ABSTRACT

HAN, QIANG. Autohydrolysis Pretreatment of Lignocellulosic Biomass for Bioethanol Production. (Under the direction of Dr. Hasan Jameel and Dr. Hou-min Chang).

Autohydrolysis, a simple and environmental friendly process, has long been studied but often abandoned as a financially viable pretreatment for bioethanol production due to the low yields of fermentable sugars at economic enzyme dosages. The introduction of mechanical refining can generate substantial improvements for autohydrolysis process, making it an attractive pretreatment technology for bioethanol commercialization.

In this study, several lignocellulosic biomass including wheat straw, switchgrass, corn stover, waste wheat straw have been subjected to autohydrolysis pretreatment followed by mechanical refining to evaluate the total sugar recovery at affordable enzyme dosages. Encouraging results have been found that using autohydrolysis plus refining strategy, the total sugar recovery of most feedstock can be as high as 76% at 4 FPU/g enzymes dosages. The mechanical refining contributed to the improvement of enzymatic sugar yield by as much as 30%.

Three non-woody biomass (sugarcane bagasse, wheat straw, and switchgrass) and three woody biomass (maple, sweet gum, and nitens) have been subjected to autohydrolysis pretreatment to acquire a fundamental understanding of biomass characteristics that affect the autohydrolysis and the following enzymatic hydrolysis. It is of interest to note that the non-woody biomass went through substantial delignification during autohydrolysis compared to woody biomass due to a significant amount of p-coumaric acid and ferulic acid. It has been

found that hardwood which has a higher S/V ratio in the lignin structure tends to have a higher total sugar recovery from autohydrolysis pretreatment.

The economics of bioethanol production from autohydrolysis of different feedstocks have been investigated. Regardless of different feedstocks, in the conventional design, producing bioethanol and co-producing steam and power, the minimum ethanol revenues (MER) required to generate a 12% internal rate of return (IRR) are high enough to discourage investors due to the high capital investment relative to low US ethanol price. Nevertheless, the economics of autohydrolysis can be substantially improved by upgrading the value of unhydrolyzed residues, such as the fuel pellets. Moreover, the utilization of proven technology and equipment renders autohydrolysis adaptable to pulp and paper industrial. Attractive economics have been found when autohydrolysis based bioethanol plant is co-located to a pulp and paper mill or the distressed pulp and paper mill is being repurposed to produce bioethanol.

An alternative to autohydrolysis combined with refining, thermomechanical pulping (TMP) process has been evaluated using corn stover as the feedstock. A significant low solids yield after the pretreatment process has been observed due to the harsh condition operated and the limitation of lab equipment. But the TMP process has great potential to be employed as a pretreatment for bioethanol production in an industrial scale if the process is optimized.

Autohydrolysis Pretreatment of Lignocellulosic Biomass for Bioethanol Production

by
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DEDICATION

To my parents - their encouragement and love in me have been constant and unconditional.

BIOGRAPHY

Qiang Han was born in Changzhou, China on March 15, 1988. He graduated from Nanjing Forestry University in 2010 with a B.S. degree in light chemical engineering. In the fall of 2010 he started his Ph.D. study at the Department of Forest Biomaterials at North Carolina State University under the guidance of Drs. Hasan Jameel, Hou-min Chang, and Richard Phillips. During his time in graduate school he interned at International Paper in Cincinnati, Ohio. He is a big fan of wolfpack sports.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1 Introduction

1. Background

The growing concerns for energy security and climate change have necessitated the need in exploring alternative energy that can replace fossil transportation fuel. Bioethanol has been one of the dominating renewable fuels in the transport sector due to its capability to be the fuel additives with gasoline or used as motor fuel in dedicated engines (Hahn-Hägerdal et al., 2006). Current bioethanol is mainly produced from sugar (Brazil) and starch (USA) but there is considerable debate about its competiveness against animal feed and human needs, and its impact on greenhouse gas emissions (Agbor et al., 2011). In this regard, bioethanol from lignocellulosic biomass has drawn remarkable interest over the world because lignocellulosic biomass: 1) is regarded as the most abundant renewable biomass; 2) does not compete with food crops; 3) is less expensive than conventional agricultural feedstock; 4) has positive impact on environment; 5) provides more employment in rural and industrial areas (Alvira et al., 2010; Demirbas, 2009; Fargione et al., 2008).

2. Lignocellulosic biomass characteristics

Lignocellulosic biomass is mainly composed of three types of polymers: cellulose, hemicellulose, and lignin. Those polymers are associated with each other and generally contribute to more than 70% of the total biomass.

2.1 Cellulose

Cellulose, mainly located in the secondary wall, is a homopolysaccharide consisting of β-D-glucopyranose moieties linked via β-(1,4) glucosidic bonds (Fig. 1.1) (Sjöström, 1993). The degree of polymerization of cellulose chain in nature can be as low as 10,000 glucose residues in wood and as high as 15,000 glucose residues in cotton (Sjöström, 1993). The disaccharide cellubiose unit, with a length of 1.03 nm is often regarded as the repeating unit of cellulose chain (Fengel & Wegener, 1983). Microfibrils, which are formed by aggregation of cellulose molecules, can bundle together to form cellulose fibers. The cellulose fibers consist of highly ordered crystalline regions and less ordered amorphous regions as a result of intra-and intermolecular hydrogen bonding (Sjöström, 1993). The crystallinity structure and strong hydrogen bonds render cellulose relatively stable towards chemical and enzyme attack.

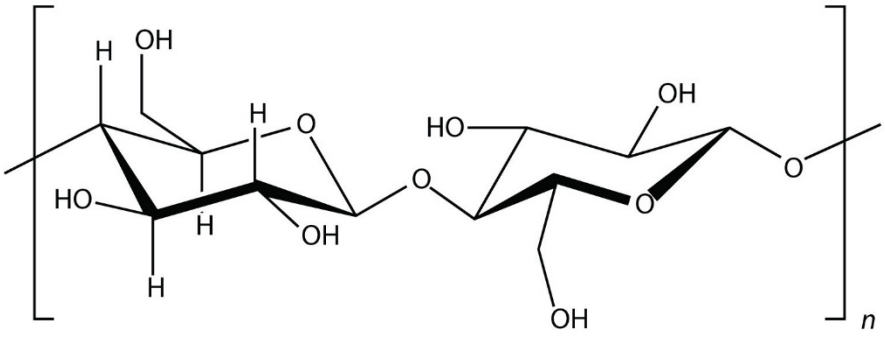

Fig. 1.1 Structure of cellulose

2.2 Hemicelluloses

Hemicelluloses, a highly branched heterogeneous polysaccharides, are composed of a wide variety of moieties such as pentoses (xylose and arabinose), hexoses (glucose, galactose, mannose, and rhamnose), and sugar acids (glucuronic acid, 4-O-methyl glucuronic acid, and galacturonic acid) (Sjöström, 1993). The amount of hemicelluloses in lignocellulosic materials can range from 20 to 40% of the dry biomass weight (Saha, 2003). The principal component of hemicelluloses in softwood is glucomannan, while in hardwood and herbaceous plants such as grasses and straw, is xylan (Fengel & Wegener, 1983).

It has been reported that hemicelluloses are linked to cellulose through intermolecular hydrogen bonding and van der Waals forces, and to lignin via cinnamate acid ester linkages, and to other hemicelluloses through covalent and hydrogen bonds (Decker et al., 2008; Sjöström, 1993). The degree of polymerization (around 200) and molecular weight of hemicelluloses are much lower compared to those of cellulose (Fengel & Wegener, 1983; Sjöström, 1993).

The xylan of hemicellulose can be easily extracted in an acid or alkaline environment, while glucomannan is more resistant to acid hydrolysis and a stronger alkaline environment is needed for the extraction of glucomannan than that of xylan (Balaban & Ucar, 1999; Fengel & Wegener, 1983). Hemicelluloses are often regarded as the most thermal-chemically sensitive components among the key constitutions of lignocellulosic biomass (Agbor et al., 2011; Hendriks & Zeeman, 2009).

2.3 Lignin

Lignin is the most abundant polymeric organic substance in the plant world. Generally, normal hardwood contains 20-28% lignin and normal softwood contains 26-32% lignin (Sjöström, 1993). Herbaceous biomass usually has a lower lignin content (10-25%) compared to woody biomass (Yu, 2013).

Lignin is an amorphous heteropolymer, built up by phenylpropane units including coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol (Fig. 1.2). The respective aromatic constituents of these alcohols in the lignin polymer are called guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H). The principle component of softwood lignin is typical guaiacyl unit whereas in hardwood the ratio of syringyl and guaiacyl unit can range from 1.1-3.1 depending on different species (Santos et al., 2012). In contrast, the lignin of herbaceous plants contains all three units (S, G, H) in significant amounts with different ratios (Buranov & Mazza, 2008).

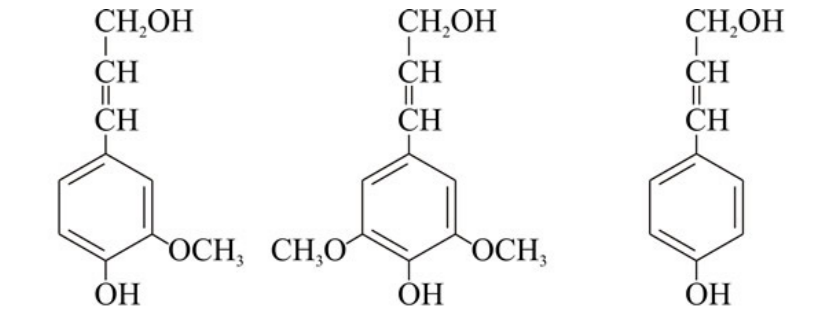

Fig. 1.2 The building units of lignin

The concentration of lignin is high in the middle lamella and low in the secondary wall. But more than 70% of the total lignin is located in the secondary wall because of its thickness (Sjöström, 1993). The lignin located in the secondary wall is mainly syringyl unit whereas more guaiacyl unit can be found in the middle lamella (Sjöström, 1993).

In order to quantify lignin content in lignocellulosic materials, a two-stage sulfuric acid hydrolysis can be applied to remove the polysaccharides and retain insoluble solids as so-called Klason lignin (Sluiter et al., 2008). But this method extensively changes the lignin native structure. To acquire a good understanding of lignin structure, two lignin isolation methods have been widely used: 1) milled wood lignin (MWL): the extractive free biomass meal is ground in a ball mill and the lignin is further extracted with a dioxane-water mixture (Björkman, 1956); 2) cellulolytic enzyme lignin (CEL): the extractive wood meal is first milled in a vibratory ball mill followed by addition of enzymes to remove polysaccharides, and the lignin is extracted with a dioxane-water mixture (Chang et al., 1975). Both MWL and CEL are the best lignin preparations now available to study the native structure of lignin. The existence of so-called lignin-carbohydrates complex (LCC) makes it impossible to isolate the lignin sample free of polysaccharides residues (Fengel & Wegener, 1983). It is evident that the interactions between lignin and polysaccharides are not only physical associations (hydrogen bonds, van der Waals forces, and chemosorption), but also chemical linkages (Fengel & Wegener, 1983).

3. Overall process of bioethanol production

The production of bioethanol from lignocellulosic materials mainly includes four unit operations: pretreatment, enzymatic hydrolysis, fermentation, and distillation and dehydration. Unlike sugar or starch based ethanol production, the recalcitrance of lignocellulosic biomass calls for pretreatment to break down the biomass matrix and provide an accessible substrate for enzymes attack. The objective of enzymatic hydrolysis is to depolymerize carbohydrate polymers to produce fermentable sugars by means of cellulolytic enzymes (Margeot et al., 2009). The resulting sugars are subjected to fermentation where yeasts can metabolize those sugars to ethanol. The ethanol was purified to meet fuel specifications through distillation and dehydration.

4. Biomass recalcitrance

Lignocellulosic biomass has evolved complex structural and chemical mechanisms to resist microbial and enzymatic deconstruction, and such natural resistance is collectively known as "biomass recalcitrance" (Himmel et al., 2007). The biomass recalcitrance can be expressed in several ways to limit enzymatic hydrolysis including:

4.1 Cellulose crystallinity

The cellulose crystallinity has been regarded as one of the most important factors that influence its enzymatic digestibility because the highly ordered regions are very compact and resistant not only to enzymes, but also to acids, and swelling in water (Sinitsyn et al., 1991). It has been found that the amorphous cellulose has a much faster hydrolysis rate compared to crystalline cellulose (Lynd et al., 2002; Zhang & Lynd, 2004), especially to the initial

hydrolysis rate (Mittal et al., 2011). It is believed that the enzymes favorably hydrolyze amorphous cellulose prior to digest more recalcitrant crystalline regions (Zhao et al., 2012a). As the crystallinity increases with the proceeding of enzymatic hydrolysis, cellulose becomes increasingly resistant to further hydrolysis (Fan et al., 1981). However, it needs to be cautious when lignocellulosic material instead of pure cellulose is employed to interpret the correlations of enzymatic hydrolysis and crystallinity index because the removal of amorphous lignin and hemicelluloses can increase the crystallinity index of the pretreated solids (Park et al., 2010).

4.2 Cellulose degree of polymerization (DP)

The enzymatic hydrolysis is a depolymerization process of cellulose by cellulase components. The endoglucanase randomly attack internal bonds at low crystallinity region and create new chain end. The exoglucanase is responsible for producing cellobiose from the ends of the exposed chains generated by endoglucanase. The resulting cellobiose is further hydrolyzed by betaglucosidase to produce glucose. In this regard, it is considered that the shorter cellulose chain, the faster the cellulose is hydrolyzed (Zhao et al., 2012a). It has been reported that the extent of enzymatic hydrolysis can be improved by the reduction in DP but those results should be interpreted with caution because the alter of DP is always accompanied by the change of other factors like crystallinity (Martínez et al., 1997; Puri, 1984; Zhao et al., 2012a).

4.3 Hemicellulose content

The hemicellulose is often regarded to limited enzyme accessibility by its physical barrier but the impact is not as important as the effect of lignin and cellulose crystallinity. The evidence of such influence can be found on acid hydrolysis pretreatment or liquid hot water pretreatment, where the removal of hemicelluloses increases the biomass enzymatic hydrolysis efficiency (Jeoh et al., 2007; Kabel et al., 2007; Kim et al., 2009a; Yang & Wyman, 2004). Moreover, it can be evidenced that the addition of hemicellulase can substantially improve the enzymatic digestibility of pretreated biomass (García-Aparicio et al., 2007; Yoshida et al., 2008).

4.4 Lignin barrier

Substantially studies have been focused on the negative impact of lignin to enzymatic hydrolysis efficiency. Such impacts can be summarized in three aspects (Yu, 2013): 1) lignin act as a physical barrier that restricts the access of enzymes to cellulose; 2) lignin may irreversibly adsorb enzymes and prevent their further actions; 3) the soluble lignin may deactivate the enzymes. Many pretreatment technologies such as alkali pretreatment, organosolv pretreatment, ozonolysis pretreatment, and oxygen delignification, are aimed at removing a significant amount of lignin to increase the enzymatic digestibility of the biomass. However, the removal of lignin is not necessary in some cases to improve enzymatic hydrolysis efficiency of the biomass. For instance, the dilute acid pretreatment and liquid hot water pretreatment only remove a part of lignin, but the cellulose digestibility of

the pretreated substrate can be largely improved due to the removal of hemicellulose and the change of lignin distribution and chemistry (Trajano et al., 2013).

4.5 Other factors

Other factors that can influence the enzymatic digestibility of lignocellulosic biomass includes: feedstock particle size, pore volume, specific surface area, and acetyl group content.

5. Pretreatment

As a consequence of biomass recalcitrance, pretreatment is essential to the conversion of lignocellulosic biomass to ethanol. The objective of pretreatment is to alter the lignocellulosic cell wall and render cellulose accessible to the action of enzymes. Many pretreatment technologies have been studied generally on the basis of physical, biological, chemical or physico-chemical actions.

5.1 Biological pretreatment

Biological pretreatment employs micro-organisms mainly brown, white, and soft-rot fungi to selectively degrade lignin and hemicelluloses thus to increase the enzymatic digestibility of remaining solids. It has been reported that white-rot fungi are the most effective fungi to degrade the lignin via the production of lignin-degrading enzymes such as peroxidases and laccases (Alvira et al., 2010; Sun & Cheng, 2002). Lignin degradation by those fungi can be very selective and effective but the rate of hydrolysis in most biological pretreatment process is too low for the industrial process (Agbor et al., 2011; Sun & Cheng, 2002).

5.2 Mechanical pretreatment

Mechanical pretreatment is aimed at reducing particle size and crystallinity of lignocellulosic by a combination of chipping, grinding and milling. Some mechanical pretreatments are a prerequisite step to get a suitable particle size prior to chemical or biological pretreatment. Some extensive mechanical pretreatments such as ball milling can stand alone as a pretreatment method to improve the digestibility of biomass via breaking down cellulose crystallinity (da Silva et al., 2010). However, the high power consumption of milling deters this process to be economically feasible.

5.3 Chemical pretreatment

5.3.1 Dilute acid pretreatment

Dilute acid pretreatment has been extensively studied over the years. The objective of dilute acid pretreatment is to solubilize mainly hemicellulose and render cellulose more accessible to enzymes attack. In the meantime, the degradation compounds such as furfural, hydroxymethyl furfural, and aromatic lignin degradation compounds can be formed, which can affect the microorganism metabolism in the fermentation step (Larsson et al., 1999; Palmqvist & Hahn-Hägerdal, 2000). The dilute acid pretreatment can be carried out in a wide range of temperatures (120-200 °C) for a period of time depending on the pretreatment severity required. Although the dilute acid pretreatment can achieve high reaction rate and hydrolysis efficiency, it has several disadvantages including corrosion of equipment, high amount of degradation products, and process complexity due to the need for neutralization and waste water treatment.

5.3.2 Alkaline pretreatment

Alkaline pretreatment is typically carried out using alkali such as NaOH, KOH, $Ca(OH)_2$, ammonium, etc. at room temperature for longer time or high temperature for shorter time. The objective of alkaline pretreatment is to cause biomass swelling, increase the internal surface area, reduce cellulose crystallinity, remove and disrupt lignin structure, and remove acetyl group and uronic acid substitutions (Agbor et al., 2011; Zhao et al., 2012b). Alkaline pretreatment has been shown to be more effective with low lignin content biomass like agricultural residues but become less effective on high lignin content biomass like wood (Agbor et al., 2011). The disadvantages of alkaline pretreatment include chemical recovery and neutralization or extensively wash of pretreated biomass prior to enzymatic hydrolysis.

5.4 Oxidative pretreatment

Oxidative pretreatment is aimed at remove mainly lignin and hemicellulose by using oxidants such as oxygen, ozone, and hydrogen peroxide to increase cellulose digestibility. The mechanisms for degradation of lignin in oxidative pretreatment vary depending on oxidants used and reaction conditions. For oxygen delignification, the phenolate ion, which is formed when a phenolic hydroxyl group in lignin reacts with alkali, reacts with oxygen to form a reactive intermediate called hydroperoxide. This intermediate then undergoes fragmentation by several possible pathways to form lignin fragments that are mostly water soluble (McDonough, 1989). For ozone pretreatment, ozone is highly reactive towards compounds with conjugated double bonds and functional groups with high electron densities. Therefore, lignin which is enriched of unsaturated bonds is most likely to be oxidized by

ozone to form soluble acidic compounds with less molecular weight including aliphatic carboxylic acids and aromatic acids (García-Cubero et al., 2009; Sannigrahi et al., 2012). Despite the high selectivity and positive environmental impact of ozone pretreatment, the cost of ozone and the mass transfer issues largely limit this pretreatment.

5.5 Organosolv pretreatment

Organosolv pretreatment utilizes organic solvent including methanol, ethanol, acetone, ethylene glycol, etc. or their mixture with or without catalysts to pretreat lignocellulosic biomass to extensively remove lignin and hemicellulose, thus increase cellulose accessible surface area and pore volume (Alvira et al., 2010; Zhao et al., 2012b). A relatively pure lignin and carbohydrates can be isolated as solids and syrup respectively, and further being recovered as byproducts (Zhao et al., 2009). But due to the volatility of organic solvent, the chemical recovery issue prevents this technology being applied at an industrial scale.

5.6 Ionic liquids pretreatment

Ionic liquids, which are typically composed of large organic cations and small inorganic anions, can dissolve carbohydrates and lignin via competing with lignocellulosic components for hydrogen bonding, thus disrupt the intricate network of non-covalent interactions among cellulose, hemicellulose, and lignin (Agbor et al., 2011; Alvira et al., 2010). It has great potential for future lignocellulosic biorefinery process, but the development of efficient chemical recovery methods and the potential toxicity of solvent to

enzymes and yeasts need to be addressed prior to a large-scale application (Alvira et al., 2010).

5.7 Physico-chemical pretreatment

5.7.1 Steam explosion

Steam explosion is a hydrothermal pretreatment in which the biomass is subjected to pressurized steam for a period of time ranging from seconds to minutes, and then suddenly depressurized (Alvira et al., 2010). The steaming process promotes the hydrolysis of acetyl groups to release organic acids which further catalyze the hydrolysis of hemicellulose. The sudden pressure drop causes explosive decompression inside fibers and leads to the separation of fibers. The combined chemical and mechanical actions result in increased accessibility of cellulose to enzymes. The steam explosion has many advantages including high solids input, chemical free process and lower energy input, but the generation of toxic compounds to yeasts and technical risk upon scaling up need to be addressed before this technology moving forwards to large scale projects.

5.7.2 Ammonia fiber expansion (AFEX)

Ammonia fiber expansion (AFEX) pretreatment is a promising physic-chemical pretreatment in which lignocellulosic biomass is treated with liquid ammonia at low temperature (< 90 °C) and high pressure for a period of time. The sudden release of pressure leads to the rapid expansion of ammonia gas, resulted in swelling of biomass, decrease of cellulose crystallinity, deacetylation of hemicellulose, and disruption of lignin carbohydrates linkages (Alvira et al., 2010; Laureano-Perez et al., 2005). It has been reported that AFEX is

more effective on biomass with lower lignin content such as herbaceous plants than high lignin content feedstock such as wood (Wyman et al., 2005). The problems for AFEX pretreatment are the cost of ammonia and the environmental concern coupled with (Agbor et al., 2011).

6. Autohydrolysis pretreatment

Autohydrolysis is a hydrothermal process in which lignocellulosic biomass is pretreated in a water only medium at elevated temperatures to solubilize mainly hemicellulose and disrupt biomass structure for an improved enzymatic digestibility. This process has been described as liquid hot water pretreatment, hydrothermal pretreatment, aqueous pretreatment, water prehydrolysis, hydrothermolysis, and hot compressed water pretreatment as well in many other studies (Garrote et al., 1999; Mosier, 2013; Ruiz et al., 2013). It has long been studied as a prehydrolysis step to separate hemicelluloses in a modified Kraft pulping process. Nowadays autohydrolysis has drawn substantial attentions as a pretreatment technology for bioethanol production.

Autohydrolysis offers several attractive features as a pretreatment method including: 1) water is only reaction medium and the whole process is environmental friendly; 2) no chemical recovery units required; 3) less degradation products compared to acid process; 4) less equipment corrosion due to the mild pH of the reaction medium; 5) sulfur free unhydrolyzed residues yield the potential to upgrade high value-added byproducts; 6) low technical risk owing to the use of proven equipment and technology; 7) low capital cost because of the process simplicity.

In an autohydrolysis process, acidic hydronium ions are generated by water autoionization at high temperature. They act as catalyst and attack the susceptible ether bonds of hemicelluloses, leading to both splitting of acetyl groups and generation of oligosaccharides (Garrote et al., 1999). The resulting acetic acid makes the solution more acidic and further catalyzes the depolymerization of biomass. It has been suggested that other organic acids such as uronic acid, formic acid and levulinic acid may also contribute to the generation of hydronium ions, but their effects are not well established (Garrote et al., 1999; Mosier, 2013). Depending on the pretreatment severity, the dissolved sugars (pentoses and hexoses) can be further degraded to form furfural and hydroxymethylfurfural, which combined with aromatic degradation products from lignin may affect the yeast metabolism in the fermentation step (Palmqvist & Hahn-Hägerdal, 2000).

6.1 Effect of autohydrolysis on hemicellulose

Autohydrolysis is an effective approach for depolymerization of hemicellulose. Under selected conditions, hemicelluloses can be almost totally removed from the lignocellulosic biomass (Garrote et al., 1999). The kinetics of hemicelluloses hydrolysis during autohydrolysis have been relatively well studied (Grénman et al., 2011; Mittal et al., 2009; Nabarlatz et al., 2004). Generally, pseudo-first order kinetic has been employed for modeling hemicellulose hydrolysis. Take the hydrolysis of xylan for an example, xylan is first depolymerized to form xylan oligosaccharide, which is further hydrolyzed to form xylose. Xylose can be degraded to form furfural, which is also susceptible to be degraded to insoluble organic compounds (Fig. 1.3) (Mosier, 2013; Weingarten et al., 2010). Mosier has

summarized the kinetic constants and activation energies of xylan hydrolysis and degradation during autohydrolysis (Table 1.1) (Mosier, 2013). Under same condition, the rate of xylan oligosaccharide hydrolysis to produce xylose is much faster than that of depolymerization of xylan to xylan oligosaccharides. On the other hand, the degradation of xylose has higher activation energy than that of hydrolysis of dissolved xylan oligomers, indicating that xylan oligosaccharides are easier to hydrolyze but the xylose degradation is more sensitive over the same temperature range. Thus, in order to maximize the xylose and xylan oligosaccharides yield, it is recommended to pretreat biomass at a relatively high temperature for a short period of time to avoid the degradation reactions.

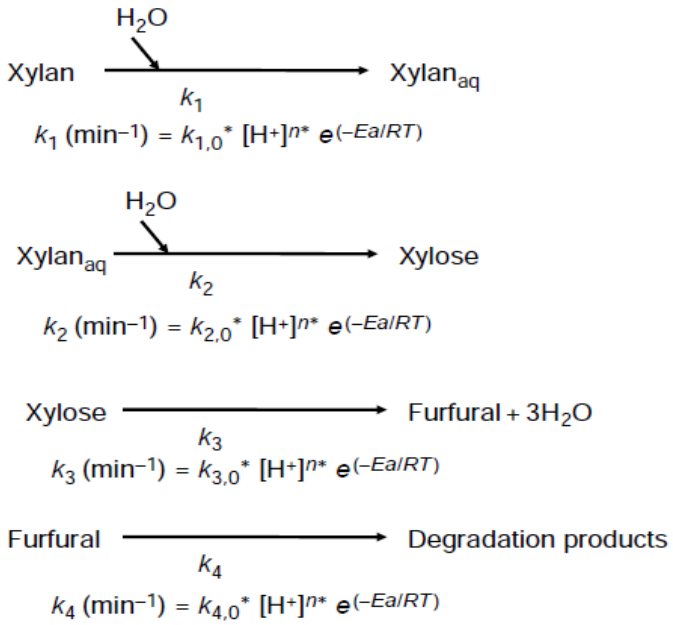

Fig. 1.3 Schematic for kinetic model of xylan hydrolysis (Mosier, 2013)

Table 1.1 Kinetic constants and activation energies for hemicellulose degradation during autohydrolysis.

Reaction	K (at 200 °C) hr^{-1}	Ea (kj/mol)
1 Xylan hydrolysis	7-25	113-156
2 Xylan oligosaccharide hydrolysis	13-30	95-120
3 Xylose degradation	0.5-5	120-160
4 Furfural degradation	1-11	67-73

6.2 Effect of autohydrolysis on lignin

Lignin change during autohydrolysis may go through a complex mechanism that involves both morphological changes and chemical reactions. Donohoe and Decker (Donohoe et al., 2008) have observed and determined lignin droplets on and within cell walls of dilute acid and hydrothermal pretreated biomass by employing FTIR, NMR, antibody labeling, and cytochemical staining. They put forward a hypothesis that those lignin droplets are a result of phase transition products after reaching lignin's glass transition temperature and they may go from glassy state to rubbery state, followed by coalesce, migration, and extrusion from the cell wall. This hypothesis has been applied to explain the droplets formation during hydrothermal and dilute acid pretreatment of many other feedstocks, including wheat straw, Tamarix ramosissima, and switchgrass (Kristensen et al., 2008; Pingali et al., 2010; Xiao et al., 2011).

In addition to morphological changes, chemical reactions occur on lignin during pretreatment. As illustrated in Fig. 1.4, acidic condition during autohydrolysis leads to the formation of carbonium ion intermediates by elimination of water (ether, ester) from benzylic

position (Li & Gellerstedt, 2008). The carbonium ion may react with the cleavage of the β-ether linkage to form Hibbert ketones through acidolysis reaction. Due to the high temperature applied in the autohydrolysis, homolytical cleavage of β-O-4 linkage from original lignin, carbonium ion, or pronated quinone methide may occur (Li et al., 2000). Both homolytical cleavage and acidolysis leads to the depolymerization of lignin during autohydrolysis. Evidence of depolymerization of lignin during autohydrolysis including the loss of β-O-4 linkages (El Hage et al., 2010; Leschinsky et al., 2008; Trajano et al., 2013), an increase of phenolic hydroxyl groups due to the cleavage of aryl-ether bond (El Hage et al., 2010; Leschinsky et al., 2008), and a decrease in the molecular weight of lignin in the autohydrolysis pretreated residues (Leschinsky et al., 2008; Lora & Wayman, 1980; Xiao et al., 2012). In the meantime, due to the high affinity for nucleophiles, carbonium ion may react with other electron-rich carbon atoms such as the C-2 and C-6 in guaiacyl and syringyl rings, which leads to the repolymerization of lignin (Chua & Wayman, 1979a; Lora & Wayman, 1980). Evidence of repolymerization of lignin during autohydrolysis includes an increase of carbon-carbon bonds (El Hage et al., 2010; Leschinsky et al., 2008; Lora & Wayman, 1980; Trajano et al., 2013), and a reduced yield in nitrobenzene oxidation (Wayman & Chua, 1979). It has been reported that during autohydrolysis syringyl units are more preferentially extracted as low molecular weight material to leave a syringyl-deficient type lignin in the pretreated solids (Chua & Wayman, 1979b; Trajano et al., 2013).

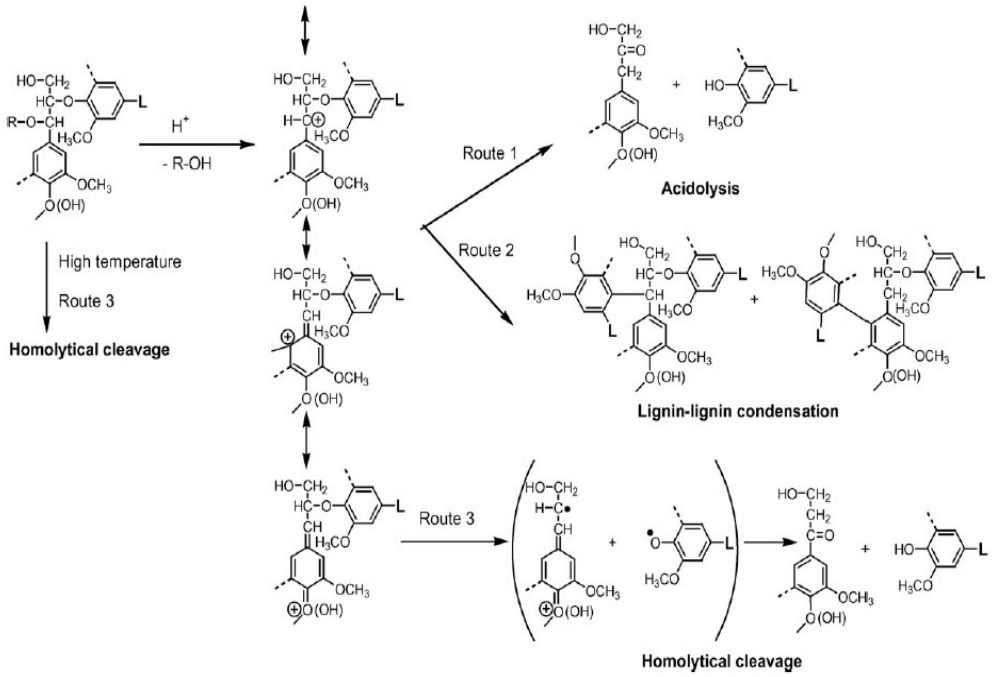

Fig. 1.4 Proposed lignin reactions during autohydrolysis (Li & Gellerstedt, 2008)

It is suggested that part of lignin which is linked to hemicellulose may go along with the removal of hemicellulose during the autohydrolysis. Several researchers have reported that the presence of polysaccharides promotes the removal of lignin (Liu & Wyman, 2003; Liu & Wyman, 2004; Trajano et al., 2013). On the other hand, the carbohydrates degradation products such as furfural may react with lignin to form condensed compounds at high temperature (Wayman & Chua, 1979). An increase of lignin content in autohydrolysis pretreated substrate has been reported, which was rationalized by the formation of "pseudo-lignin" materials resulting from a condensation reaction between lignin and polysaccharide degradation products (El Hage et al., 2010; Sannigrahi et al., 2011).

6.3 Effect of autohydrolysis on cellulose

Compared to hemicellulose and lignin, minimal impact has been found on cellulose in an autohydrolysis process because cellulose is more thermal resistant and only little cellulose degradation occurs at the temperatures below 230 °C (Garrote et al., 1999). But the reactivity of cellulose towards enzymes has been significantly improved mainly due to the removal of hemicellulose and the change of lignin distribution and chemistry. Discrepant results have been reported on the effect of autohydrolysis to cellulose crystallinity because the interference of the removal of amorphous hemicellulose and lignin (Mosier, 2013). Depending on autohydrolysis condition, the partially hydrolysis of cellulose indicates the decrease of DP of the cellulose chains. But the capability to completely dissolve cellulose in DP measurement will largely affect the results (Mosier, 2013).

6.4 Effect of autohydrolysis conditions on total sugar recovery

Autohydrolysis has been widely studied as a pretreatment technology for bioethanol production on a wide range of feedstocks. However, some studies only focused on the effect of autohydrolysis pretreatment to the enzymatic digestibility, without taking account of sugar and solids recovery yield during pretreatment process. From a more practical point of view, total sugar recovery is recommended to better evaluate the performance of autohydrolysis process for bioethanol production. Total sugar recovery is defined as the percentage of total sugar recovered from both autohydrolysis filtrate and enzymatic hydrolyzate on the total carbohydrates in the raw material. It can directly indicate the adaptability of autohydrolysis

to the feedstock under certain operation condition and enzyme dosages, regardless of the total carbohydrates content in the original biomass.

Table 1.2 shows the total sugar recovery using autohydrolysis as a pretreatment method for bioethanol production under selected conditions and different feedstocks. Comparisons of those results are difficult to make because the size of feedstock, the type of reactor, the consistency of cooking, the type of enzymes, and the enzymes dosages vary significantly among those studies. Generally, the harsh condition of autohydrolysis facilitates the digestibility of pretreated biomass but leads to considerably degradation of hemicellulose during the pretreatment. Many studies have reported that a total sugar recovery higher than 70% can be achieved when employing autohydrolysis process on a wide range of feedstocks such as barley husks, corn stover, switchgrass, sweet sorghum bagasse, poplar, and eucalyptus globulus (Ares-Peón et al., 2011; Kim et al., 2009b; Kumar et al., 2011; Mosier et al., 2005; Romaní et al., 2010; Yu et al., 2011). But those results are usually based on considerably high enzyme dosages, which are not practical in an industrial process considering the high price of enzymes (Klein-Marcuschamer et al., 2012).

It is noted that there are two key points for implementing autohydrolysis as a commercial process for bioethanol production: (1) to maximize dissolution of polysaccharides during autohydrolysis; (2) to fully disrupt biomass structural and render biomass more susceptible to the actions of enzymes. Previous developers of autohydrolysis pretreatment have often been deterred by failure to accomplish both goals because the former requires mild conditions to avoid sugars degradation and the latter need more severe

conditions to open up the biomass structure. Therefore, autohydrolysis process needs to be improved either by optimizing the process condition or introducing an efficient and compatible post-treatment.

Table 1.2 The total sugar recovery using autohydrolysis as the pretreatment method for bioethanol production under different conditions and feedstocks.

Substrate	Temperature, °C	Holding time, min	Solids recovery, %	Sugar recovered in filtrate, %	Cellulase	Enzyme dosages, FPU/g substrate	Sugar recovered in enzymatic hydrolyzate, %	Total sugar recovery, %	Reference
Wheat straw	200	0	75	22	Celluclast 1.5L	15	45	67	(Pérez et al., 2007)
Wheat straw	214	3	61	5	Celluclast 1.5 L	15	51	56	(Pérez et al., 2008)
Barley husks	204	0	53	35	Celluclast 1.5L	8	37	72	(Ares-Peón et al., 2011)
Corn stover	190	15	63	18	Spezyme CP	28	69	87	(Mosier et al., 2005)
Rice straw	180	30	60	12	Acremonium cellulase	40	53	65	(Yu et al., 2010)
Bermuda grass	150	60	67	23	Celluclast 1.5 L	30	43	66	(Lee et al., 2009)
Switchgrass	190	20	60	34	Spezyme CP	15	44	78	(Kumar et al., 2011)
Sweet sorghum bagasse	184	18	73	31	-	40	51	82	(Yu et al., 2011)
Hybrid poplar	200	10	74	17	Spezyme CP	68	57	74	(Kim et al., 2009b)
Eucalyptus globulus	210	0	70	25	Celluclast 1.5 L	10	69	94	(Romaní et al., 2010)

7. Post-treatment to enhance the enzymatic digestibility of autohydrolysis pretreated biomass

One approach to obtain the high sugar yield in both the autohydrolysis filtrate and enzymatic hydrolyzate at affordable enzyme dosages is to apply an efficient post-treatment after a relatively mild autohydrolysis process. Such type of post-treatment should be compatible with the autohydrolysis without adding complexity to the whole process. Mechanical refining is widely used in pulp and paper industrial to develop pulp fibers for the demand of the particular papermaking strength. The major effects of refining includes fiber shortening, external fibrillation and foliation, removal of primary wall, fiber swelling, breaking some of intra-fiber bonds and replacement by water-fiber hydrogen bonds (Smook, 1992).

Mechanical refining has been reported to generate significant improvements on enzymatic digestibility of the pretreated material, including green liquor pretreated loblolly pine (Wu et al., 2012; Wu et al., 2010) and hardwood (Jones et al., 2013; Koo et al., 2011), kraft hardwood pulp (Jones et al., 2013), sulfite pretreated softwood (Zhu et al., 2010), dilute acid pretreated corn stover (Chen et al., 2013; Chen et al., 2012b; Tao et al., 2012), and recovered office printing paper (Chen et al., 2012a). Generally, a 20-30% increase of enzymatic sugar yield can be obtained when mechanical refining is applied as a post-treatment depending on pretreatment severity and feedstock. Such improvement can be attributed to the shearing action during the mechanical refining which facilitates more internal delamination and surface fibrillation, disrupts the cellulose crystallinity structure,

and increases the accessible area for enzymes attack (Jones et al., 2013). It is of interest to point out that mechanical refining exerts its optimum effect on the moderate pretreated substrate because the refining improvement is limited by the biomass recalcitrance for less pretreated substrate and maximum conversion for severely pretreated substrate (Jones et al., 2013).

Mechanical refining has the great potential to be integrated into the autohydrolysis process. This simple and well proven technology does not hamper the simplicity and cleanliness of the autohydrolysis process. It can help to lower the pretreatment severity to increase the sugar recovery in the autohydrolysis filtrate and minimize the degradation of sugars, but also sustain a high enzymatic digestibility of pretreated material at affordable enzymes dosages.

8. Objective of this study

The objective of this study is to 1) develop optimum autohydrolysis conditions for converting multiple lignocellulosic biomass including wheat straw, switchgrass, corn stover, and waste wheat straw to bioethanol; 2) acquire fundamental understanding of biomass characteristics that affect autohydrolysis and the following enzymatic hydrolysis; 3) investigate the economic feasibility of developed process for ethanol production.

