ethanol production. Lignin stiffens and surrounds the fibers of polysaccharides (Fan et al., 1987). These three components form a recalcitrant structure of lignocellulose. A pretreatment step is necessary to break up the structure, making cellulose and hemicellulose more accessible to hydrolytic enzymes for fermentable sugar production. Pretreatment technologies have been intensively studied to improve the enzymatic digestibility of lignocellulose (Alizadeh et al., 2005; Chang et al., 1997; Negro et al., 2003; Piskorz et al., 1989; Sun and Cheng, 2005). Alkaline pretreatment is known for its less energy input and low cost (Chang et al., 2001). The major mechanism of alkaline pretreatment is the

saponification of intermolecular ester bonds that crosslink hemicellulose and other components, leading to a disruption of lignin structure, an increase in internal surface area, and a decrease in cellulose crystallinity (Sun and Cheng, 2002).

1.2 RESEARCH OBJECTIVES

Our previous study showed that adding cheap lime ($\text{Ca}(\text{OH})_2$) in the NaOH pretreatment of switchgrass helped to reduce the requirement for the more costly NaOH without negatively affecting the pretreatment efficiency. Based on the previous work, in the first part, the combination of the two alkaline reagents was investigated in this study to treat corn stover at room temperature for improved sugar production. Since calcium oxide (CaO) has even more desirable properties, it was used in place of $\text{Ca}(\text{OH})_2$ in this study. CaO, or commonly referred to as quicklime, is not only much cheaper than $\text{Ca}(\text{OH})_2$ in the chemical market, per unit weight of CaO is also able to provide more alkalinity. Moreover, the heat energy CaO produces when forming the hydrate ($\text{Ca}(\text{OH})_2$) in water is favorable for reaction acceleration.

Based on previous studies and first part experiments, compared with switchgrass, corn stover is more susceptible to NaOH pretreatment at room temperature (Zhang et al., 2011; Xu et al., 2010a). For switchgrass pretreatment, 2% NaOH was required to maximize sugar production, while 1% NaOH was sufficient for corn stover.

The general goal of this research is to explore the corn stover performance on combination alkaline pretreatment and switchgrass-derived black liquor pretreatment.

Specific objectives are as follows:

1) Optimize the processing conditions (NaOH loading, CaO loading, and residence time) using the combination of sodium hydroxide and calcium oxide to improve the cost-effectiveness of alkaline pretreatment at room temperature.

2) Compare with the results with those of NaOH pretreatment to evaluate the comparative advantages of this technology in treating corn stover.

3) Study the performance of black liquor from the NaOH pretreatment of switchgrass at the previously determined best conditions used for the pretreatment of corn stover at room temperature.

4) Compare with those of the pretreatments using water and NaOH solutions of different concentrations to better understand the performance of the black liquor.

5) Study and optimize the enzyme loadings applied in the subsequent enzymatic hydrolysis.

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CHAPTER 2
CONVERSION OF LIGNOCELLULOSIC MATERIALS
FOR SUGRAR PRODUCTION: A REVIEW

2.1 INTRODUCTION

Biomass-based ethanol is more and more essential nowadays as the energy consumption has rising rapidly all over the world. Lignocellulosic is regarded as a promising feedstock for bioethanol mainly because its renewable nature and availability compared to corn (starch based bioethanol) or sweet sorghum (sugar based bioethanol) (Farrell et al., 2006; Maclean et al., 2000). Reduction in net greenhouse gas emissions is also a key element compared to the first-generation bioethanol (Farrell et al., 2006; Maclean et al., 2000). In the background of world financial crisis, renewable energy technology development has been a catalyst for economic resurgence as it can improve rural employment and diversify rural economies. As the gap of gasoline supply is critical around the world, developing the renewable energy is urgent for stabilizing energy security for America (Ziyu Wang., 2009).

The annual production of lignocellulose exceeds 10^{10} MT in the world so it is the most popular renewable biomass (Sánchez and Cardona, 2008). Because of the technical obstacles, the application of the lignocellulosic feedstocks is still not commercialized.

The steps of the ethanol production from lignocellulose include pretreatment, hydrolysis, sugar fermentation, separation of residue and finally recovery and purification of fuel-grade ethanol. Removing the lignin and breaking up the crystalline structure of cellulose for enhancing enzymes accessibility to the cellulose during the hydrolysis is the key task of the pretreatment (Mosier et al., 2005b).

It is necessary to adopt suitable pretreatment technologies based on the lignocellulosic biomass properties of each raw. More importantly, different kind of pretreatments have different impact on the subsequent steps, such as generation of toxic compounds potentially

inhibitory for yeast, stirring power requirement, energy demand in the downstream process (Galbe and Zacchi, 2007). Pentose recovery, chip size required, concentration of toxic compounds formed during pretreatment are all important factors in an effective pretreatment (Sun and Cheng, 2002).

2.2 COMPOSITION OF LIGNOCELLULOSE

Cellulose, hemicellulose and lignin are the main components of lignocellulosic materials. Cellulose and hemicellulose are macromolecules formed by different kind of sugars. However, lignin is an aromatic polymer which is difficult to degrade. Different plants have quite different proportions of these compounds (Prasad et al., 2007; McKendry, 2002; Malherbe and Cloete, 2002; John et al., 2006; Stewart et al., 1997; Reguant and Rinaudo, 2000; Perez-Diaz et al., 2005).

2.2.1 Cellulose

Cellulose is formed by D-glucose subunits linked by β -1, 4 glycoside bonds. These subunits formed linear polymer long chains linked by hydrogen bonds and van der Waals forces. Most of the cellulose is presented as a crystalline form and only a small amount of non-organized cellulose chains forms amorphous cellulose.

2.2.2 Hemicellulose

Hemicellulose has a lower molecular weight than cellulose. It is also a polysaccharide but formed with D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, D-galacturonic,

D-glucuronic acids and 4-O-methyl-glucuronic. β -1, 4-and sometimes β -1, 3-glycosidic bonds link the sugars together. Hemicellulose has branches with short lateral chains consisting of different sugars (Carmen Sanchez, 2008).

2.2.3 Lignin

Both hemicellulose and cellulose are linked to lignin. This structure forms a physical seal in the plant cell wall which would give structural support and resistance against microbial attack. The phenyl propane units joined together by non-hydrolysable linkages constructs an amorphous heteropolymer, non-water soluble and optically inactive characteristics. Free radicals released in the peroxidase-mediated dehydrogenation of three phenyl propionic alcohols form the polymer. The three phenyl propionic alcohols are: coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (p-hydroxyphenyl propanol), and sinapyl alcohol (syringyl propanol). C–C and aryl-ether linkages, with aryl-glycerol β -aryl ether being the predominant structures are the main component of heterogeneous structure (Carmen Sanchez, 2008).

2.3 EFFECTIVE PARAMETERS IN PRETREATMENT OF LIGNOCELLULOSES

The properties of the lignocellulosic material make them resistant to enzymatic attack (Chang and Holtzapfle, 2000). Pretreatment is the way to change the properties to be ready for the subsequent enzymatic degradation. Different pretreatments deal with different kinds of materials.

2.3.1 Crystallinity

Both crystalline and amorphous regions are cellulose. Two thirds of the total cellulose is in the crystalline form (Chang and Holtzapple, 2000). Cellulase hydrolyzes the more accessible amorphous portion of cellulose and the enzyme is less effective in degrading the much tougher crystalline portion. Crystallinity index is not strongly related with the rate of hydrolysis. Kim and Holtzapple (2008) reported an increase degree of crystallinity of corn stover from 43% to 60% by calcium hydroxide delignification, which is caused by the removal of amorphous components. However, the yield of enzymatic hydrolysis is not affected even though the crystallinity of pretreated material increased. Fan et al. (2008) illustrated the effect of ball milling on surface area and crystallinity of cellulose. A great increase in crystallinity of cellulose by reducing the size of cellulose by milling was observed.

2.3.2 Effect of accessible surface area

A positive correlation between the pore volume and the enzymatic digestibility of lignocellulosic materials is shown by several studies. Cellulose accessible surface area is the main reason for improvement in enzymatic hydrolysis by removing lignin and hemicellulose (Zhang and Lynd, 2004). Surface area represents by crystallinity, lignin protection and hemicellulose presentation. An enzymatic hydrolysis process consists of three steps: first step is the adsorption of cellulase enzymes from liquid phase onto the surface of cellulose; second one is the biodegradation of cellulose to simple sugars, mainly cellobiose and oligomers, and the last step is the desorption of cellulase to the liquid phase (Zhang and Lynd, 2004). The surface area of lignocellulosic material and the interaction would be a limiting factor for

enzymatic hydrolysis. External and internal surface areas are two different types for lignocellulosic materials. Size and shape of the particles determines the external surface area, while the capillary structure of cellulosic fibers determines internal surface area. Dry cellulosic fibers have about 15 to 40 μm in size and therefore they obtain a relatively large external specific surface area, e.g. 0.6–1.6 m^2/g (Chandra et al., 2007). On the other hand, the internal surface area is relatively small than the external surface area. A very large internal can be created by swelling of lignocelluloses with water and polar solvents

2.3.3 Effect of lignin

Integrity, structural rigidity and prevention of swelling of lignocelluloses are the main functions for lignin (Mansfield, 1999). That is why lignin is the well-known factor for recalcitrance of lignocellulosic materials to enzymatic degradation. The lignin after dissolution can be an inhibitor for the cellulase, xylanase and glucosidase. The lignin type would result in different recalcitrance. Compared with syringyl lignin, guaiacyl lignin restricts more fiber swelling and enzyme accessibility (Mansfield, 1999).

2.3.4 Effect of hemicellulose

Hemicellulose is also a factor that can prevent cellulose from enzymatic attack. Most of the pretreatment methods are able to remove the hemicellulose. Hemicellulase can hydrolyze hemicellulose. However, in some dilute-acid treatment, the removal of hemicellulose would reduce the need for use of hemicellulase enzymes for the degradation of the biomass.

2.4 PRETREATMENT TECHNOLOGIES FOR LIGNOCELLULOSIC BIOMASS

In the last few decades different pretreatment technologies have been invented and most of them focused on a specific type of biomass (Zhang and Lynd, 2004). The pretreatment methods include biological, physical, chemical, and physical-chemical pretreatments.

2.4.1 Biological pretreatment

The biological pretreatment is known as an environmentally friendly and low cost technology. Microorganisms such as brown, white, and soft-rot fungi are known for degrading lignin and hemicellulose and very little cellulose (Sanchez, 2009). The most effective microorganism is probably the white-rot fungus. During the degradation, the fungus can secrete peroxidases and laccases (Kumar et al., 2009a).

Different lignocellulosic biomass can be treated with different white-rot fungi such as *Phanerochaete chrysosporium*, *Ceriporia lacerata*, *Cyathus stercoleris*, *Ceriporiopsis subvermispora*, *Pycnoporus cinnabarinus* and *Pleurotus ostreatus*. Most of fungi show high efficiency (Kumar et al., 2009a; Shi et al., 2008).

2.4.2 Physical pretreatments

2.4.2.1 Mechanical comminution

The mechanical pretreatment can not only reduce the particle size, but also can reduce the lignocellulosic crystallinity; these changes can increase the specific surface and reduce the

degree of polymerization. Combination of chipping, grinding or milling depending on the requirement of the size of biomass is used (10–30 mm after chipping and 0.2–2 mm after milling or grinding) (Sun and Cheng, 2002). Different milling processes (ball milling, two-roll milling, hammer milling, colloid milling) would work as a tool to improve the enzymatic hydrolysis of lignocellulosic material (Taherzadeh and Karimi, 2008). However, mechanical comminution pretreatment is not economically feasible as the size requirement would lead to great energy cost (Hendriks and Zeeman, 2009).

2.4.2.2 Extrusion

During the extrusion pretreatment, biomass is subjected to heating, mixing and shearing, which would lead to both physical and chemical modifications. Lignocellulose structure is disrupted during the screw extrusion and heating. Defibrillation, fibrillation and shortening of the fibers would make the enzymatic attack more efficient (Karunanithy et al., 2008).

