January 2014

Effect Of Bronsted And Lewis Acids On Biochemical Conversion Of Various Lignocellulosic Feedstocks Into Biofuels And Chemicals

Srinivas Reddy Kamireddy

EFFECT OF BRONSTED AND LEWIS ACIDS ON BIOCHEMICAL CONVERSION OF VARIOUS LIGNOCELLULOSIC FEEDSTOCKS INTO BIOFUELS AND CHEMICALS

by

Srinivas Reddy Kamireddy
Bachelor of Science, Acharya Nagarjuna University, 2005
Master of Science, San Jose State University, 2009

A Dissertation

Submitted to the Graduate Faculty

of the

University of North Dakota

In partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Grand Forks, North Dakota
August
2014

This dissertation, submitted by Srinivas Reddy Kamireddy in partial fulfillment of the requirements for the Degree of Doctor of Philosophy from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work was performed, and is hereby approved.

Yun Ji

Brian Tande

Robert Wills

Evguenii Kozliak

Alena Kubátová

This dissertation is being submitted by the appointed advisory committee as having met all of the requirements of the Graduate School at the University of North Dakota and is hereby approved.

Wayne Swisher
Interim Dean of the Graduate School

Date: July 24, 2014

PERMISSION

Title Effect of Bronsted and Lewis Acids on Biochemical Conversion of Various Lignocellulosic Feedstocks into Biofuels and Chemicals

Department Chemical Engineering

Degree Doctor of Philosophy

In presenting this dissertation in partial fulfillment of the requirements for a graduate degree from the University of North Dakota, I agree that the library of this University shall make it freely available for inspection. I further agree that permission for extensive copying for scholarly purposes may be granted by the professor who supervised my dissertation work or, in his absence, by the Chairperson of the department or the dean of the Graduate School. It is understood that any copying or publication or other use of this dissertation or part thereof for financial gain shall not be allowed without written consent. It is also understood that due recognition shall be given to me and to the University of North Dakota in any scholarly use which may be made of any material in my dissertation.

_____
Srinivas Reddy Kamireddy

Date 07/09/2014

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

LIST OF ABBREVATIONS

ppm…….....parts per million

CSF…….combined severity factor

BBD…….Box-Behnken Design

t……reaction time

T_H…….reaction temperature

T_R……..reference temperature

X……xylan content

X_0……..initial xylan concentration

Xm…….xylose monomer

F……furfural

k_1……rate of xylose formation

k_2……rate of furfural formation

A……pre-exponential factor

Ea……..activation energy

R……universal gas constant

T……....temperature

C……concentration of acid

ni……....reaction order

ACKNOWLEDGMENTS

PhD studies were a very rewarding, challenging time for me. There were many individuals throughout the years of my PhD research and study at the University of North Dakota that I am indebted to and without them this daunting task was not possible. First and foremost, my gratitude goes to my advisor, Prof. Yun Ji and also to Prof. Evguenii Kozliak. For their invaluable insights during discussions of the research, their patient mentoring and support as I navigated the nuances of being a researcher and surviving the challenges of the PhD process. They were both very generous with their time, expertise, encouragement and support.

Next, I would like to thank my committee members, Prof. Brian Tande, Prof. Robert Wills, and Prof. Alena Kubátová who agreed to read and give comments on my dissertation and probe my knowledge of the subject matters involved in the research. Their comments and suggestions were very helpful in refining the dissertation.

I would also take this opportunity to thank Mr. Joe Miller who helped me in many ways by fixing the reactor and HPLC many times. I deeply appreciate his support and kindness. I would also like thank my dearest friends Alireza and Hassan Abdul Sater for supporting and helping me with their valuable suggestions

Last but not the least it is my parents who have been a great source of inspiration and motivation throughout my life. I give them my deepest gratitude

ABSTRACT

A biochemical process was investigated in the conversion of lignocellulosic biomass into biofuels and value added chemicals. Kenaf, two species of sorghum (brown mid rib (BMR) & non brown mid rib (NBMR)), sunn hemp, sunflower hulls, and cornstover were used as feedstocks in this study.