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Chapter 2 Autohydrolysis pretreatment combined with mechanical refining of multiple feedstocks for bioethanol production

Chapter 2.1 Enzymatic hydrolysis of autohydrolyzed wheat straw followed by refining to produce fermentable sugars

Abstract

Wheat straw was pretreated using an autohydrolysis process with different temperatures (160-200ºC) and times (10-20 min) in order to allow the recovery of hemicellulose in the filtrate and help open up the structure of the biomass for improved accessibility of enzymes during enzymatic hydrolysis. Autohydrolysis at 190°C for 10 min provided the highest overall sugar (12.2 g/100 g raw wheat straw) in the autohydrolysis filtrate and recovered 62.3% of solid residue. Before enzymatic hydrolysis, the pulps obtained from each pretreatment condition were subjected to a refining post-treatment to improve enzyme accessibility. Enzymatic hydrolysis was performed for all the pretreated solids with and without refining post-treatment at the enzyme loadings of 4 and 10 FPU/g oven dry substrate for 96 h. A total of 30.4 g sugars can be recovered from 100 g wheat straw at 180°C for 20 min with 4 FPU/g enzyme charge.

Keywords: Enzymatic hydrolysis; Bioethanol; Autohydrolysis; Wheat straw; Fermentable sugar

1. Introduction

The usage of fossil fuels to provide energy for the world economy has negative environmental consequences. Therefore, there is great interest in utilizing alternative energy sources to supply energy and chemical feedstock demands. Lignocellulosic biomass is an abundant renewable biomaterial representing half of the organic carbon in the biosphere and as such, a potential candidate to replace a great majority of products derived nowadays from fossil fuels (Moreira et al., 2011; Lee et al., 2008). Lignocellulosic biomass, mainly composed of cellulose, hemicellulose and lignin in different proportions, is the most abundant biomaterial in nature (Juhasz et al., 2005).

Lignocellulosic biomass can be converted to bioethanol with the following steps: pretreatment, hydrolysis, fermentation and distillation. The sugars released during the enzymatic hydrolysis have many applications in various industries (Gu et al., 2012). Lignocellulosic biomass pretreatment is needed to facilitate the low efficiency of hydrolyzing biomass to fermentable sugars using enzymes for bioethanol production. Enzymatic hydrolysis is one of the effective and economically applicable methods to generate fermentable sugars due to high yields of sugars that can be produced (Lee et al., 2009; Farrell et al., 2006). Pretreatment is a crucial step in order to generate fermentable sugars by breaking down the polymeric structures of the carbohydrates and lignin in the lignocellulosic biomass and enhance the accessibility of enzymes to solid substrate during enzymatic hydrolysis step. Many treatment approaches such as acid, alkali, ammonia and steam explosions have been studied to prepare lignocellulosic substrates from a wide range of raw

materials for subsequent bioconversion (Weil et al., 1998; Kim and Lee, 1996; Chang et al., 1998; Lee et al., 2009; Dale et al., 1996).

Among all the pretreatment methods, autohydrolysis, a method that treats lignocellulosic biomass in chemical-free, water only media has several advantages compared to other pretreatment processes because it is a simple, low-cost and environmental friendly process. In addition less undesirable sugar degradation inhibitors are produced (Garrote et al., 1999; Garrote et al., 2003; Tan et al., 2008). Autohydrolysis pretreatment predominantly depolymerizes hemicellulose in biomass and assists solubility of hemicellulose into the prehydrolyzate, and also enhances the accessibility of enzymes to solid substrates during the subsequent enzymatic hydrolysis to mono-sugars (Fernandez-Bolanos et al., 2001). The severity of autohydrolysis pretreatment is often expressed as "severity factor" (Overend and Chornet, 1987). The severity factor is a combination of temperature and residence time in the autohydrolysis pretreatment process and can be used to quantify autohydrolysis.

Mechanical refining has been widely applied in pulp and paper industry in order to improve the bonding ability of the fiber and increase paper strength. It has also been used to generate microfibrils, shorten long fibers, and develop the porosity and internal surface area (up to 10%) of fiber. It has been reported that refining significantly improved enzymatic conversion, while enzyme loading was reduced by up to 50% (Chen et al., 2012).

Wheat straw is one of the most plentiful lignocellulosic biomass that is produced in huge amounts around the world every year. The straw yield is an average of ratio 1.3 kg

straw per kg grain, depending on the specific wheat varieties harvested and climatic factors (Ruiz et al., 2011). Hence, it is considered that wheat straw has an important potential as a lignocellulosic feedstock for bioethanol production. A simple process that consists of only autohydrolysis followed by refining is being proposed for wheat straw. In the past this combination has not been studied for wheat straw. If high sugar recoveries can be achieved at low enzyme dosages, the proposed process can be implemented with a low capital and operating cost. The aim of this study was to determine the optimal autohydrolysis and refining conditions for wheat straw and investigate its effect on overall sugar recovery using enzymes. An additional objective was to perform a material balance on this bioconversion process.

2. Materials and Methods

2.1. Raw material

Wheat straw was purchased from Lowe's Market (Cary, NC). Wheat straw with initial moisture content of ~5% was cut into 2-3 cm sizes and stored in plastic containers at room temperature prior to experiment. The straw was further ground by a Wiley Laboratory Mill (Model No. 4, Thomas Scientific, Philadelphia, PA) and sieved through a 20 mesh screen for composition analysis.

2.2. Compositional analysis

The moisture, Klason lignin (acid-insoluble lignin) and acid-soluble lignin contents of original and pretreated raw materials were determined by National Renewable Energy

Laboratory's (NREL) Laboratory Analytical Procedures (Ehrman, 1994, 1996; Templeton and Ehrman, 1994). First stage hydrolysis of 0.3 g samples was performed with 3.0 mL of 72% (w/w) H_2SO_4 for 2 h at room temperature. Hydrolysates were diluted to 4% (w/w) H_2SO_4 with 84 mL deionized water (DI) and autoclaved for 1 h at 121ºC. Mono-sugars were analyzed by a Dionex ICS-300 ion chromatography (IC) system. The IC system was equipped with an autosampler, a gradient pump (GP-40 model), an eluent generator (EG-2 model), a guard column (CarboPac PAI 2×50mm), an ion-exchange column (CarboPac PAI 2×50mm) and an electrochemical detector (ED-40 model). The mobile phase was Mili-Q water and 400 mN NaOH (Fisher Scientific, Fair Lawn, NJ) at a flow rate of 0.3 mL/min. A 200 mN NaOH (Fisher Scientific, Fair Lawn, NJ) was added to post column in order to optimize the baseline stability and detector sensitivity. Fucose (Sigma, Saint Louis, MO) was added to each sample as an internal standard and all the samples were filtered through a 0.20 μm nylon syringe filter (Millipore, Billerica, MA) before analysis. Sugar contents were quantitatively determined by comparison with standard sugars. All experiments were duplicated and the cellulose and hemicellulose contents were calculated using following equations (2.1, 2.2).

$$\text{cellulose (\%)} = \text{glucose(g)} \times 0.9 / \text{sample dry weight(g)} \times 100 \quad (2.1)$$

$$\text{hemicellulose (\%)} = \{(\text{xylose} + \text{arabinose})(\text{g}) \times 0.88 + \text{galactose(g)} \times 0.9\} / \text{sample dry weight(g)} \times 100 \quad (2.2)$$

where the value of 0.9 and 0.88 is the correction coefficient for hydration (Iyer and Lee, 1999).

2.3. Autohydrolysis pretreatment

Autohydrolysis pretreatments were carried out in a 1.0 L alloy C-276 reactor (Parr Instruments Company, Moline, IL). For each batch of cook, 60 g of oven dry wheat straw samples were placed in the reactor and supplemented with the proper amount of deionized water in order to set water to solid ratio of 4:1. The media was heated to desired temperatures in the range of 160 to 200ºC for 10 to 20 min (after the reactor was pre-heated to desired temperature for 20 min). The reactor was cooled to room temperature with running tap water, and pretreated samples were filtered through cheese cloth. After the filtration, filtrate was collected in a plastic vial and stored in a refrigerator at 4ºC for pH measurement, sugar, and byproduct analyses. Mono-sugars of the separated filtrate were analyzed by the IC system as outlined earlier and after acid hydrolysis of samples by using 4% (w/w) H_2SO_4 for 1 h at 121ºC. The filtrates were filtered through a 0.20 μm nylon syringe filter prior to analysis. All autohydrolysis pretreatments were performed in duplicate.

Severity factor expressing the influence of temperature and time on autohydrolysis process was calculated using equation (2.3) (Overend and Chornet, 1987).

$$\text{severity factor}=\log_{10}[t1\times\exp(T1-100/14.75)] \quad (2.3)$$

where t1 and T1 are the pretreatment time (min) and temperature (ºC), respectively. The value of 14.75 is an empirical parameter related to temperature and activation energy. Severity factor was calculated in the range of 2.77-4.25, depending on pretreatment temperature and time.

The remaining solid residues were washed under running tap water and soaked for 2 h. After that, the solid residues were centrifuged to achieve relatively uniform moisture content. The moisture contents of solid residues were tested by (NREL) Laboratory Analytical Procedures (Ehrman, 1994). Pretreated solid residues were refined using a refiner having a plate opening of 0.005 inches. Obtained pulps were centrifuged and their moisture contents were tested. Approximately 24 g of oven dried pulps with the consistency of 10% were subsequently subjected to a PFI mill refining post-treatment at 6000 revolutions. The post-treated pulp was centrifuged and fluffed in order to determine the consistency. The refined and post-treated samples were stored in a refrigerator at 4ºC for enzymatic hydrolysis.

2.4. Byproduct analysis

Acetic acid, formic acid, hydroxymethylfurfural (HMF) and furfural were analyzed by a HPLC system (Dionex UltiMate 3000, Sunnyvale, CA) equipped with a Bio-Rad Micro-Guard column, a refractive index detector and a multi wavelength ultraviolet detector (UVD170U). Compounds were successfully separated by using a 300 mm × 7.8 mm BioRad Aminex HPX-87H column, eluted at 65ºC with 5mM H_2SO_4 at a flow rate of 0.6 mL/min.

Acetic acid and formic acid contents of samples were analyzed at 210 nm and HMF and furfural at 277 nm. Standard curves were made for each byproduct compound by using a 5-point calibration. All the samples were filtered through a 0.20 μm filter before HPLC analysis.

2.5. Enzymatic hydrolysis

Enzymatic hydrolysis of refined and post-treated substrates was performed with a mixture of Cellic CTec2 and Cellic HTec2 (Novozymes, Franklinton, NC). The activity of CTec2 was tested to be 139 FPU/g (filter paper unit, described as μmol of glucose produced per minute with filter paper as a substrate) (Ghose, 1987). Enzyme dosages of 4, 10 FPU/g oven dry substrate were applied. Two grams of oven dry substrate was supplemented with enzyme mixture and then added into 50 mM acetate buffer (pH=4.8) to achieve a 5% (w/w) the consistency of solution. Sodium azide (0.1%, w/w) was used in the media to inhibit microbial contamination (Lee et al., 2010a). All samples were incubated at 50ºC in an air incubator shaker (Series 25, New Brunswick Scientific Co., NJ) at 180 rpm for 96 h.

After the enzymatic hydrolysis, samples were immediately placed into boiled water for 3 min and then centrifuged. Supernatants were filtered through a 0.20 μm filter to be recovered for sugar analysis. The solid residues were filtered through a pre-weighed filter paper (Whatman No.1). The filter papers along with solid residues were dried in an oven at 105ºC to determine the weight losses of the substrates. All the experiments were performed in duplicate. Sugar concentrations of enzymatic hydrolysates were determined by a HPLC

system (Agilent 1200, Agilent, Santa Clara, CA), including a Shodex SP0810 column (8 mm × 300 mm, Showa Denko, Tokyo, Japan). All samples eluted at 80ºC with Mili-Q water at a flow rate of 0.5 mL/min with peak detection using a refractive index detector, set at 35ºC.

3. Results and discussion

3.1. Chemical composition of wheat straw

The chemical composition of wheat straw is shown in Table 2.1. The holocellulose from wheat straw accounts for 57.0% of the total weight and consists of glucan 35.4%, xylan 17.8%, arabinan 2.7%, and galactan 1.0%. Also, wheat straw consists of 1.5% acetyl groups and 13.3% other components such as protein, uronic acids, lipids and so on. The chemical composition of wheat straw is in good agreement with other values reported in the literature (Carvalheiro et al., 2009; Ruiz et al., 2011; Pronyk and Mazza, 2012).

Table 2.1 Yield and chemical composition of raw material and autohydrolyzed wheat straw as functions of severity factor. Results based on 100 g of oven dry original raw material. The range and average of duplicate measurements is reported.

	Raw material	160ºC		170ºC		180ºC		190ºC		200ºC	
Time (min)		10	20	10	20	10	20	10	20	10	20
Severity factor		2.77	3.07	3.06	3.36	3.36	3.66	3.65	3.95	3.94	4.25
Holocellulose	57.0±0.3	52.9±0.9	54.1±0.1	52.9±0.2	47.9±3.5	45.7±0.8	41.6±0.6	39.1±0.2	37.4±0.1	36.4±0.1	33.6±0.1
Hemicellulose	21.6±0.1	18.3±0.0	18.8±0.1	17.7±0.2	14.3±1.6	11.6±0.3	7.6±0.2	5.2±0.1	2.8±0.0	1.9±0.0	1.3±0.0
Glucan	35.4±0.2	34.6±0.8	35.3±0.0	35.1±0.0	33.6±1.9	33.9±0.5	33.9±0.4	33.9±0.1	34.5±0.1	34.5±0.1	32.3±0.1
Xylan	17.8±0.1	16.4±0.0	17.1±0.1	16.3±0.2	13.8±0.9	11.6±0.3	7.6±0.2	5.2±0.1	2.8±0.0	1.9±0.0	1.3±0.0
Arabinan	2.7±0.0	1.9±0.0	1.7±0.0	1.4±0.0	0.5±0.7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Total lignin	22.6±0.1	17.9±0.3	17.0±0.3	16.8±0.2	16.5±0.3	15.1±0.2	13.8±0.0	13.9±0.1	13.3±0.2	12.6±0.4	13.2±0.3
Klason lignin	19.8±0.1	16.5	15.8	15.7	15.6	14.3	13.2	13.5	12.9	12.3	12.8
ASL[a]	2.8±0.0	1.4	1.2	1.1	0.9	0.8	0.6	0.5	0.4	0.4	0.3
Extractives	1.2±0.0	0.8	0.8	0.9	1.1	1.3	2.2	2.7	4.3	5.4	6.8
Ash	4.4±0.1	0.5±0.0	0.2±0.1	0.3±0.1	0.4±0.1	0.3±0.0	0.6±0.0	0.8±0.0	0.6±0.0	0.6±0.0	0.5±0.1
Others[b]	13.3±0.1	14.5	12.6	10.6	11.6	8.2	6.7	5.8	4.1	3.4	1.9
Yield[c]	100	86.7±0.9	84.7±1.0	81.4±1.5	77.4±1.2	70.6±0.4	64.9±0.0	62.3±0.4	59.7±2.2	58.4±0.3	56.0±1.9

[a] Acid soluble lignin. [b] By difference. [c] Solid recovery yield.

3.2. Influence of autohydrolysis on the composition of wheat straw

The yield and chemical composition of wheat straw after autohydrolysis pretreatment are shown in Table 2.1. As shown in Table 2.1, the yield of solid residue decreases from 86.7% to 56.0% with increasing severity factor from 2.77 (160°C, 10 min) to 4.25 (200°C, 20 min). The reason of decrease of solid yield is due to hemicellulose solubilization and byproducts formation, including acetic acid, formic acid, HMF and furfural. Cellulose content of solid residue remains significantly unchanged in the autohydrolyzed solids because the polymer chains in cellulose are tightly packed in highly crystalline structures. On the other hand, hemicellulose and lignin contents of solid content decrease steadily from 18.3% to 1.3% and 17.9% to 12.6% as severity factor increases from 2.77 to 4.25, respectively. Autohydrolysis affects predominantly the hemicellulose components, with 93.9% solubilization of the original hemicelluloses at a severity of 4.25. The remaining xylan in the solid residue is likely more tightly bound to the lignin, which makes it less susceptible to hydrolysis under the severest condition used in this study (Pronyk and Mazza, 2012). Approximately 42% of original lignin is solubilized at the severest condition. Similar tendencies have been observed for Coastal Bermuda grass (Lee et al., 2010a) and barley husks (Ares-Peon et al., 2011).

3.3. Influence of autohydrolysis on pH (acidity)

The pH profile during autohydrolysis indicates treatment severity and is related to the solubility of the hemicelluloses into the liquid phase, in turn, allows for the recovery of carbohydrates as soluble mono and oligo-sugars (Carvalheiro et al., 2004). Fig. 2.1 shows the

effect of autohydrolysis conditions on the pH of the filtrate. As seen in Fig. 2.1, the pH of the autohydrolysis filtrate for 10 min and 20 min decreases from 5.4 to 3.8 at 160°C and 5.1 to 3.7 at 200°C, respectively. During autohydrolysis process, acetyl groups present in raw material are cleaved to generate acetic acid during depolymerization of the hemicellulose (Garrote and Parajo, 2002; Lee et al., 2010b). Also, hydronium ions produced from water autoionization and from the ionization of acids (i.e. formic acid and acetic acid) further catalyze a series of autohydrolysis reactions (Palmqvist and Hahn-Hagerdal, 2000; Lee et al., 2009; Lee et al., 2010b). The lowest pH of the filtrate is found to be 3.7 at 200ºC for 20 min, which indicates that higher severity conditions promote the cleavage of acetyl groups into acetic acid. Similar results have been observed in previous study (Carvalheiro et al., 2009).

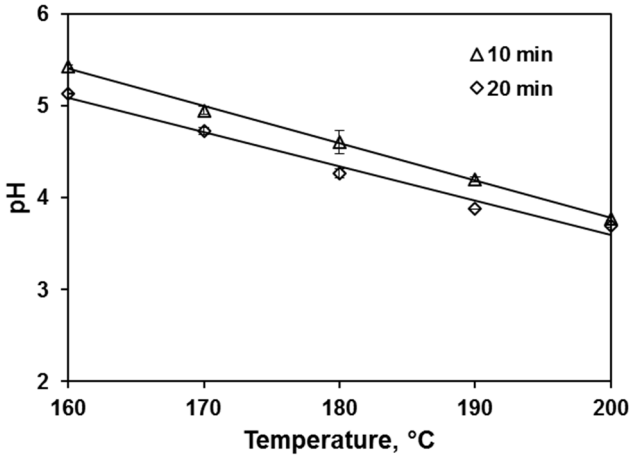

Fig. 2.1 The pH of the autohydrolysis filtrate at different pretreatment conditions. Error bars indicate the upper and lower test results.

3.4. Influence of autohydrolysis on byproducts

During autohydrolysis pretreatment, acetic acid and formic acid are produced by hydrolysis of acetyl groups in hemicellulose (Ligero et al., 2011; Zhang et al., 2010) and further degradation of sugar byproducts (i.e. hydroxymethylfurfural and furfural) (Mills et al., 2009), respectively. Fig. 2.2 shows the effects of autohydrolysis pretreatment on the byproducts including acetic acid, formic acid, HMF and furfural. Byproduct compounds not only cause lower sugar yields of hemicelluloses, but also could potentially inhibit the formation of ethanol during the fermentation process (Hu and Ragauskas, 2012). As seen in Fig. 2.2, 0.37-2.14 g acetic acid, 0.08-1.01 g formic acid, 0.00-0.36 g HMF and 0.00-2.21 g furfural are generated in the autohydrolysis filtrate with increasing severity factor from 2.77 to 4.25. Acetic acid (2.14 g), generated from hydrolysis of hemicellulose, and furfural (2.21g) a sugar degradation product from pentoses (xylose and arabinose) had the highest concentrations in the filtrate at conditions with the highest severity when compared with the other byproducts (Mills et al., 2009). Also, the increase in the concentrations of byproducts leads to decrease in pH of the filtrate. The results indicate a relationship between acid generation and hemicellulose solubilization. Similar observations have been reported in the literature (Carvalheiro et al., 2009; Lee et al., 2009).

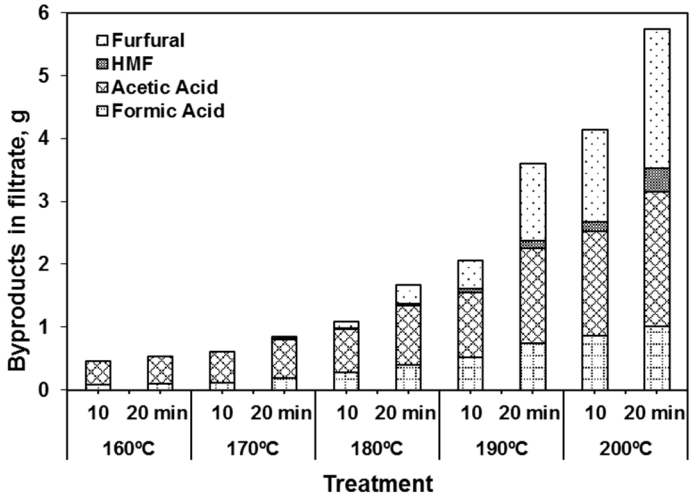

Fig. 2.2 Effect of different autohydrolysis conditions on the byproducts in the autohydrolysis filtrate.

3.5. Sugars in the autohydrolysis filtrate

Fig. 2.3 shows mono-sugars and oligo-sugars in the filtrate before and after acid hydrolysis under different autohydrolysis conditions. All results are reported in grams based on 100 g of raw material. During the autohydrolysis pretreatment, water molecule under high temperature and pressure penetrates into biomass, resulting in partial hydrolysis of hemicellulose and cellulose, and dissolution of a portion of the lignin (Thaerzadeh et al., 1997). As seen in Fig. 2.3a, arabinan is the main mono-sugars in the filtrate under lower severity conditions, while xylan is the main component at higher severities. Xylan in the filtrate is at a maximum of 1.5 g at 200ºC for 10 min, compared to the other mono-sugars. The overall mono-sugars detected in the filtrate are in the range of 0.3 to 2.1 g. The results

indicate that 4.8-17.6% of mono-sugars based on carbohydrate are recovered from the autohydrolysis filtrate.

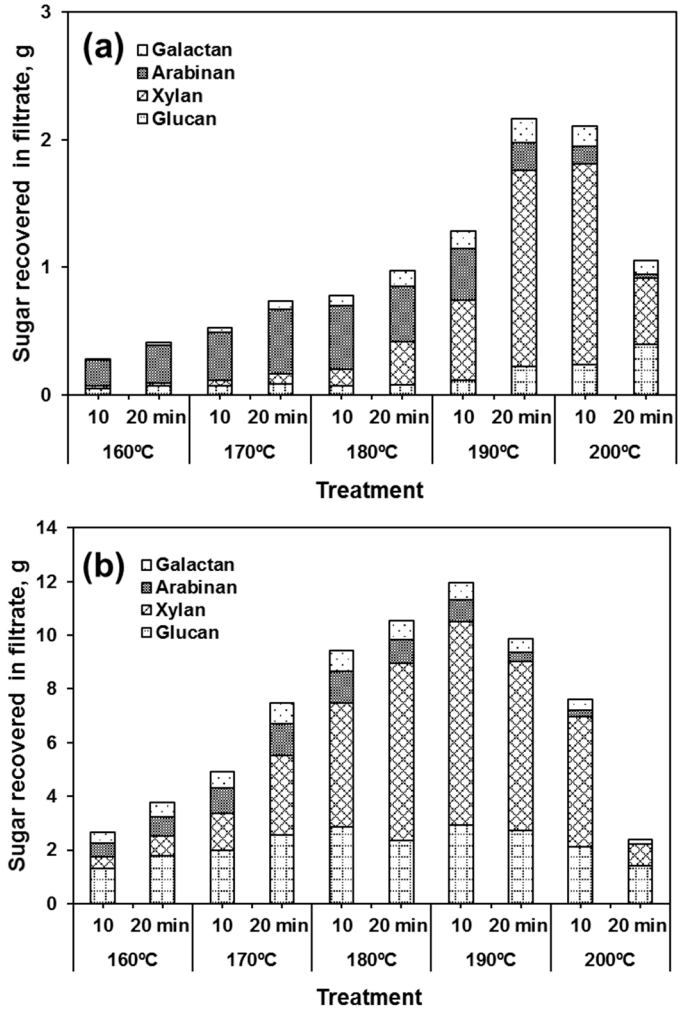

Fig. 2.3 Sugars in the autohydrolysis filtrate: (a) before acid-treated hydrolysis; (b) after acid hydrolysis with 4% H_2SO_4.

Since the sugars are dissolved as both mono and oligo-sugars, in this study, 4% (w/w) sulfuric acid is used to hydrolyze all the oligo-sugars into mono-sugars (Fig. 2.3b). It is assumed that oligo-sugars can be further hydrolyzed to mono-sugars by enzymes and recovered for the production of ethanol. As shown in Fig. 2.3b, the total sugars in the filtrate are in the range of 2.4 to 12.2 g per 100 g of starting biomass, depending on severity factor of autohydrolysis pretreatment. The maximum level of total sugars in the filtrate is found to be 12.2 g at 190ºC for 10 min, followed by 180ºC for 20 min (10.8 g) Total sugars amounts significantly decrease with increasing severity factor from 3.65 to 4.25. Compared with the byproducts formation in Fig. 2.2, this phenomenon may be due to further degradation of mono-sugars to byproducts (i.e. furfural and HMF) at the highest severity conditions. The highest total sugar in the filtrate is 68.2% of the total hemicelluloses at 190ºC for 10 min.

3.6. Enzymatic hydrolysis of autohydrolyzed solid residues

Enzymatic hydrolysis was carried out using the solid residues from each pretreatment. Fig. 2.4 shows the enzymatic hydrolysis of the substrates with and without PFI refining post-treatment from each autohydrolysis condition at the enzyme loading of 4 FPU/g oven dry substrate. As seen in Fig. 2.4a, the enzymatic hydrolysis of cellulose to glucose increases with increasing time and PFI refining post-treatment at each autohydrolysis temperature. The maximum glucose yield after enzymatic hydrolysis for substrate pretreated with PFI is found to be 24.9 g per 100 g of starting biomass at autohydrolysis condition of 180ºC for 20 min which means about 73.4% of cellulose in the solid residue from this condition is converted to fermentable glucose by enzymatic hydrolysis which is equivalent to 43.7% of carbohydrate

based on the starting raw material). The glucose yield of the substrate post-treated with PFI from 180ºC autohydrolysis for 20 min is 24.9 g while the glucose yield of the substrate without PFI from this condition is 16.2 g (28.4% of carbohydrate in the raw material). Refining is a critical step for the commercialization of autohydrolysis, since it allows for high sugar recovery at low enzyme dosages. For a process to be economically viable enzyme dosages need to be about 4-5 FPU/g of substrate, due to the high cost of enzymes.

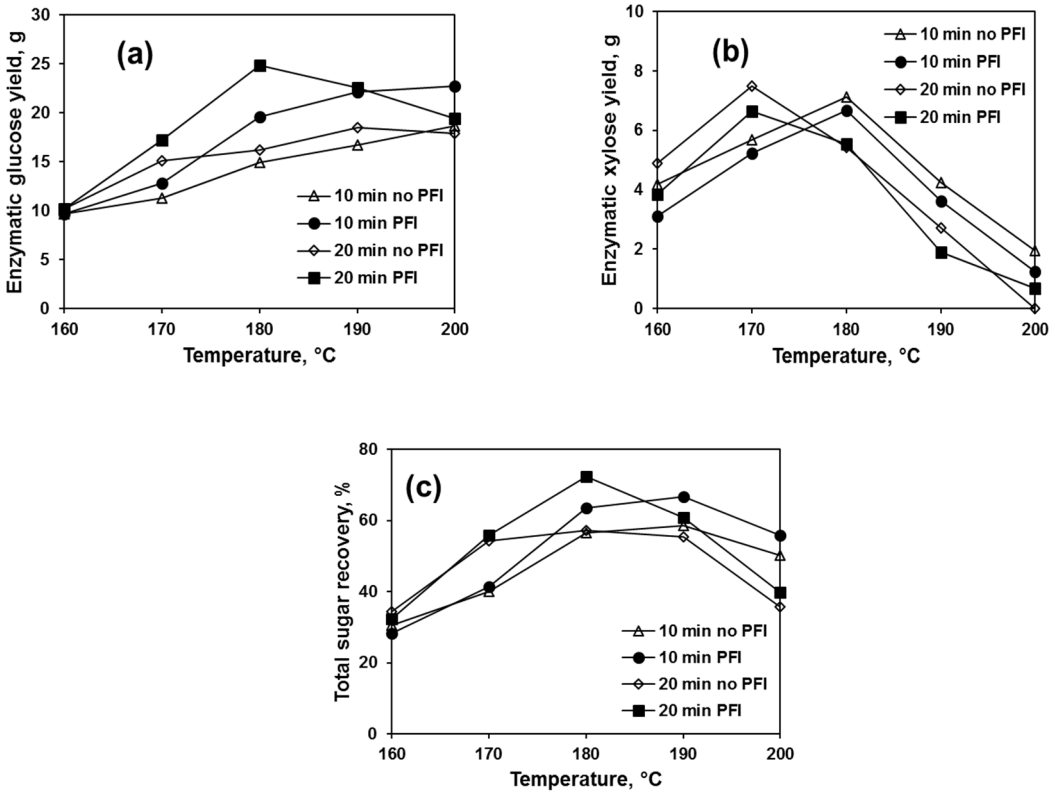

Fig. 2.4 Enzymatic hydrolysis of untreated and treated (PFI) solid residue from each pretreatment condition at 4 FPU/g substrate enzyme loading for 96 h: (a) enzymatic glucose yield; (b) enzymatic xylose yield; (c) total sugar recovery.

The highest xylose yield is found to be 7.5 g (52.4% of hemicellulose in the solid residue from this condition) after enzymatic hydrolysis of the substrate without PFI treatment from 170ºC for 20 min. On the other hand, 6.7 g xylose is generated from the substrate treated with PFI refining from 180°C for 10 min. (Fig. 2.4b). Xylose yield increases with increasing treatment time at lower temperatures, but sharply decreases at higher temperature. The reason for this is most likely due to further degradation of xylose to byproducts (i.e. furfural and HMF) at higher severity conditions.

As seen in Fig. 2.4c, with enzyme loading of 4 FPU/g, total sugar recovery for substrate treated with PFI from 180ºC for 20 min shows that 72.3% of carbohydrate in the raw material has been successfully converted to mono-sugars by enzymatic hydrolysis, 10.8 g (18.9%) of mono-sugars in the prehydrolyzate and 30.4 g (53.4%) in the enzyme hydrolyzate.

Fig. 2.5 shows the enzymatic hydrolysis of the substrates with and without PFI post-treatment from each autohydrolysis condition at the enzyme loading of 10 FPU/g oven dry substrate. This 10 FPU/g enzyme dosage was evaluated to understand the sugar recovery potential of the substrate if enzymes become cheaper in the future. PFI refining, elevated temperature and increased time increase the enzymatic hydrolysis of cellulose to glucose. The maximum glucose yield is found to be 28.3 g per 100 g of starting biomass (82.0% of cellulose in the solid residue from this condition) after enzymatic hydrolysis of the substrate with PFI treatment from 200ºC for 10 min. (Fig. 2.5a). Glucose yield of the substrate with

PFI from at 200ºC for 10 min increases from 22.7 to 28.3 g with increasing enzyme loading from 4 to10 FPU/g. As shown in Fig. 2.5b, pretreatment at 170ºC for 20 min results in the highest xylose yield of 7.4 g per 100 g of starting biomass, which means about 51.7% of hemicellulose in the solid residue from this condition is converted to fermentable xylose by enzymatic hydrolysis. Similar to enzyme loading of 4 FPU/g, xylose yield increases with increasing treatment time at lower temperatures, but sharply decreases at higher temperature because of byproducts formation. As shown in Fig. 2.5c, with enzyme loading of 10 FPU/g, total sugar recovery for substrate treated with PFI from 190ºC for 10 min shows that 75.5% of carbohydrate in the raw material has been successfully converted to mono-sugars by enzymatic hydrolysis. Substrate generated from 190ºC for 10 min autohydrolysis pretreatment combined with PFI post-treatment shows the best enzymatic hydrolysis performance in terms of total sugar recovery. Moreover, total sugar recovery increases by 15% by employing the PFI post-treatment to pulp from solid residue, depending on pretreatment temperature and time.

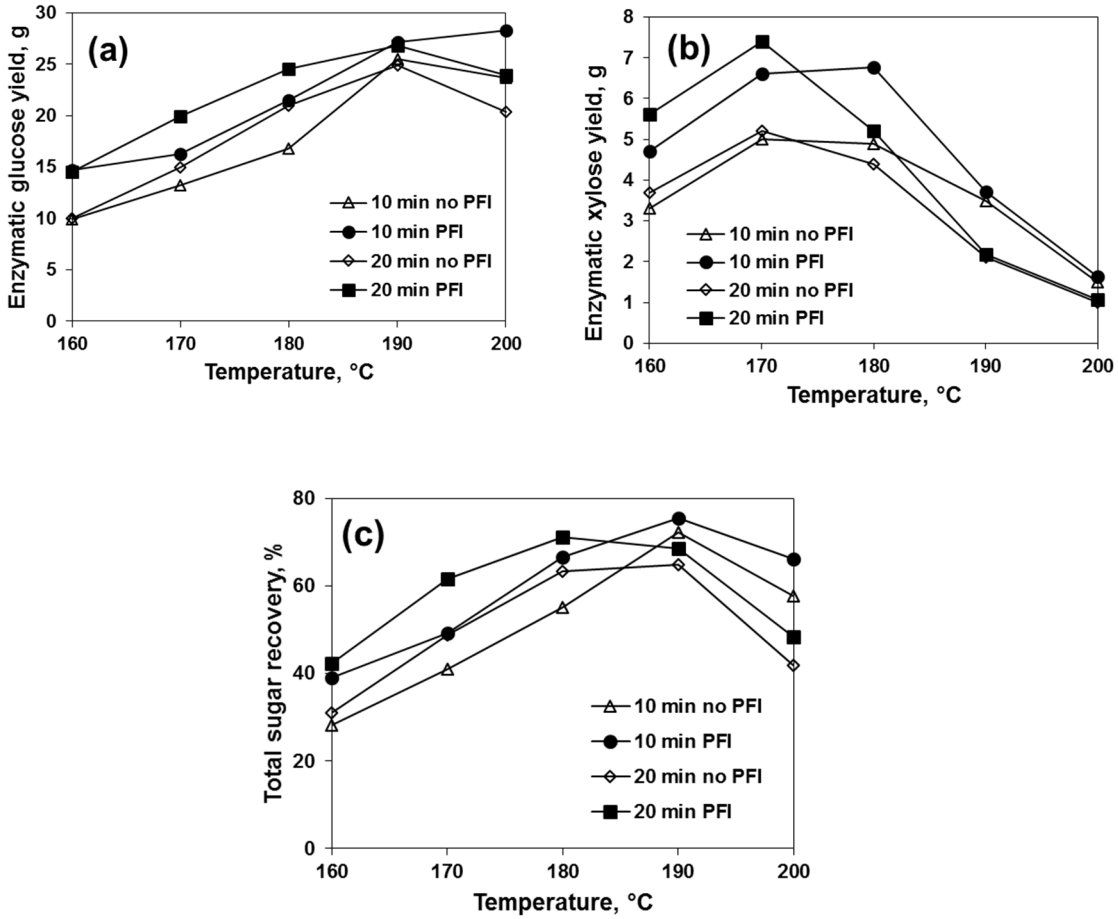

Fig. 2.5 Enzymatic hydrolysis of untreated and treated (PFI) solid residue from each pretreatment condition at 10 FPU/g substrate enzyme loading for 96 h: (a) enzymatic glucose yield; (b) enzymatic xylose yield; (c) total sugar recovery.

3.7. Material balance

Material balances of autohydrolysis pretreatment with and without PFI and subsequent enzymatic hydrolysis carried out all pretreatment and enzyme hydrolysis conditions are shown in Table 2.2 and Table 2.3, respectively. The material balance shows

higher than 86% of the solid recovery through the process for the lowest severity condition. The minimum solid recovery was 56.0% at the highest severity. This decrease in the solid recovery indicates that higher severity conditions generate byproducts including acetic acid, formic acid, furfural and HMF.

The total amount of sugar in the filtrate after autohydrolysis pretreatment increases gradually with increasing severity factor from 2.77 to 3.65 but decreases sharply at the most severe conditions because of further sugar degradation (Table 2.2 and Table 2.3). This phenomenon causes less sugar generation from hemicellulose with enzymatic hydrolysis after autohydrolysis pretreatment (Lee et al., 2010b). On the other hand, glucose generation from enzymatic hydrolysis increases in general with increasing severity factor. Autohydrolysis pretreatment combined with PFI post-treatment at 190ºC for 10 min with an enzyme loading of 10 FPU/g provides the maximum total sugar recovery, in total 43.0 g of sugar, which means 75.5% of carbohydrate in the raw material (Table 2.2). On the other hand, the highest total sugar recovery of 41.2 g was recovered at 180 for 20 min with refining at 4 FPU/g enzyme loading, which is 72.3% of the sugar yield. However, pretreatment without PFI at 190ºC for 10 min with a 10 FPU/g enzyme charge provides 72.4% of total sugar recovery (Table 2.3). The results indicate that PFI post-treatment of the pulp from each pretreatment solid residue except the lowest severity condition shows desired enhancement of enzymatic hydrolysis performance.

Table 2.2 Material balances from autohydrolysis pretreatments with PFI treatment followed by enzymatic hydrolysis.

Temperature (ºC)	Time (min)	Severity factor	Solid Recovery (%)	Sugars in filtrate (g)			FPU	Enzyme hydrolyzate (g)			Sugar recovery	
				G[a]	H[b]	T[c]		G[a]	H[b]	T[c]	(g)[d]	(%)[e]
160	10	2.77	86.7	1.3	1.5	2.8	4	9.7	3.6	13.3	16.1	28.3
							10	14.7	4.7	19.4	22.2	39.1
	20	3.07	84.7	1.8	2.2	4.0	4	10.2	4.3	14.5	18.5	32.4
							10	14.6	5.6	20.2	24.2	42.4
170	10	3.06	81.4	2.0	3.2	5.2	4	12.8	5.5	18.3	23.5	41.3
							10	16.3	6.6	22.9	28.1	49.3
	20	3.36	77.4	2.6	5.1	7.7	4	17.2	6.8	24.0	31.7	55.8
							10	19.9	7.4	27.3	35.0	61.6
180	10	3.36	70.6	2.9	6.8	9.7	4	19.6	6.9	26.4	36.1	63.4
							10	21.5	6.8	28.3	38.0	66.7
	20	3.66	64.9	2.3	8.5	10.8	4	24.9	5.5	30.4	41.2	72.3
							10	24.6	5.2	29.8	40.6	71.2
190	10	3.65	62.3	2.9	9.3	12.2	4	22.1	3.7	25.8	38.0	66.6
							10	27.1	3.7	30.8	43.0	75.5
	20	3.95	59.7	2.7	7.4	10.1	4	22.6	1.9	24.5	34.6	60.8
							10	26.8	2.2	29.0	39.1	68.7
200	10	3.94	58.4	2.1	5.7	7.8	4	22.7	1.3	24.0	31.8	55.9
							10	28.3	1.6	29.9	37.7	66.2
	20	4.25	56.0	1.4	1.1	2.5	4	19.4	0.7	20.1	22.6	39.7
							10	24.0	1.1	25.1	27.6	48.4

[a] G: released glucose.
[b] H: released xylose and other monosugars.
[c] T: total sugars.
[d] Sum of sugars in filtrate + enzyme hydrolyzate.
[e] Percentage of sugar recovery, calculated by (sugar recovery (g) / carbohydrate in the raw material (g)).

Table 2.3 Material balances from autohydrolysis pretreatments without PFI treatment followed by enzymatic hydrolysis.