2.4.3. Chemical pretreatments

2.4.3.1 Alkali pretreatments

Cellulose digestibility and lignin solubilization is greatly increased by alkali pretreatments compared to acid or hydrothermal processes (Carvalho et al., 2008). Alkali pretreatment is very flexible on time from seconds to days and it only requires room temperature. Compared to acid pretreatment, it is more effective on agricultural residues than on wood materials (Kumar et al., 2009a). Alkaline pretreatments include sodium, potassium, calcium and ammonium hydroxides. NaOH can increase the internal surface of cellulose and

decrease the degree of polymerization and crystallinity, which accelerates the lignin structure disruption (Taherzadeh and Karimi, 2008). Lime pretreatment is also widely used and it can remove amorphous substances such as lignin. Acetyl groups produced by hemicellulose can be removed by lime pretreatment (Mosier et al., 2005b). Lime has been proven successful at temperatures from 85 to 150°C and for 3–13 h with corn stover (Kim and Holtzapfle, 2006) or poplar wood (Chang et al., 2001). Compared with NaOH and KOH, pretreatment with lime is economically feasible and the $\text{Ca}(\text{OH})_2$ could be recovered by reaction with CO_2 in hydrolyzate (Mosier et al., 2005b). Oxidant agent can also help alkali improve the performance by enhancing lignin removal (Carvalho et al., 2008).

2.4.3.2 Acid pretreatment

Acid pretreatment is mainly to solubilize the hemicellulosic fraction of the biomass and make the cellulose more accessible to enzymes. Most of the acid pretreatment use diluted acid as the concentrated acid pretreatment results in large amount of inhibiting compounds and corrosion to the equipment. The recovery of concentrated acid is also a problem.

Diluted acid has been studied for wide range of lignocellulosic biomass and become more welcomed by industrial applications and different types of reactors are used for the pretreatment of lignocellulosic materials (Taherzadeh and Karimi, 2008).

H_2SO_4 has been studied for the acid pretreatment. Hydrochloric acid, phosphoric acid and nitric acid have also been tested to see the efficiency of acid pretreatment (Mosier et al., 2005a). Saccharification yield as high as 74% was achieved when wheat straw was subjected to 0.75% v/v of H_2SO_4 at 121°C for 1 h (Saha et al., 2005). Olive tree biomass was pretreated

with 1.4% H₂SO₄ at 210°C, resulting in 76.5% of hydrolysis yields (Cara et al., 2008).

Recently, ethanol yield as high as 0.47 g/g glucose was achieved in fermentation tests with cashew apple bagasse pretreated with diluted H₂SO₄ at 121°C for 15 min (Rocha et al., 2009).

2.4.3.3 Ozonolysis

Ozone is well known with high delignification efficiency, and the lignin removal increases the sugar yield in enzymatic hydrolysis (Sun and Cheng, 2002). Room temperature and normal pressure is enough for ozone pretreatment and no formation of inhibitory compounds generated for the subsequent hydrolysis and fermentation (García-Cubero et al., 2009). Many feedstocks are tested by ozone and a key drawback is a high consumption of, which is unfeasible economically (Sun and Cheng, 2002).

2.4.4 Physico-chemical pretreatments

2.4.4.1 Steam explosion:

Steam explosion is widely used in Physico-chemical pretreatment for lignocellulosic biomass. Lignocellulosic material is immersed in pressured steam for seconds to minutes and then depressurized quickly. Both mechanical forces and chemical effects are combined to the hydrolysis of acetyl groups in hemicellulose. High temperature promotes the auto hydrolysis to form acetic acid from acetyl groups. Water can act as an acid at high temperatures. The sudden depressure can result in fibers separated. During depressure, the hemicellulose hydrolyzed and solubilized and the lignin is removed from the material (Pan et al., 2005). Both the degree of enzyme accessibility and the cellulose surface are increased during the

explosion.

Particle size, temperature, residence time and combined effect of both temperature (T) and time (t) are most important factors affecting the steam explosion. The relationship of T and t is described by the severity factor (Ro) [$Ro = t * e^{[T-100/14.75]}$]. The optimal conditions for maximum sugar yield have a severity factor between 3.0 and 4.5 (Alfani et al., 2000).

Steam explosion has a low environmental impact and high energy efficiency (Avellar and Glasser, 1998). A good aspect which needs a mention is that large chip size could be used.

2.4.4.2 Liquid hot water

In liquid hot water pretreatment, water is kept in the liquid state at elevated temperatures from 160 to 240°C to cause structure disruption of lignocellulosics. Liquid hot water pretreatment does not require rapid decompression and does not need any catalyst or chemicals.

Liquid hot water is mainly to solubilize the hemicellulose and make the cellulose accessible and avoid inhibitor formation. The mixture of pretreated lignocellulosic material should be divided to two parts: a solid cellulose-enriched fraction and a liquid fraction rich in hemicellulose derived sugars. The pH should be kept between 4 and 7 to avoid the formation of inhibitors (Mosier et al., 2005a).

More than 80% of the hemicellulose is removed by liquid hot water pretreatment which would help the enzymatic digestibility of pretreated material (Mosier et al., 2005a). More hemicellulose and lignin can be removed by flow through system than batch systems (Wyman et al., 2005a).

2.4.4.3 Ammonia fiber explosion (AFEX)

In a typical AFEX process, biomass is treated in the liquid anhydrous ammonia with a high pressure at a temperature between 60 and 100°C. When the pressure is released, a rapid expansion of ammonia gas causes swelling and physical disruption of the biomass fibers and decrystallization. AFEX decreases crystallinity of cellulose and disrupts the linkages between lignin and carbohydrates (Laureano-Pérez et al., 2005). Only small amount of solid material is solubilized and nearly no hemicellulose and lignin is degraded (Wyman et al., 2005a). Acetyl groups from certain lignocellulosic materials can be removed by AFEX (Kumar et al., 2009b). AFEX pretreatment can increase the biomass digestibility and both cellulases and hemicellulases would be required for the subsequent enzymatic hydrolysis (Galbe and Zacchi, 2007). Ammonia has a characteristic of volatility but it is still feasible to recovery and recycles (Teymouri et al., 2005). However, the high cost of construction and complexity of the system make it difficult to commercialize the AFEX pretreatment technology (Eggeman and Elander 2005; Mosier et al., 2005b).

2.4.4.4 Microwave pretreatment

Both thermal and non-thermal effects are included in microwave-based pretreatment, so it is considered as a physicochemical process. In microwave pretreatment, biomass was first immersed in the chemical reagents and pretreated with microwave radiation for 5 to 20 min (Keshwani, 2009). Some studies concluded that alkalis are ideal reagents for microwave-based pretreatment (Zhu et al., 2006).

2.5 CONCLUSION

Ethanol produced from lignocellulosic materials has a great potential to replace the current petroleum-based gasoline for transportation. Different methods of pretreatment are reviewed above and both the advantages and drawbacks are illustrated. Through the review and analysis we have concluded that among all kinds of pretreatment, chemical and thermochemical methods are hopeful for large scale applications.

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CHAPTER 3

**PRETREATMENT OF CORN STOVER FOR ETHANOL PRODUCTION BY USING
THE COMBINATION OF ALKALINE REAGENTS**

ABSTRACT

Corn stover pretreatment using the combination of sodium hydroxide (NaOH) and calcium oxide (CaO) was investigated in this study at room temperature for improved cost-effectiveness of biomass-to-sugar conversion. The effects of NaOH concentration, CaO loading, and residence time on enzymatic hydrolysis were studied and the total reducing sugar yield in enzymatic hydrolysis was used to evaluate pretreatments. The results show that, compared with NaOH pretreatments, pretreatments with the combination of NaOH and CaO resulted in similarly high sugar productions but at substantially lower costs. The addition of CaO not only increased the alkalinity, which favored biomass digestibility improvement, but also contributed to better biomass preservation. Based on sugar production and cost-benefit considerations, the two recommended pretreatment conditions were 3h, 0.5% NaOH, 0.1 g CaO g⁻¹ raw biomass and 6h, 0.5% NaOH, 0.05 g CaO g⁻¹ raw biomass, at which the total reducing sugar yields were respectively 529.0 and 538.9 mg g⁻¹ raw biomass, at overall carbohydrate conversions of 75.6 and 77.0%, respectively.

3.1 INTRODUCTION

Fuel ethanol is considered as one of the best alternative/supplementary fuels to gasoline to reduce US dependence on foreign oil. It not only can be produced on a renewable basis from a variety of biomass feedstocks, but also contributes to the reduction of automobile tailpipe emissions by promoting more complete fuel combustion. Corn grain is currently the predominant feedstock for the production of fuel ethanol in the US. In 2010, the US ethanol industry produced 13 billion gallons of fuel ethanol from 4.65 billion bushels of corn grain, at an increase of 23% over the previous year (Renewable Fuels Association, 2011). However, due to the limitation in the agricultural land and resources needed for food and feed production, corn conversion for energy purposes negatively affects the food/feed provision (Sun, Y.; Cheng, J., 2002). The growing concerns over the sustainability of corn ethanol have stimulated explorations for non-edible biomass feedstocks. Corn stover refers to stalks, leaves, and cobs that remain in corn fields after the grain harvest and is considered as a potential biomass feedstock for ethanol production due to its great amount and easy access. Corn stover has a carbohydrate content of about 60% of the dry matter (National Renewable Energy Laboratory) and it is expected that, if universal no-till production of corn is adopted in the US, over 100 million tons of corn stover could be collected annually without causing erosion to exceed the tolerable soil loss (Graham, R.L. et al., 2007).

Like all other lignocellulosic feedstocks, the conversion of corn stover to ethanol is more challenging than that of corn grain because of the complex structure of lignocellulosic materials. Lignocellulose consists of three major components: cellulose, hemicellulose, and lignin. They form a recalcitrant structure that resists the access of hydrolytic enzymes to

carbohydrates, mainly cellulose and hemicellulose, for fermentable sugar production. An effective pretreatment step, therefore, is required to reduce this recalcitrance by altering the chemical and structural features of the biomass. Sodium hydroxide (NaOH) pretreatment at high temperatures ($>100^{\circ}\text{C}$) has been extensively studied to facilitate the enzymatic hydrolysis of lignocellulosic biomass and proven effective on a variety of feedstocks (Xu, J. et al., 2010). At alkaline conditions, the lignin, which stiffens and surrounds the fibers of polysaccharides, is effectively removed, the hemicellulose is partially degraded, and the recalcitrance of lignocellulose is considerably reduced through solvation and saponification reactions (Xu, J. et al, 2010;Hendriks, A.T.W.M.; Zeeman, G., 2009). However, at reduced temperatures, research shows that substantially higher chemical loadings and extended residence times are required for effective pretreatments (Graham, R.L., 2007) which would inevitably compromise the cost-effectiveness of the process and affect the application perspective of this technology.

Our previous study showed that adding cheap lime ($\text{Ca}(\text{OH})_2$) in the NaOH pretreatment of switchgrass helped to reduce the requirement for the more costly NaOH without negatively affecting the pretreatment efficiency (Xu, J.; Cheng, J.J., 2011). Although $\text{Ca}(\text{OH})_2$ barely dissolves in water and was not very effective in treating lignocellulose at room temperature (Xu, J., Cheng, J.J. et al., 2010), it not only replaces a significant portion of NaOH alkalinity at a low cost, but also helps to maintain a high pH throughout the pretreatment by gradually dissolving into the liquid phase to make up for the alkalinity consumed during pretreatment. In addition, the high solid loss that normally occurs during NaOH pretreatment can be prevented due to the presence of calcium ions. These positive

ions (each carries two positive charges) provide protection to biomass against solubilization under NaOH attack by forming linkages between negatively charged biomass components (Xu, J., Cheng, J.J. et al., 2010). Based on the previous work, the combination of the two alkaline reagents was investigated in this study to treat corn stover at room temperature for improved sugar production. Since calcium oxide (CaO) has even more desirable properties, it was used in place of Ca (OH)₂ in this study. CaO, or commonly referred to as quicklime, is not only much cheaper than Ca(OH)₂ in the chemical market (US Geological Survey) per unit weight of CaO is also able to provide more alkalinity. Moreover, the heat energy CaO produces when forming the hydrate (Ca (OH)₂) in water is favorable for reaction acceleration. The effects of NaOH loading, CaO loading, and residence time on the sugar production in enzymatic hydrolysis and the compositional change of biomass were investigated. The results were also compared with those of NaOH pretreatment to evaluate the comparative advantages of this technology in treating corn stover.