Lignocellulosic biomass primarily consists of three different components, cellulose, hemicellulose and lignin. In order to separate different fractions, and acid pretreatment is generally employed. This was achieved by using a 300 ml internal volume batch reactor. The heating source used was steam. During acid pretreatment most of the hemicellulose is hydrolyzed into the liquid fraction. The remaining solid fraction that is rich in cellulose and lignin is subjected to enzymatic hydrolysis. The maximum hemicellulose hydrolysis for four feed stocks (two species of sorghum, sunn hemp and kenaf) ranged between 72 wt% to 95 wt%. The maximum enzymatic hydrolysis yield ranged between 68 wt% to 90 wt%.

In the case of acid pretreatment of sunflower hulls, the maximum hemicellulose and cellulose yield were observed to be 59 and 53.5wt%, respectively. This difference was explained by a high lignin and wax content of the hulls cell walls, which could act as a barrier to the hydronium ions resulting in lower yields.

1. INTRODUCTION

1.1. Background

Energy is vital for social and economic development across the globe (Baños *et al.* 2011). The rapid development of the global economy, especially in the developing countries, has influenced the consumption of energy in vast quantities. Firstly, this demand led to an increase in fossil fuel prices (Vine 2008). Secondly, the increase in the concentrations of greenhouse gases especially carbon dioxide in the atmosphere has increased. According to the study conducted by (Chèze *et al.* 2013) at this current rate it is expected to increase from 400 ppm (parts per million) in 2013 to 445-490 ppm in 2025. Two strategies are employed to mitigate these effects. First, reduced dependence on fossil resources is based on the reduction in energy demand and increasing energy efficiency in industrial and domestic fields (Lee, Chen 2009). The second strategy is to develop renewable sources of energy that are sustainable and economical (Zhou *et al.* 2010).

1.2. Motivation

Traditional fossil fuels such as petroleum and coal are not considered as sustainable since they have limited availability. In the current economies, it is imperative to develop fuels from renewable sources to meet our energy requirements. Renewable sources such as solar, wind, geothermal, and bioenergy contribute about 8.1% of the total U.S energy consumption (USDA

Report 2011). A number of studies which investigated the potential contribution of renewables have indicated that in the second half of the 21st century their contribution might be as high as 20% (Akella *et al.* 2009). Energy generated from different renewable sources has its fair share both advantages and disadvantages. So far, the energy from biomass is the leading contender since its source is not intermittent as compared to wind, solar and geothermal as shown in Figure 1-1. Additionally, lignocellulose can also be used to produce value added products such as furfural, hydroxyl methyl furfural, acetic acid, succinic acid, levulinic acid. These chemicals can serve as building blocks in the production of cosmetics, pharmaceutical drugs and other industrial applications.