Temperature (ºC)	Time (min)	Severity factor	Solid Recovery (%)	Sugars in filtrate (g)			FPU	Enzyme hydrolyzate (g)			Sugar recovery	
				G[a]	H[b]	T[c]		G[a]	H[b]	T[c]	(g)[d]	(%)[e]
160	10	2.77	86.7	1.3	1.5	2.8	4	9.7	4.8	14.5	17.3	30.4
							10	9.9	3.3	13.2	16.0	28.1
	20	3.07	84.7	1.8	2.2	4.0	4	10.1	5.6	15.7	19.7	34.6
							10	10.0	3.7	13.7	17.7	31.0
170	10	3.06	81.4	2.0	3.2	5.2	4	11.3	6.4	17.7	22.9	40.2
							10	13.2	5.0	18.2	23.4	41.1
	20	3.36	77.4	2.6	5.1	7.7	4	15.1	8.0	23.1	30.8	54.1
							10	15.0	5.2	20.1	27.8	48.9
180	10	3.36	70.6	2.9	6.8	9.7	4	15.0	7.5	22.5	32.2	56.5
							10	16.8	4.9	21.7	31.4	55.1
	20	3.66	64.9	2.3	8.5	10.8	4	16.2	5.6	21.8	32.6	57.2
							10	21.0	4.4	25.4	36.2	63.4
190	10	3.65	62.3	2.9	9.3	12.2	4	16.7	4.4	21.1	33.3	58.4
							10	25.5	3.5	29.0	41.2	72.4
	20	3.95	59.7	2.7	7.4	10.1	4	18.5	3.0	21.5	31.6	55.4
							10	24.9	2.1	26.9	37.0	65.0
200	10	3.94	58.4	2.1	5.7	7.8	4	18.7	2.1	20.8	28.6	50.2
							10	23.7	1.5	25.1	32.9	57.8
	20	4.25	56.0	1.4	1.1	2.5	4	17.9	0.0	17.9	20.4	35.8
							10	20.4	1.0	21.4	23.9	42.0

[a] G: released glucose.
[b] H: released xylose and other monosugars.
[c] T: total sugars.
[d] Sum of sugars in filtrate + enzyme hydrolyzate.
[e] Percentage of sugar recovery, calculated by (sugar recovery (g) / carbohydrate in the raw material (g)).

4. Conclusions

Autohydrolysis combined with refining is a simple and efficient pretreatment method for ethanol production. A maximum of 21.4% of total sugars were released into the prehydrolyzate at 190°C for 10 min. However, the highest total sugar recovery of 72.3% was achieved at 180°C for 20 min with refining at 4 FPU enzyme dosages. A simple process consisting of only autohydrolysis with water followed by refining is able to achieve high sugar recoveries at low enzyme dosages comparable to other process options using chemicals in the pretreatment stage. This proposed process can be implemented with a low capital and operating cost.

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Chapter 2.2 Autohydrolysis combined with mechanical refining to extract fermentable sugars from switchgrass

Abstract

Switchgrass was pretreated using autohydrolysis pretreatment with different temperatures (160 - 200 °C) and times (10 and 20 min), followed by mechanical refining as post-treatment and further hydrolyzed with enzymes to evaluate its total sugar recovery. A maximum total sugar recovery of 75.9% (23.4% from autohydrolysis filtrate and 52.5% from enzymatic hydrolyzate) can be achieved when switchgrass was pretreated at 190 °C for 20 min followed by 6000 revolutions PFI refining at 4 FPU/g enzyme dosages, along with 4.4 g/100 g raw switchgrass degradation products formed. The mechanical refining has been proved to be able to significantly enhance the digestibility of autohydrolysis pretreated switchgrass at low enzyme dosage.

Keywords: switchgrass, autohydrolysis, enzymatic hydrolysis, refining, sugar recovery

1. Introduction

The majority of fuel and chemical products utilized today are derived from fossil sources through refinery operation. However, the continuous price increase of fossil fuel, the uncertainty in fossil fuel reserve, and environmental concerns of their exploitation necessitate the development of alternative and sustainable sources (Cherubini and Strømman 2011). Lignocellulosic biomass has drawn remarkable interest as an alternative resource for fuel and

chemical production due to its great abundance, competitive feedstock price, sustainable supply, and positive environmental impacts (Perlack, Wright et al. 2005). Switchgrass (*Panicum virgatum* L.) is one of the promising lignocellulosic biomass for biofuel and biochemical production as a result of high production yield (up to 23 tons/hectare per year), adaption to drought and high temperature, modest fertilizer required for growth, and high net energy yield as feedstock for biofuel production (540% more energy produced than consumed during production process) (McLaughlin and Adams Kszos 2005; Schmer, Vogel et al. 2008; Secter 2008).

Fermentable sugars are primary intermediates for biofuel and biochemical production from lignocellulosic biomass. Enzymatic conversion is the most favorable method to extract fermentable sugars because of its highly selectivity, environmental compatibility, and potential to achieve the theoretical yield of sugar. Unlike starch/sugar-based feedstock, pretreatment is an essential step towards an efficient conversion of lignocellulosic biomass to fermentable sugars owing to the native recalcitrance of lignocellulosic biomass to enzymes attack. The objective of pretreatment is to break the lignin seal, disrupt cellulose crystallinity structure, and thus render lignocellulosic biomass more susceptible to actions of enzymes (Mosier, Wyman et al. 2005). Various types of pretreatment methods have been investigated, but few of them can be successfully employed in a commercial biorefinery process because many researchers only aimed at high fermentable sugar yield without taking account into the impact of pretreatment to the economic efficiency of the whole biorefinery process including chemical recovery, capital cost of the technology, and waste water treatment.

Autohydrolysis pretreatment, so-called liquid hot water or hydrothermal pretreatment, employs only water as reaction medium at elevated temperature 160 to 220 °C (Gullón, Romaní et al. 2012). It is regarded as an attractive pretreatment process leads to extraction of oligomeric and monomeric sugars during reaction and increased enzymatic digestibility of pretreated biomass without additional chemicals. It has been reported that autohydrolysis pretreatment is suitable for a wide range of lignocellulosic biomass which have enriched hemicellulose content and modest lignin content, including hardwood, wheat straw, rice straw, corn stover, sugarcane bagasse, coastal bermuda grass, and so on (Garrote and Parajó 2002; Laser, Schulman et al. 2002; Mosier, Hendrickson et al. 2005; Lee, Shi et al. 2009; Jing, Jin et al. 2010; Ertas, Han et al. 2013). The major advantages of autohydrolysis are the low cost of solvent, negligible environmental pollution, and most importantly, lower capital cost due to its process simplicity.

The main hurdle to hamper autohydrolysis pretreatment to become a commercial process for biofuel and biochemical production is the modest sugar yield recovered from original biomass at an affordable enzyme dosage. It has been reported that mechanical refining, which has been widely used in pulp and paper industry to promote fibrillation of fibers and increase paper strength, can largely enhance the enzymatic digestibility of pretreated substrate including corn stover (Chen et al., 2012b), hardwood (Jones et al., 2013), softwood (Wu, Chang et al. 2012), and recovered office printing paper (Chen et al., 2012a). The previous work shows that a 40% of enzymatic sugar yield increase can be obtained when autohydrolysis pretreated wheat straw was subjected to 6000 revolutions PFI refining (Ertas

et al., 2013). The objective of this study is to identify the most efficient pretreatment conditions to maximize total sugar recovery and minimize degradation products from switchgrass and to check how mechanical refining can improve the digestibility of autohydrolysis pretreated biomass.

2. Materials and Methods

2.1. Raw material

Switchgrass was collected from local farmland in North Carolina, USA. The material was air-dried to have ~ 5% moisture content, hand-cut into 2-3 cm sizes, and then stored in air tight container at room temperature prior to pretreatment. Part of the switchgrass was further milled in a Thomas Wiley Laboratory Mill (Model No.4 Thomas Scientific, Philadelphia, PA) to pass through a 20 mesh screen for compositional analysis.

2.2. Autohydrolysis pretreatment of switchgrass

Autohydrolysis pretreatment was performed in a 1.0 L alloy C-276 parr reactor (Parr Instrument Company, Moline, IL, USA). For each batch of cook, 60 dry grams of switchgrass samples were loaded into the reactor and supplemented with proper amount of deionized water to make a final solid to liquor ratio of 1:4. Evacuation on the loaded reactor was carried out to minimize the false pressure generated by air. A set of temperatures (160 to 200 °C) and 2 pretreatment times (10 and 20 min) were investigated. The time to reach the target temperature ranges from 20 to 29 min depending on selected temperatures. During the reaction, the samples were agitated at a speed of 30 revolution/min. After the reaction, the

reactor was quickly quenched in a cold water bath to room temperature, and pretreated samples were filtered through cheese cloth. The filtrate was centrifuged and stored in a refrigerator at 4°C for pH measurement, sugar analysis, and byproducts analysis. The solids were washed several times with deionized water, centrifuged and fluffed to achieve a uniform consistency. Experiments were performed in duplicate.

2.3. Mechanical refining

All pretreated samples first passed through a disk refiner (Bauer 148-2, the Bauer brothers company, Springfield, Ohio) at an opening of 0.005 inch for size reduction. The pulps were then centrifuged and their consistency was tested. Part of pulp was further subjected to mechanical post-treatment in a PFI mill (Hamjern Maskin A/S, Hamar, Norway), where 24 grams of oven dry samples at 10% consistency were refined at 6000 revolutions. The refined samples were centrifuged, fluffed, and stored in a refrigerator at 4 °C for enzymatic hydrolysis. It is noted that a disk refiner is aimed at disintegrating the fiber, while the PFI post-treatment is to create microfibrils and loosen fiber internal structure.

2.4. Enzymatic hydrolysis

Autohydrolysis pretreated samples with and without mechanical refining were tested for enzymatic digestibility using a mixture of Cellic CTec2 and Cellic HTec2 (Novozymes, Franklinton, NC, USA). The activity of CTec2 (filter paper unit, expressed as μmol of glucose produced per minute with filter paper as a substrate) was determined to be 139 FPU/g according to procedure described by Ghose (Ghose, 1987). CTec2 enzyme dosages of

4 and 10 FPU/g od substrate were applied to 2 dry grams of substrate supplemented with 50 mM acetate buffer (pH 4.8) to achieve a 5% (w/v) solids loading. HTec2 was charged at a ninth of CTec2 dosage according to the recommendation from Novozymes. Sodium azide (0.1% w/v) was added into the mixture to prevent microorganism contamination during enzymatic hydrolysis. Samples were incubated in an environmental incubator shaker (New Brunswick Scientific, Edison, NJ, USA) at 50 °C, 180 rpm for 96 h. The reaction was stopped by immersing samples into boiled water bath for 10 min and then centrifuged. Supernatants were filtered through 0.2 μm nylon filters for sugar analysis.

2.5. Analytic methods

The extractives content was determined using acetone extraction in soxhlet apparatus for 24 h. The solids content, ash content, acid insoluble lignin content, and acid soluble lignin content of untreated and pretreated biomass were measured according to National Renewable Energy Laboratory's (NREL) Analytical Procedures (Sluiter et al., 2008a; Sluiter et al., 2005; Sluiter et al., 2008b). The concentration of mono-sugars after two-stage, 72% w/v (2 h at room temperature) followed by 4% w/v (1h at 121 °C) sulfuric acid hydrolysis was analyzed by a high performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) (Dionex ICS-3000, Dionex Corporation, Sunnyvale, CA, USA). The HPAE-PAD was equipped with an eluent generator (EG-2 model), a guard column (CarboPac PAI 4×50 mm), an ion exchange column (CarboPac PAI 4×250 mm), and an electrochemical detector (ED-40 model). The column was operated at 18 °C with Mili-Q water and 400 mN sodium hydroxide solution as the mobile phase at a flow rate of 0.3 ml/min. A 200 mN

sodium hydroxide solution was added to post column for optimal baseline stability and detector sensitivity. Fucose was added to each sample as internal standard and sugar concentration was determined based on a proportional relationship between internal standard and individual sugar in samples. The mono-sugars in autohydrolysis filtrate were analyzed directly by HPAE-PAD system. The total sugar in prehydrolyzate was measured according to NREL's Analytical Procedures (Sluiter et al., 2006), where prehydrolyzate was hydrolyzed with 4% w/v sulfuric acid at 121 °C for 1 h, and the resulting mono-sugars were analyzed by HPAE-PAD system.

Four species of byproducts including acetic acid, formic acid, furfural, and hydroxymethylfurfural (HMF) were analyzed by a high performance liquid chromatography (HPLC) system (Dionex UltiMate 3000, Dionex Corporation, Sunnyvale, CA, USA). The HPLC is equipped with a Bio-Rad Aminex HPX-87H column (300 mm x 7.8 mm), a Bio-Rad Micro-Guard column, and a refractive index detector. The analytical column was operated at 65 °C with 0.005 M sulfuric acid as the mobile phase at a flow rate of 0.6 mL/min.

Sugar concentration of enzymatic hydrolyzate was determined by a high performance liquid chromatography (HPLC) system (Agilent 1200, Agilent, Santa Clara, CA, USA). The sugar samples passed though Shodex SP0810 column (8×300 mm, Showa Denko, Tokyo, Japan) at the temperature of 80 °C using Milli-Q water as mobile phase at a flow rate of 0.5

mL/min. A refractive index detector was used to quantify all the sugar concentrations at the temperature of 50 °C. All the experiments were carried out in duplicate.

3. Results and discussion

3.1. Impact of autohydrolysis on the composition of switchgrass

The results of solids recovery yield and composition of the pretreated biomass after autohydrolysis are illustrated in Fig. 2.6. The original switchgrass comprises glucan 33.6%, xylan 23.6%, galactan 0.9%, arabinan 3.1%, acid insoluble lignin 18.0%, acid soluble lignin 2.9%, extractives 2.1%, acetyl groups 3.0%, and ash 3.0%. After autohydrolysis, the solids recovery yield dropped quickly with elevated temperatures and longer times. Depending on pretreatment severity, the solids recovery yield decreased from 90.6% to 47.1% based on 100 g raw switchgrass.

The xylan removal dominates the reduction of the solids recovery yield after autohydrolysis. Both higher temperatures and longer residence times help to solubilize xylan into autohydrolysis filtrate. It is observed that nearly 100% xylan removal could be achieved when the biomass was autohydrolyzed at 200 °C for 20 min. The minor sugars such as galactan, arabinan showed a great reduction as well and no significant minor sugars were detected in the residue under sever conditions. On the other hand, the cellulose content in the biomass was nearly unchanged under autohydrolysis conditions investigated in this study. It is noted that remarkable lignin removal took place when switchgrass was autohydrolyzed under harsh conditions. The lignin content decreased from 20.9 g/100 g raw switchgrass in

the untreated switchgrass to 8.2 g/100 g raw switchgrass after a 20 min autohydrolysis at 200 °C, about 61% deduction in lignin content. The substantial lignin reduction during autohydrolysis has been reported on wheat straw (Ertas et al., 2013), birch wood (Borrega, et al., 2011), and corn stover (Liu & Wyman, 2003) as well. An increased amount of acetone extractable materials from autohydrolysis residues were observed, indicating a remarkable depolymerization occurred on switchgrass under sever conditions.

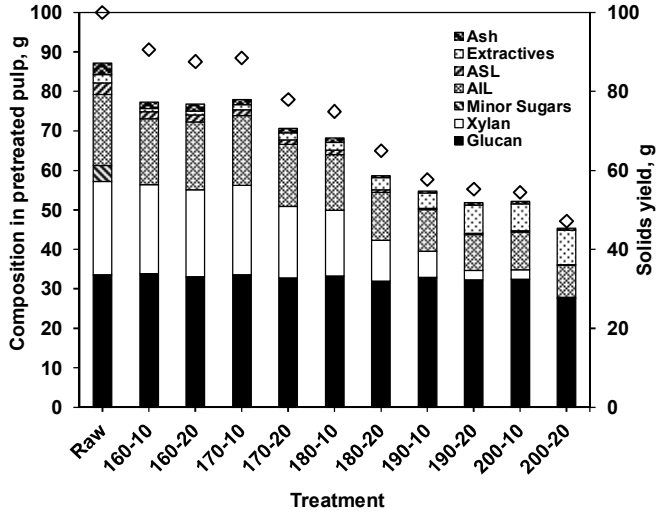

Fig. 2.6 Solids yield and composition of the autohydrolysis pretreated switchgrass, as g of component recovered per 100 g of raw switchgrass.

3.2. Impact of autohydrolysis on pH and byproducts

The pH profile indicates the severity of autohydrolysis pretreatment. As shown in Fig. 2.7, elevated pretreatment temperatures and longer residence times facilitated the decrease of

pH value in the autohydrolysis filtrate, which is largely attributed to the release of acetic acid from acetyl groups and other organic acids such as formic acid and levulinic acid formed under sever conditions.

During autohydrolysis, the formation of byproducts such as organic acids, furan derivatives, and phenolic compounds not only hamper the sugar yield from hemicellulose, but also could potentially inhibit the yeast activity during fermentation. Acetic acid is liberated from hydrolysis of acetyl groups in the hemicellulose. At high temperature and pressure, furfural is generated by the degradation of xylose and 5-hydroxymethyl furfural (HMF) is formed from hexose degradation (Dunlop 1948; Ulbricht 1984). Formic acid is generated mainly through the further degradation of furfural and HMF (Palmqvist, 2000). The partially break down of lignin yields the formation of phenolic compounds which have also been suggested to exert inhibition effect in the fermentation process (Palmqvist, 2000).

Fig. 2.7 displays the effect of autohydrolysis conditions on the byproducts generation including acetic acid, formic acid, furfural, and HMF. The higher cooking temperature and longer residence time contributed to the formation of more degradation products. The increased amount of organic acids further lowers the pH of the autohydrolysis filtrate. As shown in Fig. 2.7**,** the total amount of byproducts increased from 0.35 g/100 g raw switchgrass at 160 °C for 10 min to 8.39 g/100 g raw switchgrass at 200 °C for 20 min. Acetic acid is the major byproduct generated from autohydrolysis of switchgrass, but the formation of furfural increased substantially at higher temperature.

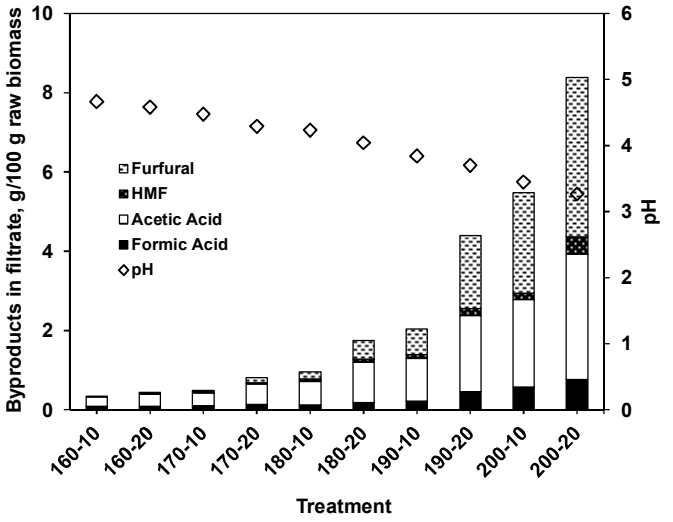

Fig. 2.7 The pH of the autohydrolysis filtrate and byproducts generation at different pretreatment conditions.

3.3. Sugars recovered in the autohydrolysis filtrate

The release of total sugars including mono-sugars and oligo-sugars in the autohydrolysis filtrate was shown in Fig. 2.8. All the sugar amounts were reported as polysaccharides form. The amount of total sugar was measured by employing 4% sulfuric acid hydrolysis to break down all oligo-sugars into monomeric forms. The Fig. 2.8a shows that elevated temperature and longer residence time can significantly improve the recovery of mono-sugars, especially xylose in the autohydrolysis filtrate. A maximum xylose recovery of 4.1 g can be obtained under 200 °C, 10 min pretreatment. Substantial reduction of mono-sugars was observed when switchgrass was pretreated at 200 °C for 20 min. This might be

due to the degradation of mono-sugars to furfural, HMF, or other byproducts under high temperatures and long residence times.

Fig. 2.8b displays the total sugars released in the autohydrolysis filtrate. The autohydrolysis at 190 °C for 10 min generated highest level of total sugars, followed by 180 °C for 20 min and 190°C for 20 min. It is noted that the maximum total sugars (14.7 g total sugars including 2.3 g mono-sugars and 12.4 g oligo-sugars) released in the autohydrolysis account for 24.3% sugar recovery based on total carbohydrates in the raw switchgrass without employing a significant amount of enzyme dosage. Xylose is the major sugars that released in the autohydrolysis filtrate which is in agreement with the large deduction of xylan content in pretreated solids.

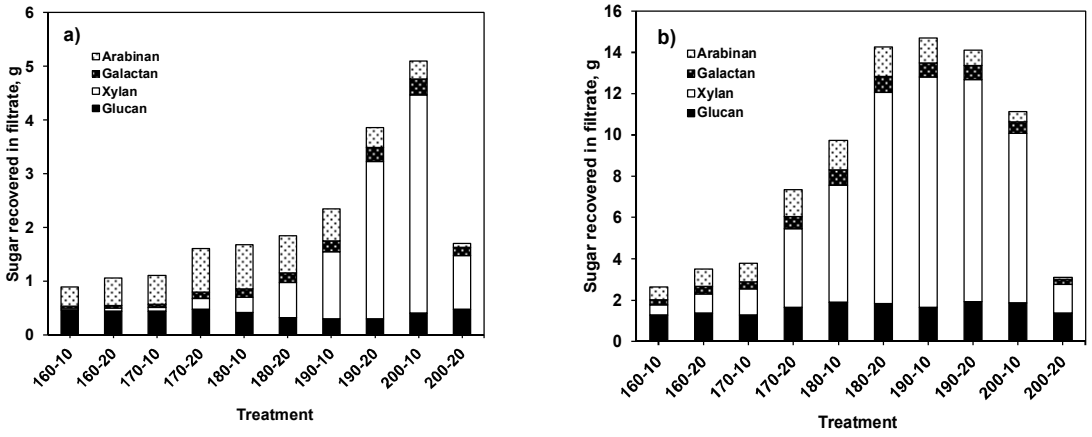

Fig. 2.8 Release of sugars in the autohydrolysis filtrate: a) before acid hydrolysis; b) after acid hydrolysis with 4% sulfuric acid, based on 100 g raw switchgrass.

3.4. Sugar recovered in the enzymatic hydrolyzate

Autohydrolysis combined with mechanical refining treated switchgrass was subjected to enzymatic hydrolysis to evaluate the effect of autohydrolysis condition and mechanical refining to enzymatic digestibility of switchgrass. As seen in Fig. 2.9, the cellulose digestibility was significantly improved with elevated temperatures and residence times. The highest glucan yield 19.7 g/100 g raw switchgrass can be achieved when switchgrass was pretreated at 200 °C for 10 min without refining post-treatment. It is important to point out that the mechanical refining can generate substantial improvements on digestibility of autohydrolysis pretreated substrate. An increase of 10.4 g glucan/100 g raw switchgrass was obtained when mechanical refining was applied to 190 °C, 20 min pretreated switchgrass, corresponding to a 53% increase on cellulose digestibility of autohydrolysis pretreated switchgrass. The maximum glucan yield after enzymatic hydrolysis of pretreated substrate is found to be around 30.3 g/100 g raw switchgrass at autohydrolysis condition of 200 °C for 10 min followed by mechanical refining, which means about 93.5% of cellulose in autohydrolysis pretreated samples is converted to fermentable glucose at 4 FPU/g substrate enzyme dosage.

Compared to enzymatic glucan yield, the enzymatic xylan yield is much lower because a large portion of xylan has already been dissolved in the autohydrolysis filtrate. The maximum enzymatic xylan yield turns out to be 7.8 g/100 g raw switchgrass when the switchgrass was pretreated at 180 °C for 10 min followed by mechanical refining. It is noted that the mechanical refining can enhance the xylan digestibility of autohydrolysis pretreated

substrate as well, where an average of 19.2% improvement on xylan digestibility can be achieved depending on pretreatment temperatures and residence times. The enzymatic xylan yield drops sharply when the temperature is higher than 180 °C. This is probably due to the dramatic degradation of xylose to byproducts at server conditions. The highest total xylan recovery from autohydrolysis filtrate and enzymatic hydrolyzate is found to be 17.2 g/100 g raw switchgrass at autohydrolysis conditions of 180 °C, 20 min followed by mechanical refining, about 72.9% of xylan can be recovered from raw switchgrass as fermentable xylose.

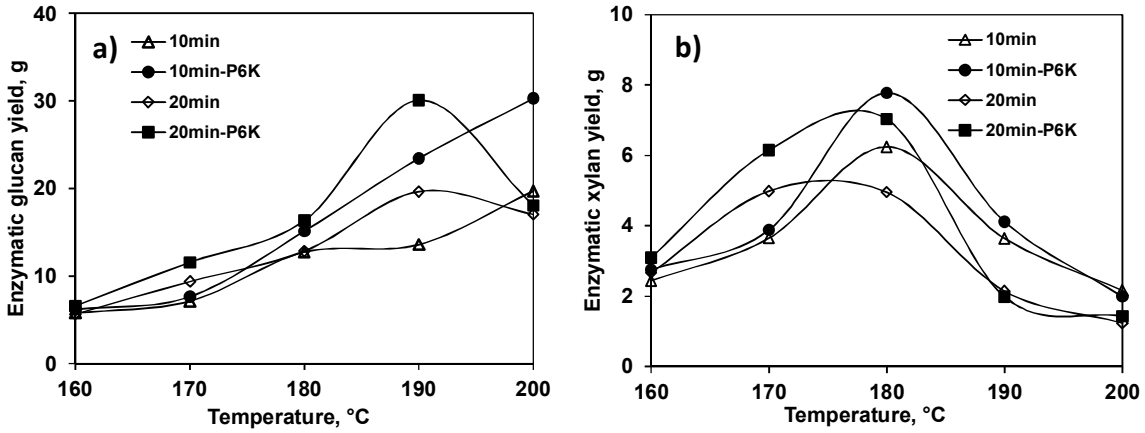

Fig. 2.9 Sugar recovered from enzymatic hydrolysis of pretreated solids at 4 FPU/g substrate enzyme loading for 96 h: a) enzymatic glucose yield; b) enzymatic xylose yield. The yield is based on 100 g raw switchgrass.

Fig. 2.10 displays the enzymatic hydrolysis of autohydrolysis pretreated switchgrass at 10 FPU/g substrate to evaluate the maximum sugar recovery from enzymatic hydrolyzate.

It is of interest to point out that the higher enzyme dosage generated substantial improvements on both glucan and xylan digestibility of non-refined substrate. However, no significant improvements were found on enzymatic digestibility of autohydrolysis pretreated pulp if the mechanical refining was applied as a post-treatment.

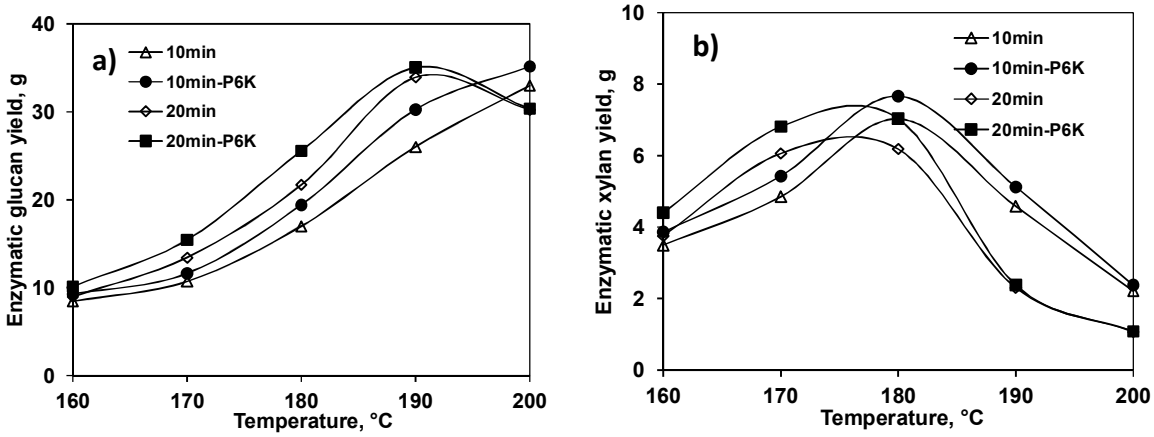

Fig. 2.10 Sugar recovered from enzymatic hydrolysis of pretreated solids at 10 FPU/g substrate enzyme loading for 96 h: a) enzymatic glucose yield; b) enzymatic xylose yield. The yield is based on 100 g raw switchgrass.

3.5. Total sugar recovery

The total sugar recovery is expressed as the sum of total sugar recovered from autohydrolysis filtrate and enzymatic hydrolyzate over the total carbohydrates content in the raw switchgrass. As seen in Fig. 2.11, the highest total sugar recovery at 4 FPU/g enzyme

dosage turns out to be 75.9% (24.3% comes from autohydrolysis filtrate, and 52.5% comes from enzymatic hydrolyzate) when switchgrass was pretreated at 190 °C for 20 min followed by 6000 revolutions PFI as the post-treatment. Higher enzyme charge enhanced the enzymatic digestibility of autohydrolysis pretreated pulp, thus provided higher total sugar recovery. It is observed that the highest total sugar recovery at 10 FPU/g enzyme dosages was 85.0 % when switchgrass was pretreated at 190 °C for 20 min followed by mechanical refining. It is of interest to point out that a substantial improvement on total sugar recovery was made from mechanical refining at lower enzyme dosage, but no significant improvement was obtained when high enzyme dosage was employed. Accordingly, it can be concluded that the mechanical refining can largely reduce enzyme dosage without hampering enzymatic digestibility of the autohydrolysis pretreated substrate.

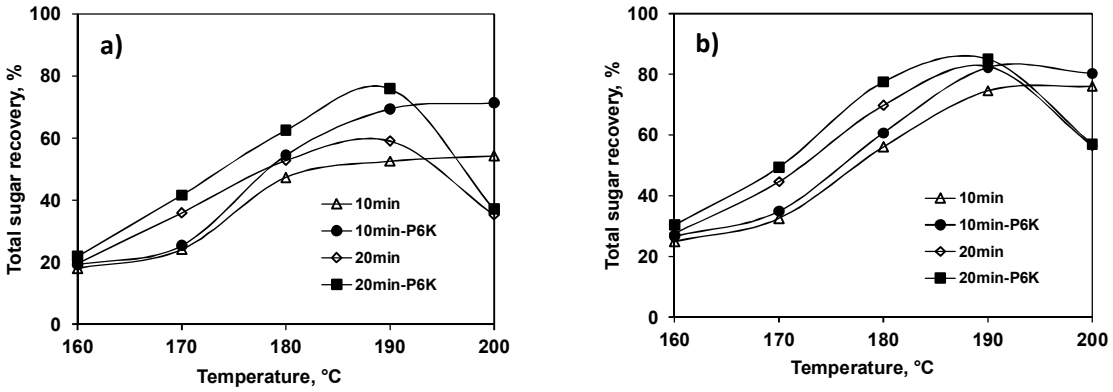

Fig. 2.11 Total sugar recovery from autohydrolysis filtrate and enzymatic hydrolyzate: a) enzymatic hydrolysis at 4 FPU/g substrate; b) enzymatic hydrolysis at 10 FPU/g substrate. All the data are based on total carbohydrates in raw switchgrass.

Conclusions

Switchgrass was subjected to autohydrolysis pretreatment with different temperatures and times, the pretreated biomass was further post-treated by mechanical refining and hydrolyzed with enzyme to evaluate the total sugar recovery. The results show that a total amount of 2.6 to 14.7 g sugars/100 g raw switchgrass can be extracted from autohydrolysis filtrate, along with 0.4 to 8.4 g byproducts/100 g raw switchgrass formed as a result of degradation reactions under different pretreatment conditions. A maximum of 32.3 g total sugars/100 g raw switchgrass can be obtained in the enzymatic hydrolyzate when switchgrass was pretreated at 200 °C for 10 min followed by mechanical refining at 4 FPU/g enzyme dosages. The highest total sugar recovery of 75.9% can be achieved at 190 °C for 20 min with refining at 4 FPU/g enzyme dosages, with 4.4 g/100 g raw switchgrass degradation products formed. The mechanical refining was found to generate substantial improvements (up to 47.5% of increase on enzymatic sugar yield) on enzymatic digestibility of autohydrolysis pretreated switchgrass.

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Chapter 2.3 Autohydrolysis combined with mechanical refining of corn stover for bioethanol production

Abstract

Corn stover was pretreated using an autohydrolysis process with different temperatures (160 to 200 °C) and times (10 and 20 min), followed by mechanical refining as post-treatment, and the treated biomass were enzymatically hydrolyzed to evaluate the total sugar recovery. The results show that the increased temperature and time improved the dissolution of hemicellulose to autohydrolysis filtrate, but led to more degradation reactions as well. The total sugar yield from enzymatic hydrolysis was substantially improved by mechanical refining and higher enzyme dosages. Autohydrolysis at 180 °C for 10 min followed by mechanical refining provided the highest overall sugar recovery for the entire process, where 70.6% of total carbohydrates in the raw corn stover can be recovered as fermentable sugars for bioethanol production at 10 FPU/g enzyme dosages, along with 2.3 g of degradation products generated based on 100 g raw corn stover.

Keywords: Autohydrolysis, refining, corn stover, sugar, bioethanol, enzymatic hydrolysis

1. Introduction

The growing demand for energy for transportation, heating and industrial processes, and increasing greenhouse gas emissions necessitate the development of alternative, non-fossil fuel energy sources. Extensive efforts from industry and governments worldwide have

been made on exploring, developing and commercializing technology for alternative transportation fuels over the past decades. The European Commission has a plan to substitute progressively 20% of conventional fossil fuels with alternative fuels in the transport sector by 2020 (Hahn-Hägerdal et al., 2006). In 2006, the US president set the goal for America to replace more than 75% of imported oil with alternative fuels by the year 2025 (Herrera, 2006). Liquid biofuels from renewable resources, particularly from lignocellulosic materials, will have a substantial role in meeting these goals.

Cellulosic ethanol is wildly regarded as one of the most promising renewable biofuels in the transport sector in terms of its compatibility with gasoline and non-competiveness against animal and human feed. Production of cellulosic ethanol mainly consists of four unit operations: pretreatment, hydrolysis, fermentation, and product separation/purification. Pretreatment is essential in this process to alter the lignocellulosic biomass structure and render cellulose accessible to the action of hydrolytic enzymes. Many pretreatment technologies have been investigated generally on the basis of combined physical, biological and/or chemical actions. However, few of them can be successfully scaled up for commercialization due to many factors such as high capital cost coupled with, low conversion yield, high enzyme loading, low chemical recovery efficiency, and environmental pollutions.

Autohydrolysis, also known as liquid hot water treatment, is a hydrothermal process treating lignocellulosic biomass in water at elevated temperature to promote the release of

acetic acid from mainly hemicellulose which further catalyzes the hydrolysis of biomass. It is an attractive pretreatment method for commercial bioethanol production because of low cost of solvent, no chemical recovery unit operation required, positive environmental impact, and low capital cost. During autohydrolysis, hemicelluloses are depolymerized and converted to soluble sugars in both oligomeric and monomeric forms. Lignin is partially depolymerized and solubilized as well due to the hydrolysis of ether and ester bonds in acidic conditions (Li & Gellerstedt, 2008; Trajano et al., 2013). Cellulose is almost untouched during the autohydrolysis, but the removal of hemicellulose and alteration of lignin largely enhance cellulose reactivity with enzyme (Donohoe et al., 2008). In addition, the formation of degradation byproducts (organic acids, furan derivatives, and phenolic compounds) from monomeric sugars at an elevated temperature can lower the yield of fermentable sugars and more importantly, inhibit the yeast activity during the fermentation (Palmqvist & Hahn-Hägerdal, 2000).

Autohydrolysis has often been abandoned for commercial bioethanol production due to the relatively low sugar recovery at economic enzyme dosages. Mechanical refining can be incorporated into autohydrolysis process and produce a substrate that is more amenable to enzymatic hydrolysis. Mechanical refining is often used in papermaking process to improve paper properties by externally fibrillating and internal swelling of the fibers. It has been reported to improve the enzymatic digestibility of pretreated biomass and reduce the pretreatment severity and inhibitory compounds generated (Jones et al., 2013). The main objective of this study is to identify the optimum autohydrolysis conditions combined with

mechanical refining to maximize the fermentable sugars recovery and minimize the degradation products using corn stover as feedstock.

2. Materials and Methods

2.1. Raw material

Corn stover was collected from a corn field in Iowa State. It was air-dried to about 5% moisture content at room temperature for one week. The stalk and leaves were separated, hand-cut into 2-3 cm sizes, and stored in sealed plastic bag at room temperature prior to pretreatment. The corn stover (stalk: leave = 7:1 by weight) was further ground by a Wiley Laboratory Mill (Model No. 4, Thomas Scientific, Philadelphia, PA) to pass through a 20 mesh screen for composition analysis.

2.2. Compositional analysis

The solids content was determined by the weight difference of sample before and after drying in a convective oven at 105 °C for 24 h. The measurement of extractives content was carried out using acetone solvent in soxhlet extraction apparatus for 24 h. The ash content was analyzed using muffle furnace cauterized at 575 °C according to National Renewable Energy Laboratory's (NREL) Laboratory Analytical Procedures (Sluiter et al., 2008a). The acid soluble lignin and acid insoluble lignin content was measured according to National Renewable Energy Laboratory's (NREL) Laboratory Analytical Procedures (Sluiter et al., 2008b), where a two-stage acid hydrolysis (72% of sulfuric acid at room temperature for 2 h followed by 4% sulfuric acid at 121 °C for 1 h) was employed on 300 mg of

extractive free samples. The resulting sugars were analyzed by a high performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) (Dionex ICS-3000, Dionex Corporation, Sunnyvale, CA, USA), equipped with an eluent generator (EG-2 model), a guard column (CarboPac PAI 4×50 mm), an ion exchange column (CarboPac PAI 4×250 mm), and an electrochemical detector (ED-40 model). The column was operated at 18 °C using Mili-Q water and 400 mN sodium hydroxide solution as the mobile phase at a flow rate of 0.3 ml/min. A 200 mN sodium hydroxide solution was added to post column for optimal detector sensitivity and baseline stability. Fucose was added to each sample as internal standard.

2.3. Autohydrolysis pretreatment

Corn stover (60 dry grams) with stalk to leave ratio 6:1 (w/w) was added into a 1.0 L alloy C-276 Parr reactor (Parr Instrument Company, Moline, IL, USA), supplemented with deionized water to make a final liquor to solid ratio 4:1. After loading, the reactor was vacuumed to remove the air so as to improve the heat transfer efficiency. The reactor was heated up to desired temperature (160 to 200 °C) and stayed for 10 and 20 min with stirring at a speed of 30 revolution/min. The average heating up time to reach the target temperature ranges from 22 to 30 min. The reaction was terminated by quenching the reactor in a water tank with running tap water. Liquor and solids were separated using cheese clothes. The solids were washed thoroughly with tap water, centrifuged and stored in 4 °C cold room for future use. The filtrate was centrifuged and stored in 4 °C cold room for pH, sugar, and byproducts analysis.

The monomeric sugars (glucose, xylose, galactose, mannose, and arabinose) in autohydrolysis filtrate were measured directly by HPAE-PAD system. The total sugar was measured by 4% sulfuric acid hydrolysis of filtrate at 121°C for 1 h according to NREL's Analytical Procedures (Sluiter et al., 2006). The resulting monomeric sugars were then analyzed in HPAE-PAD system.

Byproducts including acetic acid, formic acid, furfural, and hydroxymethyl furfural (HMF) were analyzed by a HPLC system (Dionex UltiMate 3000, Sunnyvale, CA) equipped with a Bio-Rad Aminex HPX-87H column (300 mm x 7.8 mm), Bio-Rad Micro-Guard column, a refractive index detector and a multi wavelength ultraviolet detector (UVD170U). The analytical column was operated at 65 °C with 0.005 M sulfuric acid as the mobile phase at a flow rate of 0.6 mL/min.

2.4. Mechanical refining

The pretreated pulp was integrated by a disc refiner (Bauer 148-2, the Bauer brothers company, Springfield, Ohio) at an opening of 0.005 inch. The resulting pulp was centrifuged and fluffed to measure the moisture content. The PFI refining was carried out by employing 24 dry grams disintegrated pulp with 10% consistency in a PFI refiner (Hamjern Maskin A/S, Hamar, Norway) and refined for 6000 revolutions. The refined samples were centrifuged, fluffed, and stored in a refrigerator at 4 °C for enzymatic hydrolysis.

2.5. Enzymatic hydrolysis

The enzymatic hydrolysis of pretreated pulp was carried out in an environmental incubator shaker (New Brunswick Scientific, Edison, NJ, USA) at 50 °C, 180 rpm for 96 h. The enzyme cocktail was a mixture of Cellic CTec2 and Cellic HTec2 (Novozymes, Franklinton, NC) with CTec2 activity of 139 FPU/g measured according to procedure described by Ghose (Ghose, 1987). Two CTec2 enzyme loadings 4 and 10 FPU/g substrate were employed supplemented with 1/9 HTec enzymes recommended by Novozymes. The hydrolysis consistency was controlled at 5% with 50 mM acetate buffer (pH = 4.8) and sodium azide (0.1%, w/w) was used in the media to inhibit microbial contamination.