3.2 MATERIALS AND METHODS

3.2.1 Biomass Preparation.

The corn stover was collected in early October, 2009 from a farm belonging to Novozymes North America, Inc. in Franklinton, North Carolina. The corn variety is: AgVenture Variety R9534VBW. The stover was air dried and ground using a Thomas Wiley Laboratory Mill (Model No. 4) fitted with a 1 mm screen. The prepared biomass was collected in plastic bags, sealed, and stored at room temperature. The chemical composition

of the raw biomass was as follows: glucan 36.2%, xylan 20.1%, galactan 1.45%, arabinan 3.00%, lignin 21.2%, ash 1.56%, and others 16.5%.

3.2.2 Pretreatment.

Corn stover was pretreated at 21°C (room temperature) in serum bottles. Three g of dry biomass and a desired amount of CaO powder (0, 0.05, or 0.1 g g⁻¹ dry biomass) were mixed with 30 ml of NaOH solution of a specific concentration (0.25, 0.5, or 1%, w/v) in a serum bottle. All serum bottles were sealed and crimped before pretreatment to prevent water evaporation. The pretreatment lasted for 3, 6, or 9 h, after which the pretreated biomass was recovered by filtration and washed with 300 ml of deionized water to remove excess alkali and dissolved byproducts that might be inhibitory to the subsequent enzymatic hydrolysis. About 1 g of pretreated biomass (on a dry basis) was dried at 70°C to constant weight for composition analysis, and the rest was stored in a sealed plastic bag at 4°C for enzymatic hydrolysis.

3.2.3 Enzymatic Hydrolysis.

Enzymatic hydrolysis was carried out in 50 mL plastic tubes in a controlled reciprocal shaking water bath (Model C76, New Brunswick Scientific, Edison, NJ) at 50°C, 150 rpm for 72 h. A total of 0.5 g pretreated biomass (on a dry basis) was mixed with desired volume of 0.05 M sodium citrate buffer to reach a total volume of 15 mL and a pH of about 4.8. Cellic CTec 2 (aggressive cellulases and high level of β-glucosidase) at an enzyme loading of 40 FPU (filter paper unit) g⁻¹ dry biomass and Cellic HTec 2 (endoxylnase) at an enzyme loading of 75 FXU (fungal xylanase unit) / g dry biomass were added to the slurry. The enzyme loadings applied were made excessive to eliminate the impact of enzyme limitation

on sugar production. Both enzymes were obtained from Novozymes North America, Inc. (Franklinton, North Carolina, USA) and the activities of Cellic CTec 2 and Cellic HTec 2 were respectively 115.6 FPU/ml and 1090 FXU/ml. After the addition of enzymes, sodium azide (0.3%, w/v) was added to inhibit microbial growth during hydrolysis. After the hydrolysis time elapsed, the hydrolyzate was centrifuged at 8×10^3 g and the supernatant was collected and stored at -20 °C for sugar analysis at a later time.

3.2.4 Analytical Methods.

Total solids, ash, structural carbohydrates, and lignin of raw and pretreated biomass were determined using Laboratory Analytical Procedures (LAP) established by National Renewable Energy Laboratory (NREL) (Sluiter, A.; Hames, B. Et al, 2005). Total reducing sugars in the hydrolyzate were measured by the DNS (3, 5-dinitrosalicylic acid) method using glucose as the standard (Miller, G.L., 1959; Ghose, T.K., 1987). The major monomeric sugars present in the hydrolyzate, which include glucose and xylose, were determined using high performance liquid chromatography (HPLC). The HPLC system was equipped with a Bio-Rad Aminex HPX-87H column (300mm \times 7.8mm), a Bio-Rad Micro-Guard column, a thermostatic autosampler, an isocratic analytical pump, and a refractive index detector. The analytical column was operated at 80 °C with 0.005N H₂SO₄ as the mobile phase at a flow rate of 0.6 ml/min. The sugar profiles of raw and pretreated biomass were determined by measuring the monomeric sugars derived from composition analysis using Ion Chromatography (IC). The IC (Dionex ICS-5000, Dionex Corporation, Sunnyvale, CA) was equipped with a pulsed electrochemical detector. The column used was a CarboPac PA1 (4 x 250 mm) column which was operated at 18 °C with 0.018 M potassium hydroxide as the

mobile phase at a flow rate of 0.9 ml/min. All treatments in this study were conducted in triplicate. The GLM (General Linear Model) procedure with Tukey adjustment in SAS 9.1 software (SAS Institute Inc., Cary, NC) was used for all data analysis.

3.3 RERULTS AND DISCUSSION

3.3.1 Enzymatic Hydrolysis Improvement.

When CaO was reacted with water to form Ca (OH)₂ in the pretreatment slurry, the heat energy generated was not sufficient to considerably increase the pretreatment temperature. At the greatest CaO loading of 0.1 g g⁻¹ raw biomass, the slurry temperature increased by less than 1°C and dropped back to room temperature within just a few minutes. It is, therefore, expected that the heating effect of CaO-to-Ca (OH)₂ conversion can be neglected during corn stover pretreatment.

Solid recovery is an important parameter to evaluate pretreatment performance as it determines the total amount of biomass that can be potentially converted to sugars in enzymatic hydrolysis. Although the solubilization of biomass components including valuable carbohydrates is inevitable under alkaline attack, using the combination of NaOH and CaO could substantially reduce its extent. Table 3.1 shows that the solid recovery significantly ($P < 0.05$) decreased with the increase of NaOH concentration, while the change of residence time was not significantly ($P > 0.05$) associated with solid recovery. When CaO was used with NaOH, the solid recoveries were much higher. At room temperature, the solid recovery with CaO addition ranged from 76.4 to 88.7%, which was considerably higher than without CaO addition (71.2 - 82.3%). In addition, with the increase of CaO loading, the solid recovery

increased accordingly. This indicates that adding CaO, although causes an increase of alkalinity in the system, provided some extent of biomass preservation. This is consistent with the observation in our previous study on lime ($\text{Ca}(\text{OH})_2$) pretreatment of switchgrass (Xu, J., Cheng, J.J. et al, 2011). The formation of calcium linkages within the biomass structure provided a plausible explanation to this phenomenon, and more research is required to obtain a mechanistic understanding.

After pretreatment, the recovered biomass was subjected to enzymatic hydrolysis for sugar production. The total reducing sugar yield was used to evaluate the overall pretreatment performance. Since glucose and xylose are the dominant monomeric sugars in the hydrolyzate and can well represent the conversions of cellulose and hemicellulose, respectively, their yields were also determined to understand the impacts of pretreatment conditions on different carbohydrate components. Figure 3.1a shows that the total reducing sugar yield generally increased with the increase of NaOH concentration. However, the application of the highest NaOH concentration of 1% didn't necessarily result in the maximum sugar productions, which was probably because of the elevated carbohydrate solubilization. Adding CaO successfully increased sugar production, which demonstrates the effectiveness of supplementing CaO in NaOH pretreatment. However, applying the greatest CaO loading of 0.1 g g^{-1} raw biomass did not necessarily favor sugar production, and the sugar production even decreased with the increase of CaO loading from 0.05 to 0.1 g g^{-1} raw biomass at 1% NaOH. A plausible explanation is that, at an already intense pretreatment environment created by 1% NaOH, excessive alkalinity addition would cause more carbohydrate solubilization in spite of the improved biomass preservation by more available

calcium ions. Similarly, although extending residence time should favor the digestibility improvement of biomass, it was not always positively associated with the total reducing sugar yield due to the increased biomass solubilization at longer residence times. Based on the total reducing sugar yield and the potential pretreatment cost, 3 h, 0.5% NaOH, 0.1 g CaO g⁻¹ raw biomass and 6 h, 0.5% NaOH, 0.05 g CaO g⁻¹ raw biomass were selected as the recommended conditions for corn stover pretreatment at room temperature. At these conditions, the total reducing sugar yields in enzymatic hydrolysis were respectively 529.0 and 538.9 mg g⁻¹ raw biomass, at overall carbohydrate conversions of 75.6 and 77.0%, respectively. Further economic analysis weighing the impacts of residence time and CaO loading on the cost of ethanol production is required before the best pretreatment conditions for practical application can be determined.

The effects of pretreatment conditions on both glucose and xylose yields were similar to those on total reducing sugar yield (Figure 3.1b, 3.1c). At the recommended pretreatment conditions of 3 h, 0.5% NaOH, 0.1 g CaO g⁻¹ raw biomass, the glucose and xylose yields were respectively 320.1 and 137.4 mg g⁻¹ raw biomass, at overall glucan and xylan conversions of 79.5 and 60.2%, respectively. At the recommended pretreatment conditions of 6 h, 0.5% NaOH, 0.05 g CaO g⁻¹ raw biomass, the glucose and xylose yields were respectively 331.6 and 143.7 mg g⁻¹ raw biomass, at overall glucan and xylan conversions of 82.4 and 62.9%, respectively. The overall conversions of xylan were lower than those of glucan at all pretreatment conditions, which is due to the structural difference between hemicellulose and cellulose. Hemicellulose is amorphous, heterogeneous, branched, and has

low molecular weight, making it more susceptible to solubilization than semicrystalline cellulose during alkaline attack.

Due to the limited solubility of $\text{Ca}(\text{OH})_2$ in water, part of the $\text{Ca}(\text{OH})_2$ would remain as solids throughout the pretreatment period and be washed and wasted after biomass recovery. To reduce chemical waste, more accurate investigations are required to determine the best CaO loading. Based on the recommended pretreatment conditions (3 h, 0.5% NaOH, 0.1 g CaO g^{-1} raw biomass and 6 h, 0.5% NaOH, 0.05 g CaO g^{-1} raw biomass), CaO loadings of 0.075 - 0.125 and 0.025 - 0.075 g g^{-1} raw biomass was studied respectively (Figure 3.2). The results show that the best CaO loadings were 0.1 and 0.05 g g^{-1} raw biomass respectively for pretreatments at 3 h, 0.5% NaOH and 6 h 0.5% NaOH.

Pretreatments involving $\text{Ca}(\text{OH})_2/\text{CaO}$ usually raise concerns over their effects on the hydrolysis pH. It was noticed that, even after biomass washing, the residual $\text{Ca}(\text{OH})_2$ particles could still increase the pH of the subsequent enzymatic hydrolysis beyond the optimal range, thus reducing the sugar production (Xu, J.; Cheng, J.J., 2011). To investigate the effect of CaO-induced pH rise on the enzymatic hydrolysis of pretreated corn stover, the changes of hydrolysis pH were monitored. Figure 3.3 shows the pH change during the enzymatic hydrolysis of the corn stover pretreated using 1% NaOH for 9 h at different CaO loadings. Throughout the 72-h hydrolysis period, the pHs at different CaO loadings were quite stable, and there was a positive correlation between the CaO loading applied and the average pH. Although sodium citrate buffer (pH 4.8) was used for pH stabilization, the residual $\text{Ca}(\text{OH})_2$ could still significantly ($P < 0.05$) increase the hydrolysis pH. Nonetheless, the pH change was not expected to affect sugar production in a substantially way as the

Novozymes product data sheet indicated that, for practical application, the optimal pH is around 5.0 for Cellic CTec and 4.5-6.0 for Cellic HTec. Although it is theoretically possible to remove all the residual $\text{Ca}(\text{OH})_2$ particles through intensive biomass washing, increasing wash intensity inevitably results in greater solid loss, which would compromise sugar production in hydrolysis.