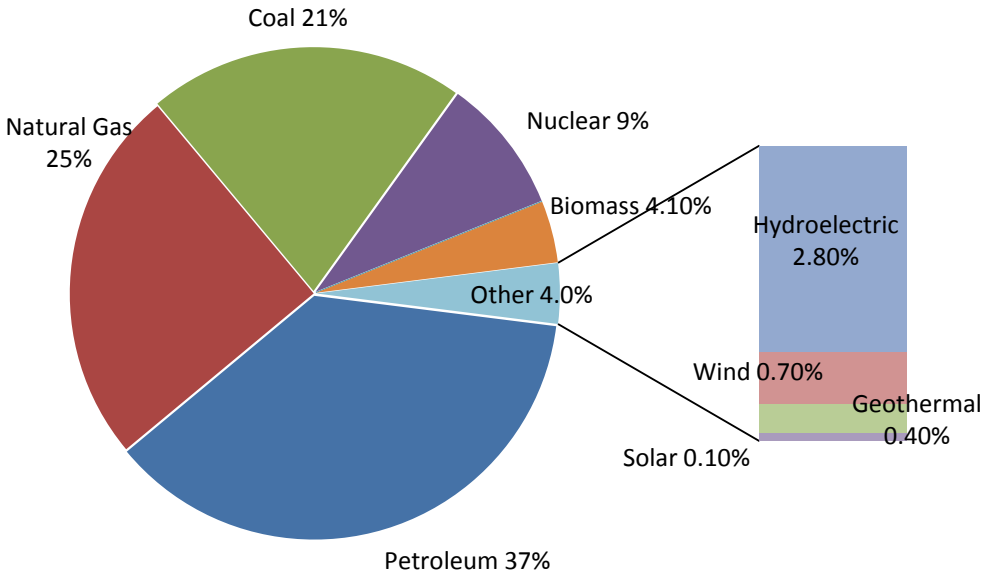

Figure 1-1 Total energy consumption in 2010 in terms of percentage (USDA Report 2011).

1.3. Biofuel Energy Overview

Bio-based industrial development was pushed by the US Congress, initially in 2000. In the USA it is intended that by 2020 at least 25% of organic-carbon-based industrial feedstock chemicals and 20% of liquid fuels will be produced by bio-based industry (RFA 2011). The biofuels produced from biomass have led to a decrease in petroleum imports from 61% to 41% in the US since 2008 (RFA 2011).

Biomass has long been harnessed as an energy resource since ancient times. Wood has been harvested both in small and large quantities for energy generation through combustion (Bowyer *et al.* 2003).

Fuels from biomass have been divided into first-generation and second-generation biofuels. First-generation biofuels are derived from starch, sugar and oil. The main agricultural crops associated with first-generation biofuels are corn, sugarcane, soybean, oil palm and rapeseed (Fargione *et al.* 2010). Ethanol from corn is one of the most well-known first generation biofuels. Though first-generation technologies are the most mature, they have been deemed unsustainable due to their direct competition with food. The production of bio-fuel from food crops increases the price of food commodities as well as the potential for adverse ecological issues such as an increased burden on water use (Fargione *et al.* 2010).

Second generation bio-fuel included the fuels from lignocellulosic biomass, including agricultural and forest residues and nonfood energy crops such as switchgrass. Moreover, second-generation fuels do not directly compete with the use of food sources. Hence, conversion of

lignocellulosic biomass into transportation fuels presents a powerful opportunity for energy security, reduction of the trade deficit and improved agricultural economy (Wyman 1999a).

Biomass is a renewable and readily available resource. Moreover, due to its ability to reduce greenhouse gases from the atmosphere its utilization as an energy resource was reported to give a net positive change in emissions compared with fossil fuels (Sims *et al.* 2010). In 2005, Oak Ridge National Laboratory (ORNL) reported that about 1.3 billion ton of biomass is available annually for the use as biofuel in the US (Perlack *et al.* 2005). This includes biomass (crops and residues) from both forest and agricultural crops.

The commercialization of bio-fuel production will lead to more jobs and will boost the GDP of U.S.(RFA 2011). Moreover, the production of ethanol from biomass will benefit the farmers thus improving the local economy. Similar economic benefits can be expected for bio-fuel produced from forest residues.

1.4. Forest and Agricultural Residues

Forest residues are defined as the biomass material remaining in forests that have been harvested for wood and paper industry. Typically, forest residues are either left in the forest or disposed of via open burning through forest management programs. The process of harvested forest products, such as saw logs and pulpwood, generates significant quantities of mill residues and pulping liquors (Perlack *et al.* 2005). These residues generated in the processing of forest products account for 50 percent of the current biomass (Perlack *et al.* 2005). These materials are

used by the forest products industry to manage residue streams, produce energy and recover important chemicals.

Agricultural residues are the biomass material left after harvesting agricultural crops. The major agricultural residue includes corn stover, sweet and wheat straw. According to the study conducted by (Werther *et al.* 2000), the potential for producing bio-fuels and chemicals from agricultural residues is higher than from forest residues as the amount of biomass available is almost 3 times that of the forest residues as seen from Figure 1-2.