The hydrolysis reaction was stopped by soaking samples in the boiling water for 10 min. The samples were centrifuged and the supernatants were filtered through 0.2 μm nylon filters for sugar analysis. The sugar concentration was analyzed by a high performance liquid chromatography (HPLC) system (Agilent 1200, Agilent, Santa Clara, CA, USA), equipped with Shodex SP0810 column (8×300 mm, Showa Denko, Tokyo, Japan) and a refractive index detector. The column was operated at the temperature of 80 °C using Milli-Q water as mobile phase at a flow rate of 0.5 mL/min. All the experiments were carried out in duplicate.

3. Results and discussion

3.1. Chemical composition of corn stover

The chemical composition of corn stover is illustrated in Table 2.4. The total carbohydrate accounts for 64.5% of the total weight, including glucan 38.9%, xylan 20.3%,

galactan 1.3%, and arabinan 4.1%. The corn stover also comprises 13.6% acid insoluble lignin, 2.4% acid soluble lignin, 4.3% ash content, and 2.4% acetyl groups. The total mass balance is only around 90% because some other components such as uronic acid, and protein content which may exist in the corn stover but was not measured in this study.

Table 2.4 Percent dry weight composition of corn stover.

Glucan	Xylan	Galactan	Arabinan	AIL[a]	ASL[b]	Extractives	Ash	Acetyl
38.9	20.3	1.3	4.1	13.6	2.4	2.1	4.3	2.4

[a] AIL: acid insoluble lignin
[b] ASL: acid soluble lignin

3.2. Impact of autohydrolysis on composition of corn stover

The solids recovery yield and composition after autohydrolysis is illustrated in Fig. 2.12. Elevated temperature and longer time contributed to the depolymerization of lignocellulosic biomass and resulted in decreased solids yield from 89.7% (160 °C for 10 min) to 51.2% (200 °C for 20 min). The decreased solids yield is mainly due to the hemicellulose solubilization, formation of degradation products, and partially depolymerization of lignin.

The xylan removal during autohydrolysis had the largest impact on the solids recovery yield. It is well known that autohydrolysis features removal of acetyl groups from hemicellulose and the resulting acetic acid generated from acetyl groups further catalyzes the

hydrolysis of hemicellulose to form soluble sugars (Garrote et al., 1999; Ruiz et al., 2013). Substantial hemicellulose dissolution is observed when corn stover was pretreated under harsh conditions. For instance, only 1.2 g xylan/100 raw corn stover remained in the pretreated solids when the biomass was pretreated at 200 °C for 20 min, corresponding to a 92.6% deduction based on xylan content in original corn stover. The minor sugars such as galactan and arabinan underwent dramatically dissolution as well and no significant minor sugars were detected when the temperature is higher than 170 °C. Unlike hemicellulose, the cellulose in the biomass is more resistant to hydrothermal treatment and more than 85% of cellulose can be recovered in the pretreated solids under server conditions.

It is of interest to point out that a significant amount of lignin was dissolved during autohydrolysis as well. A complete removal of acid soluble lignin can be achieved when the temperature is higher than 170 °C. For acid insoluble lignin, a 5.9% to 45.6% of lignin removal can be obtained when corn stover is pretreated under different conditions, which is consistent with results reported by Ertas (Ertas et al., 2013). The reason of lignin removal during autohydrolysis might be due to a significant amount of ester and ether linkages existing in corn stover lignin which are liable to acid hydrolysis even under weak acidic condition (Li & Gellerstedt, 2008). An increased amount of acetone extractable materials was observed at elevated temperatures and longer times, which might be due to the formation of degradation products at small molecular weights that are readily extracted by organic solvent.

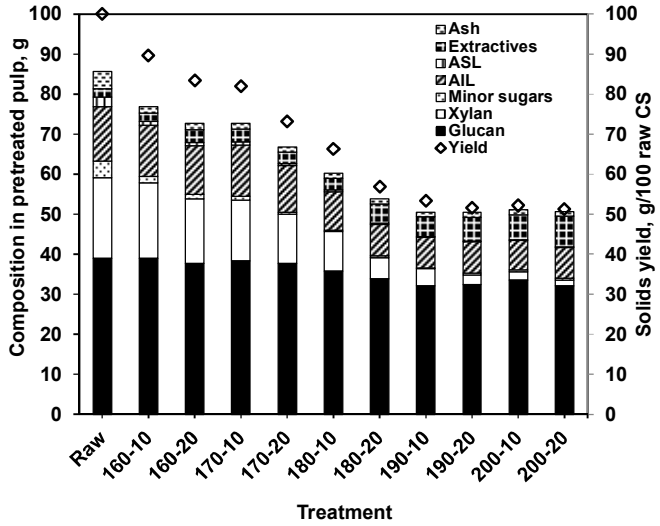

Fig. 2.12 Composition of autohydrolysis pretreated corn stover under different autohydrolysis conditions, based on 100 g raw corn stover.

3.3. Impact of autohydrolysis on pH

When water is heated up to high temperatures (160 - 200 °C), the hydronium ions from autoionization of water act as catalysts and promote the depolymerization of lignocellulosic biomass. Organic acids, mainly acetic acid and uronic acid, generated during autohydrolysis can further catalyze the hydrolysis and lower the pH of the solution. The pH profile during autohydrolysis can indicate the cooking severity and hemicellulose solubilization. As shown in Fig. 2.13, the pH drops steadily with increased temperatures and cooking times. The lowest pH of the filtrate is found to be 3.6 at 200 °C for 20 min, where the largest amount of hemicellulose removal can be observed from Fig. 2.12, indicating a mutual effect of acid generation and hemicellulose solubilization.

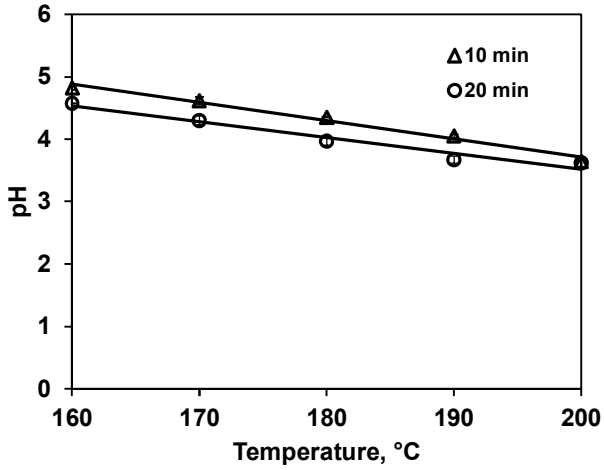

Fig. 2.13 The pH of autohydrolysis filtrate at different autohydrolysis conditions.

3.4. Impact of autohydrolysis on byproducts

The effect of autohydrolysis conditions on byproducts formation is investigated because those degradation products might be potential inhibitors during yeast fermentation process (Larsson et al., 1999). As shown in Fig. 2.14, the total amount of byproducts is strongly related to the severity of autohydrolysis. A maximum amount of byproducts 7.7 g/100 g raw corn stover was obtained when corn stover was pretreated at the highest severity (200 °C for 20 min). Acetic acid, which is produced by hydrolysis of acetyl groups from hemicellulose, is the major byproduct generated during autohydrolysis (Palmqvist & Hahn-Hägerdal, 2000). When the temperature is higher than 190 °C, furfural starts to form in a significant amount due to the degradation of pentose. In contrast, hydroxymethyl furfural (HMF), which is produced from hexose degradation does not show substantially increase

under sever conditions because of a limited amount of hexose produced from cellulose in autohydrolysis filtrate. The harsh condition also contributed to the increase of formic acid, which is formed mainly through the degradation of furfural and HMF (Dunlop, 1948; Ulbricht et al., 1984).

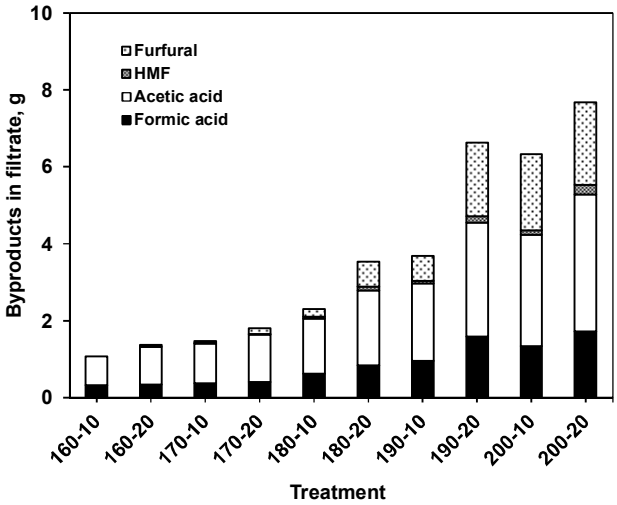

Fig. 2.14 Byproducts in autohydrolysis prehydrolyzate, based on 100 g raw corn stover.

3.5. Sugar in autohydrolysis filtrate

One of the advantages for autohydrolysis pretreatment is to recover a significant amount of oligo-sugars or mono-sugars in the autohydrolysis filtrate without employing enzymes. Fig. 2.15a shows the mono-sugars produced in the autohydrolysis filtrate. Only a small amount of mono-sugars (0.8 - 2.1 g/100 g raw corn stover) were released in the autohydrolysis filtrate. The major component of mono-sugars released was xylose at

temperature higher than 170 °C. When the temperature was higher than 190 °C, the mono-sugars started to decrease due to the degradation products formed at sever conditions.

The total sugars in autohydrolysis filtrate was measured by 4% sulfuric acid hydrolysis of filtrate at 121 °C for 1 h to break down all oligo-sugars to monomeric form. A total amount of sugars in autohydrolysis filtrate varied from 1.5 - 11.0 g/100 g raw corn stover depending on different temperatures and cooking times. The xylose accounted for the largest portion of total sugars released in autohydrolysis filtrate, which is in agreement with the decreased amount of xylan in pretreated solids as shown in Fig. 2.12. The highest total sugars recovered from filtrate is found to be 11.0 g/100 raw corn stover when corn stover was pretreated at 190 °C for 10 min. It is noted that a substantial deduction of total sugars in filtrate is observed when the cooking time was elongated to 20 min at 190 °C, indicating a remarkable degradation of sugars to byproducts, which can be proved in Fig. 2.14 as well.

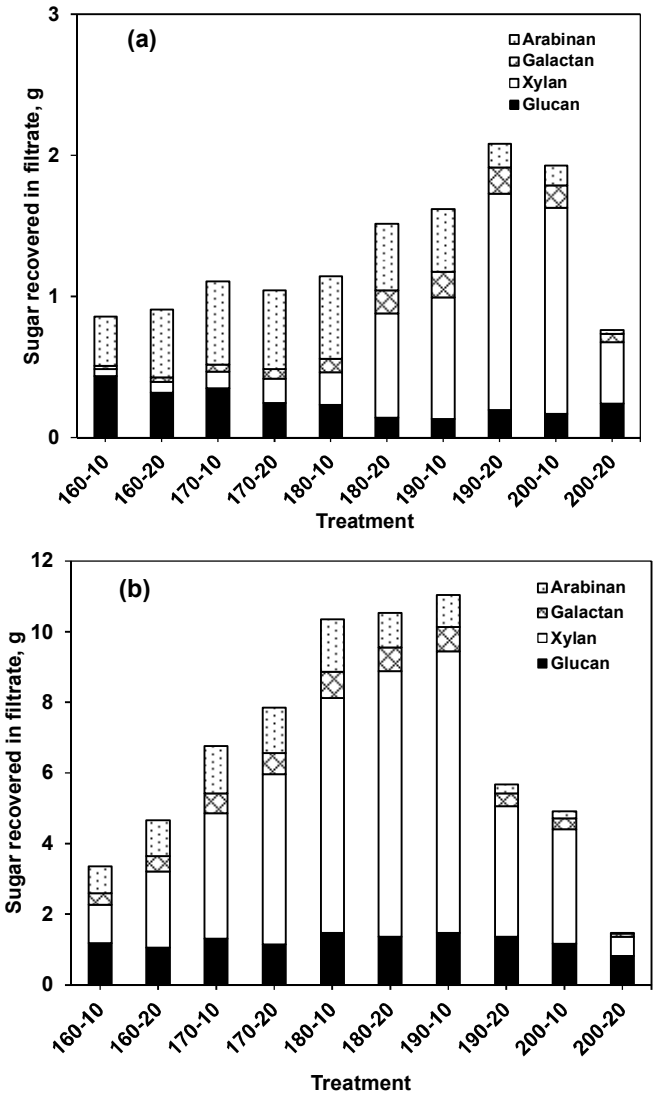

Fig. 2.15 Release of sugars in the autohydrolysis filtrate: a) before acid hydrolysis; b) after acid hydrolysis with 4% sulfuric acid, based on 100 g raw corn stover.

3.6. Enzymatic hydrolysis of autohydrolysis pretreated solids

Enzymatic hydrolysis with two different enzyme loadings (4 and 10 FPU/g dry substrate) was applied to autohydrolysis pretreated solids before and after mechanical

refining to investigate the effect of autohydrolysis conditions and mechanical refining to enzymatic digestibility of pretreated corn stover. As seen in Table 2.5a, no significant improvement was observed on enzymatic hydrolysis of cellulose to glucose with increase of temperature and cooking time at 4 FPU/g enzyme dosages. The maximum glucose yield at 4 FPU/g enzyme dosages after enzymatic hydrolysis of pretreated corn stover is found to be 20.8 g/100 g raw corn stover at autohydrolysis condition of 170 °C for 10 min. The enzymatic xylose yield at 4 FPU/g enzyme dosages has a maximum value of 7.3 g/100 g raw corn stover when corn stover was pretreated at 170 °C for 10 min, and then starts to decrease with elevated temperature and time because of a limited amount of xylan remain in the pretreated corn stover under harsh conditions. The highest total enzymatic sugar yield at 4 FPU/g enzyme dosages is found to be 28.6 g/100 g raw corn stover at autohydrolysis condition of 170 °C for 10 min.

Increased amount of enzyme loading substantially improved the cellulose digestibility of autohydrolysis pretreated corn stover. As shown in Table 2.5a, when 10 FPU/g enzyme dosages was applied to pretreated corn stover, the glucose yield increased by 3.4 – 12.8 g/100 g raw corn stover compared to enzymatic hydrolysis at 4 FPU/g enzyme dosages. It is of interest to point out that such improvement is strongly associated with the cooking severity. For instance, 3.4 g incremental glucose yield was obtained at autohydrolysis condition of 160 °C for 10 min, compared to 12.8 g incremental glucose yield achieved at autohydrolysis condition of 200 °C for 20 min. The maximum total sugar yield from enzymatic hydrolysis

turns out to be 35.0 g/100 g corn stover when corn stover was pretreated at 170 °C for 10 min without mechanical refining and enzymatic hydrolyzed at 10 FPU/g enzyme dosages.

Mechanical refining has been found to be effective in improving enzymatic digestibility of pretreated biomass by lowering the cooking severity or decreasing enzyme dosage (Jones et al., 2013). The enzymatic digestibility of autohydrolysis pretreated corn stover with mechanical refining was illustrated in Table 2.5b. It is observed that the mechanical refining increased the total sugar yield from enzymatic hydrolysis by an average of 3.1 g/100 g raw corn stover at 4 FPU/g enzyme dosages and 2.1 g/100 g raw corn stover at 10 FPU/g enzyme dosages. The largest incremental enzymatic total sugar 4.5 g/100 g raw corn stover can be achieved by mechanical refining when corn stover was treated at 180 °C for 10 min at 4 FPU/g enzyme dosages. The overall maximum total sugar yield from enzymatic hydrolysis is found to be 38.4 g/100 g raw corn stover when corn stover was pretreated at 170 °C for 10 min, followed by mechanical refining and 10 FPU/g enzymatic hydrolysis.

Table 2.5 Enzymatic sugar yield based on 100 g raw corn stover.

a) without mechanical refining

Temperature, °C	Times, min	Cellulase (FPU/g)	Sugar, g		
			Glucan	Xylan	Total sugar [a]
160	10	4	18.7	6.7	26.3
		10	22.1	7.9	31.0
	20	4	18.8	6.7	26.2
		10	22.3	7.7	30.6
170	10	4	20.8	7.3	28.6
		10	25.8	8.6	35.0
	20	4	20.2	6.3	26.7
		10	26.3	7.5	34.2
180	10	4	18.6	5.3	24.1
		10	26.1	6.5	32.9
	20	4	18.4	2.7	21.1
		10	27.3	3.6	31.1
190	10	4	17.5	2.2	19.7
		10	26.8	3.0	29.9
	20	4	18.5	1.1	19.6
		10	29.8	1.7	31.5
200	10	4	19.0	0.8	19.8
		10	31.5	1.4	32.9
	20	4	17.5	0.5	18.0
		10	30.2	1.0	31.2

[a] Total sugar is the sum of glucan, xylan, and arabinan. The data of arabinan are not listed.

Table 2.5 Continued. b) with mechanical refining

Temperature, °C	Times, min	Cellulase (FPU/g)	Sugar, g		
			Glucan	Xylan	Total sugar [a]
160	10	4	18.8	6.9	26.5
		10	22.6	8.0	31.4
	20	4	20.8	7.3	28.7
		10	24.8	8.4	33.8
170	10	4	23.2	8.2	31.8
		10	28.5	9.4	38.4
	20	4	22.6	6.8	29.6
		10	27.8	7.9	36.0
180	10	4	22.6	5.9	28.6
		10	28.1	6.8	35.1
	20	4	23.3	3.3	26.6
		10	29.5	4.0	33.7
190	10	4	20.5	2.5	23.0
		10	29.3	3.4	32.8
	20	4	21.4	1.2	22.6
		10	30.9	1.8	32.7
200	10	4	22.0	1.0	22.9
		10	33.0	1.5	34.5
	20	4	20.4	0.6	21.0
		10	31.8	1.1	32.9

[a] Total sugar is the sum of glucan, xylan, and arabinan. The data of arabinan are not listed.

3.7. Total sugar recovery

Total sugar recovery is defined as the percentage of the total fermentable sugar recovered from autohydrolysis filtrate and enzymatic hydrolyzate in the total carbohydrates in the raw corn stover. As seen in Fig. 2.16a, the highest total sugar recovery at 4 FPU/g enzyme dosages is found to be 60.5% when corn stover was pretreated at 180 °C for 10 min followed by mechanical refining. When temperature is higher than 180 °C, the total sugar recovery starts to decrease because of degradation reactions occurred under sever conditions.

It is noted that at same temperature, longer cooking time leads to lower total sugar recovery. Such deduction on total sugar recovery can be explained by a significant increase of degradation products generated at longer pretreatment times as shown in Fig. 2.14, which hampers fermentable sugar recovery. An average of 4.8% total sugar recovery can be obtained when mechanical refining was applied to boost the efficiency of autohydrolysis pretreatment. Higher enzyme loading significantly enhanced the total sugar recovery from corn stover. The Fig. 2.16b shows that the maximum total recovery is found to be 70.6% (16.2% from autohydrolysis filtrate and 54.4% from enzymatic hydrolyzate) when corn stover was pretreated at autohydrolysis condition of 180 °C for 10 min followed by mechanical refining and then subjected to 10 FPU/g enzymatic hydrolysis.

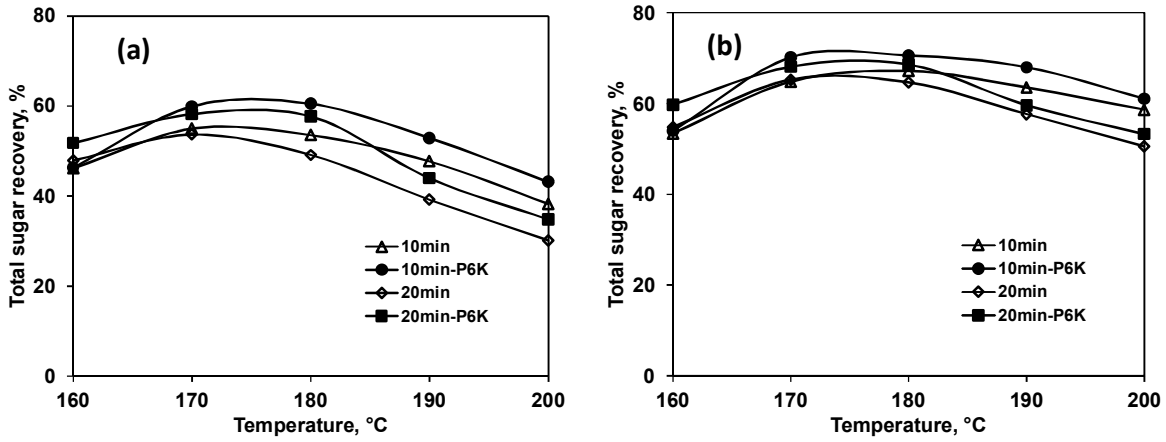

Fig. 2.16 Total sugar recovery from autohydrolysis filtrate and enzymatic hydrolyzate: a) enzymatic hydrolysis at 4 FPU/g substrate; b) enzymatic hydrolysis at 10 FPU/g substrate. All the data were based on total carbohydrates in raw corn stover.

4. Conclusions

Autohydrolysis combined with mechanical refining provides a simple and efficient pretreatment method for bioethanol production. A maximum of 17.4% of total sugars were released into the autohydrolysis filtrate at 190 °C for 10 min. However, the highest total sugar yield from enzymatic hydrolysis turns out to be 31.8 g/100 g raw corn stover at 4 FPU/g enzyme dosages and 38.4 g/100 g raw corn stover at 10 FPU/g enzyme dosages when corn stover was pretreated at 170 °C for 10 min followed by mechanical refining. Autohydrolysis at 180 °C for 10 min followed by mechanical refining provided the highest overall sugar recovery for the entire process, where 70.6% of total carbohydrates in the raw corn stover can be recovered as fermentable sugars for bioethanol production at 10 FPU/g enzyme dosages, along with 2.3 g of degradation products generated based on 100 g raw corn stover.

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Chapter 2.4 Autohydrolysis pretreatment of waste wheat straw for cellulosic ethanol production in a co-located straw pulp mill

Abstract

Waste wheat straw is the waste product from feedstock preparation process in a straw pulp mill. It has a significant annual production rate and no commercial value has been explored on this material. In this study waste wheat straw was pretreated using an autohydrolysis process followed by mechanical refining, and the pretreated materials were further enzymatically hydrolyzed to evaluate the total sugar recovery for bioethanol production. Results show that autohydrolysis at 170°C for 40 min followed by 6000 revolution PFI refining provided the best result in this study, where a total sugar recovery (total sugars in autohydrolysis filtrate and enzymatic hydrolyzate over total carbohydrates on raw WWS) of 70% at 4 FPU/g od substrate enzyme charge could be obtained. The economic evaluation of this biorefinery process indicates that cellulosic ethanol production from autohydrolysis of WWS is a very profitable business, with 28.4% of internal rate of return can be achieved based on current ethanol wholesale price in China.

Keywords: autohydrolysis, wheat straw, enzymatic hydrolysis, refining, economics

1. Introduction

The growing concern for the energy security and the greenhouse gas emissions, combined with the need for finding better strategies to handle municipal, industrial and agricultural residues, continues to motivate a great deal of interest in conversion of

lignocellulosic biomass to fuels and chemicals. Wheat straw is one of the most abundant agriculture residues that is produced in large amounts all over the world. Like many other lignocellulosic biomass, wheat straw is mainly composed of cellulose (33-40% by dry weight), hemicellulose (20-25%), and lignin (15-20%) (Prasad et al., 2007). The abundance of carbohydrates in wheat straw renders it a promising candidate for bioethanol production.

The enzymatic conversion of lignocellulosic biomass to ethanol comprises two major processes: hydrolysis of cellulose and hemicellulose to fermentable reductive sugars and fermentation of the free sugars to ethanol. The efficiency of this technology is limited by the complex chemical and physical structure of lignocellulosic biomass. Thus pretreatment is essential to open up the matrix structure of biomass and provide an enzyme accessible substrate. Various pretreatment technologies, including physical, chemical, and biological pretreatment have been extensively investigated, but few of them have been successfully scaled up for commercialization due to high capital cost coupled with, low conversion yield, high enzyme loading, low chemical recovery efficiency, or environmental pollutions.

Autohydrolysis, also known as liquid hot water pretreatment, is a hydrothermal process which treats the lignocellulosic biomass in a water-only media at elevated temperature (160-240 °C). This chemical-free process provides a simple, low-cost and environmental friendly way for generation of ethanol from bagasse, agriculture residue, energy crops and hardwood (Ertas et al., 2013; Jing et al., 2010; Laser et al., 2002; Lee et al., 2009; Treasure et al., 2012). The objective of autohydrolysis is to solubilize mainly

hemicellulose, provoke disruption of lignocellulosic structure, and thus making the cellulose more accessible to enzymatic attack. During the autohydrolysis hemicelluloses are depolymerized and converted into soluble sugars and lignin is partially depolymerized and solubilized as well but the re-condensation of lignin soluble components onto biomass surface makes complete delignification impossible (Alvira et al., 2010). It is revealed that the removal of hemicellulose and change in lignin structure dramatically open up the cell wall matrix structure and expose more cellulose accessible surface to enzyme attack (Donohoe et al., 2008). In addition, the formation of degradation byproducts (organic acids, furan derivatives, and phenolic compounds) from monomeric sugars at an elevated temperature can lower the yield of fermentable sugars and may inhibit the yeast activity during the fermentation. The amount of degradation products generated in autohydrolysis is largely driven by the severity of the reaction.

Mechanical refining has been widely used in pulp and paper industry to promote fibrillation of fiber and increase paper strength. Refining of pretreated biomass has been shown to improve significantly the efficiency of enzymatic hydrolysis (Wu et al., 2010). It has been found be able to shorten fiber length, create microfibrils on fiber surface, promote fiber swelling, and loosen fiber internal structure (Jones et al., 2013). It has been reported that mechanical refining could generate substantial improvements on enzymatic digestibility of corn stover (Chen et al., 2012b; Tao et al., 2012), hardwood (Koo et al., 2011), and recovered office printing paper (Chen et al., 2012a).

In this study autohydrolysis combined with mechanical refining of a special wheat straw (refers to waste wheat straw) which is generated from raw material preparation process in a straw pulp mill was investigated. If the biorefinery mill can be co-located with the pulping mill, the zero cost of waste wheat straw will render this waste material very attractive as a bioethanol feedstock candidate. Therefore, this study is aimed at (1) identifying the best autohydrolysis condition for waste wheat straw to maximize the fermentable sugars yield and minimize the degradation byproducts; (2) determining the financial value of bioethanol production from autohydrolysis of waste wheat straw in China.

2. Materials and Methods

2.1. Raw material

Wheat straw is one type of widely used raw materials for pulping and paper making in China. To keep good quality of the pulp, wheat straw is usually cut, crushed and screened to remove dirt, sand, ash and leaf debris. The reject comprise fair amount of carbohydrates but have no commercial application yet. The reject used in this study was provided by a Chinese pulp and paper company, which has a pulp production rate of 400,000 metric ton per year. The mill has a plan to expand its annual production to 800,000 metric ton in the near future. For every ton of pulp produced, 0.6 tons of reject is generated. The reject has a very high ash content of 58% and contains largely of dirt and sand. The reject is screened in a laboratory 20 mesh screen. Most of the dirt and sand passed through the screen. The reject retained by the 20 mesh screen (about 50% of the original reject) is used for this study and is referred as waste wheat straw (WWS). The screening of the reject was kindly done by Prof.

Yongcan Jin of Nanjing Forestry University and only WWS was shipped to our laboratory for this study. The raw WWS with initial moisture content of 10% was stored in air tight plastic bag at room temperature without any physical treatment prior to composition analysis and treatment.

2.2. Autohydrolysis

Autohydrolysis was carried out in a 300 ml Alloy C276 parr reactor (Parr Instrument Company, Moline, IL, USA). WWS and water were mixed in the vessel at a ratio of 4:1 (w/w) (72 g water and 18 dry g WWS). The residence time at target temperature was set at 10 min, 20 min, 40 min and 60 min for 170 °C, 10 min, 20 min, and 40 min for 180 °C. Only 10 min residence time was investigated for 190 °C and 200 °C. The vessel was vacuumed before heating up to minimize the effect of false pressure generated by air. The ramping time to reach the target temperature varies from 18 to 23 min depending on the target temperature. The vessel was equipped with a stirrer operated at a speed of 30 revolution/min. Triplicate runs were performed. After reaction, the whole reactor was quenched in a cold water bath and then pretreated samples were taken out and squeezed using cheese cloth. The filtrate was collected for solids content, pH measurement, sugar analysis and byproducts measurement. The remaining solids were washed with excess water, centrifuged, and stored in sealed plastic bag in a 4°C cold room for solids yield measurement, composition analysis, PFI refining, and enzymatic hydrolysis. The moisture content of the pretreated samples was measured in a 105 °C convection oven by weight difference of wet sample and oven dry sample. The total solids yield of pretreated samples was calculated by equation (1).

% Solids yield = "wet sample weight – moisture in wet sample" /"dry sample weight into pretreatment" × 100% (1)

For integrated process, the pretreated samples were directly collected in sealed plastic bags for PFI refining and enzymatic hydrolysis without solids and liquor separation.

2.3. PFI refining

Part of the pretreated samples was subjected to a 6,000 revolution refining in a PFI mill (Hamjern Maskin A/S, Hamar, Norway) using 24 g of dry biomass at 10% consistency according to Tappi method T248 sp-00. The samples were then centrifuged with cheese cloth, fluffed, and sealed in plastic bags in cold room. For integrated process, the refined samples were directly stored in sealed bags for future use without centrifuging.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis was performed in 50 ml centrifuge tubes in an environmental incubator shaker (New Brunswick Scientific, Edison, NJ, USA) set at 50 °C, 180 rpm for 96 h. Two oven dry g of pretreated samples were immersed into 50 mM acetate buffer (pH 4.8) to achieve a 5% (w/v) solids loading. Sodium azide (0.1% w/v) was added in the mixture to inhibit microbial growth during the hydrolysis (Zhou et al., 2011). A commercial cellulase complex including a cellulase mixture (C-tec2) and a xylanase (H-tec2) was generously provided by Novozymes (Franklinton, NC). The activity of the cellulase (C-tec2) was determined to be 139 FPU/mL according to method described by Ghose (Ghose, 1987). A dosage of 4 FPU/od g of substrate (5.2 mg enzyme protein per g of substrate) was added into

the mixture supplemented with a ninth of the C-tec2 which was recommended by Novozymes. In addition, a high enzyme dosage of 10 FPU/od g of substrate was also investigated. The enzymatic hydrolysis was stopped by soaking the samples into the boiling water for 10 min and then centrifuged. The supernatants were filtered through 0.2 μm filter for sugar analysis. All the measurements were duplicated.

2.5. Analytical methods

The determination of extractives was carried out according to TAPPI method T 204 cm-97. The total solids, ash content, acid soluble lignin, acid insoluble lignin of untreated and pretreated straw, and sugar analysis were measured according to National Renewable Energy Laboratory's (NREL) Analytical Procedures (Sluiter et al., 2008a; Sluiter et al., 2005; Sluiter et al., 2008b). The elemental analysis of WWS ash was carried out by the Department of Soil Science at North Carolina State University using a Perkin-Elmer 2400 analyzer. The concentration of sugars (glucose, xylose, galactose, arabinose, and mannose) was quantified by a high performance liquid chromatography (HPLC) system (Agilent 1200, Agilent, Santa Clara, CA, USA). The sugar samples were filtered by 0.2 μm filter and passed through Shodex SP0810 column (8×300 mm, Showa Denko, Tokyo, Japan) at the temperature of 80 °C. The mobile phase for the column was Milli-Q water at a flow rate of 0.5 mL/min. A refractive index detector was used to quantify all the sugar concentrations at the temperature of 50 °C. The byproducts (acetic acid, formic acid, furfural, and HMF (5-hydroxymethyl furfural)) were determined using a high performance liquid chromatography (HPLC) system (Dionex UltiMate 3000, Dionex Corporation, Sunnyvale, CA, USA), equipped with a Bio-

Rad Aminex HPX-87H column (300 mm x 7.8 mm), a Bio-Rad Micro-Guard column, and a refractive index detector. The analytical column was operated at 65 °C with 0.005 M H2SO4 as the mobile phase at a flow rate of 0.6 mL/min. The acid hydrolysis of the prehydrolyzate was carried out to break down all the oligo-sugars released in prehydrolyzate according to the NREL's Analytical Procedure (Sluiter et al., 2006). The prehydrolyzate was hydrolyzed with 4% w/v sulfuric acid at 121 °C for 1 h. The yields of total sugars, monomeric sugars, and sugar degradation products were calculated based on per g of raw biomass.

2.6. Process simulation

A complete process model was developed using WinGEMS V.5.3, which is widely used in pulp and paper industry for mass and energy balance. The proposed process for cellulosic ethanol production from WWS is illustrated in Fig. 2.17. WWS is subject to autohydrolysis at 170 °C for 40 min. After blowing, the blow heat is recovered to preheat the make-up water in the digester, and the slurry is diluted to 4% and sent to mechanical refining. The refined pulp goes through a screw press, where liquor-solid separation takes place. The liquor is heat exchanged and detoxified by an ion exchange resin and recycled back as dilution liquor for mechanical refining feed. The solids are diluted to 20% consistency and sent to a multiple-stage enzymatic hydrolysis system (Xue, 2011). A five-carbon and six-carbon separated fermentation is carried out, where a 95% conversion efficiency for hexoses and 80% for pentoses is assumed. After distillation, the beer column tops are further dehydrated to 99.5% cellulosic ethanol as final product and the beer column bottoms pass through a clarifier to separate the liquor and solids. The liquor is recycled back as dilution

water, while the solids are sent to fertilizer plant as raw material for fertilizer production because of high potassium and lignin content in the residues (Jun-he, 2004). It is noted that due to the high ash content, the residues are not considered a good fuel for biomass boiler.

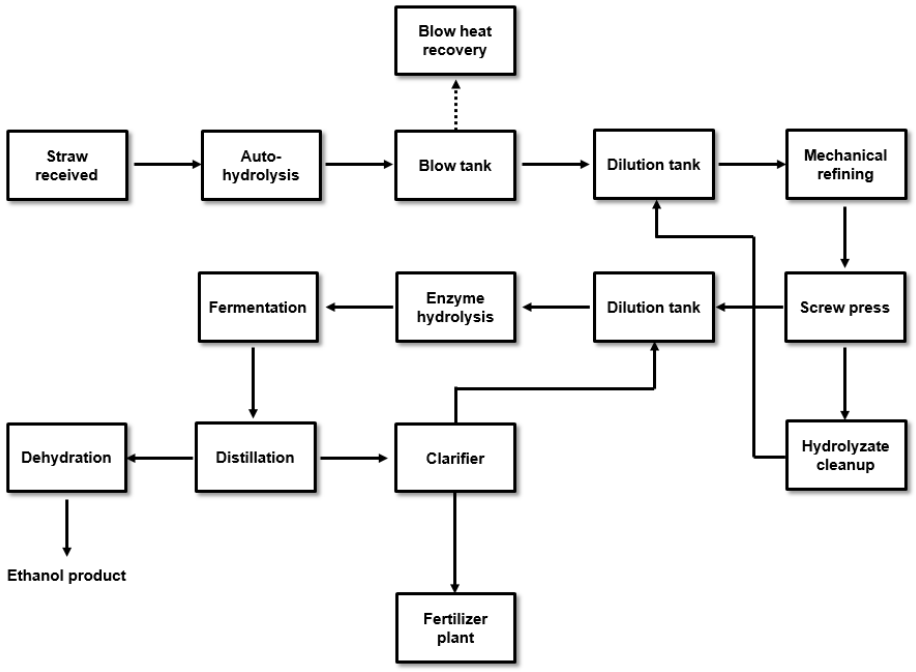

Fig. 2.17 Process description of cellulosic ethanol production from waste wheat straw

2.7. Economic analysis

Economic model was built based on the outputs of simulation results. Major assumptions applied in the financial analysis are listed in Table 2.6. Briefly, the project life was set at 15 years with a feedstock supply of 200,000 dry metric tons per year. 10 years

straight line depreciation schedule was applied and the discount rate was set at 12%. The tax rate was set at 25% with enterprise income tax only because of the tax subsidy on cellulosic ethanol in China. A terminal value in year 15 of five times of year-15 EBITDA (Earnings Before Interest, Taxes, Depreciation and Amortization) was assumed. The maintenance expense was estimated as a function of the Replacement Asset Value (RAV), where the investment cost to replace the original asset escalates annually in cost at 2%. Capital reinvestment and other mill fixed costs were estimated at 1% and 3% of replacement asset value, respectively. The overhead was assumed to be 3% of annual sales. The enzyme price is assumed at $1 per kg enzyme product which equals to $5.7 per kg enzyme protein according to methodology developed by Phillips (Phillips et al., 2013). All the other prices were set according to 2013 Chinese market prices.

Table 2.6 Operative and financial assumption and key simulation results used in the economic analysis.

Description	Value	Description	Value
Startup year	2015	Hours per year	8400
Terminal year	2030	Excess spending in startup year,% of direct cost	15%
Feedstock supply, dry tons per year	200,000	Ethanol revenue, $ per liter	0.8
% of CAPEX spending in year -2	30%	Ethanol yield, liter per dry ton biomass	213
% of CAPEX spending in year -1	50%	Power cost, $ per MWH	98.3
% of CAPEX spending in year -0	20%	Steam cost, $ per ton	23.6
% of Nominal capacity, project year 1	50%	Enzyme cost, $ per kg enzyme product	1.0
% of Nominal capacity, project year 2	80%	Yeast cost, $ per liter ethanol	0.004
Working capital on materials, % of direct cost	10%	Hourly and Administrative Staff, $ per Year	342,000
Working capital on product, % of revenue	10%	Salaried Staff, $ per year	80,000
Years depreciation schedule	10-S/L[a]	Maintenance expense, % of RAV[c]	2%
Tax rate, with tax loss carryforward	25%	Capital reinvestment, % of RAV[c]	1%
Discount rate	12%	Other fixed costs, % of RAV[c]	3%
Terminal value, year 15 EBITDA[b] multiple	5.0	Sales and other overhead, % of sales	3%

Note: All prices are expressed as US dollars and based on Chinese market.
[a]10-S/L = 10 years straight line depreciation schedule
[b] EBITDA = Earnings Before Interest, Taxes, Depreciation and Amortization
[c] RAV = Replacement Asset Value

3. Results and discussion

3.1. Chemical composition of waste wheat straw

The composition analysis of WWS is summarized in Table 2.7. The total carbohydrates of WWS are 47.3% of the total weight, including glucan 28.2%, xylan 13.1%, and other minor sugars 6.0%. It is noted that the ash content of WWS is very high, reaching up to 25% of the total weight. The elemental analysis on ash content (Table 2.8) shows that 19.6% of ash comes from silicon and no heavy metal contamination was detected. It is of interest to know that the high amount of potassium which is essential for cells metabolism was found in WWS ash, making the final residue a potential raw material for fertilizer production. It is obvious that the WWS has a distinct composition difference compared to that of regular wheat straw. The value of WWS is not as high as regular wheat straw in terms of lower carbohydrates content and high ash content. However, considering the zero cost of the WWS feedstock, the production of bioethanol from WWS would be economically feasible if an efficient biorefinery process is applied.

Table 2.7 Percent dry weight composition of waste wheat straw (WWS) vs. wheat straw.

Feedstock	Glucan	Xylan	Galactan	Arabinan & Mannan	Lignin	Extractives	Ash
WWS	28.2	13.1	0.6	5.4	19.1	1.9	24.6
Wheat straw [a]	38.2	21.2	0.7	2.8	23.4	13	10.3

[a] Data obtained from a reference (Wyman 1996)

Table 2.8 Percent dry weight composition of ash content in waste wheat straw (WWS).

Si	K	Ca	Fe	S	Mg	P	Na	Mn	Others
19.6	7.1	6.4	1.6	1.4	1.3	0.3	0.3	0.1	61.9

3.2. Effect of autohydrolysis conditions on pH and solids yield

Autohydrolysis is catalyzed by hydronium ions from the water autoionization and acetic acid released from acetyl groups in hemicellulose (Garrote et al., 2001; Mittal et al., 2009). During the autohydrolysis, the cleavage of acetyl groups in raw material lowers the pH of the filtrate to between 3 and 4. To some extent the pH value of the autohydrolysis filtrate is related to the reaction of the hemicellulose and indicates the severity of the treatment. As shown in Fig. 2.18, the acidity of the filtrate is higher with an increase of the autohydrolysis temperature and time, indicating a higher temperature and a longer time promote the cleavage of acetyl groups in hemicellulose. Nevertheless, the pH of the WWS filtrate is relatively higher than that of other feedstock in similar treatment condition (Ertas et al., 2013; Huijgen et al., 2012; Lee et al., 2009; Pérez et al., 2008). This is probably due to the high ash content in WWS neutralizes the acid generated during autohydrolysis. It is noteworthy that the high pH of the prehydrolyzate contributes to minimizing the formation of degradation byproducts and potentially eliminating the need for conditioning chemicals before enzymatic hydrolysis.