3.3.2 Lignin Reduction.

Lignin is a complex aromatic polymer present in plant cell wall, which confers structural support, impermeability, and resistance against microbial attack and oxidative stress (Pérez, J.; Muñoz-Dorado, J. et al, 2002). Since lignin is a major physical barrier limiting enzyme access to cellulose and hemicellulose, its removal/degradation is of great importance to the improvement of enzymatic hydrolysis. Figure 3.4 shows that higher NaOH concentrations generally resulted in greater lignin reductions. This is in agreement with the previous reports that NaOH is a strong base and can effectively remove lignin from lignocellulosic biomass (Silverstein, R.A., 2007). CaO addition caused reduced lignin removal, especially at higher NaOH concentrations when lignin was under more intense alkaline attack. The observed lignin preservation caused by CaO addition is consistent with the reports that divalent calcium ions from $\text{Ca}(\text{OH})_2$ dissociation have high affinity for lignin and can effectively crosslink lignin molecules (Sundin, J.; Hartler, N. et al, 2000). Residence time didn't have a significant ($P > 0.05$) effect on lignin reduction. A plausible explanation is that, lignin removal from corn stover occurred during the initial stage of pretreatment (probably less than 3 h) when the high initial pH restrained the dissociation of

Ca(OH)₂ to produce Ca²⁺ and form calcium linkages among lignin molecules for their preservation.

3.3.3 Material Balances.

Material balances were performed on raw biomass and the biomass pretreated using the recommended pretreatment conditions (3 h, 0.5% NaOH, 0.1 g CaO g⁻¹ raw biomass and 6 h, 0.5% NaOH, 0.05 g CaO g⁻¹ raw biomass). After pretreatment at the recommended conditions, the total dry weight of biomass was determined and the biomass components including glucan, xylan, galactan, arabinan, lignin, ash, and others were determined and compared with those of the raw biomass. Figure 3.5 shows that the pretreatment using the combination of NaOH and CaO resulted in satisfying carbohydrate preservation. After pretreatment at 3 h, 0.5% NaOH, 0.1 g CaO g⁻¹ raw biomass and 6 h, 0.5% NaOH, 0.05 g CaO g⁻¹ raw biomass, the glucan reductions were respectively 6.07 and 2.21%, while the xylan reductions were respectively 13.7 and 11.8% which were higher than those of glucan due to the more susceptible structure of hemicellulose. Changes of galactan and arabinan were similar to that of xylan. It can be concluded that, although the total amount of carbohydrates in the biomass were not substantially changed after pretreatment, the biomass structure had been remarkably altered, which led to a great improvement of enzymatic hydrolysis. The impact of pretreatment on non-carbohydrate components was more significant. Lignin, ash, and other undefined components including protein, waxes, fats, resins, gums, and chlorophyll (Kuhad, R.C.; Singh, A., 1993; Sluiter, A. Et al, 2005) were all considerably removed.

3.3.4 A Comparison with NaOH Pretreatment.

Since the total reducing sugar yield of the pretreatment using 1% NaOH for 9 h was comparable with those at recommended conditions, a comparison was conducted between the pretreatment using the combination of NaOH and CaO and that using NaOH alone. Table 3.2 shows that the pretreatment using 1% NaOH resulted in a much greater lignin reduction. A high overall carbohydrate conversion could also be achieved at the expense of a more intensive biomass solubilization. However, the potential cost of NaOH pretreatment was much higher than those of using the combination of NaOH and CaO. Assuming that the market price of NaOH is 4.5 times as much as that of CaO (Lerner, I., 2007) to produce similar amount of sugar for ethanol fermentation, the chemical cost of the pretreatment using 1% NaOH for 9 h is 38.5 and 63.6% higher than those of the pretreatments respectively at 3 h, 0.5% NaOH, 0.1 g CaO g⁻¹ raw biomass and 6 h, 0.5% NaOH, 0.05 g CaO g⁻¹ raw biomass. Moreover, the more extended residence time required in NaOH pretreatment will result in much higher capital investment and operational cost in practical applications. The preliminary comparison indicates that corn stover pretreatment using the combination of NaOH and CaO is a more promising technology than NaOH pretreatment at room temperature.

3.4 CONCLUSIONS

Compared with NaOH pretreatment at room temperature, the pretreatment of cover stover using the combination of NaOH and CaO was proven effective in reducing the pretreatment cost without compromising the sugar yield in enzymatic hydrolysis. After

pretreatment at recommended conditions of 3 h, 0.5% NaOH, 0.1 g CaO g⁻¹ raw biomass and 6 h, 0.5% NaOH, 0.05 g CaO g⁻¹ raw biomass, the overall conversions of glucan in raw biomass were respectively 79.5 and 82.4%, and those of xylan respectively reached 60.2 and 62.9%. The application of CaO resulted in substantially reduced lignin reductions. Nonetheless, the sugar yield in hydrolysis was still high despite the presence of a large amount of lignin in the biomass. It can be concluded that, instead of total lignin reduction, the structural alternations of lignin and the breakup of lignin-carbohydrate complex at alkaline conditions were responsible for the improved enzyme access to carbohydrates. However, report shows that lignin limits enzyme access to carbohydrates not only through posing physical barrier, but also by causing unproductive binding of enzymes (Palonen, H., 2004). It is necessary, therefore, to further investigate the impact of this pretreatment technology on enzyme loading in the future work.

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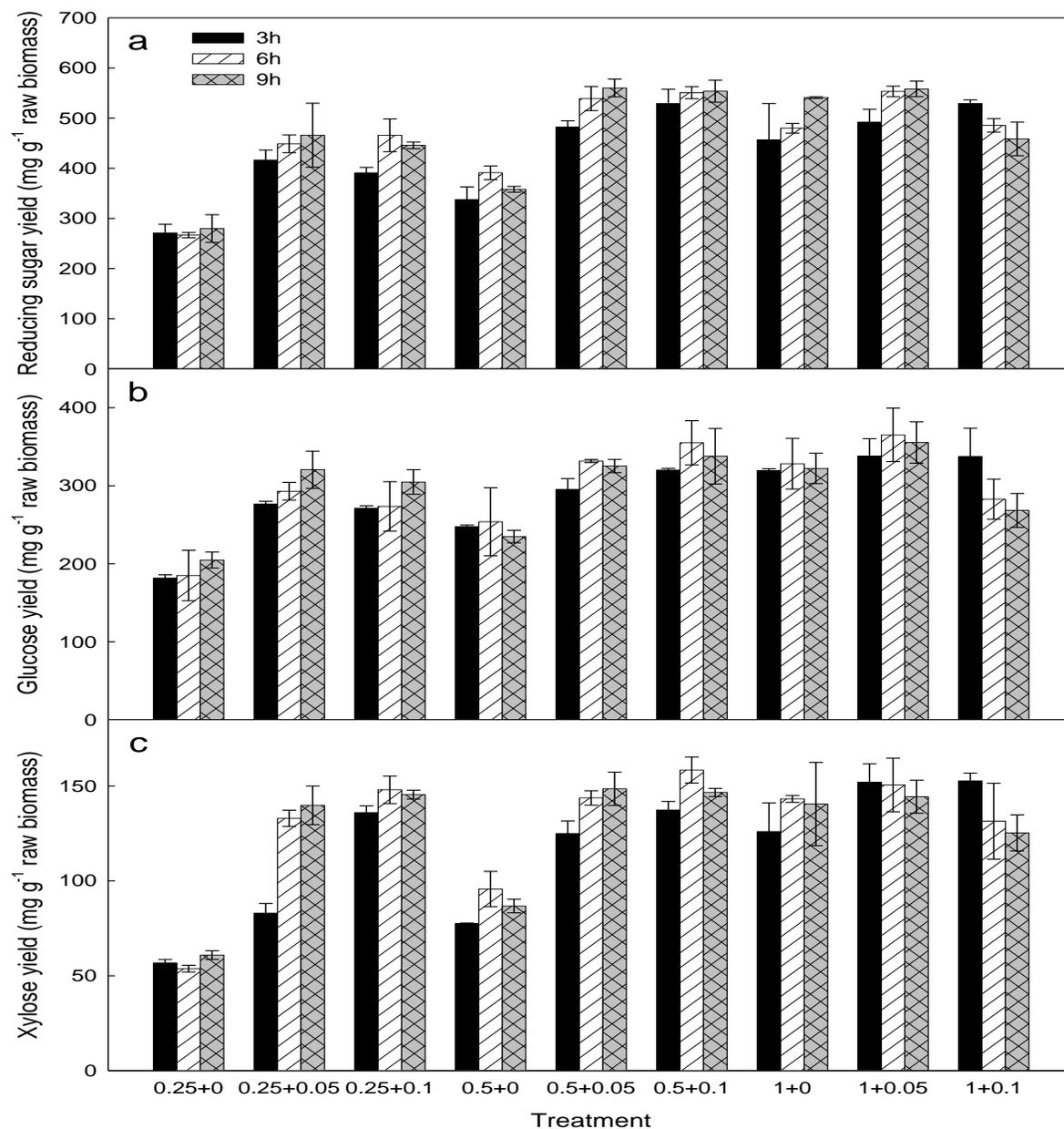


Figure 3.1 Yields of (a) total reducing sugars, (b) glucose, and (c) xylose in enzymatic hydrolysis of corn stover pretreated using the combination of NaOH and CaO at room temperature (the tick label of the horizontal axis: A + B represents A% NaOH + B g CaO g⁻¹ raw biomass).

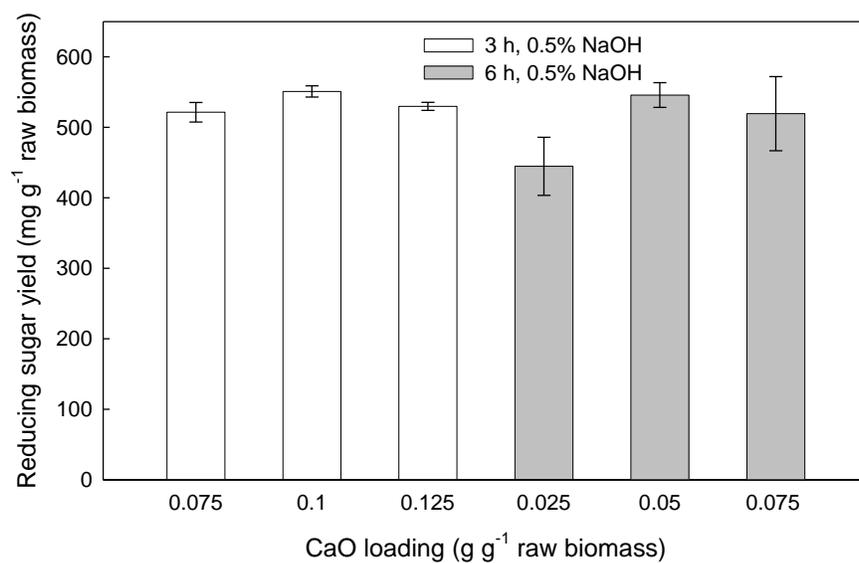


Figure 3.2 Total reducing sugar yields in the enzymatic hydrolysis of corn stover pretreated at recommended residence times and NaOH concentrations, and different CaO loadings.