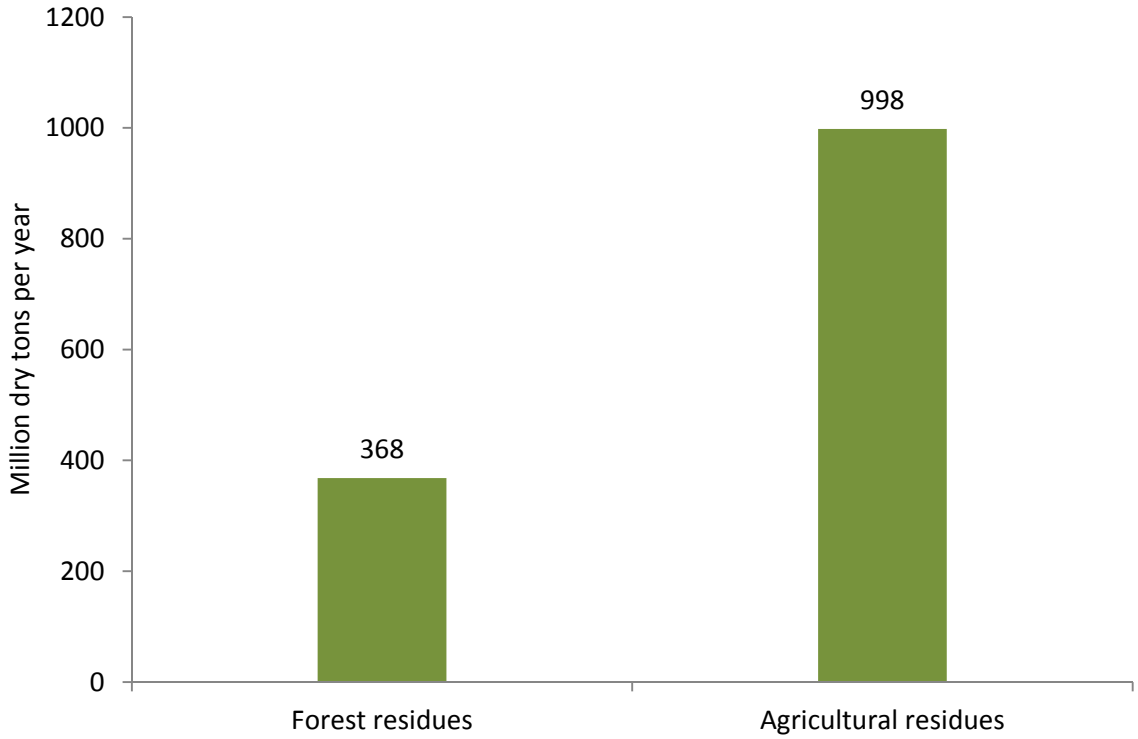

Figure 1-2. Annual biomass resource potential.(Perlack *et al.* 2005)

1.5. Challenges

The complexity of the lignocellulosic material poses several problems that hinder commercialization (Wyman *et al.,* 2005a). Pretreatment is needed to disrupt the hemicellulose and lignin structure and expose the cellulose to hydrolysis (Mosier *et al.,* 2005). The pretreatment step is expected to account for a third of the total processing costs in second-generation lignocellulosic biorefineries (Wyman *et al.,* 2005b) despite over two decades of active research examining multiple pretreatment methods. Pretreatment research focuses on developing processes that enhance conversion rates, reduce the need for hydrolytic enzymes, and increase biofuel yields (Mosier *et al.,* 2005). Defining a single most efficient method of pretreatment is not feasible due to the diverse nature of lignocellulosic biomass (Mosier *et al.,* 2005) thus crop-specific research is needed in order to promote the commercialization of second generation biofuels. This dissertation evaluates two pretreatment approaches of dilute acid and Lewis acid and aids in gathering information that can guide in the commercialization process.