As illustrated in Fig. 2.18, elevated temperature and residence time contributed to lower solids recovery yield. The solids yield decreased from 74.0 g/100 g raw WWS after 10 min pretreatment at 170 °C to 46.6 g/100 g raw WWS after 10 min autohydrolysis at 200 °C. The decrease of solids recovery yield is in agreement with the drop of pH in the prehydrolyzate, indicating an increased depolymerization of WWS under severe autohydrolysis conditions.

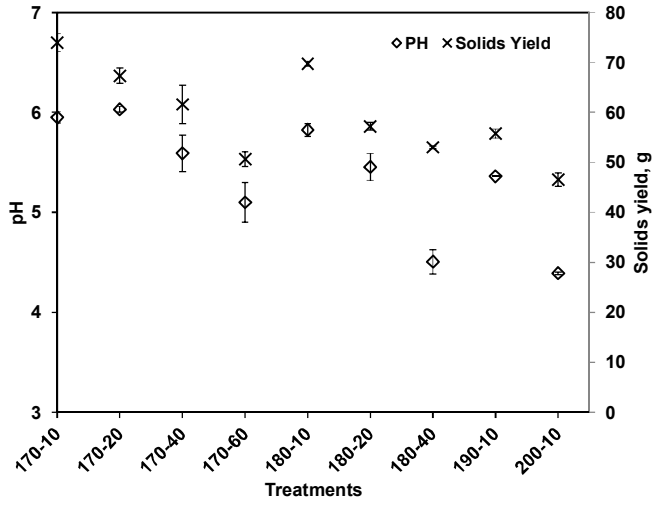

Fig. 2.18 The pH of the prehydrolyzate and solids yield based on 100 g raw WWS under different autohydrolysis conditions.

3.3. Effect of autohydrolysis on composition of waste wheat straw

The components of autohydrolysis residues at different conditions are shown in Table 2.9. The solubilization of ashes dominated the reduction of solid residues, buffering the

acidity of the prehydrolyzate. Increased amount of extractives under higher temperature indicates more extractable small molecular substances were produced during the treatment, showing a notable influence of temperature on the depolymerization of biomass. Higher temperature and longer residence time promoted the removal of lignin during autohydrolysis. It is observed that nearly 40% of lignin in the raw WWS was removed after autohydrolysis at 200 °C for 10 min.

Xylan is the major hemicellulose in waste wheat straw. Higher temperature and longer residence time helps to solubilize hemicellulose. Both 40 min autohydrolysis at 180 °C and 10 min autohydrolysis at 200 °C reduced the xylan content from 13.1 g/100 g (in untreated WWS) to 2.0 g/100 g raw WWS. The minor sugars such as galactan, arabinan and mannan showed a greater reduction as well under harsh condition and no significant minor sugars were detected in the residue. The cellulose was nearly untouched during the autohydrolysis, resulting in a cellulose enriched substrate. It is observed that the glucan content of 200 °C and 10 min pretreated WWS residue accounted for 50.9% of the total solids, which provided an excellent cellulose enriched substrate for subsequent enzymatic hydrolysis.

Table 2.9 Solids recovery yield and composition of the solid residues after autohydrolysis treatment as g of component recovered per 100 g of raw WWS. All the results are duplicated.

	Raw	170 °C				180 °C			190 °C	200 °C
		10 min	20 min	40 min	60 min	10 min	20 min	40 min	10 min	10 min
Glucan	28.2	25.1	25.5	26.1	24.6	25.1	25.6	25.6	24.0	23.7
Xylan	13.1	13.0	10.4	8.0	5.6	11.4	5.3	2.1	6.0	2.0
TL[a]	19.1	18.6	13.7	12.4	11.9	19.9	12.3	12.2	15.6	11.6
Ash	24.6	6.8	6.3	5.6	6.0	6.7	6.2	5.7	6.4	5.7
Extractives	1.9	1.4	1.1	1.5	1.4	1.5	2.0	3.1	2.3	3.7
Yields	100	74.0	67.3	61.6	50.6	69.8	57.2	53.0	55.8	46.6

[a] TL: Total lignin including acid insoluble lignin and acid soluble lignin.

3.4. Sugars and byproducts in autohydrolysis filtrate

Autohydrolysis contributes to the recovery of mainly hemicellulose in the prehydrolyzate. In this study autohydrolysis filtrates were hydrolyzed using 4% sulfuric acid to decompose all oligomeric sugars into monomeric forms. The total sugar released into prehydrolyzate showed a pronounced increase from 3.4 g/100 g raw WWS in untreated WWS to a maximum of 8.5 g/100 g raw WWS when WWS was treated at 180 °C for 20 min or 190 °C for 10 min (Fig. 2.19). When biomass was treated at 170 °C, the longer residence time helps to release more sugars, especially xylan into the prehydrolyzate. However, at 180 °C the sugars released in treated prehydrolyzate started to decrease after the maximum sugar amount was reached at 20 min. It is probably due to the degradation of monomeric sugars to furfural, HMF and other byproducts under severe conditions.

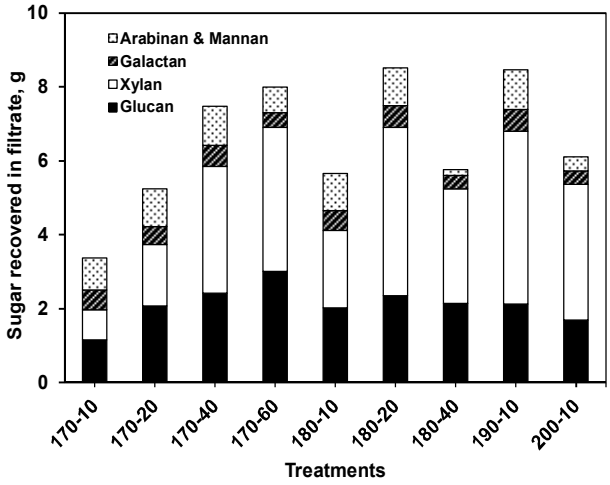

Fig. 2.19 Release of sugar (after hydrolysis with 4% sulfuric acid treatment) in autohydrolysis filtrate, based on 100 g raw WWS.

Sugar degradation products from autohydrolysis, such as organic acids, furfural, and HMF (5-hydroxymethyl furfural), which are known to inhibit microorganisms during fermentation process (Kim et al., 2009; Palmqvist & Hahn-Hägerdal, 2000) were investigated as well. Fig. 2.20 shows that higher temperatures and longer residence times increased the total amount of byproducts. However, the total amount of byproducts generated in this study is substantially less than that in other study at similar conditions (Ertas et al., 2013). It is probably because high pH value in the filtrate can help minimizing the degradation of monomeric sugars to various byproducts. The autohydrolysis at 180 °C for 40 min generated highest amount of byproducts which is in agreement with the decline in the sugar content in the prehydrolyzate as discussed above.

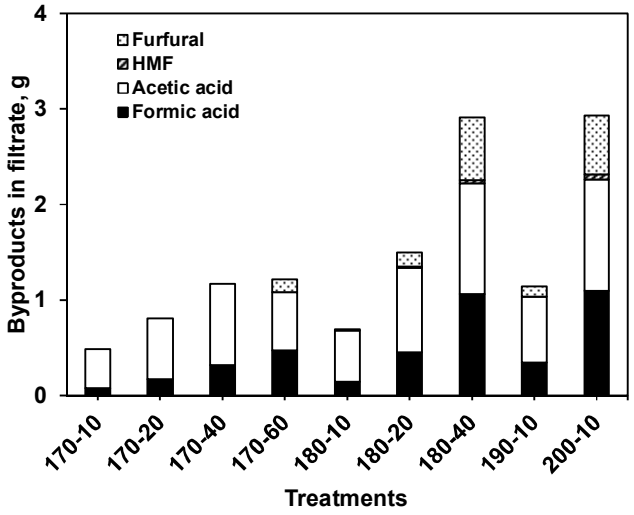

Fig. 2.20 Byproducts in autohydrolysis prehydrolyzate, based on 100 g raw WWS.

3.5. Effect of autohydrolysis conditions on enzymatic hydrolysis

Table 2.10 displays the material balance from autohydrolysis followed by enzymatic hydrolysis at different enzyme dosages. The autohydrolysis treated WWS exhibited a substantial increase by 2-3 folds of total enzymatic hydrolysis sugar yield in contrast to untreated raw material (Table 2.10a). The enzymatic digestibility of cellulose increased progressively with elevated temperatures and longer residence times. The highest glucan yield 21.1 g/100 g raw WWS after enzymatic hydrolysis is observed when WWS was pretreated at 200 °C for 10 min without mechanical refining. In contrast, the enzymatic efficiency of xylan displayed a notable reduction at harsh conditions. A large amount of xylan was released to the prehydrolyzate and some were degraded to form byproducts under harsh condition, leaving a xylan deficient substrate for enzymatic hydrolysis.

It is widely acknowledged that the recalcitrance of the lignocellulosic biomass originates from the lignin and hemicelluloses' strong molecular framework which protects the cellulose from the attack of cellulolytic enzymes (Bidlack et al., 1992; Pérez et al., 2007). In this study, a couple of reasons can be speculated to explain the improvement of digestibility of autohydrolysis treated WWS. Firstly, the solubilization of hemicellulose and acetyl groups may remove the physical barriers and increase the accessibility of the cellulose to enzyme. This is consistent with results obtained in this work where the residue xylan amount in pretreated pulp is proportional to the enzymatic digestibility (Fig. 2.21). Secondly, the partially solubility of lignin after autohydrolysis facilitated the digestibility of the biomass by removing the physical barrier caused by lignin (Öhgren et al., 2007) or reducing the non-productive adsorption of cellulase to lignin (Berlin et al., 2005; Esteghlalian et al., 2001). It is noted that the autohydrolysis does not feature largely delignification effects, but the redistribution of lignin has been reported on maize cell walls, which opens up the structure of the cell wall and therefore improves the accessibility of the cellulose microfibrils (Donohoe et al., 2008).

Table 2.10 Material balance from autohydrolysis followed by enzyme treatment:

a) at 4 FPU/g

Treatment	Pretreated Solids (g) [a]	Sugars in filtrate (g)	By-products (g)	Refining [b]	Enzyme hydrolyzate (g)				Total sugar recovery (%) [c]
					Glucan	Xylan	Minor Sugars	Total	
Raw	-	-	-	-	5.8	1.9	0.5	8.1	17.2
170-10	74.0	3.4	0.48	w/o	10.0	4.8	0.5	15.3	39.4
				w	12.3	6.6	0.5	19.4	48.1
170-20	67.3	5.2	0.80	w/o	10.9	6.8	0.4	18.2	49.5
				w	14.1	7.9	0.4	22.4	58.5
170-40	61.6	7.5	1.17	w/o	13.8	6.6	0.3	20.7	59.5
				w	17.4	7.7	0.2	25.3	69.3
170-60	50.6	8.1	1.21	w/o	15.1	3.7	0.1	18.9	56.9
				w	18.5	4.2	0.0	22.7	65.0
180-10	69.8	5.7	0.69	w/o	12.8	6.6	0.4	19.9	53.9
				w	15.7	8.0	0.3	24.1	62.8
180-20	57.2	8.5	1.49	w/o	15.1	5.4	0.2	20.6	61.6
				w	17.9	5.8	0.0	23.7	68.1
180-40	53.0	5.8	2.91	w/o	19.3	3.5	0.1	22.8	60.3
				w	21.8	3.9	0.1	25.8	66.7
190-10	55.8	8.5	1.14	w/o	15.6	4.8	0.2	20.7	61.5
				w	18.9	5.7	0.1	24.7	70.0
200-10	46.6	6.1	2.93	w/o	21.1	2.2	0.1	23.4	62.4
				w	21.7	2.5	0.1	24.3	64.2

Table 2.10 Continued. b) at 10 FPU/g

Treatment	Pretreated Solids (g) [a]	Sugars in filtrate (g)	By-products (g)	Refining [b]	Enzyme hydrolyzate (g)				Total sugar recovery (%) [c]
					Glucan	Xylan	Minor Sugars	Total	
Raw	-	-	-	-	8.4	2.5	0.7	11.6	24.4
170-10	74.0	3.4	0.48	w/o	12.6	5.8	0.7	19.0	47.4
				w	15.5	8.0	0.6	24.1	58.0
170-20	67.3	5.2	0.80	w/o	14.5	8.0	0.4	22.9	59.5
				w	17.5	9.2	0.5	27.2	68.6
170-40	61.6	7.5	1.17	w/o	17.3	7.4	0.0	24.7	68.0
				w	21.2	8.9	0.2	30.3	79.8
170-60	50.6	8.1	1.21	w/o	19.2	4.3	0.1	23.6	66.9
				w	21.5	4.7	0.1	26.3	72.7
180-10	69.8	5.7	0.69	w/o	17.1	8.0	0.6	25.7	66.3
				w	19.2	9.3	0.4	28.9	73.1
180-20	57.2	8.5	1.49	w/o	19.3	6.2	0.2	25.7	72.3
				w	20.7	6.5	0.0	27.3	75.6
180-40	53.0	5.8	2.91	w/o	22.0	4.0	0.0	26.1	67.2
				w	23.6	4.1	0.0	27.7	70.8
190-10	55.8	8.5	1.14	w/o	20.2	5.7	0.3	26.2	73.2
				w	22.1	6.5	0.2	28.8	78.7
200-10	46.6	6.1	2.93	w/o	23.4	2.5	0.2	26.0	67.9
				w	22.9	2.7	0.1	25.7	67.1

[a] Solids yield is based on 100 g original WWS.
[b] w/o: without refining; w: with refining
[c] Sum of sugar recovery from filtrate and enzyme hydrolyzate

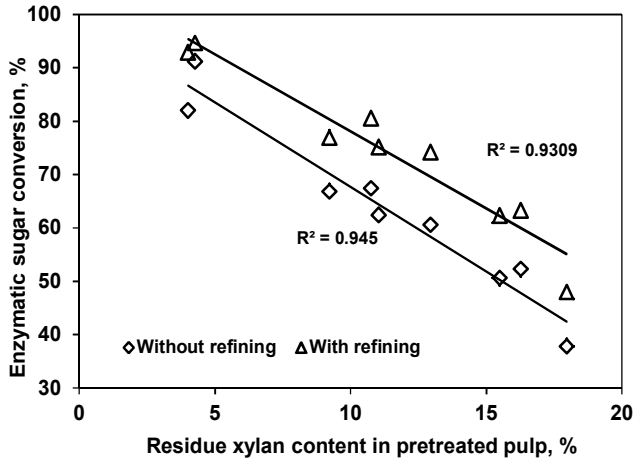

Fig. 2.21 Relationship between residue xylan content and enzymatic digestibility.

3.6. Effect of refining on enzymatic digestibility of autohydrolysis treated WWS

The PFI refining significantly improved the cellulose conversion during enzymatic hydrolysis. As shown in Table 2.10a, an average increase of 20% glucan conversion was achieved when autohydrolysis pretreated WWS was refined. The major effects of refining on the substrate can be summarized as fiber shortening, fiber fibrillation, swelling (penetration of water into the cell wall) and hydration (breaking of some intra-fiber bonds and replaced with water-fiber hydrogen bonds) (Smook, 1992). It is speculated that refining improved the enzymatic digestibility via swelling of the fiber and internal fibrillation. It is noted that almost no improvement was made by refining for 200 °C autohydrolysis treated biomass, indicating that a severe disruption of biomass structure under this condition allows enzymes to attack cellulose easily without additional post-treatment.

3.7. Effect of enzyme charge on enzymatic digestibility

Effect of higher enzyme dosage on enzymatic hydrolysis was also evaluated to see the maximum sugar recovery yield that can be achieved from autohydrolysis pretreated substrate (Table 2.10b). The 10 FPU/g enzyme dosages improves enzymatic sugar yield by 1.4 to 5.8 g/100 g raw WWS compared to that at 4 FPU/g enzyme charge. No significant improvement has been observed with higher enzyme charge for severely treated substrate such as 180°C for 40 min and 200 °C for 10 min combined with refining. This might be due to the limited amount of sugar content remains in the pretreated biomass after a significant amount of sugar released to the prehydrolyzate. It is observed that autohydrolysis pretreated WWS at 170 °C for 40 min followed by refining showed a maximum enzymatic sugar yield of 30.0 g/100 g raw WWS when 10 FPU/g od enzyme was charged compared to 25.3 g/100 g raw WWS enzymatic sugar yield at 4 FPU/g enzyme dosages. It is speculated that either increasing enzyme loading or applying efficient autohydrolysis pretreatment can help improving enzymatic digestibility.

3.8. Total sugar recovery

The total sugar recovery was calculated by the sum of sugars released in autohydrolysis prehydrolyzate and enzymatic hydrolyzate over total carbohydrates in the raw WWS. Table 2.10a shows that the higher temperature and longer residence time enhanced the enzymatic sugar yield but hampered the sugar yield in the filtrate due to the sugar degradation. The results indicate that refining improved the total sugar recovery by an average of 8%. The highest total sugar recovery around 70% could be achieved when WWS

was pretreated at 170 °C for 40 min, or 180 °C for 20 min, or 190 °C for 10 min followed by refining. But autohydrolysis at 170 °C for 40 min was recommended for bioethanol production because of its lower steam pressure requirement for reactor feeding system.

3.9. Effect of solids and liquor separation after autohydrolysis on total sugar recovery

After autohydrolysis, pretreated pulp and prehydrolyzate were separated and pulp was further washed to remove all the non-structure sugar attached to the fiber surface. The major purpose of this operation is to acquire a better understanding of biomass characteristics after autohydrolysis and the ratio of sugar yield from autohydrolysis filtrate and enzymatic hydrolyzate. However, in a commercial process, all the slurry after autohydrolysis would be subjected to enzymatic hydrolysis to reduce complexity of the process and maintaining a high sugar concentration. Therefore, the effect of solids and liquor separation after autohydrolysis on total sugar recovery was investigated. The Fig. 2.22 displays that the solid and liquor separation after autohydrolysis has a slightly increase of total sugar recovery for most cases. The improvement may come from the removal of inhibitions like unbounded lignin and other impurities generated during autohydrolysis (Liu & Zhu, 2010). However, considering the complexity of adding wash process and increased capacity for enzyme saccharification, fermentation and ethanol purification, the integrated process seems to be more economically feasible, though a small amount of ethanol yield might be sacrificed.

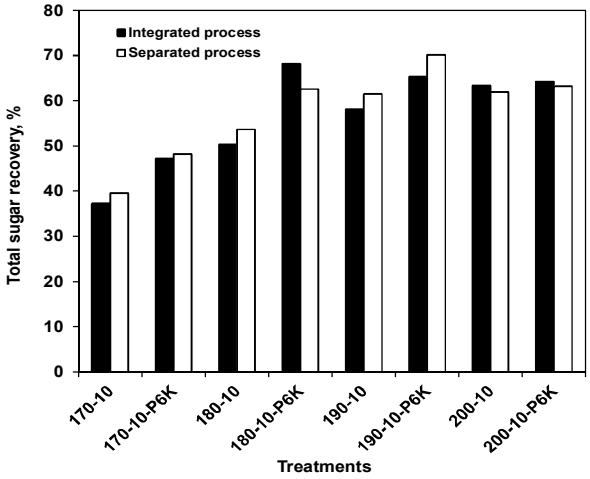

Fig. 2.22 Effects of solids and liquor separation after autohydrolysis on total sugar recovery of WWS. Enzyme dosage is 4 FPU/g od substrate.

3.10. Economic evaluation

Economic evaluation was performed on the scenario of autohydrolysis at 170 °C for 40 min followed by refining and enzymatic hydrolysis at 4 FPU/g enzyme loading, which yields the highest sugar recovery and relatively lower byproducts. The following economic indicators were evaluated including net present value (NPV), internal rate of return (IRR), and minimum ethanol revenue (MER). The NPV is defined as the sum of discounted free cash flow at target rate of return (12% in this study) at each project year. The IRR corresponds to the discount rate that gives a zero NPV and it is a widely used as a metric to evaluate the return on investment for each project. The MER is defined as the required minimum wholesale price of ethanol to achieve a specific internal rate of return.

Table 2.11 displays that the total capital investment on this project is around $37 million, corresponding to $0.86 CAPEX per liter ethanol product. It is noted that the equipment cost and installation cost are much cheaper in China than those in the US, resulting in a relatively low capital investment. The total cash cost is $0.45 per liter ethanol, amid which energy cost accounts for the highest portion, followed by enzyme cost. This biorefinery process presents a net present value of approximately $83 million at 12% discount rate and 28.4% of internal rate of return based on Chinese ethanol wholesale price, indicating a very profitable business. The MER at 12% internal rate of return is $0.56 per liter ethanol, much lower compared to $0.8 per liter ethanol wholesale price in China. Overall, the cellulosic ethanol production from autohydrolysis of WWS displays a very attractive approach for commercialization of cellulosic ethanol. The major reasons can be attributed to the zero cost of raw material and low capital cost due to process simplicity.

Table 2.11 Economic evaluation in Year-3 projection.

	Quantity	Cost per unit
WWS input, dry mt per year	200,000	-
Annual ethanol production, liter	42,655,692	-
Ethanol yield, liter per dry mt	213.3	-
CAPEX, $ per liter ethanol	$36,716,300	$0.86
Enzyme cost per liter ethanol	-	$0.12
Energy cost per liter ethanol	-	$0.26
Total direct cost per liter ethanol	-	$0.39
Total indirect cost per liter ethanol	-	$0.17
Total cash cost per liter ethanol	-	$0.45
MER, $ per liter ethanol	-	$0.56
NPV @ 12% discount rate, %	$83,540,214	-
IRR @ $0.8 per liter ethanol revenue	28.40%	-

Conclusions

Waste wheat straw from feedstock preparation process in a straw pulp mill was subjected to an autohydrolysis pretreatment with different temperatures and residence time. The pretreated materials were further refined and enzyme hydrolyzed. Results showed that during autohydrolysis, 3.4 to 8.5 g/100 g raw WWS sugar can be recovered from prehydrolyzate, and 0.5 to 2.9 g/100 g raw WWS byproducts can be generated as well under different pretreatment conditions. For the maximum enzymatic sugar yield at 4 FPU enzyme dosages, 23.4 g/100 g raw WWS enzymatic sugar was achieved when WWS was pretreated at 200 °C for 10 min without mechanical refining, and 25.8 g/100 g raw WWS enzymatic

sugar was obtained when WWS was pretreated at 180°C for 40 min followed by mechanical refining. The highest total sugar recovery including sugar recovered from autohydrolysis filtrate and enzymatic hydrolyzate based on total carbohydrates in raw WWS can be approximately 70% when WWS was pretreated at 170 °C for 40 min or 190 °C at 10 min. But considering the feed system limitations at the mill, autohydrolysis at 170 °C at 40 min followed by 6000 revolution PFI refining was recommended for bioethanol production. The economic evaluation based on the optimal condition in this study indicates that cellulosic ethanol production from autohydrolysis of WWS is a very attractive business, which can generate 28.4% internal rate of return based on current ethanol wholesale price in China.

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Chapter 3 Fundamental characteristics that affect enzymatic digestibility of autohydrolysis pretreated biomass

Abstract

Six types of lignocellulosic biomass including sugarcane bagasse, wheat straw, switchgrass, maple, sweet gum, and nitens were subjected to autohydrolysis pretreatment, followed by enzymatic hydrolysis to evaluate the impact of biomass characteristics to the autohydrolysis and the following enzymatic hydrolysis. It has been found that the lignin of non-woody biomass is easier to depolymerize during autohydrolysis compared to woody biomass because of the existence of significant amount of p-coumaric acid and ferulic acid. The lignin structure such as S/V ratio in the woody biomass plays a significant role in the total sugar recovery from autohydrolysis process where the higher S/V ratio, the higher amount of sugar can be extracted from the woody biomass.

Keywords: Autohydrolysis, lignin, enzymatic hydrolysis, sugar recovery

1. Introduction

Lignocellulosic biomass is the most abundant renewable resources on earth. In the past decades, there have been substantial studies in converting this biomass for the production of bioethanol. In order to produce bioethanol, cellulose and hemicellulose in biomass need to be enzymatic hydrolyzed to fermentable sugars, followed by fermentation into ethanol. However, the recalcitrance of lignocellulosic biomass requires expensive pretreatment operations to open up the matrix structure and provide an enzyme accessible

substrate for hydrolysis (Himmel et al., 2007). Autohydrolysis is an efficient and capital-effective method to produce commercial bioethanol from lignocellulosic biomass because its simplicity and effectiveness compared to those more complex and capital-intensive pretreatment technologies (Phillips et al., 2013).

During autohydrolysis, acidic hydronium ions are generated by water autoionization at high temperature. They act as catalyst and attack the susceptible ether bonds of hemicelluloses, leading to both splitting of acetyl groups and generation of oligosaccharides (Garrote et al., 1999). The resulting acetic acid makes the solution more acidic and further catalyzes the depolymerization of biomass. Depending on the pretreatment severity, the dissolved sugars (pentoses and hexoses) can be further degraded to form furfural and hydroxymethylfurfural, which combine with aromatic degradation products from lignin may affect the yeast metabolism in the fermentation step (Palmqvist & Hahn-Hägerdal, 2000). The enzymatic digestibility of pretreated biomass can be largely improved by the removal of hemicellulose and disrupt of lignin structure (Mosier, 2013).

Many studies have been carried out on the mechanism of hemicellulose solubilization during autohydrolysis. However, only limited data is available on understanding how the lignin changes during autohydrolysis process and how those changes affect the enzymatic digestibility of autohydrolysis pretreated biomass. In this regard, six types of lignocellulosic material including woody biomass (maple, sweet gum, and nitens), non-woody biomass (sugarcane bagasse, wheat straw and switchgrass) were investigated to acquire a fundamental

understanding of lignin structure change during autohydrolysis among different feedstock and the effect of lignin structure change to enzymatic digestibility of autohydrolysis pretreated substrate.

2. Materials and Methods

2.1 Raw material

All the raw material was air-dried at room temperature for two weeks to have moisture content around 5%. The wood chips (maple, sweet gum, and nitens) were hand-cut to have a size of 2 cm × 1 cm × 0.5 cm (length × width × thickness). The non-woody biomass (sugarcane bagasse, wheat straw, and switchgrass) were hand-cut into 2-3 cm size. All the feedstocks after cutting were stored in sealed plastic bag at room temperature prior to pretreatment. In addition, part of the feedstock were ground by a Wiley Mill (Model No. 4, Thomas Scientific, Philadelphia, PA), and the sawdust were screened to have a particle size between 20-40 mesh for composition analysis.

2.2 Composition analysis

The solids content was measured by the weight difference of samples before and after drying in a convective oven at 105 °C for 24 h. The extractives content was determined in a soxhlet extraction apparatus with acetone and water mixture at a ratio of 9:1 for 24 h. The ash content was measured by weight difference before and after cauterizing samples in a muffle furnace at 575 °C for 4 h. The acid soluble lignin and acid insoluble lignin was analyzed by a two-stage acid hydrolysis (72% of sulfuric acid at room temperature for 2 h followed by 4%

sulfuric acid at 121 °C for 1 h) according to National Renewable Energy Laboratory's (NREL) Laboratory Analytical Procedures (Sluiter et al., 2008). The resulting filtrate was filtered through 0.2 μm nylon filters. The sugar concentration was analyzed by Dionex-IC (Dionex-IC-3000, Dionex, USA), equipped with an eluent generator (EG-2 model), a guard column (CarboPac PAI 4×50 mm), an ion exchange column (CarboPac PAI 4×250 mm), and an electrochemical detector (ED-40 model). The column was operated at 18 °C using Mili-Q water and 400 mN sodium hydroxide solution as the mobile phase at a flow rate of 0.3 ml/min. A 200 mN sodium hydroxide solution was added to post column for optimal detector sensitivity and baseline stability. Fucose was added to each sample as internal standard.

2.3 Autohydrolysis pretreatment combined with mechanical refining

The autohydrolysis pretreatment was carried out in a 1.0 L alloy C-276 Parr reactor (Parr Instrument Company, Moline, IL, USA). For each run, 50 dry g of biomass was loaded into the reactor supplemented with deionized water to make a final liquid to solids ratio of 10:1. To get a better mass and heat transfer, the reactor was vacuumed before heating. The target temperature was set at 180 °C and the average ramping time was around 30 min. The target temperature was maintained for 40 min and then the reactor was quickly quenched in a water tank. After reaction, the liquid was squeezed out by using cheese clothes and stored in a 4 °C refrigerator. The solids were washed thoroughly with tap water, centrifuged and stored in a 4 °C refrigerator for future use.

The solids recovery yield was determined by measuring the total wet weight and the moisture content of pretreated samples. The pretreated samples was disintegrated by a disc refiner (Bauer 148-2, the Bauer brothers company, Springfield, Ohio) at an opening of 0.005 inch, followed by the fibrillation in a PFI refiner (Hamjern Maskin A/S, Hamar, Norway) at 10% consistency for 6000 revolutions. The refined samples were centrifuged, fluffed, and stored in a 4 °C refrigerator for enzymatic hydrolysis.

The total sugar in the autohydrolysis filtrate was analyzed according to National Renewable Energy Laboratory's (NREL) Analytical Procedures (Sluiter et al., 2006), where the aliquot of autohydrolysis filtrate was hydrolyzed with 4% w/v sulfuric acid at 121 °C for 1 h. The resulting hydrolyzate was neutralized using sodium carbonate and then filtered through 0.2 μm nylon filters. The sugar concentration was measured in a HPLC system (Agilent 1200, Agilent, Santa Clara, CA, USA), equipped with Shodex SP0810 column (8×300 mm, Showa Denko, Tokyo, Japan) and a refractive index detector. The column was operated at 80 °C with Milli-Q water as the mobile phase at a flow rate of 0.5 mL/min. The refractive index detector was operated at 50 °C.

The byproducts including acetic acid, formic acid, furfural, and hydroxymethyl furfural (HMF) were measured by a HPLC system (Dionex UltiMate 3000, Sunnyvale, CA), where a Bio-Rad Aminex HPX-87H column (300 mm x 7.8 mm) was operated at 65 °C with 0.05 M sulfuric acid as the mobile phase at a flow rate of 0.6 mL/min.

2.4 Enzymatic hydrolysis

The enzymatic hydrolysis was carried out in an environmental incubator shaker (New Brunswick Scientific, Edison, NJ, USA) set at 50 °C, 180 rpm for 96 h. The enzyme cocktail Cellic CTec 2 and Cellic HTec 2 (Novozymes, Franklinton, NC) were used in this study and the activity of CTec 2 was measured to be 139 FPU/g according to procedure described by Ghose (Ghose, 1987). The hydrolysis was performed at 5% consistency in a 50 mM acetate buffer (pH=4.8) with 5 FPU/ dry g substrate CTec 2 supplemented with HTec 2 at the amount of 1/9 of CTec 2 dosages recommended by Novozymes. Sodium azide (0.1%, w/w) was added into the mixture to avoid contamination.

The hydrolysis reaction was stopped by soaking the samples into the boiling water for 10 min. The samples were centrifuged and the supernatant were collected and filtered through 0.2 μm nylon filters for sugar analysis. The sugar analysis was carried out in the HPLC system with Shodex SP0810 column as discussed before.

2.5 Alkaline nitrobenzene oxidation

The alkaline nitrobenzene oxidation was performed in a stainless bomb reactor and the reactor was electronically heated in an aluminum block. Both untreated extractive free and pretreated biomass meal (200 mg) were mixed with 7 mL of 2 N NaOH solution and 0.4 mL of nitrobenzene in the bomb reactor and heated to 170 °C for 2.5 h. For all non-woody biomass, an additional cooking was performed at 190 °C for 4 h to obtain the yield of p-hydroxybenzaldehyde (H). During the reaction, the bomb reactor was shaken vigorously

every half an hour. Once the reaction was completed, the bomb reactor was rapidly quenched in a cold water bath and 1 mL of 5-iodovanillin solution (80 mg dissolved in 5 mL dioxane) was added as the internal standard. The mixture was extracted with 30 mL of CH_2Cl_2 for 3 times to remove the residual nitrobenzene. Then the aqueous phase was acidified with 2 N HCl to pH around 2.5. The resulting solution was extracted with 30 mL of CH_2Cl_2 for 3 times again, and the organic phase was collected and dried with anhydrous Na_2SO_4. One milliliter of the product was dried in a rotatory evaporator at 30 °C. The dried solids were dissolved in 50 of μL pyridine and 50 μL of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). The resulting solution was injected directly to gas chromatography (GC). The detail of GC analysis was described in our previous study (Santos et al., 2012).

2.6 P-coumaric acid and ferulic acid

One gram of extractive free wood meal was mixed with 20 ml 1N NaOH, purged with nitrogen gas, and placed in an environmental incubator at room temperature, 200 rpm for 48 h. After reaction, the cinnamic acid was added as internal standard and mixed vigorously. The samples were then centrifuged to remove all the unreacted solids. The liquor was acidified to pH between 2-2.5 using 2 N HCl. The resulting samples were further extracted with 10 ml ethyl acetate for three times. The extract was filtered and injected to HPLC which is equipped with Zorbax SB-C3 column, gradient pump and UV detector. A 30 min gradient was applied in which 10 mM formic acid in acetonitrile and 10 mM formic acid in water were used as the mobile phase.

3. Results and discussion

3.1 Chemical composition of raw material

The chemical composition of raw material was listed in Table 3.1. Generally the woody biomass has a higher lignin content compared to non-woody biomass. The ash content of non-woody biomass is much higher than that of woody biomass. The total carbohydrates content of all feedstocks ranges from 58.9 - 66.7%.

Table 3.1 Chemical composition of raw material, based on g per 100 g raw material.

	Glucan	Xylan	Galactan	A&M [b]	AIS [c]	ASL [d]	Ash	Ext [e]	Acetyl
SC bagasse [a]	42.3	20.9	0.9	2.6	18.2	2.3	2.7	3.9	3.3
WS	39.0	18.8	1.2	3.1	19.4	2.9	2.4	1.9	2.0
Switchgrass	34.3	24.1	0.9	3.1	18.4	2.9	3.0	2.1	3.0
Maple	43.2	13.0	0.7	3.4	22.1	3.1	0.3	2.0	3.5
Sweet Gum	40.2	15.7	0.8	2.2	21.5	3.7	0.6	1.2	4.0
Nitens	44.4	14.3	1.1	1.4	19.9	4.6	0.2	1.1	3.7

[a] SC bagasse: Sugarcane bagasse
[b] A&M: Arabinan and mannan
[c] AIS: Acid insoluble lignin
[d] ASL: Acid soluble lignin
[e] Ext: Extractives

3.2 Autohydrolysis of different types of biomass

3.2.1 Solids recovery after autohydrolysis

The components recovered as solids after autohydrolysis are shown in Fig. 3.1. The depolymerization of woody biomass was stronger than that of non-woody biomass at the

same autohydrolysis condition. Hemicellulose dominated the loss of solids for all feedstocks and around 80% of hemicellulose were either dissolved or degraded during the autohydrolysis process. Both cellulose and lignin in the woody biomass is more resistant to thermal treatment compared to that of non-woody biomass. It is of interest to point out that more than 30% of lignin in non-woody biomass can be solubilized during the pretreatment whereas only around 18% of lignin in woody biomass depolymerized into the autohydrolysis filtrate. The presence of p-coumaric acid and ferulic acid in non-woody biomass may lead to a higher degree of depolymerization in non-woody biomass lignin during autohydrolysis. The results show that the non-woody biomass has a total amount of p-coumaric acid and ferulic acid in a range of 2.2 to 7.2% of the total lignin, while no such compounds were detected in woody biomass lignin. The p-coumaric acid and ferulic acid are linked to carbohydrates in non-woody biomass through ester or ether linkages, which can be readily cleaved during hydrothermal treatment.

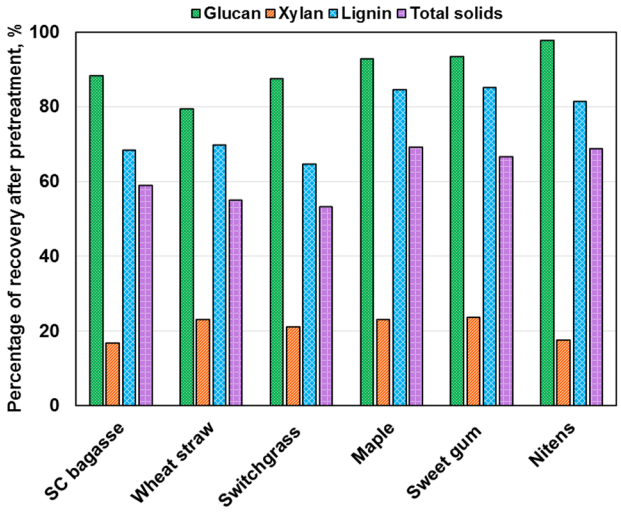

Fig. 3.1 Components recovered after autohydrolysis pretreatment.

3.2.2 Sugar released in the autohydrolysis filtrate

The percentage of sugars released in the autohydrolysis (based on the sugar component in original sugar) is presented in Fig. 3.2. The non-woody biomass has a slightly higher percentage of glucan released into the autohydrolysis filtrate compared to woody biomass, which is consistent to the previous results that lower amount of cellulose can be retained in the pretreated solids for non-woody biomass. It is obvious that the percentage of xylan recovered from autohydrolysis filtrate for woody biomass is higher than that of non-woody biomass. Considering the percentage of xylan recovered in the pretreated solids is similar, more degradation xylose might occur for non-woody biomass, resulting in a reduced amount of xylose in the autohydrolysis filtrate.

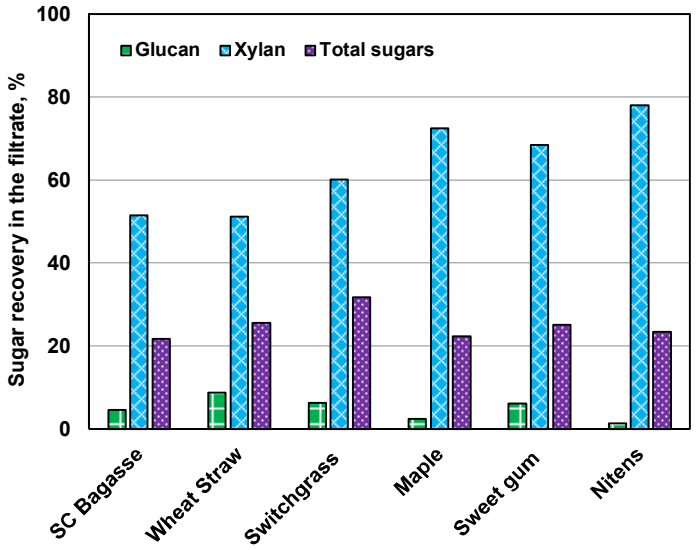

Fig. 3.2 Total sugar released in the autohydrolysis filtrate, percentage based on sugar component in raw material.

3.2.3 Byproducts formed during autohydrolysis

Under the same pretreatment severity, the amount of byproducts formed in the autohydrolysis filtrate varied significantly. As shown in Fig. 3.3, the sugars in the sugarcane bagasse are more vulnerable towards degradation during the autohydrolysis process, where the highest amount of total byproducts was obtained. In contrast, the wheat straw had the lowest amount of byproducts probably because of the lowest acetyl group in the wheat straw. The pH of the autohydrolysis filtrate is correlated to the amount of byproducts generation, which means the lower pH value, the higher amount of byproducts can be generated.