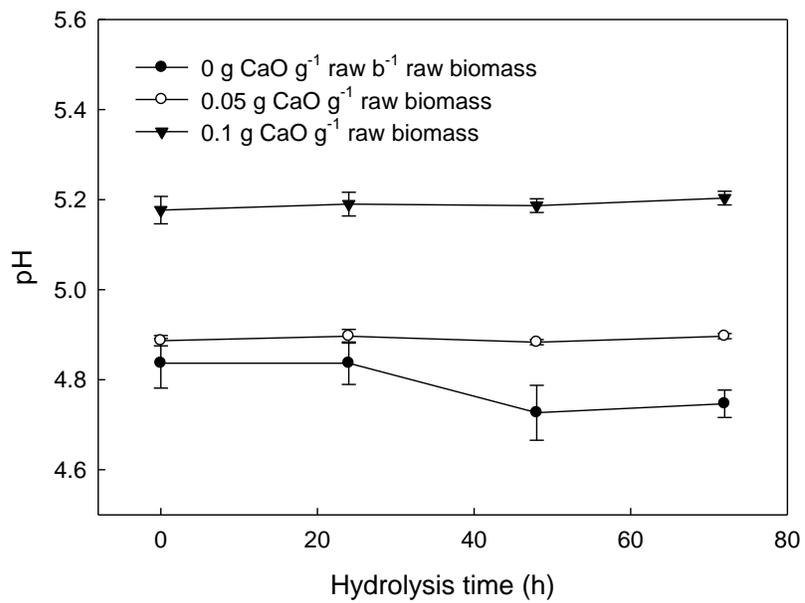


Figure 3.3 Changes of pH during enzymatic hydrolysis of the corn stover pretreated using 1% NaOH for 9 h at different CaO loadings.

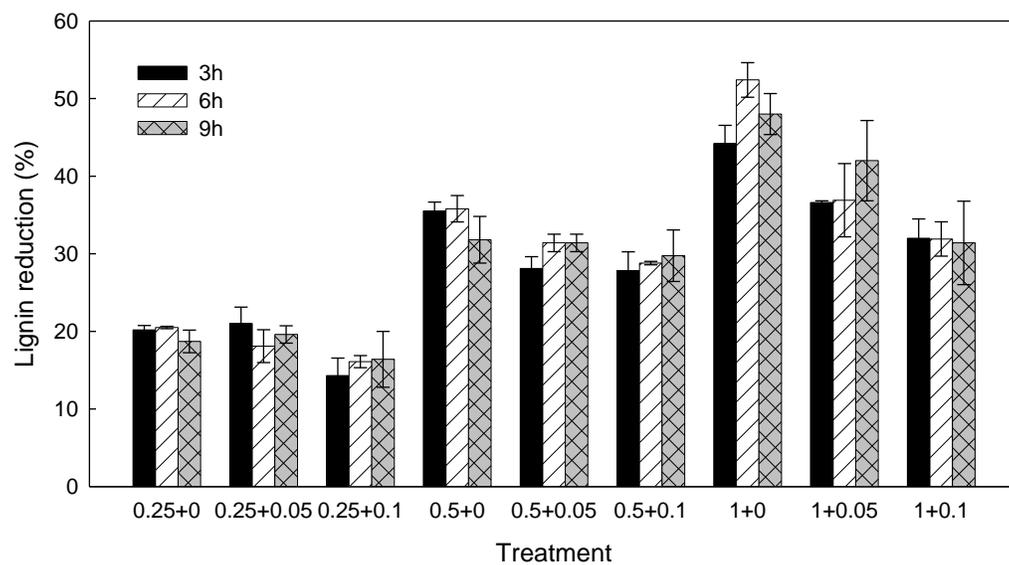


Figure 3.4 Lignin reductions of corn stover pretreated using the combination of NaOH and CaO at room temperature (the tick label of the horizontal axis: A + B represents A% NaOH + B g CaO g⁻¹ raw biomass)

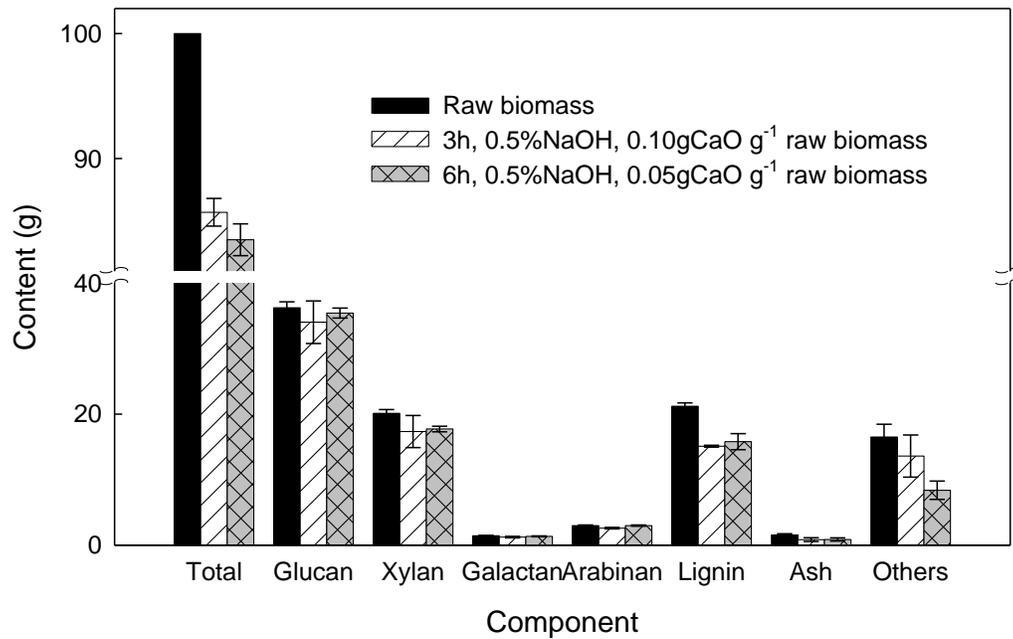


Figure 3.5 Material balances for raw corn stover and the corn stover pretreated at room temperature under the recommended conditions (3 h, 0.5% NaOH, 0.1 g CaO g⁻¹ raw biomass and 6 h, 0.5% NaOH, 0.05 g CaO g⁻¹ raw biomass).

Table 3.1 Solid Recoveries after Pretreatments with the Combination of NaOH and CaO at

Room Temperature

Residence time (h)	NaOH concentration (%, w/v)	Percent solid recovery (%)		
		CaO loading (g g^{-1} raw biomass)		
		0	0.05	0.1
3	0.25	82.3 (1.65) *	83.6 (0.52)	88.7 (1.11)
	0.5	79.3 (0.77)	82.5 (2.47)	85.7 (1.11)
	1	73.4 (0.64)	79.5 (1.25)	85.7 (2.05)
6	0.25	81.7 (0.51)	82.0 (1.30)	85.7 (1.40)
	0.5	80.1 (1.47)	83.5 (1.27)	88.5 (0.47)
	1	73.1 (1.57)	76.4 (1.41)	85.2 (2.58)
9	0.25	81.0 (0.92)	81.5 (0.42)	84.8 (1.80)
	0.5	79.0 (1.54)	79.8 (0.02)	85.4 (1.88)
	1	71.2 (1.37)	76.9 (1.95)	83.3 (0.63)

* The number in parentheses is standard deviation of triplicate samples.

Table 3.2 Comparison between pretreatments using NaOH + CaO and NaOH alone

Pretreatment	Solid recovery (%)	Glucan conversion (%)	Xylan conversion (%)	Lignin reduction (%)	Potential cost
3 h, 0.5% NaOH, 0.1g CaO g ⁻¹ raw biomass	85.7 (1.11)	79.5 (1.44)	60.2 (3.66)	27.8 (1.70)	Low
6 h, 0.5% NaOH, 0.05g CaO g ⁻¹ raw biomass	83.5 (1.27)	82.4 (3.92)	62.9 (0.11)	31.4 (2.43)	
9 h, 1% NaOH	71.2 (1.37)	80.2 (4.27)	62.4 (0.83)	48.0 (1.04)	high

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CHAPTER 4
PRETREATMENT OF CORN STOVER FOR SUGAR PRODUCTION WITH
SWITCHGRASS-DERIVED BLACK LIQUOR

ABSTRACT

Switchgrass and corn stover are two of the most intensively studied herbaceous feedstocks for sugar production. To improve the cost-effectiveness of biomass-to-sugar conversion, sodium hydroxide (NaOH) pretreatment of switchgrass was carried out at room temperature using the previously determined best conditions, and the spent alkaline liquid (black liquor) was recycled to pretreat corn stover, the feedstock exhibiting a higher susceptibility to alkaline attack, for improved enzymatic hydrolysis. The results show that, because of the high pH and the appreciable amount of carbohydrates in the initial black liquor, the sugar productions of the corn stover pretreated with the black liquor were comparable with those of the biomass pretreated with 1% NaOH in enzymatic hydrolysis. After black liquor pretreatment at the best residence time of 24 h, the total reducing sugar, glucose, and xylose yields of corn stover reached 478.5, 287.7, and 145.3 mg/g raw biomass, respectively.

4.1 INTRODUCTION

Cellulosic ethanol is a renewable and environmentally friendly transportation fuel that can be produced from a variety of non-edible lignocellulosic feedstocks including dedicated energy crops, agricultural residues, and forestry wastes (Xu et al., 2010a). The conversion of lignocellulose to ethanol includes three basic steps: pretreatment of raw biomass, enzymatic hydrolysis for sugar production, and ethanol fermentation. Great efforts have been devoted to the efficiency improvement of each of these steps to make cellulosic ethanol economically competitive. Due to the complex composition of lignocellulose and its recalcitrant structure, an effective pretreatment of raw biomass is considered essential to the success of the subsequent processes. The purpose of pretreatment is to remove lignin barrier from the lignocellulosic material and alter biomass structure so that cellulose and hemicellulose can be more accessible to enzymes in the hydrolysis for fermentable sugar production (Cheng, 2010).

Alkaline pretreatment is one of the most intensively studied pretreatment technologies and alkaline reagents including sodium hydroxide (NaOH), lime ($\text{Ca}(\text{OH})_2$), and aqueous ammonia (NH_3) have been proven effective on many biomass feedstocks (Xu and Cheng, 2011; Wang et al., 2010; Xu et al., 2010b; Kim and Lee, 2005a). Alkaline pretreatment is efficient in biomass delignification and causes saponification of intermolecular ester bonds crosslinking various biomass components (Sun and Cheng, 2002). Among the above mentioned alkaline reagents, $\text{Ca}(\text{OH})_2$ and NH_3 have attracted much attention because they can be easily recycled after pretreatment with established lime kiln technology and through evaporation, respectively (Kim and Holtzapple, 2005; Kim and Lee, 2005b). However, since

they are weak bases, it normally takes long residence times for them to effectively break up lignocellulose structure, especially at mild temperatures. In contrast, although NaOH is a strong base and has been proven effective even at mild temperatures (Xu et al., 2010a), its application prospect is compromised by the high chemical cost resulted from the difficulty in the chemical recycling. It is expected, therefore, that the procedures helping to recycle/reuse the spent NaOH solution will definitely improve the economic promise of this pretreatment technology. Fox et al. (1989) once investigated the application of spent alkaline liquid (black liquor) in the NaOH pretreatment of bagasse. The results showed that, at a NaOH loading of 0.1 g/g raw biomass, 35% of the original alkali remained after pretreatment and could be reused for another round of pretreatment. Nonetheless, NaOH supplementation was required to maintain the pretreatment effectiveness. Based on the different susceptibilities of biomass feedstocks to NaOH pretreatment, it is possible to collect the black liquor from the NaOH pretreatment of a less susceptible feedstock and use it in the pretreatment of a more susceptible feedstock to avoid supplementing NaOH and, consequently, reduce the overall chemical cost. The mix of feedstocks received by an ethanol plant also requires the development of such an economically sound pretreatment procedure that enables the utilization of recycled black liquor to reduce the cost of ethanol production.

Switchgrass and corn stover are the two most extensively studied lignocellulosic feedstocks for ethanol production. Switchgrass is a perennial grass native to North America. It is regarded as a promising dedicated energy crop due to its excellent yield and low agricultural inputs for cultivation (Keshwani and Cheng, 2009). Corn stover, on the other hand, has attracted much attention because of its abundance and readily availability (Zhang et

al., 2011). Our previous studies and preliminary experiments showed that, compared with switchgrass, corn stover is more susceptible to NaOH pretreatment at room temperature (Zhang et al., 2011; Xu et al., 2010a). For switchgrass pretreatment, 2% NaOH was required to maximize sugar production, while 1% NaOH was sufficient for corn stover. Therefore, in this study, black liquor from the NaOH pretreatment of switchgrass at the previously determined best conditions was collected and used for the pretreatment of corn stover at room temperature. The pretreatments were evaluated by the sugar production of corn stover in the subsequent enzymatic hydrolysis and the results were compared with those of the pretreatments using water and NaOH solutions of different concentrations to better understand the performance of the black liquor.