1.6. Anatomy of the Biomass

1.6.1. Cellulose

Cellulose is a highly branched polymer that consists of β-D- glucopyranose moieties linked by β-1-4 glucoside bonds as seen in Figure 1-3. The degree of polymerization varies greatly for each biomass species. The cellulose repeating units of 20 to 300 are grouped together to form microfibrils to form cellulose (Agbor *et al.* 2011). The cellulose microfibrils are mostly independent but the ultrastructure of cellulose is largely due to the presence of covalent bonds, hydrogen bonding and Van der Waals forces. Hydrogen bonding within cellulose determines the

straightness of the chain but interchain hydrogen bonds might introduce order (crystalline) or disorder (amorphous) into the structure of cellulose (Agbor *et al.* 2011).

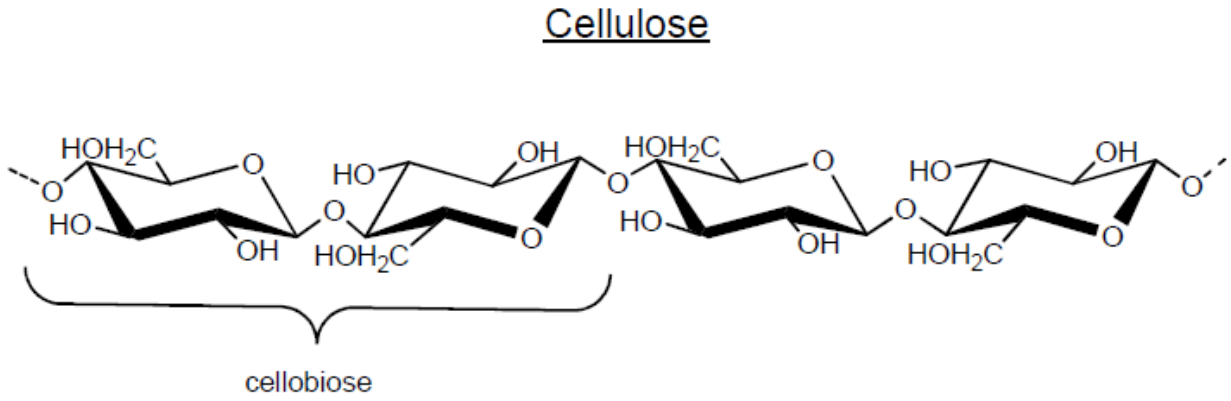

Figure 1-3. Chemical structure of cellulose microfibrils

1.6.2. Hemicellulose

Hemicellulose is the second most abundant polymer; unlike cellulose it is a hetero polymer. It mainly consists of pentoses (xylose, arabinose) hexoses (mannose, glucose, galactose) and acetylated sugars (Saha 2003). They have a lower molecular weight compared to cellulose and branches with short lateral chains that are easily hydrolyzed (Fengel, Wegener 1983). Hemicellulose in agricultural residues such as cornstover, wheat straw and sorghum, mainly consists of xylan. In many biomass residues xylans are composed of heteropolysaccharides with backbone chains of 1,4-linked β-d-xylopyranose units. In addition to xylose, xylan may contain arabinose, glucuronic acid or its 4-O-methyl ether, acetic acid, ferulic and p-coumaric acids (Chandra *et al.* 2007, Fengel, Wegener 1983) as evident from Figure 1-4.