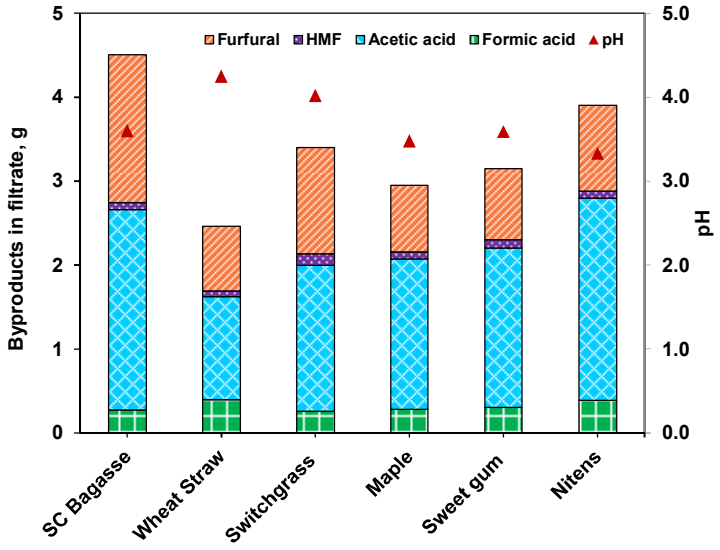

Fig. 3.3 Byproducts and pH in the autohydrolysis filtrate, g based on 100 g raw material.

3.3 The effect of autohydrolysis and mechanical refining on enzymatic hydrolysis

After autohydrolysis, the pretreated material was further mechanical refined and subjected to enzymatic hydrolysis at 5 FPU/ g enzymes dosages. The enzymatic sugar conversion based on the sugar in the pretreated pulp is shown in Fig. 3.4. Switchgrass and maple has the lowest enzymatic sugar conversion of around 70%. In contrast, all the other substrate has a total enzymatic sugar conversion higher than 80%, indicating that autohydrolysis combined with refining is a very efficient pretreatment method.

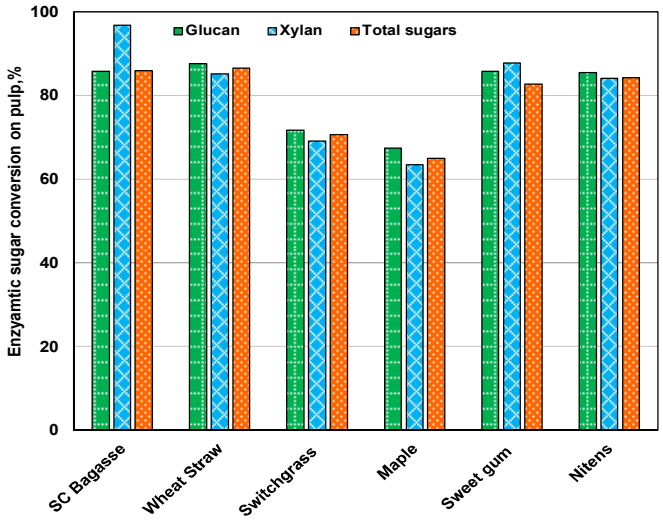

Fig. 3.4 Sugar conversion of pretreated substrate during enzymatic hydrolysis at 5 FPU/g enzyme dosages.

3.4 Total sugar recovery

The total sugar recovery including sugar recovered from autohydrolysis filtrate and enzymatic hydrolyzate is presented in Fig. 3.5. For three non-woody biomass, the amount of sugar recovered from autohydrolysis filtrate and enzymatic hydrolyzate varies but the total sugar recovery is similar. For woody biomass, the total sugar recovery is proportional to the S/V ratio in raw lignin (Fig. 3.6), indicating that the lignin structure has a substantial impact to the performance of autohydrolysis process.

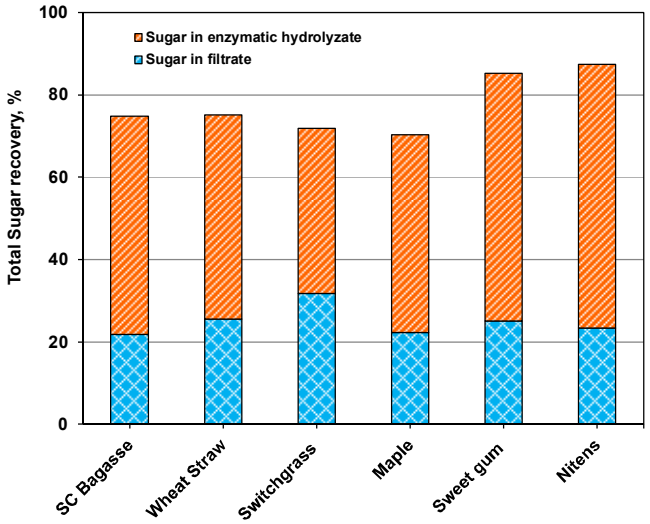

Fig. 3.5 Total sugar recovery at 5 FPU/g enzyme dosages, based on total carbohydrates in raw material.

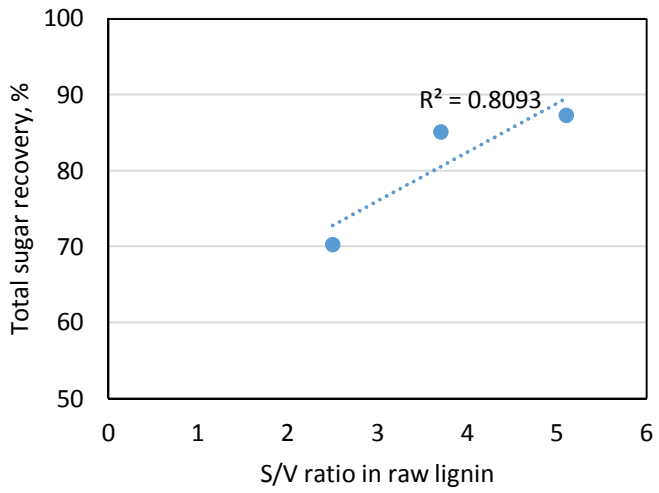

Fig. 3.6 The effect of S/V ratio in raw lignin to the total sugar recovery.

4. Conclusions

Six different types of lignocellulosic biomass were subjected to autohydrolysis pretreatment, followed by enzymatic hydrolysis to evaluate the impact of biomass characteristics to the autohydrolysis and the following enzymatic hydrolysis. It has been found that the lignin of non-woody biomass is easier to depolymerize during autohydrolysis compared to woody biomass because of the existence of significant amount of p-coumaric acid and ferulic acid. The lignin structure such as S/V ratio in the woody biomass plays a significant role in the total sugar recovery from autohydrolysis process in which the higher S/V ratio, the higher amount of sugar can be extracted from the woody biomass.

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Chapter 4 Cellulosic ethanol production from autohydrolysis of hardwood: economic trade-offs between lignin combustion or pelletization in the context of greenfield, co-location, and repurposing

Abstract

The emerging cellulosic ethanol market calls for a capital-efficient biorefinery approach to break through the economic barrier that stands in the way of many otherwise exciting approaches. Autohydrolysis combined with mechanical refining could be a particularly attractive process for cellulosic ethanol production because of simplicity and relative effectiveness. In this study, process economic analyses of bioethanol production from autohydrolysis of hardwood associated with lignin combustion versus pelletization and sale are evaluated in greenfield, co-location, and repurposing scenarios. Overall, six cases investigated in this study reflected an internal rate of return ranging from 0.2% to 17.6%. Co-production of bioethanol and residue fuel pellets appears more attractive than producing ethanol and burning residue for power and steam. The co-location and repurposing scenarios significantly improve the economics of the biorefinery by reducing the capital investment, and provide viable economic options for kraft mills for the continuing decline in domestic demand for printing papers.

Keywords: Autohydrolysis; Hardwood; Ethanol; Pellets; Economics

1. Introduction

Substantial investment at the laboratory, pilot and commercial scale have been made in the past decade to develop cellulosic ethanol as a cost-competitive fuel option, but commercial production of cellulosic ethanol is only now beginning to emerge. The major barriers that prevent investors from moving forward with large scale projects on cellulosic ethanol lie in high technical risk coupled with high capital investment cost relative to ethanol product value. Nevertheless, it is important to note that several pathways can be proposed to overcome these hurdles. Simplicity, meaning capitalizing the least number of unit operations is the main way to break through the capital investment barrier, even if ethanol yield is modest (Phillips et al., 2013). The production of marketable byproducts in addition to ethanol from cellulosic biomass may further improve the overall financial performance of the biorefinery (Arato et al., 2005; Pan et al., 2005; Treasure et al., 2012). In addition, repurposing a closed pulp mill or co-locating the biorefinery plant with an existing kraft pulp mill may largely reduce the capital investment by taking advantage of joint facilities and a consolidated biomass supply chain (Gonzalez et al., 2011c; Gu et al., 2012; Jin et al., 2010; Phillips et al., 2013).

Unlike starch and sugar based ethanol production, cellulosic ethanol production requires pretreatment to overcome the recalcitrance of lignocellulosic biomass and thus provide an enzyme accessible substrate for hydrolysis (Alvira et al., 2010; Hendriks & Zeeman, 2009; Mosier et al., 2005; Sun & Cheng, 2002; Yang & Wyman, 2008). Extensive pretreatment technologies have been investigated to achieve the target ethanol yield.

However, few of them can be successfully implemented to commercial cellulosic ethanol production because of 1) low ethanol yield; 2) high capital investment; 3) high enzyme dosage; 4) low chemical recovery efficiency; 5) environmental pollution. Autohydrolysis, which treats the lignocellulosic biomass in a water-only medium at elevated temperature combined with refining stands out as a particularly attractive approach to cellulosic ethanol production, mainly because of competitive carbohydrates recovery (percentage of carbohydrates in original wood that are converted to monomeric sugars) at low enzyme dosage (Ertas et al., 2013), negligible environmental pollution, and most importantly, the process simplicity. Moreover, many of the unit operations proposed for autohydrolysis are proven technologies that have been used in kraft pulp mills for more than a hundred years, and the mildly acidic (greater than 3) conditions can be processed in mild steel equipment typical for the pulp and paper industry. The integration of a cellulosic ethanol plant with an existing kraft pulp mill will greatly help the implementation and commercialization of cellulosic ethanol.

The European wood pellet market has become prosperous in recent years. This is a result of European countries' objective to increase their share of renewable energy consumption, but also to some extent has been an effect of the increase of oil price. It was estimated that Europe consumed 13 million tons of wood pellets in 2012 and the demand is expected to rise to 25-30 million tons per year by 2020 based on current trends (The Economist, 2013). The price of wood pellets in European market varies between areas, quality and packaging, but generally ranges as high as $160 per metric ton (FOB price)

according to published reports (Argus Biomass Markets, 2013). Process residue from autohydrolysis has a higher heating value compared to wood and does not have sulfur emission issue when it is burned in biomass boiler. Thus, the process residue from autohydrolysis process could be utilized as raw material for biomass pellets production to boost the profitability of the biorefinery process. Other products for autohydrolysis lignin may emerge, but biomass value as fuel pellets is 2 times the value as fuel in a biomass boiler.

The aim of this paper is to present the economics of cellulosic ethanol production in an autohydrolysis process using mixed southern hardwood as feedstock. Six cases were built in a combination of different treatments of the process residue (which we shall now refer to as "lignin", since that material constitutes the largest part of unhydrolyzed residue) and different project context (greenfield, co-location, and repurposing). The following economic indicators were determined to investigate the trade-offs between lignin combustion and pelletization in an integrated conversion process: internal rate of return (IRR), net present value (NPV), payback years, and minimum ethanol revenue (MER).

2. Materials and methods

2.1. Feedstock

Mixed natural southern hardwood chips were used as raw material in this analysis. The moisture content of the raw material was estimated to be about 45%. The chemical composition of the feedstock used for this study was measured in the lab at the Department of Forest Biomaterials at North Carolina State University (Table 4.1). The feedstock

delivered cost was estimated at $71 per dry metric ton based on prevailing market prices in the Southeastern United States (Gonzalez et al., 2011b).

Table 4.1 Percent dry weight composition of mixed natural southern hardwood

Feedstock	Glucan	Xylan	Galactan	Arabinan	Mannan	Rhamnan	Lignin	Ash
Hardwood	44.4	15.3	0.5	0.5	2.3	0.3	26.3	0.3

2.2. Basis for evaluation

A total of six cases were evaluated (Table 4.2), representing the combinations of different co-products (steam and power, and lignin pellets) and different scenarios (greenfield, co-location, and repurposing). The economics of co-production of bioethanol and steam and power in a greenfield plant was explored first as the base case. In the alternative context cases, the biorefinery plant is co-located with an existing pulp mill or a pulp mill is repurposed to make cellulosic ethanol. In addition, co-production of ethanol and lignin pellets in greenfield, co-location, and repurposing scenarios were investigated as additional cases.

Table 4.2 Basis for evaluation

Case	I	II	III	IV	V	VI
Product	Ethanol	Ethanol	Ethanol	Ethanol	Ethanol	Ethanol
Co-product	Power and steam	Power and steam	Power and steam	Pellets	Pellets	Pellets
Context	Greenfield	Co-location	Repurposing	Greenfield	Co-location	Repurposing

2.3. Proposed pathway

2.3.1. Processing of lignin to power and steam versus pellets fuel

The proposed technical pathway for cellulosic production from autohydrolysis of hardwood is illustrated in Fig. 4.1. Hardwood chips are fed into the cooking vessel for 1 hour residence time at 180 °C with a solid to liquor ratio of 1:3. During autohydrolysis, 4.1% of cellulose and 49.1% of hemicellulose are released into solution as monomeric and oligomeric sugars. After autohydrolysis, the slurry goes through a blow tank to release high pressure and recover blow heat for digester pre-heating. Then the pretreated biomass is diluted to 20% consistency, adjusted to pH 5 with sodium hydroxide solution, and conveyed to a mechanical refiner, which has been found to generate significant improvements in enzymatic hydrolysis efficiency (Jones et al., 2013). A screw press system is introduced to split the liquor out of the whole slurry to heat exchange the hot filtrate with chill water. In addition, the cooled screw press filtrate is passed through an ion exchange resin to control the concentration of undesired acid products that were generated during autohydrolysis, mainly acetic acid. Enzyme hydrolysis was carried out at 20% solids content with an enzyme charge of 5 FPU/

OD g solids for 96 hours in a unique multiple stage hydrolysis system (Xue, 2011). The conversion efficiency during enzymatic hydrolysis was measured in the lab as 84.8% for cellulose and 33.5% for hemicelluloses. Conversion efficiencies used for fermentation were assumed as 95% for hexoses and 80% for pentoses with a total residence time of 36 hours (Humbird et al., 2011). After distillation, the rectified beer column tops are further dehydrated to produce 99.5% cellulosic ethanol as the final product. The beer column bottoms are passed through a lignin filter to separate solids and liquor. The filtrate is recycled back to the system as dilution water, while the lignin-enriched solids are utilized for either power and steam generation or lignin pellets production after drying.

As illustrated in Fig. 4.1 (a), lignin residue can be concentrated to 60% consistency through a high efficiency pressure filter (Humbird et al., 2011) and fed into a biomass boiler to generate power and steam for the whole biorefinery plant. Alternatively, as shown in Fig. 4.1 (b), the dewatered lignin can be processed in a pellets plant to produce biomass pellets fuel. Considering the modest price of natural gas in the US, the processing steam can be generated through a package natural gas boiler and the hot flue gas can be utilized as a drying source for lignin pellets production. The trade-off between lignin combustion for power and steam and pelletization for sale is driven by several factors such as the market price of natural gas and lignin pellets, the lower capital investment for the gas boiler compared to a biomass boiler and a turbine generator.

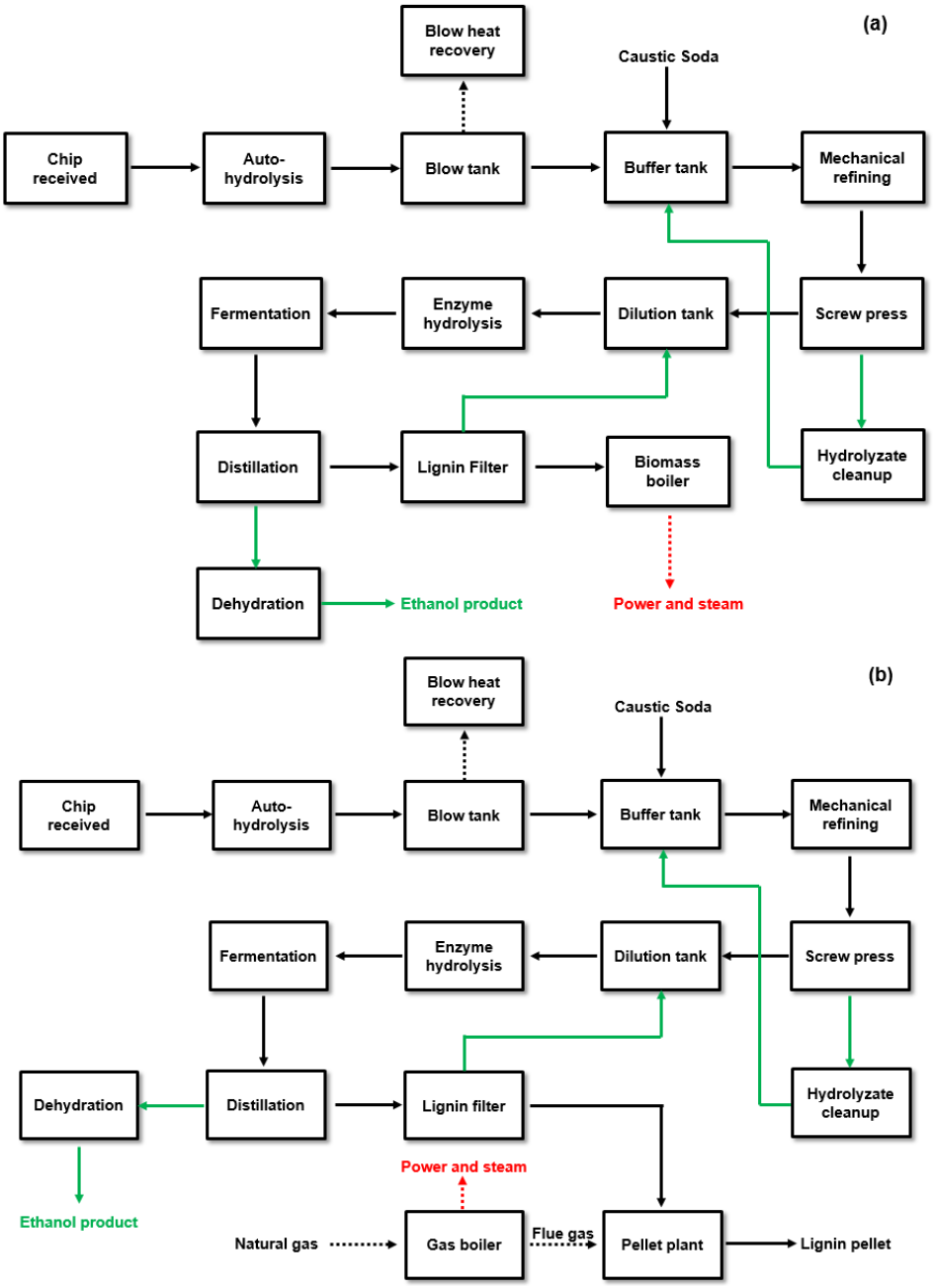

Fig. 4.1 (a) Proposed integrated system for cellulosic ethanol, power and steam production; (b) Proposed integrated system for cellulosic ethanol and lignin pellets production

2.3.2. Context of greenfield, co-location, and repurposing

Greenfield project refers to a new project started from purchase and development of land and plant infrastructure, and purchase, installation and startup of process equipment. The co-location scenario significantly reduces the financial risks by sharing the feedstock supply chain, and existing facilities such as administration buildings, warehouse, waste water plant, and power house. Fig. 4.2(a) displays how the biorefinery plant fits well with a kraft mill. The biorefinery plant takes advantage of the biomass supply chain from kraft mill, getting wood chips to produce cellulosic ethanol while sending back lignin solids to the power plant of kraft mill. Because of the cleanliness of the autohydrolysis process, the lignin could be either pelletized to make biomass pellets fuel, or fed into a biomass boiler to recover energy without adding extra burdens to the kraft recovery boiler. Moreover, the screened mill sludge, which could be readily hydrolyzed by enzyme (Duff et al., 1994), could also be fed into biorefinery plant to produce cellulosic ethanol. This option was not investigated in this analysis.

The United States forest products industry is currently in significant decline in product demand and profitability (Phillips et al., 2013). A number of permanently shut down pulp mills yield the opportunity to recapture those assets for cellulosic ethanol production. The mildly acidic (greater than pH 3) processes in autohydrolysis pretreatment can be accomplished in mild steel equipment typical for pulp and paper mills. As shown in Fig. 4. 2(b) if a kraft mill is repurposed to make cellulosic ethanol, the only new pieces of equipment are those required to process fiber through enzyme hydrolysis, fermentation and

ethanol recovery. In this regard, capital investment for the biorefinery portion of the plant can be maintained as low as corn-based ethanol plant regardless of the effect of sugar concentration on the fermentation and distillation capital investment.

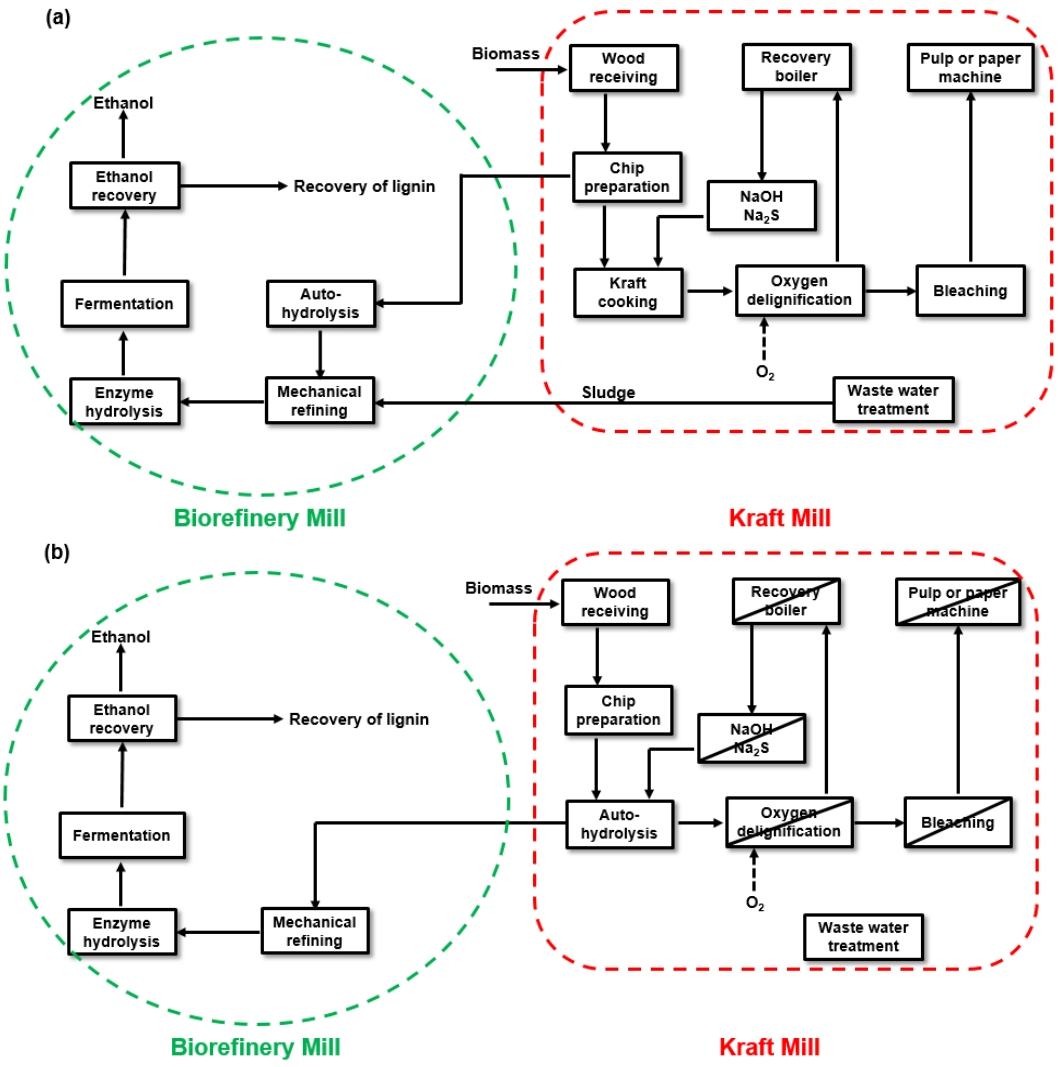

Fig. 4.2 (a) Proposed scenario of co-locating biorefinery mill with a kraft mill; (b) Proposed scenario of repurposing a kraft mill to bioethanol production.

2.4. Process simulation

A complete process model for the integrated autohydrolysis biorefinery was produced using WinGEMS V.5.3. This process simulation software is originally developed for use in pulp and paper industry and therefore has specialty blocks and unit operations particularly useful for application in pulp and paper and biorefinery mill as well (Treasure et al., 2012). A steady-state mass and energy balance for the entire process was produced and exported to a spreadsheet by interface with Microsoft Excel, where it could be referenced for the economic evaluation. Two main simulation models were built: (i) the base case in which lignin was combusted directly to generate power and steam, and (ii) the alternative case in which lignin was pelletized to make pellets fuel. The heating value of the lignin was estimated based on its composition (lignin, cellulose, and hemicellulose) according to the methodology developed by Domalski (Domalski E., 1986).

2.5. Economic analysis

Standard investment finance techniques were used and the specific parameters and some key simulation results are displayed in Table 4.3. In brief, the financial project life was set at 15 years with a steady feedstock input of 500,000 dry metric tons per year. Total operating hours were assumed to be 96% availability (8400 hours per year). A depreciation schedule of 10 years straight line was used in the analysis. The tax rate was set at 35% with tax loss carryforward where the negative profits in previous years can be carried forward to offset part of taxes in profitable years. A terminal value in year 15 of five times of year-15 EBITDA (Earnings Before Interest, Taxes, Depreciation and Amortization) was assumed. A

discount rate of 12% was set which is consistent with other studies (Gonzalez et al., 2011a; Gonzalez et al., 2011c). The maintenance and capital reinvestment was estimated as a function of the Replacement Asset Value (RAV), where the investment cost to replace the original asset escalates annually in cost at 2%. Other mill fixed costs were estimated at 3% of replacement asset value and overhead was assumed to be 2% of annual sales. A staffing plan was prepared for each alternative which, with typical salary, wage and benefit costs, establishes labor costs.

Ethanol selling price was set at $0.653 per liter according to the average FOB, Omaha, NE ethanol selling price in 2013 (Nebraska Energy Office, 2013). No ethanol subsidy or tax credit was used in this analysis. The ethanol production rate is 323 liters per dry ton biomass derived from simulation results and assumed to have a production improvement of 0.5% annually. The cost of enzyme has been regarded as a large part of the operating cost for cellulosic ethanol production (Cherry & Fidantsef, 2003; Klein-Marcuschamer et al., 2012). However, the detailed cost information of enzyme is limited to the public, so the enzyme cost in this analysis was estimated from the limited public information as follows (Phillips et al., 2013): Novozymes has indicated enzyme costs for corn stover in 2012 at $0.50 per gallon ethanol production. Assuming best literature value of 4 FPU of CTec-2 enzyme per gram of glucan and a 50% improvement in cost going forward (both efficiency and cost of enzyme production), an estimate of $0.12 enzyme cost per liter was derived with enzyme product cost of $1.00 per kilograms enzyme product (equals to $5.7 per kg enzyme protein). This assumption is consistent with study performed by Tao

(Tao et al., 2013). Lignin pellets fuel was estimated to have a moisture content of 6% and the production rate is 484 kg per dry ton biomass based on simulation results. Lignin pellets revenue was estimated based on the wood pellets FOB price from south coast of US to Europe and was assumed to increase by 3% every year. The power, natural gas, gasoline denaturant (2% on ethanol by volume) and caustic soda prices were set according to the latest US market prices.

Table 4.3 Operative and financial assumption and key simulation results used in the economic analysis.

Description	Value	Description	Value
Startup year	2015	Ethanol revenue, $ per liter	0.653
Terminal year	2030	Ethanol yield, liter per dry ton biomass	323
Feedstock supply, dry tons per year	500,000	Lignin pellets revenue, $ per ton	160
% of CAPEX spending in year -2	10%	Lignin pellets moisture content	6%
% of CAPEX spending in year -1	50%	Biomass cost, $ per dry ton	71
% of CAPEX spending in year -0	40%	Power cost, $ per MWH	65
% of Nominal capacity, project year 1	50%	Natural gas cost, $ per MMBTU	4.0
% of Nominal capacity, project year 2	85%	Gasoline denaturant, $ per liter	0.766
Working capital on materials, % of direct cost	15%	Caustic soda cost, $ per dry ton	500
Working capital on product, % of revenue	5%	Enzyme cost, $ per kg enzyme product	1.0
Years depreciation schedule	10-S/L[a]	Yeast cost, $ per liter ethanol	0.004
Tax rate, with tax loss carryforward	35%	Operating labor (lignin combustion), $ per year	5,702,750
Discount rate	12%	Operating labor (lignin pelletization), $ per year	5,942,750
Terminal value, year 15 EBITDA[b] multiple	5.0	Maintenance expense, % of RAV[c]	2%
Hours per year	8400	Capital reinvestment, % of RAV[c]	1%
Excess spending in startup year,% of direct cost	10%	Other fixed costs, % of RAV[c]	3%
Training cost, % of year-1 labor cost	105%	Sales and other overhead, % of sales	2%

[a] 10-S/L = 10 years straight line depreciation schedule

[b] EBITDA = Earnings Before Interest, Taxes, Depreciation and Amortization

[c] RAV = Replacement Asset Value

3. Results and discussion

3.1. Capital investment

Capital expenditure (CAPEX), which represents all of the investments in infrastructure and equipment, is listed in Table 4.4. The bare equipment cost was estimated according to data sources from pulp and paper mill studies, published NREL cellulosic ethanol reports (Humbird et al., 2011; Wooley et al., 1999), and the related literature (Pirraglia et al., 2010). All equipment costs have been escalated to startup year and sized for an equivalent biomass flow of 500,000 dry metric tons per year. The total installed equipment cost was derived from the capital investment methodology developed by Max S. Peters (Peters et al., 1968). Four levels (zero, low, medium, and high) of direct cost factors (erection, instruments and controls, piping, electrical, buildings, yard improvement, foundations, and service facilities) and indirect cost factors (engineering, construction, legal, contractor fee, inflation, and contingency) were applied to estimate the total installed cost in accordance with the equipment installation complexity. The total installed cost is around 3.2 multiples to bare equipment cost which is consistent with many large capital investment project estimates where an initial conservative value is warranted given the incomplete scope definition in the initial preliminary design. The CAPEX of six scenarios can be summarized:

- Lignin combustion and pelletization in the greenfield context.

The total CAPEX for lignin combustion and pelletization in greenfield is ca. $333 million and ca. $303 million respectively, corresponding to $2.06 and $1.88 CAPEX per annual liter ethanol production. The higher investment for lignin combustion option is due to

the higher cost of a biomass boiler and turbine generator relative to a gas boiler and pellets plant. The enzymatic hydrolysis system accounts for the largest portion of the capital investment, due to the size of reactors in a 20% solids enzymatic hydrolysis system. The autohydrolysis pretreatment capital which includes reactors, blow tank and blow heat recovery system only accounts for approximately 10% of the total capital. This is in agreement with other study that the autohydrolysis pretreatment, also known as liquor hot water (LHW) pretreatment, is capital cost competitive amid many other pretreatment technologies such as dilute acid pretreatment, lime pretreatment and ammonium fiber expansion due to the low cost of materials of construction and no recycling of pretreatment chemicals (Wyman, 2013).

- Lignin combustion and pelletization in the context of co-location.

It is clear that CAPEX of co-location cases are much lower than that of greenfield projects by taking advantage of existing equipment and buildings such as the roads, administration buildings, biomass supply and handling, waste water treatment plant, and power house in a kraft pulp mill. The Table 4.4 displays that the CAPEX of lignin combustion case in co-location scenario significantly drops from ca. $333 million to ca. $194 million, rendering it even lower than that of lignin pellet case in co-location scenario. The main deduction comes from the capital savings from biomass boiler and turbine generator.

- Lignin combustion and pelletization in the context of repurposing.

Owing to the compatibility of autohydrolysis technology in a kraft pulp mill, the closed kraft pulp mill can be repurposed to produce cellulosic ethanol, and the only new pieces of equipment are those required to process fiber through enzymatic hydrolysis, fermentation and ethanol recovery. In this regard, capital investment in repurposing scenario for both lignin combustion and pelletization cases turn out to be the lowest among all financial contexts discussed. A 54% and 39% of CAPEX reductions for lignin combustion and pelletization case are observed respectively in the repurposing scenario relative to greenfield project.

Table 4.4 Capital expenditure for all cases

Area	Greenfield lignin combustion (US $)	Co-location lignin combustion (US $)	Repurposing lignin combustion (US $)	Greenfield lignin pellets (US $)	Co-location lignin pellets (US $)	Repurposing lignin pellets (US $)	Source
Land purchase	1,500,000	300,000	-	1,500,000	375,000	-	1
Land grading	4,652,778	930,556	-	4,652,778	1,163,194	-	1
Roads	4,000,000	800,000	-	4,000,000	1,000,000	-	1
Offices	10,000,000	5,000,000	-	10,000,000	5,000,000	-	1
Raw water treatment	13,664,033	-	-	13,664,033	-	-	3
Waste water treatment	5,932,914	-	-	5,932,914	-	-	3
Chips preparation	36,832,890	-	-	36,832,890	-	-	3
Autohydrolysis pretreatment	32,159,708	32,159,708	-	32,159,708	32,159,708	-	1,3
Mechanical refining	5,344,729	5,344,729	-	5,344,729	5,344,729	-	1
Screw press	6,766,351	6,766,351	-	6,766,351	6,766,351	-	1
Ion exchange system	4,260,994	4,260,994	4,260,994	4,260,994	4,260,994	4,260,994	2
Enzymatic hydrolysis	69,967,536	69,967,536	69,967,536	69,967,536	69,967,536	69,967,536	1,3
Fermentation	36,198,918	36,198,918	36,198,918	36,198,918	36,198,918	36,198,918	3
Distillation	9,020,056	9,020,056	9,020,056	9,020,056	9,020,056	9,020,056	3
Molecular sieve	6,191,887	6,191,887	6,191,887	6,191,887	6,191,887	6,191,887	3
Product storage & shipment	4,056,188	4,056,188	4,056,188	4,056,188	4,056,188	4,056,188	3
Lignin filter	13,278,339	13,278,339	13,278,339	13,278,339	13,278,339	13,278,339	3
Pellets mill	-	-	-	30,719,202	30,719,202	30,719,202	4
Biomass boiler	41,850,222	-	-	-	-	-	1
Natural gas package boiler	-	-	-	4,393,835	-	-	1
Turbine generator	23,268,934	-	-	-	-	-	3
Power tie	3,769,231	-	-	3,769,231	-	-	1
Other [a]	-	-	10,000,000	-	-	10,000,000	1
Total CAPEX	332,715,709	194,275,262	152,973,918	302,709,590	225,502,103	183,693,121	
CAPEX,$ per liter ethanol	2.06	1.20	0.95	1.88	1.40	1.14	

Source: [1](Mill study), [2](NREL 1999), [3](NREL 2011), [4](Pirraglia, A. 2010) [a] Other: Repurposing capital for uncertainty.

3.2. Production costs

Production costs of greenfield projects in project year-3 including direct cost (feedstock, enzymes, yeast, denaturant and energy) and indirect costs (labor, maintenance, overhead, depreciation and other fixed costs) are illustrated in Fig. 4.3. The total cash costs of lignin combustion and pellets project are $0.48 and $0.33 per liter ethanol, respectively, both of which are lower than the ethanol wholesale price. The production costs for both scenarios are similar except for the energy cost, which is calculated based on the net energy cost of the whole process. They both have an energy credit from lignin by selling excess power or lignin pellets fuel, but the lignin pellets scenario has a substantially higher energy credit of $0.15 per liter ethanol (Revenue from lignin pellet sale minus cost for purchasing gas and power) compared to $0.01 per liter ethanol from lignin combustion (Revenue from selling excess power). Considering the high price of biomass pellets in the European market and modest price of natural gas in the US market, it appears that selling lignin as pellets fuel is a better choice than burning it directly for energy supply.

The main cost drivers for the greenfield lignin combustion scenario are biomass (33%), followed by deprecation (30%), enzyme cost (15%), and other mill fixed costs (9%), etc. (Fig. 4.3a). In contrast, the greenfield lignin pellets scenario has a biomass cost share of 42%, depreciation of 35%, enzymes cost of 20%, and other mill fixed costs of 10%, etc. (Fig. 4.3b). These results are consistent with many other studies that biomass cost dominates the cost share of the whole process (Gonzalez et al., 2011a; Gonzalez et al., 2011c; Treasure et al., 2012). The high depreciation cost is because project year-3 is still under the 10 years

straight line depreciation schedule, and it will fall down to significantly lower level after the depreciation schedule. The enzyme cost is well controlled to relatively low level because of the mechanical refining utilized after autohydrolysis pretreatment which has been proved to substantially decrease the enzyme dosage without hampering sugar recovery yield (Ertas et al., 2013; Jones et al., 2013).

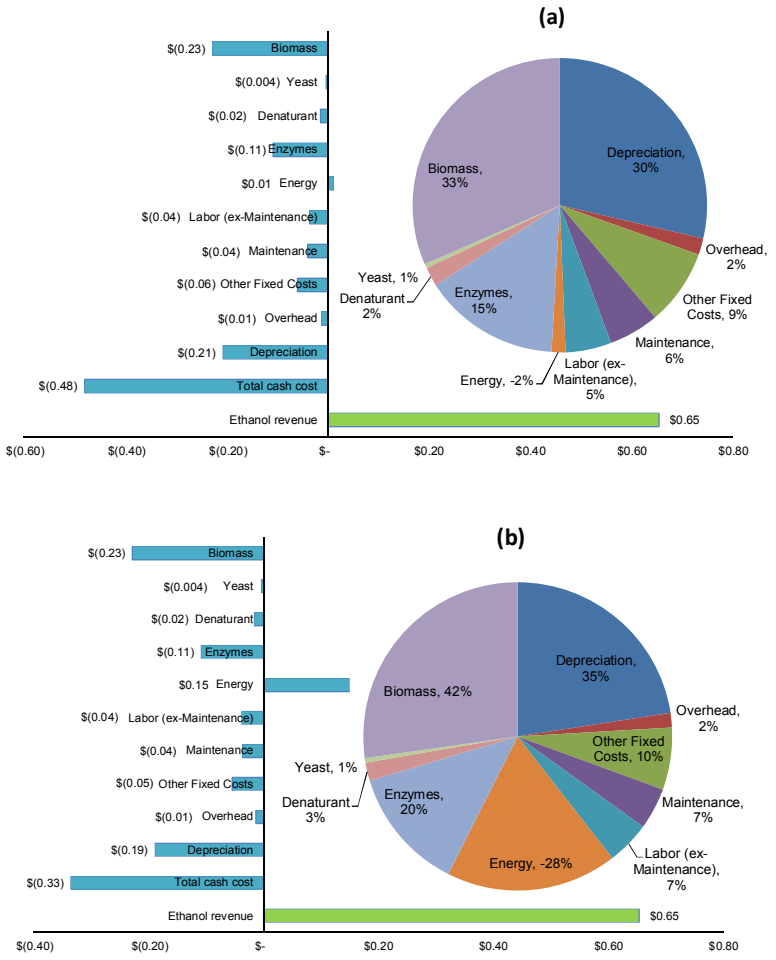

Fig. 4.3 Ethanol production costs (US$ per liter ethanol) and cost share (%) in project year-3 for: (a) greenfield lignin combustion scenario; (b) greenfield lignin pellets scenario.

3.3. Financial indicators

The respective economic merits of each scenario were compared based on the net present value (NPV), the internal rate of return (IRR), and the payback years. The NPV is defined as the sum of discounted free cash flow at target rate of return (12% in this study) at each project year. Generally, a positive NPV at target rate of return represents a profitable business and would meet investor's return criteria. The IRR corresponds to the discount rate that gives a zero NPV and it is a widely used as a metric to evaluate the return on investment for each project. The payback years, which indicates the length of time required to recover the initial capital input, is also an alternative indicator of the investment return. In this study the payback years is calculated based on the total years required for having a positive accumulated EBITDA.

Table 4.5 displays that the net present value of lignin combustion cases in all scenarios turns out to be negative at 12% discount rate mainly because of the high capital input relative to low ethanol selling price. In contrast lignin pellets cases in co-location and repurposing scenarios exhibit a positive net present value indicating a profitable business model at current ethanol market price and financial scope. From an economic point of view, pelletizing lignin seems to be a better choice than burning it for power and steam in terms of net present value, internal rate of return, and payback years, regardless of greenfield, co-location, or repurposing scenario. It is important to note that autohydrolysis is a chemical free process and the downstream lignin residue is sulfur free and has low ash content, which is an

ideal raw material for pellets fuel production and the high value of pellets fuel substantially enhances the overall financial performance of the whole biorefinery process.