4.2 MATERIALS AND METHODS

4.2.1 Biomass preparation

“Performer” switchgrass was harvested in late July 2007 from the Central Crops Research Station near Clayton, North Carolina. Corn stover (AgVenture Variety R9534VBW) was harvested in early October 2009 from a farm belonging to Novozymes North America, Inc. in Franklinton, North Carolina. Both biomass feedstocks were oven dried at 50°C for 72 hours, ground to pass a 2 mm sieve in a Thomas Wiley Laboratory Mill (Model No. 4), and stored in sealed plastic bags at room temperature.

4.2.2 Pretreatment

Switchgrass was pretreated at room temperature using 2% NaOH (w/v) for 6 h, which was the best condition determined in our previous study (Xu et al., 2010a). A total of 300 g

of dry switchgrass biomass was mixed with 3 L of NaOH solution in glass bottles for pretreatment. After the pretreatment time elapsed, the pretreated biomass was recovered by filtration and the black liquor was collected and stored at 4°C for the pretreatment of corn stover at a later time. To better evaluate the performance of black liquor, the pretreatments of corn stover were also conducted at room temperature by using deionized (DI) water, 1% NaOH, and 2% NaOH for comparison. Three grams of corn stover biomass (dry basis) was mixed with 30 mL of pretreatment liquid in a serum bottle. All serum bottles were sealed to prevent water evaporation and the samples were pretreated for 3, 6, 12, 24, and 48 h. After pretreatment, the pretreated biomass was recovered by filtration, washed with 300 mL of DI water to remove excess alkali, and stored in sealed plastic bags at 4°C before enzymatic hydrolysis.

4.2.3 Enzymatic hydrolysis

A total of 0.5 g pretreated biomass (dry basis) was mixed with desired volume of 0.05 M sodium citrate buffer in a 50 mL plastic tube to reach a total volume of 15 mL and a pH of 4.8. Cellic CTec (aggressive cellulases and high level of β -glucosidase) and Cellic HTec (endoxylnase) provided by Novozymes North America, Inc. (Franklinton, North Carolina, USA) were added to the mixture, making a final solid loading of 3.3% for hydrolysis. The activities of Cellic CTec and Cellic HTec were 115.6 FPU (filter paper unit)/mL and 1090 FXU (fungal xylanase unit)/mL, respectively, and excessive enzyme loadings of 40 FPU g/g dry biomass and 75 FXU g/dry biomass were applied, respectively, to avoid enzyme limitation. After the addition of enzymes, sodium azide (0.3%, w/v) was added to inhibit microbial growth during the hydrolysis. The samples were incubated in a controlled

reciprocal shaking water bath (Model C76, New Brunswick Scientific, Edison, NJ) at 50°C, 150 rpm for 72 h. After the hydrolysis time elapsed, the hydrolyzate was centrifuged at 8×10^3 g and the supernatant was collected and stored at -20 °C for sugar analysis at a later time. The pretreatments were evaluated and optimized based on the total reducing sugar yield in enzymatic hydrolysis. After the best conditions were determined for black liquor pretreatment, the loadings of Cellic CTec (0-40 FPU/g dry biomass) and Cellic HTec (0-75 FXU/g dry biomass) were studied and optimized.

4.2.4 Analytical methods

Total solids, structural carbohydrates, lignin, and ash of switchgrass and corn stover were determined using Laboratory Analytical Procedures (LAP) established by National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008; Sluiter et al., 2005a; Sluiter et al., 2005b). Total reducing sugars in the hydrolyzate were measured by the 3,5-dinitrosalicylic acid (DNS) method using glucose as the standard (Miller, 1959; Ghose, 1987). The monomeric sugars including glucose produced mainly from the hydrolysis of cellulose and xylose, the dominant hydrolysis product from hemicelluloses, were determined using high performance liquid chromatography (HPLC). The HPLC system was equipped with a Bio-Rad Aminex HPX-87H column (300 mm \times 7.8 mm), a Bio-Rad Micro-Guard column, a thermostatic autosampler, an isocratic analytical pump, and a refractive index detector. The analytical column was operated at 80 °C with 0.005N H₂SO₄ as the mobile phase at a flow rate of 0.6 mL/min. The samples were injected at 20 μ L and the acquisition time was 25 min. All treatments in this study were conducted in triplicate. The GLM (General Linear Model)

procedure with Tukey adjustment in SAS 9.1 software (SAS Institute Inc., Cary, NC) was used for statistical analysis.

4.3 RESULTS AND DISCUSSION

4.3.1 Biomass composition

The chemical compositions of raw switchgrass and corn stover are reported in Table 4.1. For both biomass feedstocks, the combination of glucan and xylan composes more than 92% of the total carbohydrates, which is consistent with the fact that glucose and xylose are the monomer unit and major building block of cellulose and hemicellulose, respectively. The carbohydrate content of corn stover is 13.6% higher than that of switchgrass, which makes corn stover a better feedstock for biochemical conversion to ethanol in terms of total sugar provision. The lignin contents of switchgrass and corn stover were similar and in agreement with the typical lignin contents of herbaceous crops and agricultural residues (McMillan, 1994). Compared with corn stover, switchgrass contains more ash as well as other biomass components which may include protein, waxes, fats, resins, gums, and chlorophyll (Sluiter et al., 2005c; Kuhad and Singh, 1993).

4.3.2 Study on black liquor pretreatment

After pretreatment of switchgrass at room temperature using the previously determined best conditions (2% NaOH and 6 h), the black liquor was collected for corn stover pretreatment. The sugar production of the pretreated switchgrass in enzymatic hydrolysis is not reported in this study and it is assumed that the result is the same as that obtained in our previously published work (Xu et al., 2010a). After pretreatment, the total reducing sugar

yield of switchgrass in enzymatic hydrolysis was 406.2 mg/g raw biomass, with an overall carbohydrate conversion of 67.8%.

Solid recovery is normally measured as an important parameter to evaluate the biomass preservation during pretreatment. Table 4.2 shows that, except for water pretreatment, solid recovery significantly ($P < 0.05$) decreased with the extension of residence time for all the pretreatments. At the same residence time, applying 2% NaOH resulted in the lowest solid recoveries due to the most intense alkaline conditions it created, while the solid recoveries at 1% NaOH and black liquor were considerably lower and comparable. According to the literature (Silverstein et al., 2007; MacDonald et al., 1983), although lignin is the major biomass component solubilized during NaOH pretreatment, some carbohydrates, especially hemicellulose, will also dissolve into the pretreatment liquid under intense alkaline attack, which inevitably affects the sugar production in enzymatic hydrolysis. Therefore, it is expected that the pretreatment with 2% NaOH, which maximized the sugar production of switchgrass (Xu et al., 2010a), might be too strong for corn stover and would compromise its sugar production.

After enzymatic hydrolysis of the pretreated biomass, the total reducing sugars in the hydrolyzate were measured and used to evaluate the overall pretreatment effectiveness. The yields of glucose and xylose were also determined to understand the conversions of cellulose and hemicellulose, respectively, during pretreatment. The results show that water pretreatment was not sufficient to substantially improve the scarification of corn stover, and the maximum total reducing sugar yield obtained was 208.5 mg/g raw biomass, with an overall carbohydrate conversion of 30.6% (Fig. 4.1a). Generally, the sugar yield increased

with the extension of residence time. For the pretreatments with 1% NaOH, 2% NaOH, and black liquor, the best residence times for sugar production were, 24, 12, and 24 h, respectively, at which the total reducing sugar yields were 488.3, 432.2, and 478.5 mg/g raw biomass, 71.7, 63.5, and 70.3% of the theoretical yield, respectively. The sugar productions at 2% NaOH were considerably lower than those at 1% NaOH and black liquor at any residence times longer than 3 h. This is because of the increased solubilization of carbohydrates, especially hemicellulose, during the pretreatment in an overly harsh alkaline environment created by 2% NaOH. Pretreatments using black liquor resulted in sugar yields comparable with those using 1% NaOH. The outstanding performance of black liquor in improving the enzymatic hydrolysis of corn stover could be attributed to two factors. The first factor is the high pH of black liquor. Fig. 4.2 shows the changes of pH during the pretreatment of corn stover with 1% NaOH, 2% NaOH, and black liquor. The initial pH of the slurry formed by mixing biomass and black liquor was 12.01, which, although significantly ($P < 0.05$) lower than that of the slurry made by using 1% NaOH or 2% NaOH, was sufficient to cause a substantial alteration of lignocellulose structure. After pretreatment, all the pHs were significantly ($P < 0.05$) reduced, indicating the consumptions of alkalinity during the reactions. The second factor is the carbohydrates present in the initial black liquor collected from switchgrass pretreatment. Table 4.3 shows the sugars and lignin that were derived from the initial black liquor and the black liquor collected after the pretreatment of corn stover for 24 h. After pretreatment of switchgrass at room temperature using 2% NaOH for 6 h, 48.4% of the lignin in the raw biomass was degraded and solubilized, resulting in a lignin concentration of 9.79 g/L in the initial black liquor. In the meantime, 8.2% of the

glucan and 32.3% of the xylan in the raw biomass were also solubilized. This contributed to the measurements of considerable glucose and xylose in the initial black liquor. The high carbohydrate content of black liquor is likely to increase the sugar production of corn stover in the enzymatic hydrolysis. This is because the dry corn stover was immersed in black liquor for 24 h and became saturated with the carbohydrate-rich liquid during black liquor pretreatment. Although biomass washing was conducted after pretreatment to remove excess alkali, a moderate wash intensity of 100 mL DI water/g raw biomass was applied to avoid carbohydrate loss, which might not be sufficient to wash away all the dissolved carbohydrates. This means that a considerable amount of dissolved carbohydrates contained in the pretreated corn stover, which normally has a moisture content of over 80%, could be from the initial black liquor, and these carbohydrates, together with the carbohydrates of corn stover, were converted to sugars in the subsequent enzymatic hydrolysis. Table 4.3 also shows that more sugars could be derived from the black liquor after corn stover pretreatment. This makes the second round of black liquor reutilization possible. However, alkalinity supplementation might be required due to the considerably reduced pH after the pretreatment.

The effects of pretreatment method and residence time on glucose yield were similar to those on total reducing sugar yield (Fig. 4.1b), except that the glucose yields after pretreatment using 2% NaOH were comparable with those after pretreatments using 1% NaOH and black liquor. This is because that cellulose, the linear chain of glucose, has a semicrystalline structure, which made it less susceptible to solubilization in the harsh alkaline environment. The best residence times for glucose yield were the same as those for total reducing sugar yield for 1% NaOH, 2% NaOH, and black liquor pretreatment (24, 12, and 24

h, respectively), and the corresponding glucose yields were, respectively, 294.0, 294.4, and 287.7 mg/g raw biomass, which were 73.1, 73.2, and 71.5% of the theoretical yield, respectively. Residence time did not have a significant ($P>0.05$) effect on xylose yield, which is different from its effect on glucose yield (Fig. 4.1c). This can be explained by the distinct properties between these two carbohydrates. Unlike semicrystalline cellulose, hemicellulose is a group of polysaccharides with heterogeneous, amorphous, and branched structures and has much lower molecular weights. Although extending residence time favored the reduction of cellulose crystallinity and, consequently, improved glucose yield in enzymatic hydrolysis, it caused an increase in hemicellulose solubilization. Compared with NaOH pretreatment, the xylose yields of corn stover were significantly ($P<0.05$) higher after black liquor pretreatment in most cases. Since hemicellulose-derived sugar oligomers/monomers composed most of the carbohydrates in the initial black liquor, this result is consistent with our earlier speculation that the considerable carbohydrate content of the initial black liquor is likely to have caused the high sugar productions of the pretreated corn stover. The best residence times for xylose yield were 6, 3, and 6 h for 1% NaOH, 2% NaOH, and black liquor pretreatment, respectively, and the corresponding xylose yields were, 131.0, 121.4, and 145.3 mg/g raw biomass, respectively.