It appears that all the financial indicators place the repurposing scenario as the best option, followed by co-location and greenfield. Taking lignin combustion option as an example, the IRR increases from 0.2% in greenfield scenario to 5.1% in co-location scenario, and further goes up to 7.3% in repurposing scenario. It is noted that the capital reduction in both co-location and repurposing scenarios significantly improve the economic performance of each biorefinery process. The best option in this study turns out to be repurposing lignin pellets case, where $92.9 million net present value at 12% discount rate, 17.6% of internal rate of return at current ethanol wholesale price, and 3.8 payback years can be achieved.

Table 4.5 Net present value, internal rate of return, and payback years for the six scenarios

Scenarios	lignin combustion	lignin combustion	lignin combustion	lignin pellet	lignin pellet	lignin pellet
Context	Greenfield	Co-location	Re-purposing	Greenfield	Co-location	Re-purposing
NPV, Million US$	-229.6	-87.4	-50.2	-11.0	56.5	92.9
Payback years	- [a]	8.2	6.5	6.1	4.6	3.8
IRR, %	0.2%	5.1%	7.3%	11.5%	15.0%	17.6%

[a] payback years longer than project life.

3.4. Minimum ethanol revenue

The minimum ethanol revenue (MER) is defined as the required minimum wholesale price of ethanol to achieve a specific internal rate of return. The minimum ethanol revenue required to achieve an internal rate of return of 4%, 8%, 12%, 16% and 20% for all cases are illustrated in Fig. 4.4. For lignin combustion option, most MER values are higher than the US ethanol wholesale price regardless of different financial contexts because of the low ethanol value relative to the high capital investment. In contrast, many MER values from lignin pellets option have a lower price than ethanol wholesale price indicating a profitable project under certain rate of return. Generally lignin pellets option has a lower minimum ethanol revenue price compared to lignin combustion option in all financial contexts because the high value of lignin pellets offsets a large portion of the production cost.

It is noted that the co-location scenario greatly reduces the minimum ethanol selling price especially for the lignin combustion case, where an average reduction of $0.14 per liter ethanol is observed. Furthermore, the repurposing scenario drops the MERs of lignin combustion by an average of $0.18 per liter ethanol relative to greenfield project. Overall the greenfield lignin combustion case shows the highest MER values and none of the MERs in this scenario can match the $0.65 per liter ethanol wholesale price. On the other hand, the repurposing lignin pellets option has the lowest MER value and it drives MER below the current ethanol wholesale price at even 16% of internal rate of return, indicating a low risk and high profitability of this option.

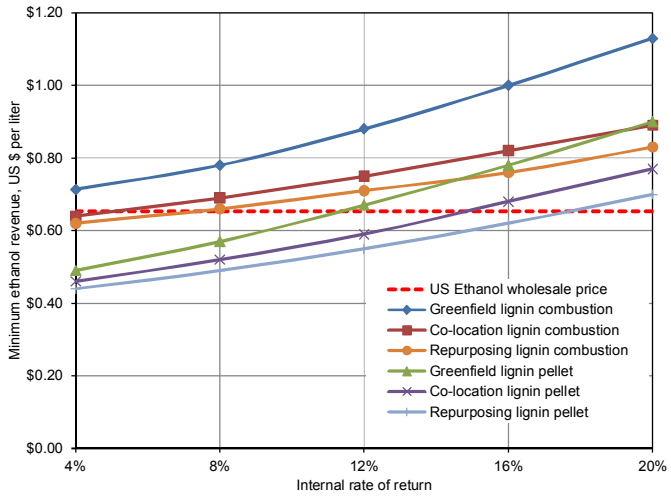

Fig. 4.4 Minimum ethanol revenue (US$ per liter ethanol) at different internal rate of return.

3.5. Sensitivity analysis

A sensitivity analysis was performed for the greenfield lignin pellets scenario to evaluate how changes in capital investment, ethanol yield/price, biomass cost, enzyme cost, energy cost including natural gas and power cost, and lignin pellets price affect the net present value of the project at 12% discount rate. This analysis was carried out with a variation of ±25% of the central values. As shown in Fig. 4.5, the net present value is influenced most significantly by changes in ethanol yield/price, followed by capital investment, lignin pellets selling price, and biomass cost. The energy cost and enzyme cost represent the set of least sensitivity to net present value.

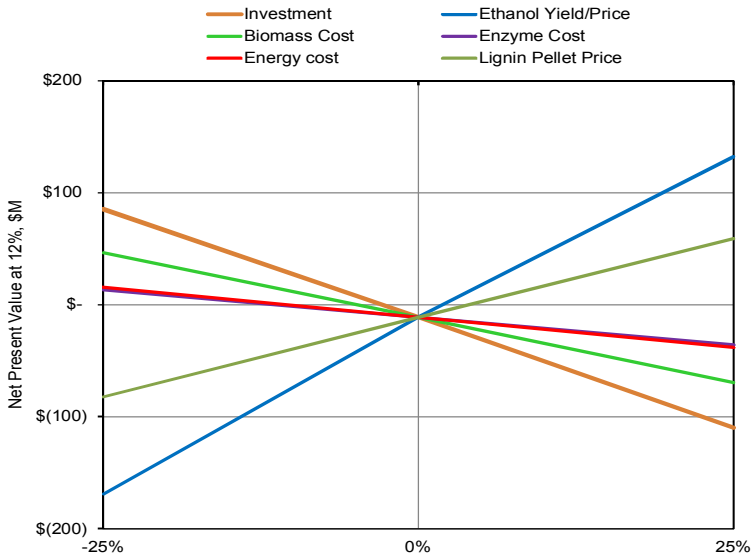

Fig. 4.5 Sensitivity analysis of net present value at 12% discount rate with variation of ± 25% for investment, ethanol yield/price, biomass cost, enzyme cost, energy cost, and lignin pellets price (case: greenfield lignin pellets)

3.6. Effect of annual ethanol selling price escalation to minimum ethanol revenue

In the absence of mandates ethanol market prices will ultimately be driven by gasoline price. A significant price increase for both ethanol and gasoline during the past decades are observed according to historic selling price for ethanol and unleaded gasoline in Omaha, Nebraska (Nebraska Energy Office, 2013). However, the unpredictability of oil price and the eruption of corn ethanol in recent years make it difficult to predict future ethanol market price. Therefore, a key assumption was made in this study that the ethanol market price will remain the same for the entire project life. Nevertheless, the financial performance of different options is most sensitive to ethanol selling price as shown in Fig. 4.5. Hence the

minimum ethanol revenues (at 12% discount rate) at different predicted trends of future ethanol market price were plotted in Fig. 4.6. It appears that the MER values for all cases have a steady decline when the annual incremental ethanol market prices are higher, where a \$0.04-\$0.06 per liter reduction of MER was observed related to 1% annual increase for ethanol selling price. When a 2% annual increase of ethanol price was assumed in the revenue model, the MER of all cases except for greenfield lignin combustion case turn out to be below current ethanol wholesale price. It is noted that the MER of greenfield lignin combustion case cannot match the ethanol wholesale price even at the assumption of 3% annual increase for ethanol selling price.

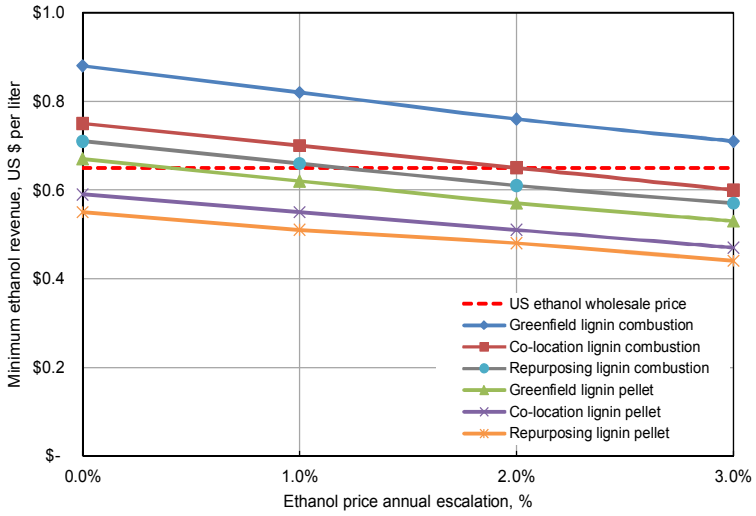

Fig. 4.6 Minimum ethanol revenue (at 12% discount rate) (US$ per liter ethanol) at different ethanol price annual escalation

3.7. Financial merits of mechanical refining adding to autohydrolysis process

Unlike many other studies on autohydrolysis, mechanical refining was incorporated into this process and has been proved to significantly improved the enzymatic hydrolysis efficiency of autohydrolysis pretreated substrate by internal fibrillation or swelling (Ertas et al., 2013; Jones et al., 2013). The possible benefits of mechanical refining can be addressed in two ways: either by alleviating the autohydrolysis intensity and thus improving pretreatment solids yield, or by reduction in enzyme dosage. Mechanical refining pretreatment for bioethanol production is often contentious for its high energy consumption. However, in this study, only mild refining is utilized upon the autohydrolysis pretreated substrate and the total cost of refining including capital and power cost (Power consumption was estimated as 150 kwh per dry ton loading) adding onto the minimum ethanol revenue is $0.03 per liter ethanol, relative to $0.16 per liter ethanol cost saving generated by refining through ethanol yield improvement. Hence it is a very attractive approach to combine autohydrolysis with mechanical refining to implement cellulosic ethanol commercialization.

3.8. Economic merits: autohydrolysis combined with refining versus steam explosion

A separate study was carried out to roughly compare the economic value of autohydrolysis combined with refining versus steam explosion in a greenfield lignin combustion scenario. The steam explosion case was simulated by assuming biomass is fed into reactor with 45% moisture content and subjected to steam explosion at 200 °C, and all the conversion efficiency factors and capital investment remain the same as those of autohydrolysis. The results show that the steam explosion has a minimum ethanol revenue of

$0.84 per liter ethanol at 12% discount rate compared with $0.88 per liter ethanol for autohydrolysis. The $0.04 per liter improvement mainly comes from the saving of processing steam due to decreased volume of water in the reactor. However, the steam explosion technology suffers a higher technical risk than batch digesters in large scale projects. In contrast, autohydrolysis is a proven technology that has been utilized in pulp and paper mills for more than one hundred years.

4. Conclusions

Autohydrolysis pretreatment combined with refining can provide a cost-effective way to produce cellulosic ethanol because of its simplicity and efficiency. Analysis shows that the lignin pellets option displays a better choice than burning lignin directly because of the higher value attached to lignin pellets fuel. The co-location and repurposing scenarios substantially improve the financial performance of a cellulosic ethanol biorefinery by largely reducing the capital burden. The best scenario in this study is to co-produce bioethanol and lignin pellets from autohydrolysis of hardwood in a repurposing scenario, where a 17.6% internal rate of return can be achieved.

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Chapter 5 Economics of bioethanol production from autohydrolysis - a simple, low capital, financeable process using proven process equipment and technology

Abstract

The process of autohydrolysis combined with mechanical refining is an attractive process for the production of bioethanol since it is environmental friendly, capital-efficient, using proven equipment and technology, and has competitive ethanol yield as more complex technologies. The economics of autohydrolysis process for bioethanol production using five feedstocks were evaluated including natural mixed hardwood, switchgrass, wheat straw, sugarcane bagasse, and corn stover. Taking advantage of the sulfur free autohydrolysis process, pelletization of unhydrolyzed residue for fuel pellets sales was evaluated as an alternative to the more conventional alternative of heat and power generation from the residues. The results indicate that with current US ethanol price, the key for commercial bioethanol production is to simplify the production process to mitigate capital burden, employ feedstock with low cost per ton of carbohydrates and less recalcitrance, and upgrade unhydrolyzed residues to high value-added products like fuel pellets, or better.

Keywords: Autohydrolysis; Refining; Bioethanol; Pellets; Economics

1. Introduction

Bioethanol from lignocellulosic biomass offers unique and desirable features: abundant and sustainable resource, positive environmental impact, and no conflict with

human food supply. Extensive efforts from private and public sectors have been made on bioethanol production towards commercialization, but there has been minimum commercial bioethanol available from lignocellulosic biomass because of poor economics. Generally, developers are focused on higher sugar yield, but more complex technology which suffers from high capital investment relative to low ethanol price and high technical risk upon scaling up.

In order to make lignocellulosic bioethanol production financeable, several criteria need to be considered (Phillips, 2014): (1) use feedstock that is abundant and low cost per ton of carbohydrates; (2) minimize capital investment by reducing complexity of operation; (3) develop low cost post-treatment steps for increasing sugar yield; (4) upgrade value of unhydrolyzed residue; (5) co-locate biorefinery with industrial plant to alleviate capital burden; (6) use industrially proven equipment and technology to reduce technical risk.

Among all the pretreatment technologies studied, autohydrolysis process stands out as an attractive pretreatment method for bioethanol production due to several advantages: (1) effective on many feedstock with relatively high hemicellulose content (Ertas et al., 2013; Mosier et al., 2005; Romaní et al., 2010); (2) low capital investment due to the simplicity of the process - low cost on construction materials and no chemical recovery required (Wyman, 2013); (3) environmental friendly since only water is used as reaction medium; (4) less corrosive process; (5) less sugar degradation products compared to inorganic acid process; (6) sulfur free unhydrolyzed residues yields the opportunity to upgrade the value of residues

like fuel pellets; (7) proven process technology and equipment in the pulp and paper industry for decades. However, autohydrolysis has often been abandoned as a financeable bioethanol process due to the relatively low yield of fermentable sugars at economical enzyme dosages, compared to other more complex and more capital-intensive alternatives. Mechanical refining, commonly used in pulp and paper industry, can be utilized to improve the enzymatic hydrolysis of autohydrolysis pretreated substrate. Mechanical refining has been reported to have a positive effect on enzymatic digestibility of dilute acid pretreated corn stover (Chen et al., 2012b), autohydrolysis pretreated wheat straw (Ertas et al., 2013), green liquor pretreated hardwood (Koo et al., 2011) and loblolly pine (Wu et al., 2012; Wu et al., 2010), and recovered office printing paper (Chen et al., 2012a). In this regard, autohydrolysis combined with refining can be a very attractive approach to produce bioethanol in an efficient and cost-effective manner.

The aim of this study is to present the economics for the production of bioethanol using autohydrolysis process followed by mechanical refining using several feedstock including mixed natural hardwood, switchgrass, wheat straw, sugarcane bagasse, and corn stover. In particular, the scenario of producing bioethanol and pelletizing the unhydrolyzed residues for fuel pellets sales was evaluated compared to heat and power generation from residues. The following financial indicators were determined to gauge the economic performance of the process: internal rate of return (IRR), net present value (NPV), payback period, and minimum ethanol revenue (MER).

2. Materials and methods

2.1 Feedstock

In this study five feedstocks were evaluated including natural mixed hardwood, switchgrass, wheat straw, sugarcane bagasse, and corn stover. The chemical compositions of the five types of biomass were measured at the lab of Department of Forest Biomaterials at North Carolina State University. As seen in Table 5.1, the total carbohydrates of the five types of feedstock range from 56.9 to 66.4%. Generally, the woody biomass constitutes higher glucan content but lower xylan content compared to non-woody biomass. The woody biomass also contains more lignin but less ash and extractives than non-woody biomass. The delivered costs for each type of feedstock presented in Table 5.1 were calculated using biomass growth, harvesting, transportation, and storage models developed by Gonzalez etc. (Gonzalez et al., 2011b; Gonzalez et al., 2011c). Delivered cost considered profit for the landowner and harvesting contractor. Transportation cost was based on market prices as of 2013.

Table 5.1 Biomass composition, moisture content, and delivered cost.

	Natural hardwood	Switchgrass	Wheat straw	Sugarcane bagasse	Corn stover
Glucan, %	44.4	33.6	35.4	40.6	38.9
Xylan, %	15.3	23.6	17.8	20.9	20.3
Minor sugars, %	3.7	4.0	3.7	4.9	5.4
Total carbohydrates, %	63.4	61.2	56.9	66.4	64.6
Lignin, %	26.3	20.9	22.6	19.6	16.0
Ash, %	0.4	3.0	4.4	2.7	4.3
Extractives, %	1.4	2.1	1.9	3.9	2.1
Acetyl, %	3.7	3.0	2.0	3.3	2.4
Other organics, %	4.8	9.8	12.2	4.1	10.6
Sum	100	100	100	100	100
Assumed moisture content	45%	16%	16%	16%[a]	16%
Delivered cost, $ per dry metric ton	$72.8	$82.1	$63.8	$48.0	$52.9
Delivered cost, $ per ton carbohydrates	$114.8	$134.1	$112.1	$88.4	$81.9

[a] Bagasse with initial 40-50% of moisture was assumed to be dried prior to transport.

2.2 Reaction conditions and yields

Data on pretreatment and enzymatic hydrolysis yields were adopted from laboratory studies at the Department of Forest Biomaterials at North Carolina State University. Best pretreatment conditions on each type of feedstock were selected in accordance with the highest total sugar recovery at affordable enzyme dosages. Two key points for the success of autohydrolysis pretreatment are: (1) to maximize dissolution of polysaccharides during autohydrolysis; (2) to fully disrupt biomass structural and render biomass more susceptible to the actions of enzymes. Previous developers of autohydrolysis pretreatment have often been

deterred by failure to accomplish both goals because the former requires mild conditions to avoid sugars degradation but the latter need more severe conditions to open up the biomass structure. One of the innovative aspects of this work is to apply mechanical refining to the autohydrolysis process so as to reduce the pretreatment severity but sustain high enzymatic conversion efficiency as well. As illustrated in Table 5.2, under the optimum autohydrolysis condition selected, the total sugar released in the autohydrolysis filtrate ranges from 8.3 to 14.2 g/100 g raw biomass, which means 13.1 to 23.2% of carbohydrates in raw biomass can be recovered as oligomers or monomers during reaction without employing enzymes. In the meantime, 44.6 to 62.3% of the carbohydrates in the raw biomass can be enzymatic hydrolyzed to fermentable sugars at affordable enzyme dosages when mechanical refining was applied to the pretreated material. The amount of degradation products was well controlled to acceptable levels especially for non-woody biomass. All enzymatic hydrolysis yields data were obtained using laboratory data at 5% insoluble solids loading in a batch hydrolysis, due to the ease of performing experiments. Twenty percent of initial insoluble solids loading were input into the simulation model by employing multiple-stage enzymatic hydrolysis, where the internal loop dilutes initial insoluble solids loading up to 10% and comparable hydrolysis efficiency has been obtained (Xue, 2011).

Table 5.2 Process conditions and yields of autohydrolysis pretreatment and enzymatic hydrolysis.

	Natural hardwood	Switchgrass	Wheat straw	Sugarcane bagasse	Corn stover
Autohydrolysis					
Temperature (°C)	180	190	180	180	180
Time (min)	60	20	20	20	10
Liquid to solids ratio	3:1	3:1	3:1	3:1	3:1
Solids yield [a] (%)	73.6	55.3	64.9	72.7	66.3
6C sugar yield [a] (%)	1.4	1.9	2.3	1.6	1.5
5C sugar yield [a] (%)	6.9	12.3	8.4	11.2	8.8
Acetic acid (%)	4.1	1.9	0.9	1.3	1.4
Furfural (%)	2.1	1.8	0.3	0.5	0.2
Enzymatic hydrolysis					
Enzyme dosages (FPU/g)	5	4	4	5	4
Insoluble solids (%)	20	20	20	20	20
Time (h)	96	96	96	96	96
6C sugars yield [a] (%)	36.1	30.1	24.9	33.1	22.6
5C sugars yield [a] (%)	3.4	2.2	5.7	5.7	6.2
Total sugars yield [a] (%)	47.8	46.5	41.3	51.6	39.1
Total sugars recovery [b] (%)	75.4	76.0	72.6	77.7	60.5

[a] Sugar yields are based on 100 g raw biomass.

[b] Total sugars recovery are based on total carbohydrates in raw material.

2.3 Process design and simulation

The techno-economic process was designed to process 500,000 dry metric tons of biomass per year, operating at 8400 hours per year (96% availability). An overview of the integrated biorefinery process is illustrated in Fig. 5.1. For woody biomass, wood chips were used as feedstock and directly conveyed to digester. For non-woody biomass, incoming biomass is first chopped at the feedstock handling unit to have a suitable particle size and

then sent to pretreatment area. The autohydrolysis pretreatment is carried out under selected conditions for each type of feedstock as discussed before. After reaction, blow heat is recovered to preheat digester make-up water. The whole slurry is conditioned to have a suitable pH (pH=5) for enzymatic hydrolysis. Since water is only used as reaction medium, the pH after reaction ranges from 3.3 to 4.4 so that only a small amount of alkali is required. Pretreated biomass is conveyed to mechanical refiner to promote fibrillation of fibers. The refined material is then dewatered at a screw press unit. Two functions can be achieved by liquid and solids separation: one is to control the undesired degradation products at a desirable concentration though an ion exchange resin; the other is to get rid of the excess heat by heat exchange of the hydrolyzate with chill water. Enzymatic hydrolysis was carried out in a multiple-stage system using conversion efficiency factors described before. The fermentation efficiency was assumed as 95% for hexoses and 80% for pentoses with a total residence time of 36 hours (Humbird et al., 2011). Ethanol product is extracted in a distillation system and further purified to achieve 99.5% concentration through molecular sieves. The unhydrolyzed residues are dewatered by a high efficiency lignin filter to reach 60% consistency (Humbird et al., 2011).

In a conventional biorefinery process, the unhydrolyzed residues - mostly lignin material - are sent to biomass boiler to generate steam and power for process demand as shown in Fig. 5.1 (a). Alternatively, since autohydrolysis is a sulfur free process, the unhydrolyzed residues can be upgraded to fuel pellets (referred as lignin pellets) which sell for 2 times the value as fuel in a biomass boiler (offset by the need to purchase natural gas

for steam generation, as well as purchased power). As presented in Fig. 5.1(b), low pressure packaged gas boiler can be used to generate process steam, taking advantage of the moderate gas price in US. The hot flue gas can be utilized as a drying source for lignin pellets production. Power can be purchased from grid.

The process simulation was carried out using WinGEMS v5.3, which is widely used in pulp and paper industry to acquire mass and energy balance. Owing to the specialty blocks and unit operations critical for biomass based bioprocess, it has gained popularity in application for many biorefinery studies (Gonzalez et al., 2011a; Gonzalez et al., 2011d; Phillips et al., 2013; Treasure et al., 2012). This simulation tool requires input data from laboratory results and critical process data including production rate, chemicals and energy consumption, and capacity of the equipment were exported to financial spreadsheet using Microsoft Excel.

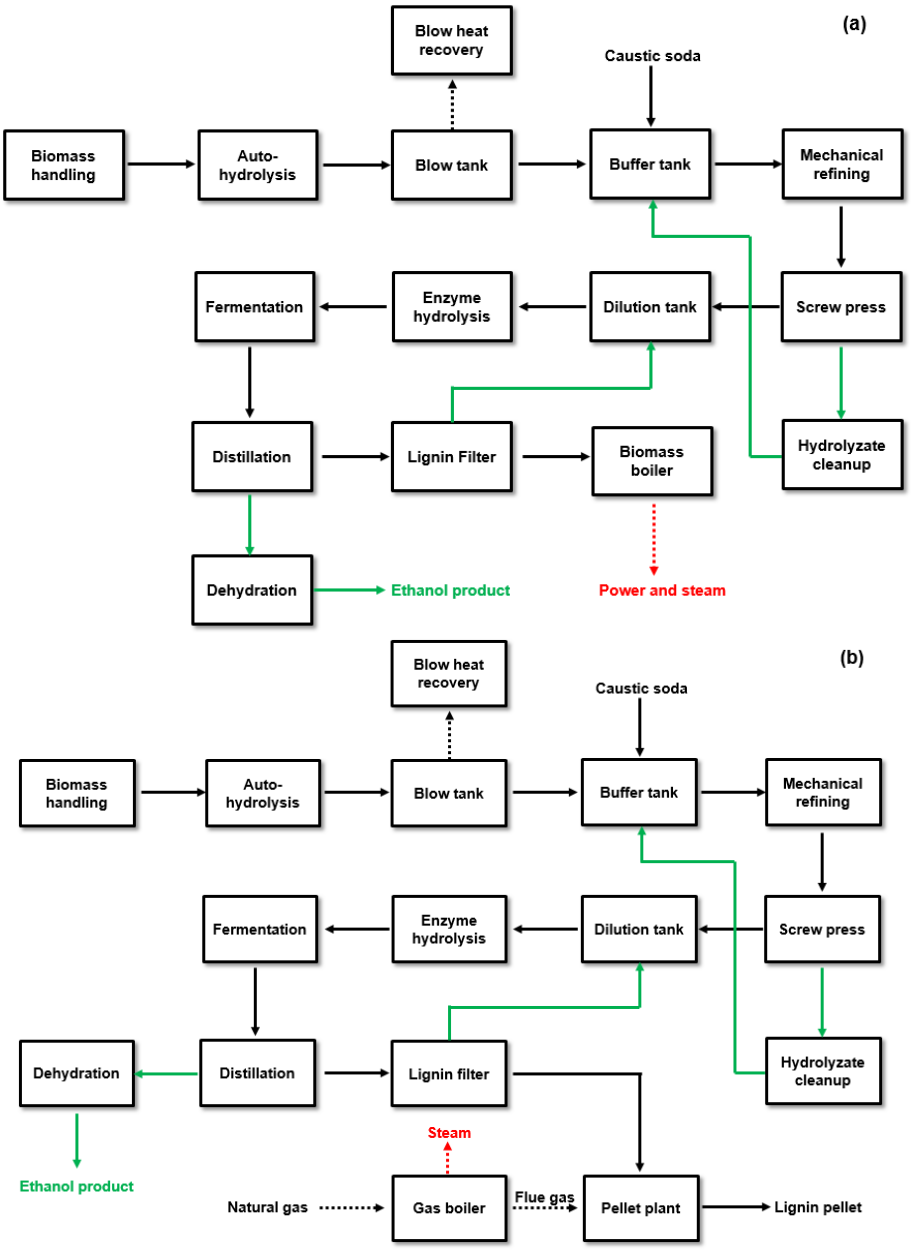

Fig. 5.1 (a) Process diagram for bioethanol, power and steam production; (b) Process diagram for bioethanol and lignin pellets production.

2.4 Capital investment

Capital investment was estimated based on data sources from pulp and paper mill studies, published NREL cellulosic ethanol reports (Humbird et al., 2011; Wooley et al., 1999), and the related literature (Pirraglia et al., 2010). All equipment costs were escalated to reference year of 2016 and sized for a capacity to process 500,000 dry metric tons of biomass per year. The total installed equipment cost was derived from the methodology developed by Max S. Peters (Peters et al., 1968), where four levels (zero, low, medium, and high) of direct cost factors (erection, instruments and controls, piping, electrical, buildings, yard improvement, foundations, and service facilities) and indirect cost factors (engineering, construction, legal, contractor fee, inflation, and contingency) were used to estimate the total installed cost in line with the complexity of equipment installation. The total installed capital cost is around 3.2 times bare equipment cost which is in agreement with many large capital investment estimates that initial conservative value is warranted given the incomplete scope in the initial design.

2.5 Financial simulation

Major assumptions used in the economic analysis were summarized in Table 5.3. In brief, the initial investments were spread over first three years including startup year 2016. The project life was set to be 15 years. The reinvestment capital, maintenance expense, and other fixed mill costs were set as 1%, 2%, and 3% of the Replacement Asset Value (RAV), where the estimated cost to rebuild the assets each future year escalates annually at 2%. Labor costs were estimated based on a stuffing plan, including typical salary, wage and

benefits costs. Tax rate was set at 35% with tax losses accumulated and carried forward to offset profits made in future year. Ten-year straight line depreciation schedule was applied. Financial indicators including Net Present Value (NPV), Internal Rate of Return (IRR), Minimum Ethanol Revenue (MER), and payback period were determined using a discounted cash flow analysis with a discount rate of 12%. Ethanol revenue was set at $0.653 per liter according to the average FOB, Omaha, NE ethanol selling price in 2013 without any subsidies (Nebraska Energy Office, 2013). Lignin pellets revenue was assumed at $160 per metric ton (6% moisture) FOB price from southeast of US to Europe (Argus, 2014). Enzyme price was derived at $1 per kg enzyme product using methodology developed by Phillips (Phillips et al., 2013). All the other raw material pricing and indices were input from chemical marketing and forecasts.

Table 5.3 Summary of financial analysis assumptions

Item	Value
Capital investment	Investments were developed for $2016, the startup year. Capital spending in each case was 10% in 2014, 50% in 2015, and 40% in 2016.
Project life	15 years
Terminal value	5 x EBITDA (Earnings Before Interest, Taxes, Depreciation and Amortization) of the terminal year (2031)
Replacement asset value (RAV)	Benchmark was calculated on the basis of the estimated cost of reproducing the assets each future year. Calculated by assuming 2% annual increase in the installation cost.
Reinvestment capital	1% of RAV reinvested as capital each year in order to maintain existing capability.
Maintenance expense	2% of RAV included annually accounting for maintenance labor and materials.
Other fixed costs	3% of RAV accounts for operating supplies, insurance and inspection.
Overhead costs	2% of annual sales
Labor costs	Labor costs were established based on a stuffing plan, with typical salary, wage and benefit costs.
Working capital on materials	15% of direct costs
Working capital on products	5% of revenue
Excess spending in startup year	10% of direct costs at startup year
Training costs	105% of year-1 labor costs
Tax	35% overall tax rate on profit, with tax losses accumulated and carried forward to offset profits made in future years.
Depreciation	Based on 10-year straight line depreciation schedule.
Discount rate	12%
Net present value	All free cash flows (Cash flows less new fixed capital and change in working capital) were discounted at 12% to the startup year.
Internal rate of return (IRR)	Corresponds to the discount rate that gives a zero NPV.
Minimum ethanol revenue (MER)	The ethanol revenue required to achieve Zero NPV at 12% internal rate of return.
Payback period	Calculated based on the total years required for having a positive accumulated EBITDA.
Ethanol revenue	$0.653 per liter according to the average FOB, Omaha, NE ethanol selling price in 2013.
Lignin pellets revenue	$160 per metric ton based on the wood pellets FOB price from southeast coast of US to Europe.
Raw material pricing	Power: $65 per MWH; Natural gas: $4.0 per MMBTU; Gasoline Denaturant: $0.766 per liter; Caustic soda: $500 per dry metric ton; Enzyme: $1 per kg enzyme product; Yeast: $0.004 per liter ethanol

3. Results and discussion

3.1 Capital investments

The total installed capital costs for different types of feedstock and options of lignin treatment including power generation (PW) and lignin pellets sales (LP) are illustrated in Fig. 5.2. The main unit operations for estimating capital investment can be divided into six areas: (1) infrastructure: land purchase and grading, roads and office construction, water plant, waste water treatment; (2) feedstock handling: truck receiving, biomass storage and reclaim, biomass chopping, transfer conveyer system; (3) pretreatment: digester, blow and heat recovery system, refining, screw press, ion exchange resin; (4) enzymatic hydrolysis: deconstruction tank, hydrolysis tank, gravity clarifier, sugar storage tank; (5) biorefinery: fermentation, beer column, rectification column, molecular sieve, product storage and shipment; (6) Power system: lignin filter, gas boiler or biomass boiler, or turbine generator, or pelletizer plant, power tie. Capital investment is more dependent on the selected feed rate of biomass and less dependent on the actual conversion to bioethanol.

Generally, the total capital investment for lignin combustion option is higher than that of lignin pelletization option regardless of feedstock types. This is a result of the higher capital cost for biomass boiler and turbine generator required to convert unhydrolyzed residues to steam and power compared to moderate investment for a pelletizer plant. With respect to cost drivers on each area, enzymatic hydrolysis accounts for a significant portion of capital investment due to the need to process saccharification at 20% consistency. It is of interest to point out that pretreatment capital is less than 20% of the total capital costs, owing

to the simplicity of the autohydrolysis process including low cost of materials of construction and no chemical recycling units required. This is in agreement with most studies that autohydrolysis is a capital-efficient pretreatment technology among many other pretreatment methods such as dilute acid pretreatment, lime pretreatment, and ammonium fiber expansion (Hamelinck et al., 2005; Kazi et al., 2010; Wyman, 2013).

In terms of different types of feedstock, woody biomass has a higher capital input in the biomass handling area because fresh wood chips carry over more moisture than non-woody biomass resulting in an increased size of feedstock handling system. The variety of pretreatment capitals is mainly in virtue of different sizes of refiner and ion exchange resin. For instance, woody biomass usually have a higher solids recovery yield under same condition so that more solids would be sent to refiner. Elevated temperatures and longer times would increase the undesired sugar degradation products like organic acids and furan derivatives, adding more capacity to ion exchange resin. The higher capital cost in enzymatic hydrolysis is mainly because more dilution liquid is introduced to maintain the same hydrolysis consistency, leading to a larger reactor size.

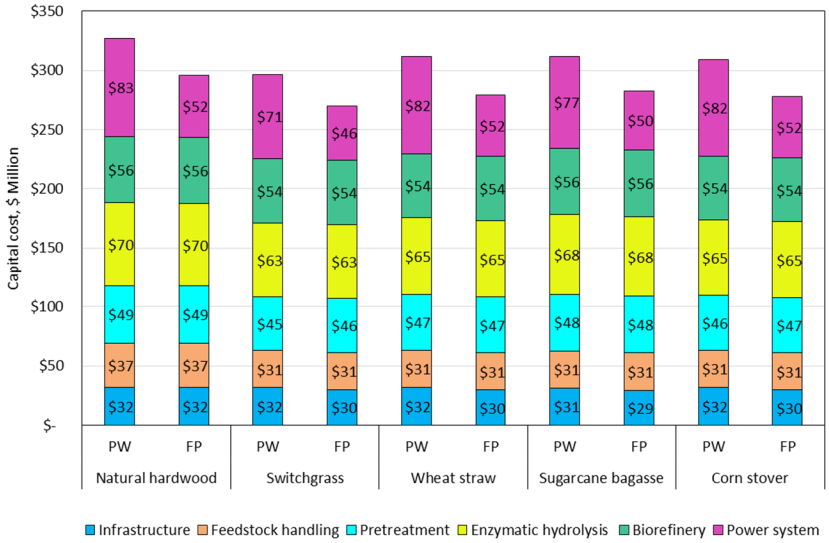

Fig. 5.2 Capital cost distributed by areas and feedstock types

3.2 Ethanol yield

Ethanol yield and Capital Expenditure (CAPEX) per liter ethanol for each type of feedstock are presented in Fig. 5.3. Higher ethanol yields (323 and 342 liter dry metric ton biomass) were found in natural hardwood and sugarcane bagasse at 5 FPU/g affordable enzymes dosages, indicating that autohydrolysis combined with mechanical refining can generate competitive ethanol yield compared to those more complex technologies (Gnansounou & Dauriat, 2010; Wyman, 2013). Wheat straw has a relatively low ethanol yield because of low carbohydrates in staring material. More future work need to be done on corn stover which shows the lowest ethanol yield mainly due to the low enzymatic hydrolysis efficiency. The lowest capital investment based on per liter ethanol product is found to be

$1.7 per liter ethanol when sugarcane bagasse is fed into autohydrolysis process and unhydrolyzed residues are pelletized for fuel pellets sales.

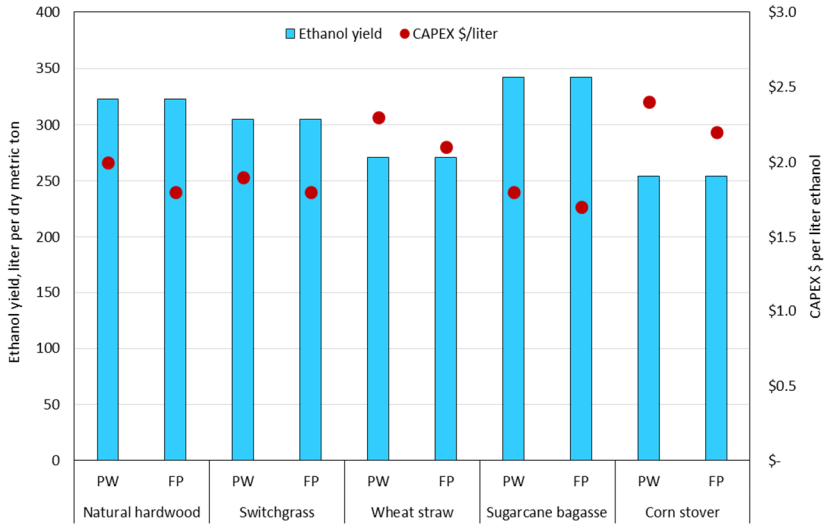

Fig. 5.3 Ethanol production yield and CAPEX per liter of ethanol for each type of feedstock and different options

3.3 Production costs

The production costs in $ per liter denatured ethanol product are depicted for each case in Table 5.4. In lignin combustion scenarios, the most costly feedstock to operate with is corn stover, which suffers from low ethanol yield and renders a high production costs in $ per liter ethanol. Sugarcane bagasse has the lowest production costs, with total costs $0.58 per liter ethanol and total cash costs $0.39 per liter ethanol. With respect to cost share of a single type of feedstock, biomass cost and depreciation on capital investments are the main

cost drivers. They combined contribute to around 60% of the total costs for all the feedstock, which is consistent with many other bioenergy studies (Gonzalez et al., 2012; Gonzalez et al., 2011d; Treasure et al., 2012; Wooley et al., 1999). Process simplicity is the best way to overcome the capital investment barrier. Exploring a type of suitable biomass with low cost per ton of carbohydrates and low recalcitrance to hydrolysis is a good recipe for feedstock barrier. The lignin combustion options for all type of feedstock are energy sufficient by burning the lignin residues for steam and power. A small amount of energy credit can be obtained by selling the excess power to grid.

Compared to lignin combustion option, the lignin pelletization option has considerably lower total costs for all the feedstocks. The cost reduction mainly comes from applying the energy credit from fuel pellets sales as a positive offset to the cost of natural gas and purchased power. Such reductions are particularly remarkable for those feedstocks with lower ethanol yield because more unhydrolyzed residues can be pelletized for sale. Since autohydrolysis is a sulfur free process, the opportunity exists to upgrade the lignin residues for pellets production. In the short run, it is a very profitable business to sell lignin pellets to Europe because of the robust biomass pellets price in Europe resulted from European countries' objective to increase their share of renewable energy consumption. It was estimated that 13 million tons of wood pellets were consumed in Europe in 2012 and the demand is expected to rise to 25 to 30 million tons per year by 2020 based on current trends (Economist, 2013). In the long run, the unhydrolyzed residues from autohydrolysis process

have the great potential to be upgraded to other high value-added products due to the cleanliness of the whole process.

Table 5.4 Production costs in Year-3 for each type of feedstock, $ per liter denatured ethanol product.

Cost drivers	Natural hardwood		Switchgrass		Wheat straw		Sugarcane bagasse		Corn stover	
	PW	FP	PW	FP	PW	FP	PW	FP	PW	FP
Biomass	0.23	0.23	0.28	0.28	0.24	0.24	0.15	0.15	0.22	0.22
Enzymes	0.11	0.11	0.09	0.09	0.10	0.10	0.10	0.10	0.11	0.11
Yeast	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.005
Denaturant	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Gas	-	0.05	-	0.05	-	0.05	-	0.04	-	0.05
Power	-0.01	0.06	-0.02	0.05	-0.02	0.06	0.00	0.05	-0.02	0.06
Fuel pellets	-	-0.27	-	-0.23	-	-0.32	-	-0.23	-	-0.34
Net energy	-0.01	-0.16	-0.02	-0.13	-0.02	-0.21	0.00	-0.14	-0.02	-0.23
Total direct costs	***0.35***	***0.20***	***0.37***	***0.26***	***0.34***	***0.16***	***0.27***	***0.13***	***0.33***	***0.12***
Labor (ex - Repair)	0.04	0.04	0.04	0.04	0.04	0.05	0.03	0.04	0.05	0.05
Maintenance and repair	0.04	0.04	0.04	0.03	0.04	0.04	0.04	0.03	0.05	0.04
Other mill fixed costs	0.06	0.05	0.06	0.05	0.07	0.06	0.05	0.05	0.07	0.06
Overhead	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.02
Depreciation	0.20	0.18	0.20	0.18	0.23	0.21	0.18	0.17	0.24	0.22
Total indirect costs	***0.35***	***0.32***	***0.34***	***0.32***	***0.40***	***0.36***	***0.32***	***0.29***	***0.42***	***0.39***
Total cash costs	0.48	0.33	0.51	0.38	0.50	0.31	0.39	0.24	0.49	0.27
Total costs	0.70	0.53	0.71	0.58	0.74	0.53	0.58	0.42	0.75	0.51
Ethanol revenue	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65

3.4 Feedstock type and profitability

The major financial indicators including net present value, internal rate of return, and payback period are presented in Fig. 5.4. The discount rate was set at 12% for all the discounted flow cash analysis, which is higher than many other bioenergy studies (Humbird et al., 2011; Littlewood et al., 2013; Treasure et al., 2012) to give investors more conservative evaluation towards risk and uncertainty of a new project. As seen in Fig. 5.4, the net present values of lignin combustion options for all types of feedstock turn out to be negative, failing to meet the target rate of return. While lignin combustion is the most common design to implement bioethanol production, the high capital investment and low co-product value coupled with this option may discourage investors move towards commercialization.

In contrast, taking better advantage of the value of unhydrolyzed residues, lignin pellets options display a better financial performance compared to lignin combustion cases. Three types of feedstock including natural hardwood, sugarcane bagasse, and corn stove present positive net present values. It is of interest to point out that though with lowest ethanol yield, the corn stove case can still be a profitable business by selling ethanol and lignin pellets in the same time. Lowest net present value for lignin pellets option is found on switchgrass because the harsh condition applied in the pretreatment liquefied a significant amount of biomass which can be hardly recovered as neither ethanol nor fuel pellets. Among all the feedstock investigated, sugarcane bagasse is the most economic feasible biomass for bioethanol and lignin pellets co-generation, with 119 million NPV, 17.0% IRR, and 4.1

payback years. The success of sugarcane bagasse can be attributed to the low cost per ton of carbohydrates and higher conversion efficiency.

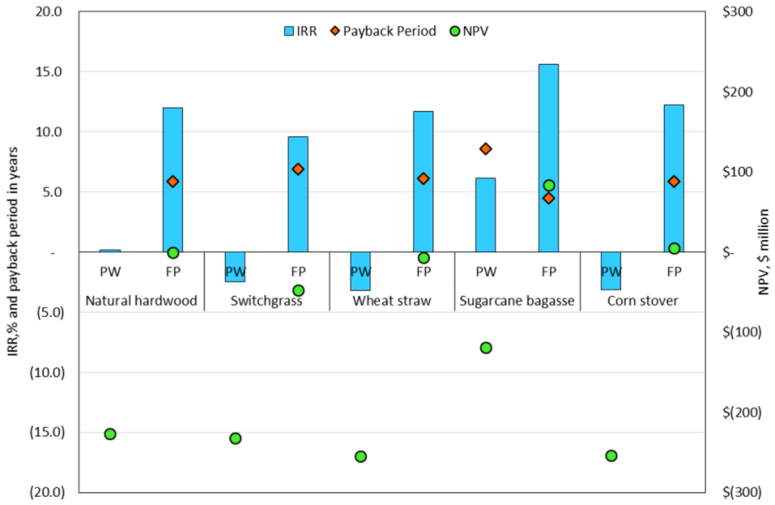

Fig. 5.4 NPV, IRR, and payback period for each type of feedstock, payback periods that longer than project life are not listed.

3.5 Minimum ethanol revenue

The minimum ethanol revenue (MER) is widely used to in bioethanol economic studies to evaluate the minimum ethanol selling price producer can provide to achieve certain amount of return. In this study, MER values were generated using a discount rate of 12%, at which the net present value of the project is zero. As shown in Fig. 5.5, all the cases with lignin combustion option have MER values higher than ethanol wholesale price, which is consistent to the results shown in the previous section. Under conventional ethanol plant

design, corn stover and wheat straw have the highest MER, close to $1 per liter; while sugarcane bagasse has the lowest MER, benefited from the high ethanol yield. If lignin pelletization is introduced, most of the feedstock except for switchgrass and wheat straw can have a lower MER compared to ethanol wholesale price. It is speculated that lowering the pretreatment severity can also place switchgrass and wheat straw in a profitable position if lignin pellets option is employed.

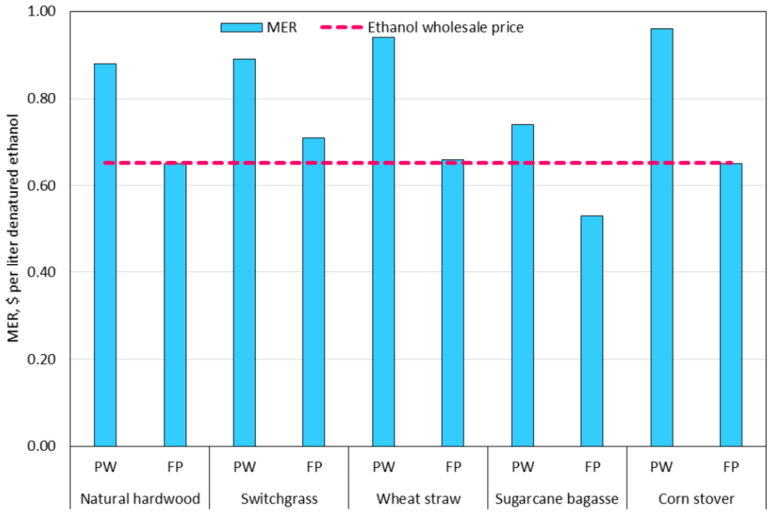

Fig. 5.5 Minimum ethanol revenue for each type of feedstock.

3.6 Sensitivity analysis

In order to understand the impacts of economic drivers on the financial indicators, a sensitivity analysis was performed varying capital investment, ethanol yield/price, biomass

cost, enzyme cost, energy cost including natural gas and power cost, and lignin pellets price using sugarcane bagasse as feedstock. This analysis was carried out with a variation of ±25% of the central values. As presented in Fig. 5.6, both lignin combustion and lignin pellets options are influenced most substantially by ethanol yield/price, followed by capital investment. The lignin pellet price also plays an important role in driving the economic performance of the process. Interestingly, both two options are not very sensitive to enzyme cost. This is probably due to the improved efficiency and price of enzymes, as well as the low amount of enzyme dosages used in this process.

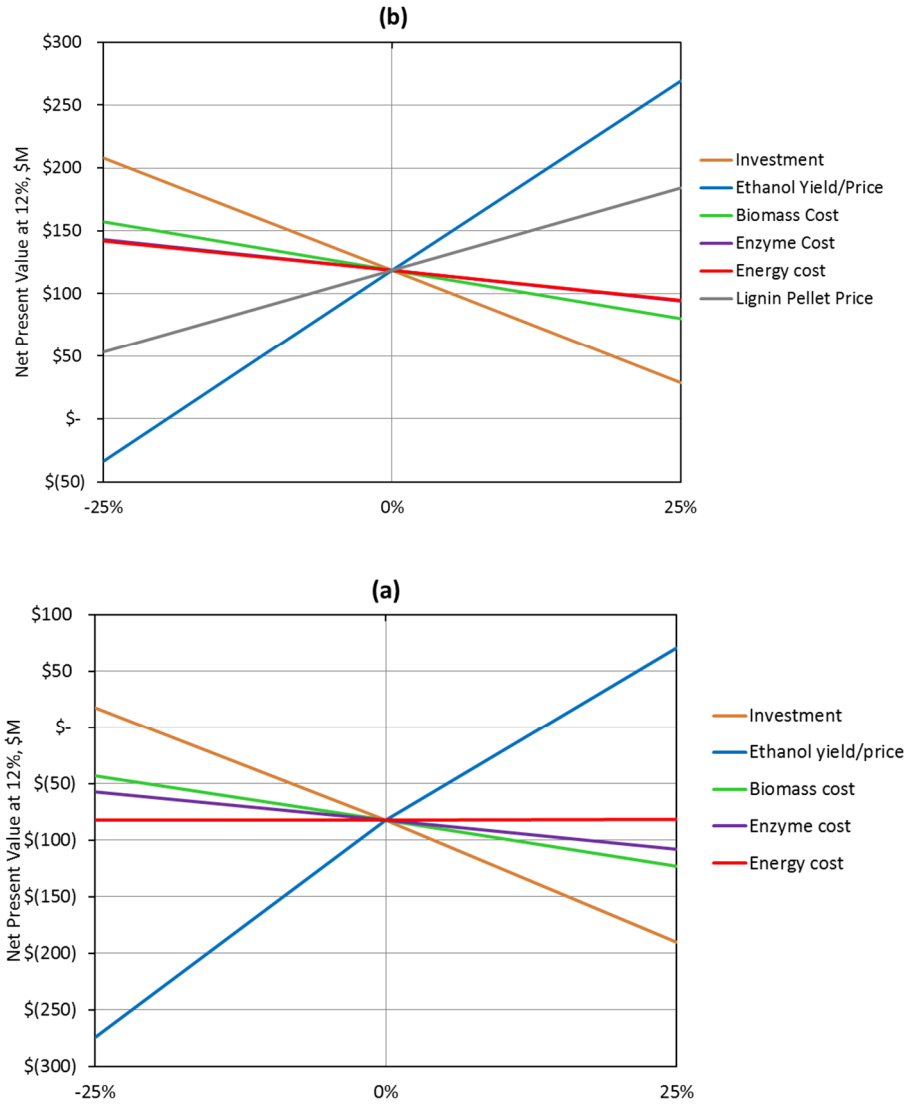

Fig. 5.6 Sensitivity analysis of net present value at 12% discount rate with variation of ± 25% for investment, ethanol yield/price, biomass cost, enzyme cost, energy cost, and lignin pellets price. (a) Lignin combustion for sugarcane bagasse; (b) Lignin pellets for sugarcane bagasse.

4. Conclusions

The process of autohydrolysis combined with mechanical refining is a potentially attractive process for the production of bioethanol since it is environmental friendly, capital-efficient, available on commercial equipment, and has competitive ethanol yield as more complex technologies. Nevertheless, in a conventional design, producing bioethanol and co-producing steam and power, the minimum ethanol revenues (MER) required to generate a 12% internal rate of return (IRR) are high enough to discourage investors due to the high capital investment relative to low US ethanol price. Owing to the sulfur free process, pelletization of the unhydrolyzed residues can be incorporated into autohydrolysis process and the economic performance was substantially boosted. Our analysis indicates that biomass with lower cost per ton of carbohydrates and less recalcitrance such as sugarcane bagasse and natural hardwood can produce attractive economics, with internal rate of return (IRR) greater than 12% in the proposed biorefinery scenario.

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Chapter 6 Thermo-mechanical pulping pretreatment of corn stover for bioethanol production

Abstract

Thermo-mechanical pulping (TMP), a well-known technology in pulp and paper industry was utilized as pretreatment for bioethanol production. TMP pretreatment at different conditions (temperature and refiner gap) and various post-treatment including PFI refining and fractionation, followed by enzymatic hydrolysis at 4 FPU/g enzyme dosages were carried out to evaluate the total fermentable sugar recovery from corn stover. The results indicate that the increased temperature facilitated the enzymatic digestibility of corn stover. PFI refining can generate significant improvements on enzymatic digestibility of TMP pretreated corn stover. No significant improvement was found on fractionation process due to the poor fractionation efficiency. The highest total sugar recovery was found to be 61.5% when corn stover was pretreated at TMP condition of 160 °C and 0.002 inch refiner gaps, followed by 8000 revolutions PFI refining at 4 FPU/g enzyme dosages.

Keywords: Corn stover, thermo-mechanical pulping, sugar, enzymatic hydrolysis, ethanol

1. Introduction

Currently, petroleum is still the primary feedstock for production of liquid fuels and industrial chemicals. However, the continuing questions about the longevity of supply and environmental impacts of its production and use, have motivated a great deal of interest to explore alternatives such as agricultural residues and woody biomass (Bozell, 2010). Corn

stover is considered a strategic feedstock for the production of biofuels and chemicals because of its abundant and widespread availability (Graham et al., 2007; Kadam & McMillan, 2003), sustainable production, economic feasibility (Von Sivers & Zacchi, 1996), and most importantly, no conflict with human food supply.

Like lignocellulosic biomass in general, corn stover has natural resistance of microbial and enzymatic deconstruction, collectively known as "biomass recalcitrance", which can be attributed to the morphological structure and chemical composition of lignocellulosic biomass, such as the crystalline structure of the native cellulose (Varga et al., 2004), the low specific surface exposed to enzyme (Zhu et al., 2009), and the poor cellulose accessibility in the presence of lignin (mainly) and hemicelluloses (Zhang et al., 2007). Thus, these difficulties must be overcome through employing a suitable pretreatment stage by means of disrupting the lignocellulosic matrix structure and removing some of the lignin and/or hemicelluloses in a cost-effective manner.

Extensive studies have been applied to development of efficient and cost-effective pretreatment technology. But many of them have only been tested in a lab-scale, accompanied with uncertainty for scale-up feasibility. The use of existing technologies in pulp and paper industry to produce cellulosic ethanol can provide several potential advantages, such as experienced workforce, proven technology and equipment, and possibility to recaptured assets from distressed mills (Gonzalez et al., 2011; Jin et al., 2010).

Thermo-mechanical pulping (TMP) is a widely used pulping technology in pulp and paper industry to produce mechanical printing papers such as newsprint as well as uncoated and coated magazine papers. This process involves with steaming the raw materials under pressure in a short period to soften the chips prior to mechanical refining (Smook, 1992). The combined steaming and mechanical refining process renders thermo-mechanical pulping a potentially pretreatment method for bioethanol production from lignocellulosic biomass. Moreover, over 30 newsprint mills have shut down over the past 5 years in Eastern Canada and Northeastern United States due to the continuing shrink of printing paper market (Santos et al., 2011). Those assets can be recaptured to produce cellulosic ethanol so as to reduce capital investment in a significant manner. The objective of this study is to identify process conditions in TMP as pretreatment method for bioethanol production. Some post-treatments such as PFI refining and oxygen delignification were investigated to boost the performance of TMP as well.

2. Materials and Methods

2.1 Raw material

Corn stover was collected from local farmland in North Carolina. It was air dried for 2 weeks to have uniform moisture content around 5%. The stalk and leaves were separated and cut into 1 inch length, and then mixed together with 1:1 ratio by weight for the following experiment. Part of corn stover was ground by a Wiley Laboratory Mill (Model No. 4, Thomas Scientific, Philadelphia, PA) to pass through a 20 mesh screen for composition analysis.

2.2 Thermomechanical pulping

Pretreatment was carried out in a TMP unit at the Department of Forest Biomaterials, North Carolina State University. Corn stover (1000 OD g) was loaded into TMP unit and steamed for 30 min at temperatures from 130 to 170 °C. Then the biomass was passed through a refiner with a disk gap from 0.002 to 0.008 inch at pressure for one time. After reaction, the whole slurry was collected and analyzed for pretreatment yield by consistency and total wet weight. Then the pretreated solids and filtrate were separated using cheese clothes. The filtrate was centrifuged and supernatant was stored in a refrigerator at 4 °C for pH analysis and sugar analysis. The solids were washed using tap water, centrifuged, and fluffed to have uniform moisture content for enzymatic hydrolysis.

2.2 PFI refining

Part of washed TMP pulp (24 OD g) was subjected to PFI refining at 10% consistency for 4000 and 8000 revolutions according to Tappi method T248 sp-00. The refined pulp was centrifuged, fluffed, and stored in a refrigerator at 4 °C for enzymatic hydrolysis.

2.3 Fractionation and oxygen delignification

Fractionation was carried out on TMP pulp to separate fines and coarse fibers. Fines were directly hydrolyzed with enzymes and coarse fibers were subjected to oxygen delignification followed by enzymatic hydrolysis. Fractionation was accomplished by dynamic sedimentation, where 40 OD g pulp was first integrated for 5 min and then

dispersed into 4 L water. The solution was mixed and stand still for 3 min, then the upper liquor which contains fines were decant and collected. The process was repeated for 3 times. The collected liquor was centrifuged and the fines were further freeze dried. The pulp left was drained using 200 mesh bucket and centrifuged to obtain coarse fibers.

Oxygen delignification was carried out in a rotatory bomb digester. Each reactor was loaded with 100 g OD coarse fibers supplemented with appropriate amount of deionized water to make a final 10% consistency. The sodium hydroxide was charged at 3% (by Na2O) of starting material and oxygen was loaded at 100 psig pressure. The reaction occurred at 110 °C for one hour. After oxygen delignification, the bomb reactor was quickly quenched in cold water. The pretreated pulp was thoroughly washed with tap water and centrifuged for future use.

2.2 Enzymatic hydrolysis

Enzymatic hydrolysis was carried out in an air shaker at 50 °C, 180 rpm for 96 hr. The enzyme mixture Cellic CTec2 and Cellic HTec2 (Novozymes, Franklinton, NC) was used for hydrolysis. The activity of CTec 2 was 139 FPU/g measured according to procedure described by Ghose (Ghose, 1987). Acetate buffer solution (50 mM, pH=4.8) was added into each aliquot to make a 5% hydrolysis consistency. Sodium azide (0.1%, w/w) was added into the samples to inhibit microbial contamination. The reaction was stopped by soaking samples into the boiling water for 10 min. The samples were centrifuged and the supernatants were filtered through 0.2 μm nylon filters for sugar analysis.

2.2 Analytic methods

The composition analysis of raw corn stover and pretreated pulp was carried out using methods described by Ertas (Ertas et al., 2013). The enzymatic sugar concentration was analyzed by a high performance liquid chromatography (HPLC) system (Agilent 1200, Agilent, Santa Clara, CA, USA), equipped with Shodex SP0810 column (8×300 mm, Showa Denko, Tokyo, Japan) and refractive index detector. The column was operated at 80 °C using Milli-Q water as mobile phase at a flow rate of 0.5 mL/min.

Fiber quality analyzer (FQA) was utilized to measure fiber length and percentage of fines. A fiber suspension (about 1 mg/L) was prepared and injected into FQA. The fiber length was reported as arithmetic length and the fines are defined as any particles that are less than 0.2 mm in size and reported as percentage of total number of particles counted.

3. Results and discussion

3.1 Chemical composition of corn stover

The chemical composition of corn stover is illustrated in Table 6.1. The corn stover has a total carbohydrates content of 59.7%, including 36.1% glucan, 19.6 xylan, 1.2% galactan, and 2.8% arabinan. In addition to carbohydrates, it also constitutes extractives 11.1%, acid insoluble lignin 14.4%, acid soluble lignin 2.9%, and ash 3.8%.

Table 6.1 Chemical composition of corn stover

Extractives	Glucan	Xylan	Galactan	Arabinan	AIL	ASL	Ash	Total
11.1	36.1	19.6	1.2	2.8	14.4	2.9	3.8	91.9

3.2 TMP pretreatment

Corn stover was subjected to TMP pretreatment at different temperatures and gap distances and the pretreated corn stover was further enzymatic hydrolyzed to evaluate the total sugar recovery.

3.2.1 Effect of TMP conditions to pulp yield, sugar in filtrate, and pH of filtrate

The pulp yield of TMP pretreatment at different temperatures (130 to 170 °C) and different refiner gaps (0.002, 0.004, 0.008 inch) is presented in Table 6.2. The pulp yield ranged from 48.7 to 82.4% depending on different pretreatment conditions. Generally the pulp yield decreased when corn stover was pretreated at higher temperature due to increased hemicellulose removal under higher temperatures. The various refiner gaps did not considerably affect the yield of TMP pretreatment. It is noted that the overall pulp yield of TMP is relatively low compared to autohydrolysis at similar temperatures (Ertas et al., 2013). This is probably because the TMP pretreatment in this study was operated in an open system, where the high pressure steam continuously hit the biomass, condensed and discharged to sewer. Those solids dissolved in the condensate cannot be recovered and leads to a low TMP yield. Another independent experiment was carried out to troubleshoot the low TMP pretreatment yield. Corn stover (100 g) was subjected to steaming at 170 °C for 30 min in the

TMP unit without refining and solids yield was measured afterwards. The results shows that only 56.4% of corn stover can be recovered after steaming 30 min at 170 °C, indicating a significant impact of steaming temperature and time to TMP yield.

The filtrate was obtained by squeezing the pretreated wet pulp using cheese cloth. Both sugar and pH were analyzed on the filtrate. As seen in Table 6.2, the pH of the filtrate dropped from 5.3 to 4.3 with increase of temperature from 130 to 170 °C, but irrelevant to refiner gaps. The total sugars in the filtrate ranged from 3.2 to 10.3 g/100 raw corn stover at different TMP conditions, but most sensitive to pretreatment temperature.

Table 6.2 Pulp yield, sugar released in filtrate and pH under different TMP conditions.

	Pulp, %	Sugar in filtrate,%	pH-end
T130-8	82.4	3.8	5.0
T130-4	76.7	3.6	5.3
T130-2	80.1	3.2	5.1
T150-8	68.3	5.2	4.6
T150-4	60.7	4.8	4.6
T150-2	65.6	4.8	4.4
T170-8	49.8	9.5	4.5
T170-4	52.8	10.3	4.3
T170-2	48.7	9.7	4.3

3.2.2 Effect of TMP conditions on fiber quality

The fiber length and fines ratio of TMP pulp were analyzed in fiber quality analyzer (FQA). The fiber length was reported as arithmetic fiber length, which is the sum of all the

individual fiber lengths divided by the total number of fibers measured. The percentage of fines was reported as an arithmetic basis, which is the number of fines divided by the total number of fibers (fines included). As seen in Fig. 6.1, the average fiber length and fines ratio of TMP pulp are strongly related to refiner gaps and irrelevant to pretreatment temperatures. The smaller refiner gaps lead to the shorter the fiber and the higher percentage of fines. It is noted that the fines ratio of TMP pulp falls between 55% and 59% for all conditions tested in this study, indicating a dramatic fiber shortening effect during TMP pretreatment.

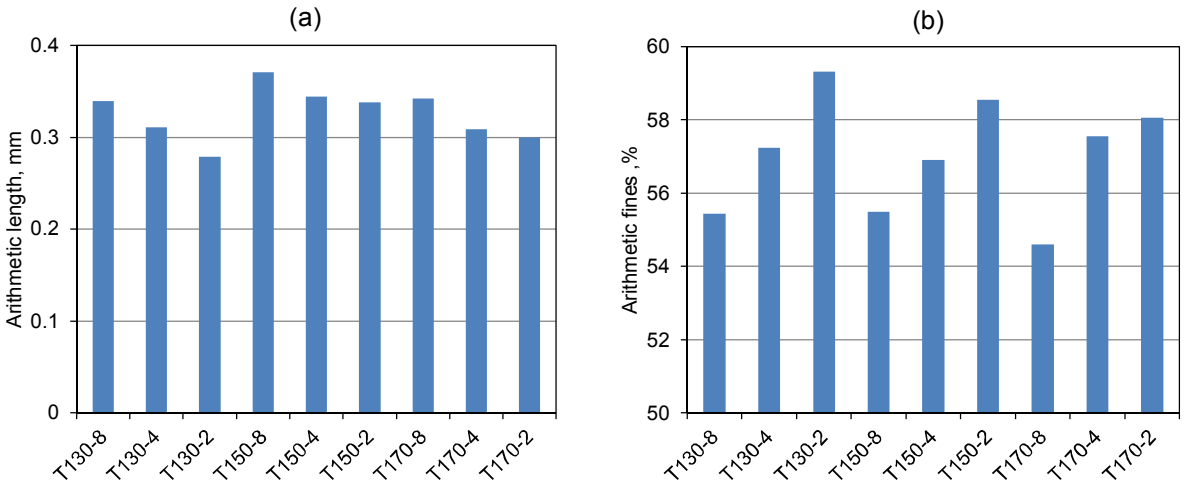

Fig. 6.1 (a) Arithmetic length of TMP fiber; (b) Arithmetic fines of TMP fiber.

3.2.3 Effect of TMP conditions on enzymatic hydrolysis

TMP pulp was subjected to enzymatic hydrolysis at 4 FPU/g enzyme dosages to evaluate its sugar yield at different TMP conditions. The Fig. 6.2 shows that with elevated

temperature, the glucan yield after enzymatic hydrolysis increased, especially when the temperature went from 150 to 170 °C. In contrast, the xylan yield increased initially when the temperature went from 130 to 150 °C, but then started to drop when the temperature further went up to 170 °C, indicating a significant xylan solubilization during the pre-steaming stage. The overall enzymatic sugar yield increased at higher temperature, but no significant impact of refiner gaps to enzymatic sugar yield was observed. The highest enzymatic sugar yield was found to be 22.1 g/100 g raw corn stover at TMP conditions of 170 °C and 0.008 inch refiner gap.

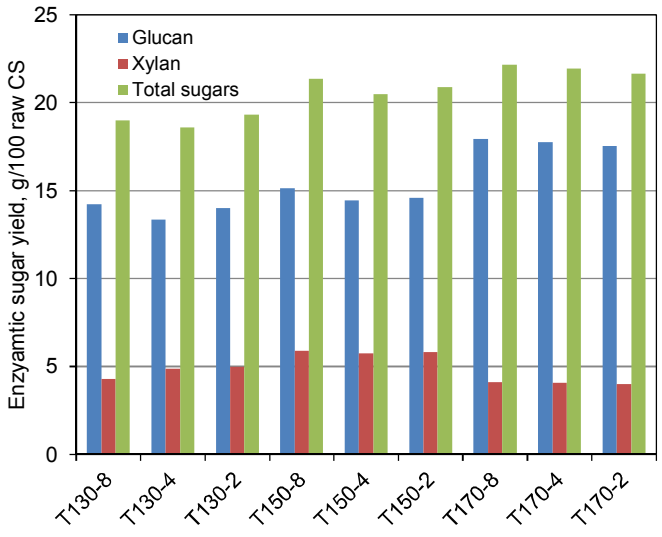

Fig. 6.2 Sugar yield in enzymatic hydrolyzate on TMP pretreated pulp.

3.2.3 Effect of TMP conditions on total sugar recovery

The total sugar recovery was calculated based on the sum of sugars from TMP filtrate and enzymatic hydrolyzate against total carbohydrates in the raw corn stover. As seen in Fig. 6.3, the total sugar recovery ranged from 37.2 to 53.9% at all TMP conditions. The majority of fermentable sugars were recovered from enzymatic hydrolyzate. Temperature played an important role on total sugar recovery, but not for refiner gaps. The highest total sugar recovery was found to be 53.9% (36.7% from enzymatic hydrolyzate at 4 FPU/g enzyme dosages and 17.3% from TMP filtrate) when the corn stover was pretreated at 170 °C with a refiner opening of 0.004 inch.

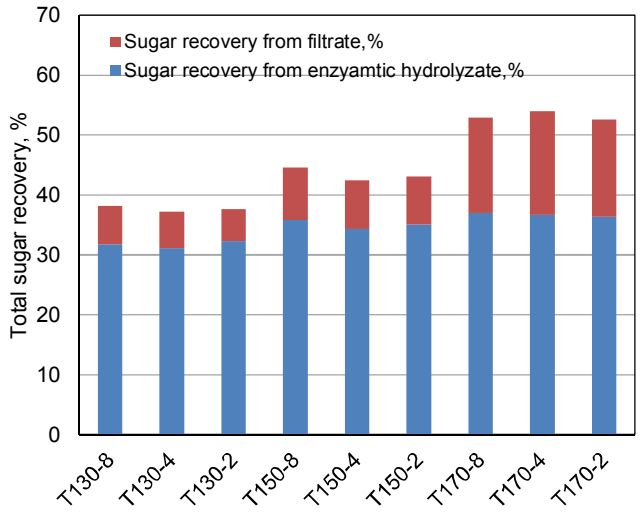

Fig. 6.3 Total sugar recovery under different TMP pretreatment conditions.

3.3 TMP pretreatment combined with PFI refining

It has been reported that during TMP process, if the refining was carried out at high temperature (>150 °C), the fiber delamination would likely occur between P and S1 layer and created a lignin-enriched fiber surface (Koljonen et al., 2003; Smook, 1992). Thus the PFI refining was introduced in order to peel off the surface lignin and improve the enzymatic digestibility of TMP pretreated pulp at higher temperature.

3.3.1 Effect of PFI refining on enzymatic hydrolysis of TMP pulp

The TMP (160 °C and 0.002 inch) pretreated pulp was subjected to FPI refining at 4000 and 8000 revolutions. The refined pulp was further enzymatic hydrolyzed at 4 FPU/g enzyme dosages to evaluate the sugar yield. Encouraging results were found in Fig. 6.4 that the PFI refining facilitated the enzymatic hydrolysis of TMP pulp. Both glucan and xylan yield in the enzymatic hydrolyzate increased with the addition of PFI refining. The total enzymatic sugar yield increased from 21.8 g/100 g raw corn stover to 28.2 g/100 g raw corn stover when TMP pretreated pulp was further refined using PFI at 8000 revolution. Though the surface lignin content was not measured in this study, the peeling of the surface lignin may be the factor that contributes to the improvement of enzymatic hydrolysis by PFI post-treatment.

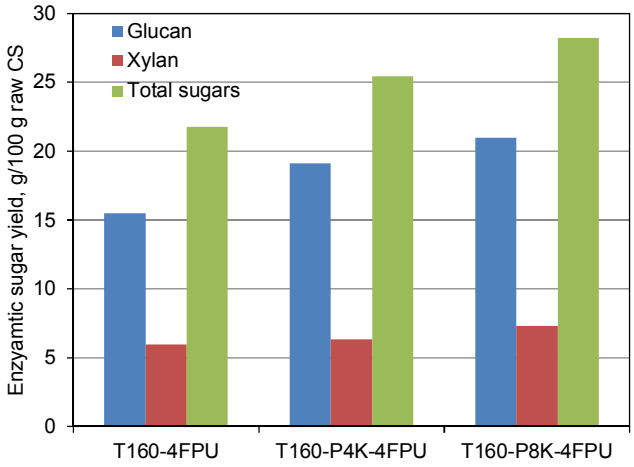

Fig. 6.4 Sugar yield in enzymatic hydrolyzate on TMP-PFI treated pulp.

3.3.2 Effect of PFI refining on total sugar recovery

The total sugar recovery at 4 FPU/g enzyme dosages of TMP pretreatment with/without PFI refining was illustrated in Fig. 6.5. The PFI post-treatment improved the total sugar recovery of TMP pretreated corn stover and such improvement is larger when the revolution increased from 4000 to 8000. The highest total sugar recovery was found to be 61.5% when the corn stover was pretreated at TMP condition of 160 °C and 0.002 inch refiner gap followed by 8000 revolution PFI refining compared to 50.7% total sugar recovery if only TMP pretreatment was applied.

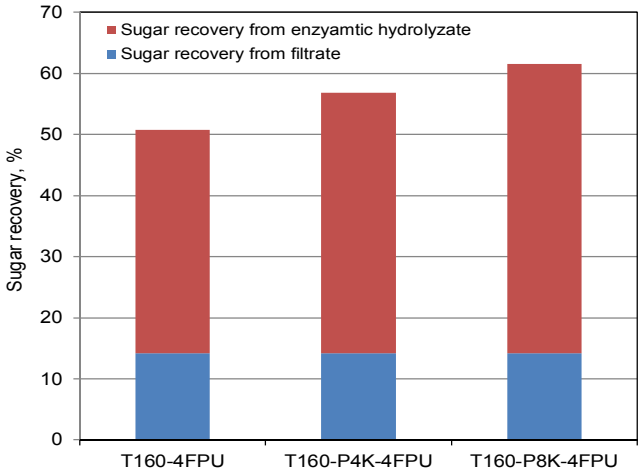

Fig. 6.5 Total sugar recovery on TMP-PFI treated corn stover.

3.4 TMP pretreatment combined with fractionation

Due to a significant amount of fines generated during TMP pretreatment, fractionation was introduced after TMP pretreatment to separate fines and coarse fibers. The fines are assumed to be readily hydrolyzed by enzymes and the coarse fibers were further subjected to oxygen delignification to improve the enzymatic digestibility of fibers.

3.4.1 The properties of fines and coarse fibers after fractionation

After fractionation, 77.0% of the TMP fibers can be collected as coarse fibers and 8.1% of fibers can be collected as fines. The total combined yield of fractionation was only 85.1% due to the fractionation method employed. It is speculated that a significant amount of fines were lost during the fractionation process and the fractionation method needs to be improved in the future. The chemical composition of TMP pulp, fines, and coarse fibers is listed in Table 6.3. The TMP pulp has a total carbohydrates content 61.2% and lignin content

20.8%. The coarse fibers have similar composition compared to unfractionated TMP pulp, with total carbohydrates content 65.8% and lignin content 20.2%. The fines have a much lower carbohydrates content (40.3%) and higher lignin content (40.3%).

Table 6.3 Chemical composition of fines and coarse fibers after fractionation, based on 100 g raw corn stover.

	Yield	Glucan	Xylan	AIL	ASL	Ash	Sum
T160	59.6	28.0	8.5	11.2	1.2	1.5	50.4
T160-C	45.9	23.5	6.7	8.5	0.8	0.3	39.8
T160-F	4.8	1.6	0.3	1.6	0.1	0.3	3.9

The fiber property of unfractionated TMP pulp, coarse fibers, and fines were listed in Table 6.4. As expected, the length of coarse fibers is longer than unfractionated TMP pulp and fines. The fines have a much higher fine ratio (84.2%) compared to unfractionated TMP pulp (54.9%) and coarse fibers (46.8%).

Table 6.4 FQA results of TMP pulp, coarse fibers, and fines

FQA	T160	T160-C	T160-F
Arithmetic Length, mm	0.37	0.43	0.15
Arithmetic Percent Fines,%	54.9	46.8	84.2

3.4.2 The effect of fractionation on total sugar recovery

After fractionation, the fines were directly subjected to enzymatic hydrolysis at 2 FPU/g enzyme dosages and oxygen delignification was conducted on coarse fibers to improve its enzymatic digestibility. As seen in Fig. 6.6, the fines are readily hydrolyzed by enzyme even at 2 FPU/g low enzyme dosages, with 75.1% glucan conversion and 97.0% xylan conversion. For coarse fibers, 38.7% of lignin was removed after oxygen delignification. The delignified pulp was then hydrolyzed at 2 FPU/g enzyme dosages. The oxygen delignified substrate has a higher glucan conversion (50.3%) and xylan conversion (80.3%) based on delignified pulp, compared to 43.1% glucan conversion and 62.7% xylan conversion based on coarse fibers when coarse fiber was directly fed to enzymatic hydrolysis. But the yield loss during oxygen delignification renders the overall sugar yield (12.9 g/100 g raw corn stover) lower than 14.6 g/100 g raw corn stover obtained from directly hydrolysis of coarse fibers. The total sugar recovery was found out to be 34.8% based on carbohydrates in the raw corn stover, which is lower than that from TMP only and TMP plus PFI refining mainly because the low yield of fractionation step. Thus, a more efficient fractionation method needs to be developed to boost the whole process. In addition, the optimization of oxygen delignification condition and enzyme loadings on fines and treated coarse fibers can improve the fractionation process as well.

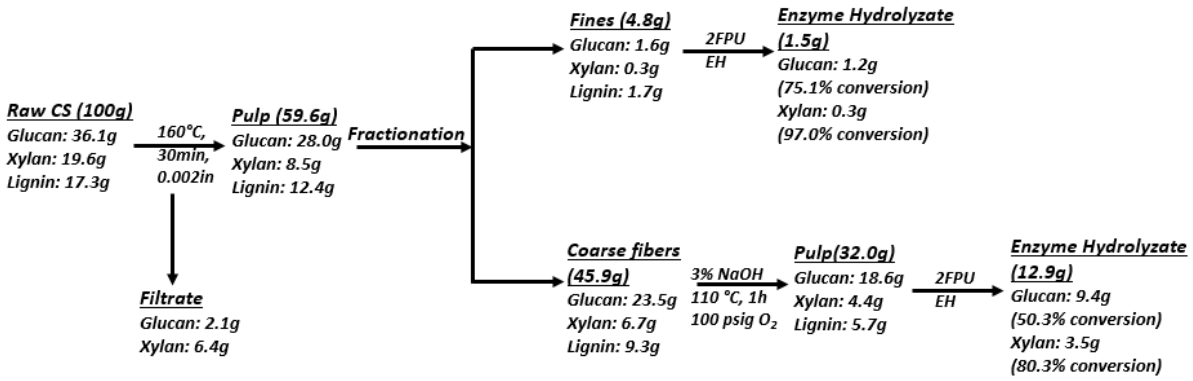

Fig. 6.6 Material balance of fractionation process on corn stover

4. Conclusions

The corn stove was subjected to TMP pretreatment at different temperatures and refiner gaps and followed by various types of post-treatment, then enzyme hydrolyzed at 4 FPU/g to evaluate the total sugar recovery. The highest total sugar recovery from TMP only was found to be 53.9% at the TMP condition of 170 °C and 0.004 inch refiner openings. Encouraging results were found when 8000 revolutions PFI refining was applied as a post-treatment on TMP pulp, where 61.5% total sugar recovery was obtained compared to 50.7% total sugar recovery without PFI refining. The fractionation was introduced to the TMP process aimed at improving enzymatic sugar yield of TMP pulp. But due to the yield loss during fractionation process, only 34.8% total sugar recovery can be achieved. The highest total sugar recovery in this study was found to be 61.5% when corn stover was pretreated at TMP condition of 160 °C and 0.002 inch refiner gaps, followed by 8000 revolutions PFI refining at 4 FPU/g enzyme dosages.

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Chapter 7 Conclusions

Several feedstocks including wheat straw, switchgrass, corn stover, waste wheat straw have been subjected to autohydrolysis pretreatment followed by mechanical refining. The total sugar recovery including sugar recovered from autohydrolysis filtrate and enzymatic hydrolyzate for most biomass can be as high as 76% at 4 FPU/g enzyme dosages. But only 60% of total sugar recovery was obtained from corn stover. The mechanical refining significantly improved the enzymatic sugar yield of autohydrolysis pretreated substrate by as much as 30%. Mechanical refining exerted its optimum effect on the moderate pretreated substrate.

When six different types of lignocellulosic biomass were pretreated at the same autohydrolysis condition, it has been found that non-woody biomass lignin are more susceptible to hydrothermal pretreatment and an average of 30% of lignin removal can be observed. The lignin structure such as S/V ratio in the woody biomass plays a significant role in the total sugar recovery from autohydrolysis process in which the higher S/V ratio, the higher amount of sugar can be extracted from the woody biomass.

Economic analysis shows that though autohydrolysis combined with refining has a low capital cost on pretreatment area and competitive ethanol yield, in the conventional design, producing bioethanol and co-producing steam and power, the minimum ethanol revenues (MER) required to generate a 12% internal rate of return (IRR) are high enough to discourage investors due to the high capital investment relative to low US ethanol price.

However, upgrading unhydrolyzed residues to high value byproducts like fuel pellets and co-location and repurposing scenarios substantially boosted the economics of autohydrolysis process, making it a financial process for bioethanol production. Our analysis indicates that biomass with lower cost per ton of carbohydrates and less recalcitrance such as sugarcane bagasse and natural hardwood can produce attractive economics, with internal rate of return (IRR) greater than 12% in the proposed biorefinery scenario.

Essentially, TMP pretreatment is an alternative to autohydrolysis combined with mechanical refining, but no encouraging results have been found on TMP pretreatment so far. However, the optimization of TMP condition and process design can make it a promising pretreatment technology for bioethanol production in an industrial scale.

Chapter 8 Future work

The poor conversion of autohydrolysis on corn stover needs to be investigated. Such limitation might be due to the great variety of corn stover including the different digestibility of stalk and leave, and the geography effect that leads to distinct characteristics of corn stover.

The effect of mechanical refining to the enzymatic digestibility of autohydrolysis pretreated biomass has not been well understood. The understanding of mechanical action that contributes to the increased enzymatic digestibility can help to design and engineer the high performance refiner which can be used in the commercial bioethanol production process.

The autohydrolysis process offers the great feature that producing unhydrolyzed residues mainly lignin without chemical contamination. In the short run, fuel pellets can be a good choice owing to the prosperity of biomass pellets market in the Europe. But in the long run, high value-added byproducts need to be explored such as resin and antioxidant from lignin compounds.

The difference of lignin structure in hardwood leads to a differential digestibility in the autohydrolysis process. It is of interest to evaluate the impact of lignin structure to the economics of bioethanol production from different hardwood species so as to provide useful criteria for hardwood plantation for fuel production.