To determine the real conversions of biomass carbohydrates facilitated by black liquor pretreatment, mass balances were performed on total reducing sugars, glucose, and xylose to exclude the contribution of the carbohydrates in the initial black liquor to the sugar production of corn stover in enzymatic hydrolysis. Table 4.4 shows that, before excluding the contributions of the carbohydrates in the initial black liquor, the conversions of total

carbohydrate and glucan were comparable between NaOH and black liquor pretreated biomass, while the conversion of xylan was considerably higher for black liquor pretreated biomass, which is due to the presence of a considerable amount of hemicellulose-derived carbohydrates in the pretreated biomass (Xylan is the most abundant carbohydrate in the initial black liquor). After the contributions of the carbohydrates in the initial black liquor were excluded, the conversions of all carbohydrates significantly ($P < 0.05$) decreased, especially xylan. This reflects the fact that the carbohydrates in the initial black liquor play an important role in achieving high sugar productions in the enzymatic hydrolysis of pretreated biomass.

4.3.3 Lignin reduction

Effective delignification is one of the major mechanisms of alkaline pretreatment that cause the improvement of enzymatic digestibility of lignocellulosic biomass. For NaOH pretreatment at room temperature, the lignin reduction increased with both the elevation of NaOH concentration and the extension of residence time (Fig. 4.3). This is in agreement with the report that there is a positive correlation between the intensity of alkaline pretreatment and the delignification efficiency (Xu et al., 2010c). At the best residence times for total reducing sugar yield, the lignin reductions were 56.0% and 66.6% for pretreatments using 1% NaOH and 2% NaOH, respectively. Although the pH of black liquor was lower than that of 1% NaOH, the delignification abilities of the two alkaline solutions were comparable. At the best residence time of black liquor pretreatment, the lignin reduction of corn stover reached 59.3%. The great delignification ability of black liquor might be attributed to the appreciable amounts of organic compounds in the initial black liquor, which improved the solvation of

lignin during corn stover pretreatment. For example, phenol is an important monophenolic product from lignin depolymerization processes (Kleinert and Barth, 2008). It has been proven effective in the delignification of wheat straw for improved enzymatic hydrolysis (Zacchi et al., 1988). Further studies, however, are required to better understand the release of organic compounds during switchgrass pretreatment and the interaction between these compounds and corn stover lignin during the reutilization of black liquor.

4.3.4 Enzyme loading study

The study on enzyme loadings was based on the black liquor pretreatment of corn stover at room temperature using the best residence time of 24 h. During the study of Cellic CTec loading (0-40 FPU/g dry biomass), the Cellic HTec loading was kept excessive at 75 FXU/g dry biomass to eliminate the impact of Cellic HTec limitation. With the increase of cellulase loading from 0 to 5 FPU/g dry biomass, the total reducing sugar yield increased by 6.55 times from 39.7 to 299.8 mg/g raw biomass, indicating the high efficiency of Cellic CTec (Fig. 4.4a). The sugar production increased with the elevation of Cellic CTec loading and generally leveled off after the enzyme loading reached 20 FPU/ g dry biomass. Similar trends were observed for both glucose and xylose yields. Although further increasing Cellic CTec loading to 40 FPU/ g dry biomass resulted in a 7.2% higher total reducing sugar yield, doubling enzyme usage might not be justified economically. Therefore, 20 FPU/ g dry biomass was recommended for Cellic CTec application. Cellic HTec loadings were studied based on the recommended Cellic CTec loading. Elevating Cellic HTec loading not only increased xylose yield, but also caused an increase of glucose yield because of the better removal of hemicellulose barriers which reduce the access of Cellic CTec to cellulose (Fig.

4.4b). It is apparent that a Cellic HTec loading of 20 FXU/g dry biomass was sufficient to maximize sugar production and should be recommended, at which the total reducing sugar yield was 32.5% higher than that without Cellic HTec addition.

The same enzyme loading study was also carried out based on the corn stover pretreated using 1% NaOH at the best residence time of 24 h for comparison. The results show that the Cellic CTec loading of 10 FPU/ g dry biomass was sufficient to maximize sugar production, which was substantially lower than the Cellic CTec requirement of black liquor pretreated biomass (Fig. 4.4a). At the best Cellic CTec loading, the total reducing sugar yield of NaOH pretreated corn stover was 445.6 mg/g raw biomass, 8.87% higher than that of the black liquor pretreated biomass at the best Cellic CTec loading. The best Cellic HTec loadings for NaOH and black liquor pretreated corn stover were the same. At the best Cellic HTec loading of 20 FXU/ g raw biomass, the total reducing sugar yield of NaOH pretreated corn stover was 430.2 mg/g raw biomass, which was statistically the same as that of the black liquor pretreated biomass (Fig. 4.4b). It seems that, although applying black liquor caused a reduction in chemical expenses for biomass pretreatment, it resulted in a higher enzyme requirement in the subsequent enzymatic hydrolysis. An economic analysis, therefore, is required before the real cost-effectiveness improvement achieved by this technology can be evaluated.

4.4 CONCLUSIONS

Black liquor recycled from NaOH pretreatment of switchgrass was effective in improving the enzymatic hydrolysis of corn stover at room temperature. Both the high pH of

black liquor and its appreciable carbohydrate content contributed to high sugar productions of corn stover, which were comparable with those of the biomass pretreated with 1% NaOH. After black liquor pretreatment for 24 h, 478.5, 287.7, and 145.3 mg of total reducing sugars, glucose, and xylose were produced from one gram of corn stover, respectively. However, the increased enzyme requirement in hydrolysis might compromise the cost-effectiveness of this technology.

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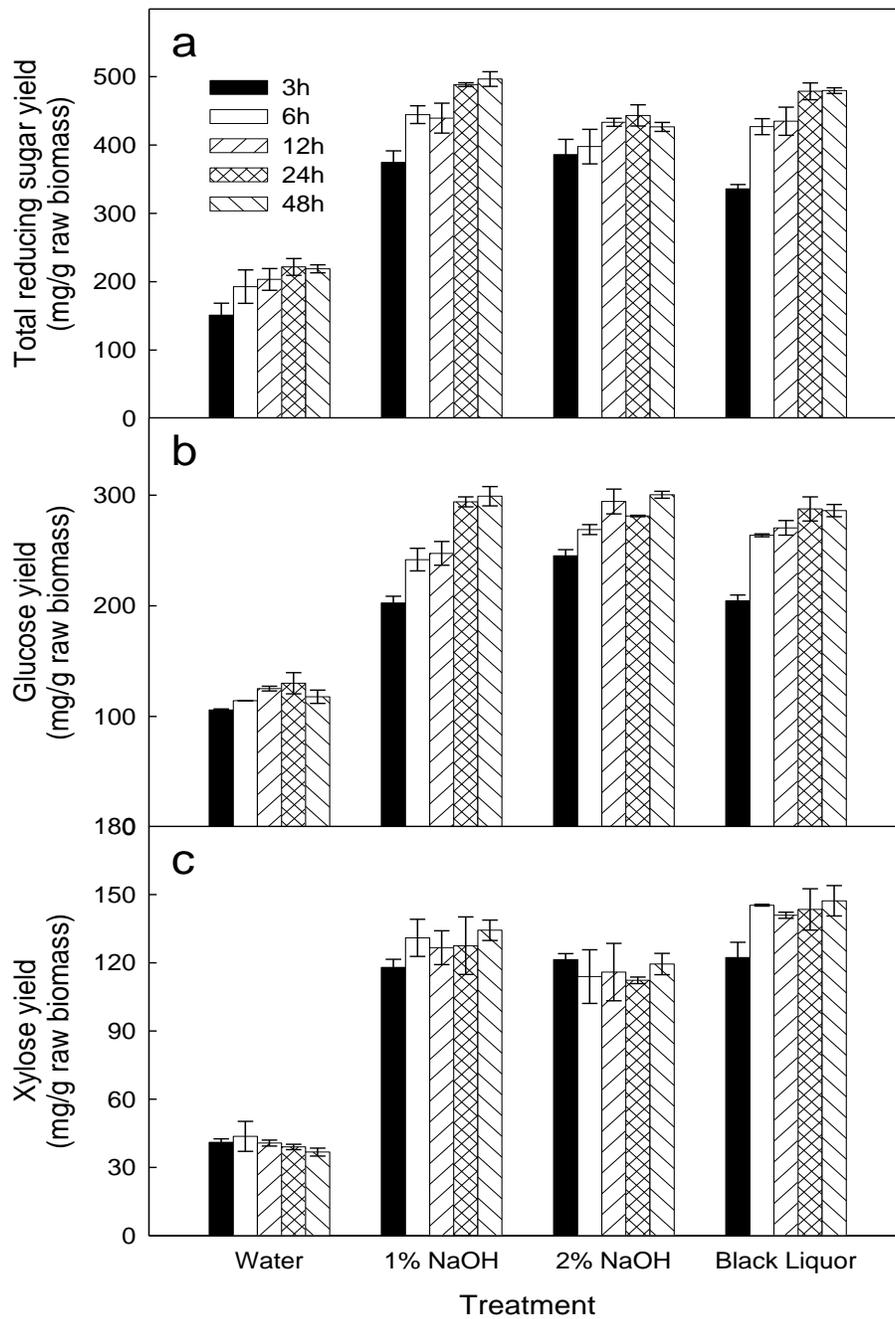


Figure 4.1 Yields of (a) total reducing sugars, (b) glucose, and (c) xylose in the enzymatic hydrolysis of corn stover pretreated at room temperature under different conditions.

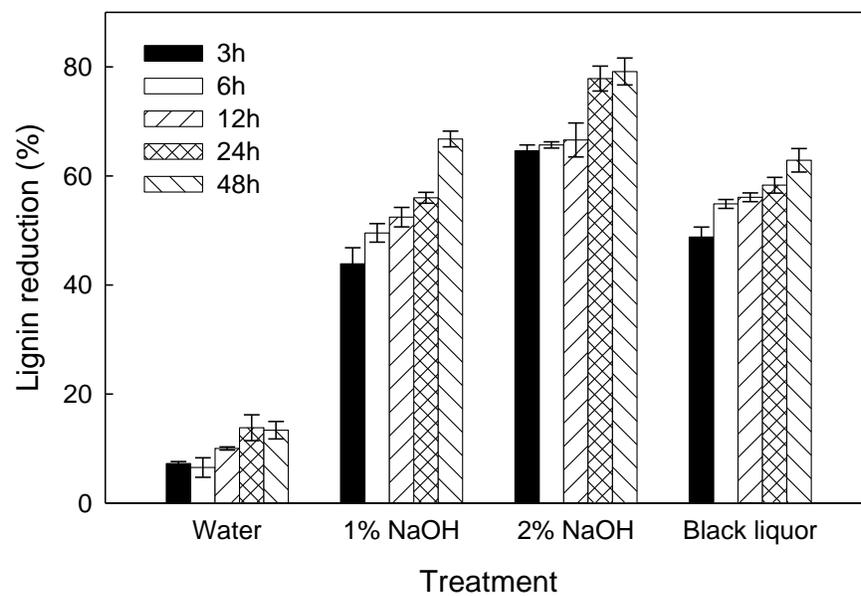


Figure 4.2 Lignin reductions of corn stover after pretreatment at room temperature under different conditions.

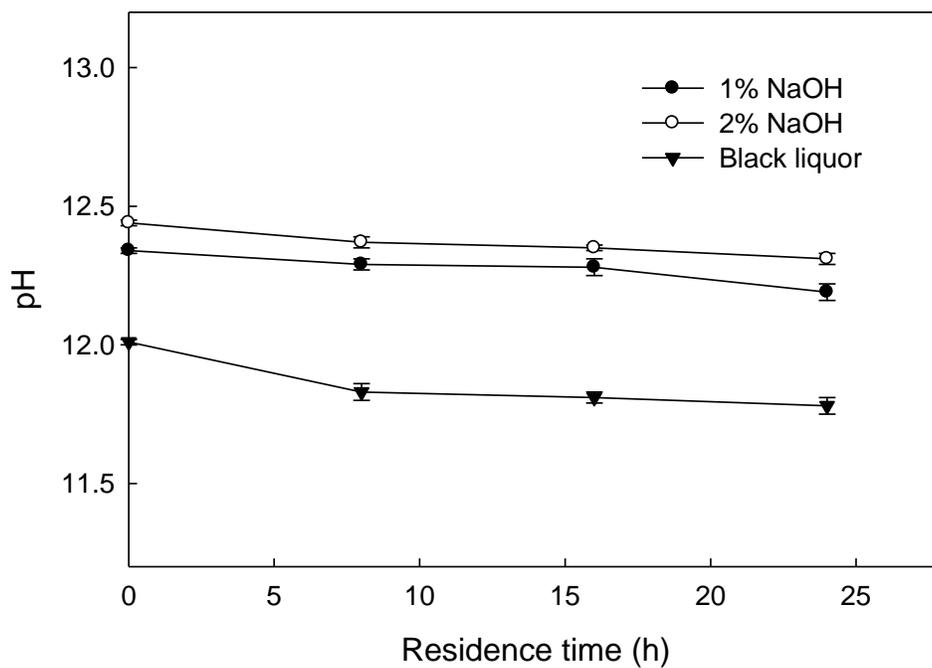


Figure 4.3 Changes of pH during the pretreatment of corn stover at room temperature using NaOH and black liquor.

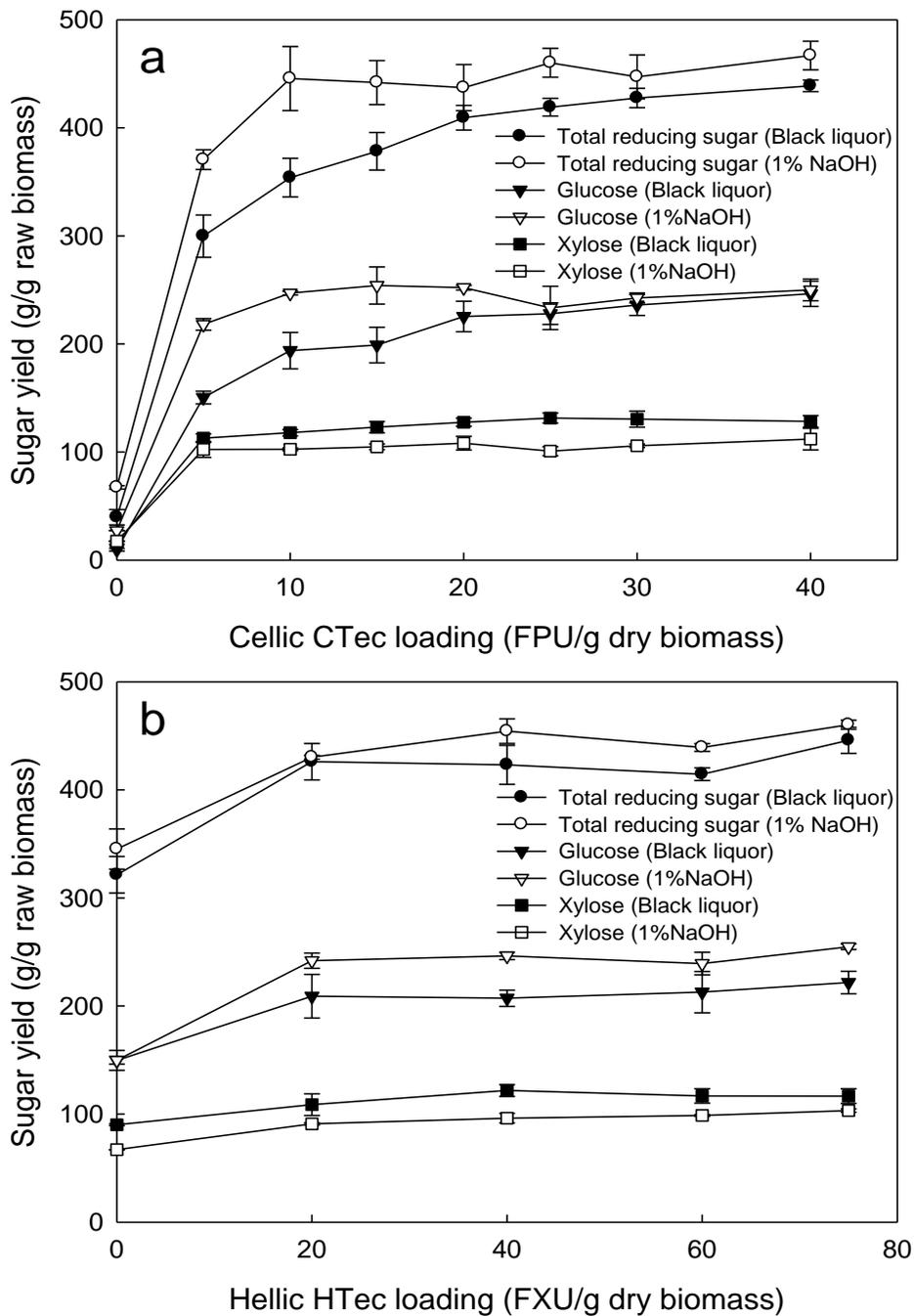


Figure 4.4 Effects of (a) Cellic CTec and (b) Cellic HTec loadings on total reducing sugar, glucose, and xylose yields of corn stover pretreated at room temperature using black liquor for 24 h and 1% NaOH for 24h

Table 4.1 Chemical compositions of raw switchgrass and corn stover.

Component	Content (%)	
	Switchgrass	Corn stover
Glucan	32.0 (1.69)*	36.2 (0.91)
Xylan	17.9 (1.04)	20.1 (0.58)
Galactan	1.73 (0.09)	1.45 (0.01)
Arabinan	1.87 (0.11)	3.00 (0.08)
Lignin	21.4 (0.34)	21.2 (0.50)
Ash	3.77 (0.12)	1.56 (0.19)
Others	21.3 (2.45)	16.5 (3.21)

* The number in parentheses is the standard deviation of triplicate samples.

Table 4.2 Solid recoveries of corn stover after pretreatments at room temperature.

Residence time (h)	Solid recovery (%)			
	Water	1% NaOH	2% NaOH	Black liquor
3	80.8 (1.35)	71.2 (3.78)	66.8 (0.82)	72.2 (2.43)
6	81.2 (1.66)	67.1 (1.79)	62.7 (0.33)	68.7 (1.17)
12	80.8 (2.28)	66.9 (0.79)	61.0 (1.14)	67.4 (0.72)
24	81.8 (0.62)	64.8 (1.29)	56.1 (1.04)	65.6 (3.30)
48	82.0 (1.79)	59.8 (1.58)	56.1 (0.23)	63.6 (1.90)

Table 4.3 Sugars and lignin derived from the initial black liquor and the black liquor collected after the pretreatment of corn stover at room temperature for 24 h.

Components	Concentration (g/L)	
	Before corn stover pretreatment	After corn stover pretreatment
Total solids	39.6 (0.05)	65.6 (0.21)
Total reducing sugars	11.8 (0.24)	17.4 (0.15)
Glucose	4.01 (0.15)	5.29 (0.08)
Xylose	6.29 (0.08)	10.2 (0.22)
Total lignin	9.79 (0.21)	20.7 (0.11)
Acid insoluble lignin	9.25 (0.23)	19.7 (0.12)
Acid soluble lignin	0.54 (0.03)	1.02 (0.08)

Table 4.4 Carbohydrate conversions of corn stover after NaOH and black liquor pretreatment at respective best conditions.

Component	Conversion (%)		
	1% NaOH	Black liquor*	
		Before exclusion	After exclusion
Total carbohydrate	71.7	70.3	66.2
Glucan	73.1	71.5	70.0
Xylan	57.6	63.6	55.3

* The carbohydrate conversions were calculated before and after the contributions of the carbohydrates in the initial black liquor to the sugar production in enzymatic hydrolysis were excluded.

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CHAPTER5
CONCLUSIONS AND FUTURE WORK

5.1 CONCLUSIONS

Corn Stover, referring to stalks, leaves, and cobs that remain in corn fields after the grain harvest, is considered as a potential feedstock for sugar production due to its great amount and easy access in the United States. Lignocellulosics, although are promising feedstocks for the production of biofuels including bioethanol and biobutanol, still have some challenges due to their recalcitrant structure. Pretreatment of the raw biomass to reduce structural recalcitrance, therefore, is essential to the improvement of enzymatic digestibility of lignocellulose for sugar production. A number of pretreatment technologies have been developed so far, among which alkaline pretreatment has attracted much attention due to its relatively low cost and effectiveness at mild temperatures. Sodium hydroxide (NaOH) and lime (CaO) are two alkali reagents which have been extensively studied in the chemical pretreatment of lignocellulosic biomass. This study focused on the effects of pretreatments on the sugar recovery after enzymatic hydrolysis with excessive enzyme doses. The combined sodium hydroxide and lime pretreatment was optimized based on a statistical analysis of the total reducing sugars yield as well as the production of glucose and xylose in the hydrolyzate.

After pretreatment at recommended conditions of 3 h, 0.5% NaOH, 0.1 g CaO g⁻¹ raw biomass and 6 h, 0.5% NaOH, 0.05 g CaO g⁻¹ raw biomass, the overall conversions of glucan in raw biomass were respectively 79.5 and 82.4%, and those of xylan, respectively, reached 60.2 and 62.9%. The application of CaO resulted in substantially reduced lignin reductions. Nonetheless, the sugar yield in hydrolysis was still high despite the presence of a large

amount of lignin in the biomass. It can be concluded that, instead of total lignin reduction, the structural alternations of lignin and the breakup of lignin-carbohydrate complex at alkaline conditions were responsible for the improved enzyme access to carbohydrates. However, some report has shown that lignin limits enzyme access to carbohydrates not only through posing physical barrier, but also by causing unproductive binding of enzymes. It is necessary, therefore, to further investigate the impact of this pretreatment technology on enzyme loading in the future work.

Black liquor is the spent alkaline liquid collected after sodium hydroxide pretreatment of lignocellulosic biomass, which is normally wasted after pretreatment. Black liquor recycled from NaOH pretreatment of switchgrass was effective in improving the enzymatic hydrolysis of corn stover at room temperature. Both the high pH of black liquor and its appreciable carbohydrate content contributed to high sugar productions of corn stover, which were comparable with those of the biomass pretreated with 1% NaOH. After black liquor pretreatment for 24 h, 478.5, 287.7, and 145.3 mg of total reducing sugars, glucose, and xylose were produced from one gram of corn stover, respectively. However, the increased enzyme requirement in hydrolysis might compromise the cost-effectiveness of this technology.

5.2 SUGGESTIONS FOR FUTURE WORK

High temperatures and pressures are routinely applied to effectively improve the digestibility of lignocellulose or much higher chemical loadings and more extended residence times are required at milder conditions which lead to the high cost of pretreatment. In this

thesis, distinct properties that NaOH and CaO showed in biomass structure alteration, lignin removal, and carbohydrate loss prevention make it possible to manipulate these two alkali reagents to make them work together in lignocellulose pretreatment to reduce temperature, chemical loading, and residence time requirements. In the future, the combined NaOH and CaO pretreatment technology should be tested for corn stover at higher temperatures, e.g. 40-60°C.

Our research results showed that, after sodium hydroxide pretreatment of switchgrass using the best conditions obtained in our previous study the alkalinity of the black liquor collected was high enough to justify the second round usage. In addition, the appreciable carbohydrate content of the black liquor would also contribute to the sugar production of the pretreated biomass during enzymatic hydrolysis. In future studies, to increase the cost-effectiveness of alkaline pretreatment, the black liquor collected after sodium hydroxide pretreatment of switchgrass and corn stover at room temperature should be tested for reuse for the three-round pretreatment with/without alkalinity supplementation.