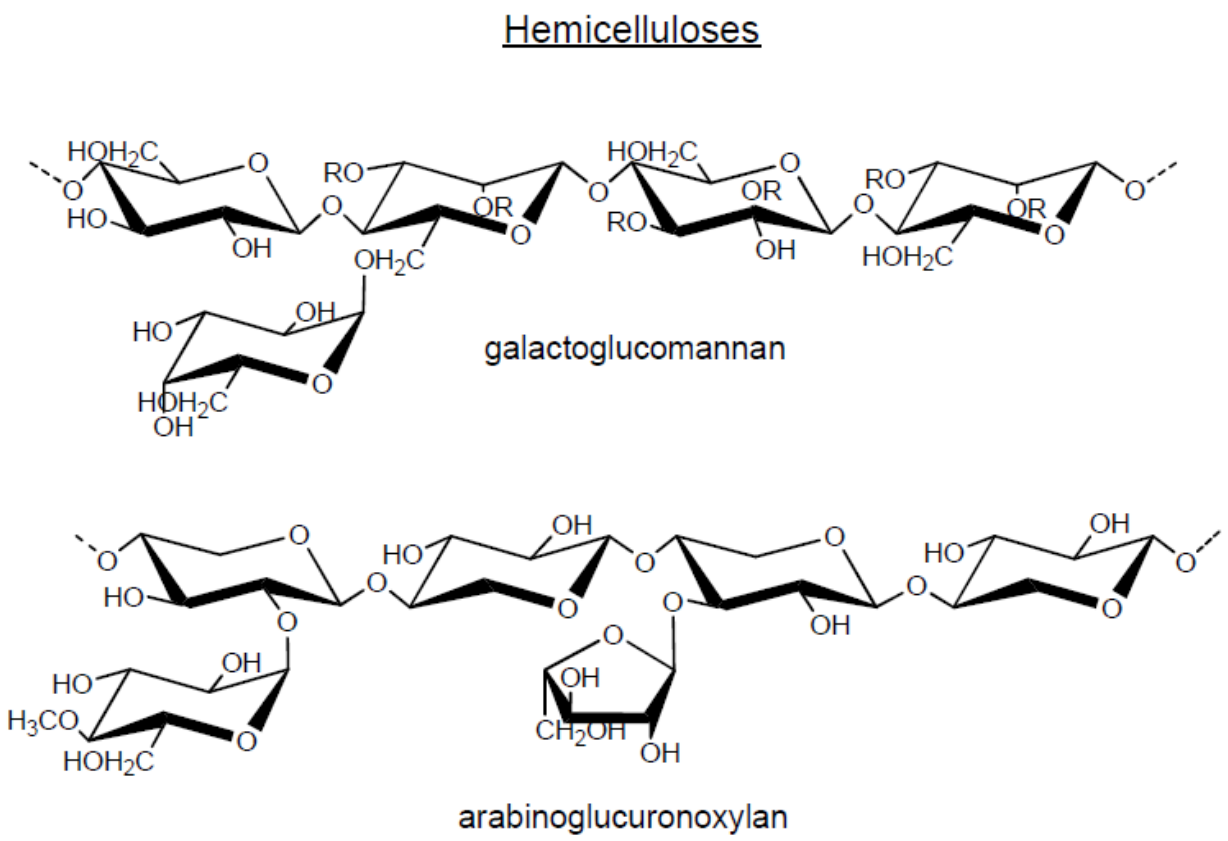

Figure 1-4. Chemical structure of hemicellulose

1.6.3. Lignin

Lignin is the third most abundant polymer that is found in the cell walls of the agricultural residues. Lignin is hydrophobic in nature and binds to different components of the biomass (Chang, Holtzapple 2000). The major function of lignin is to provide resistance to microbial attacks and oxidative stresses (Agbor *et al.* 2011). Lignin is an amorphous aromatic polymer primarily composed of phenyl propane units (p-coumaryl, coniferyl and sinapyl alcohol) held together by different linkages (Hendriks, Zeeman 2009a) as seen in Figure 1-5. Based on the type of the biomass the structure of lignin varies. Softwoods (gymnosperms) mostly consists of

coniferyl alcohol-derived constituents and hardwoods (angiosperms) have a mixture of syringyl and coniferyl type structures. Grasses usually contain mainly p-coumaryl groups coupled with the other types (Hon, Shiraishi 2000). The various combinations of mono-lignols lead to complicated inter unit linkages mainly composed of C-C and C-O types.

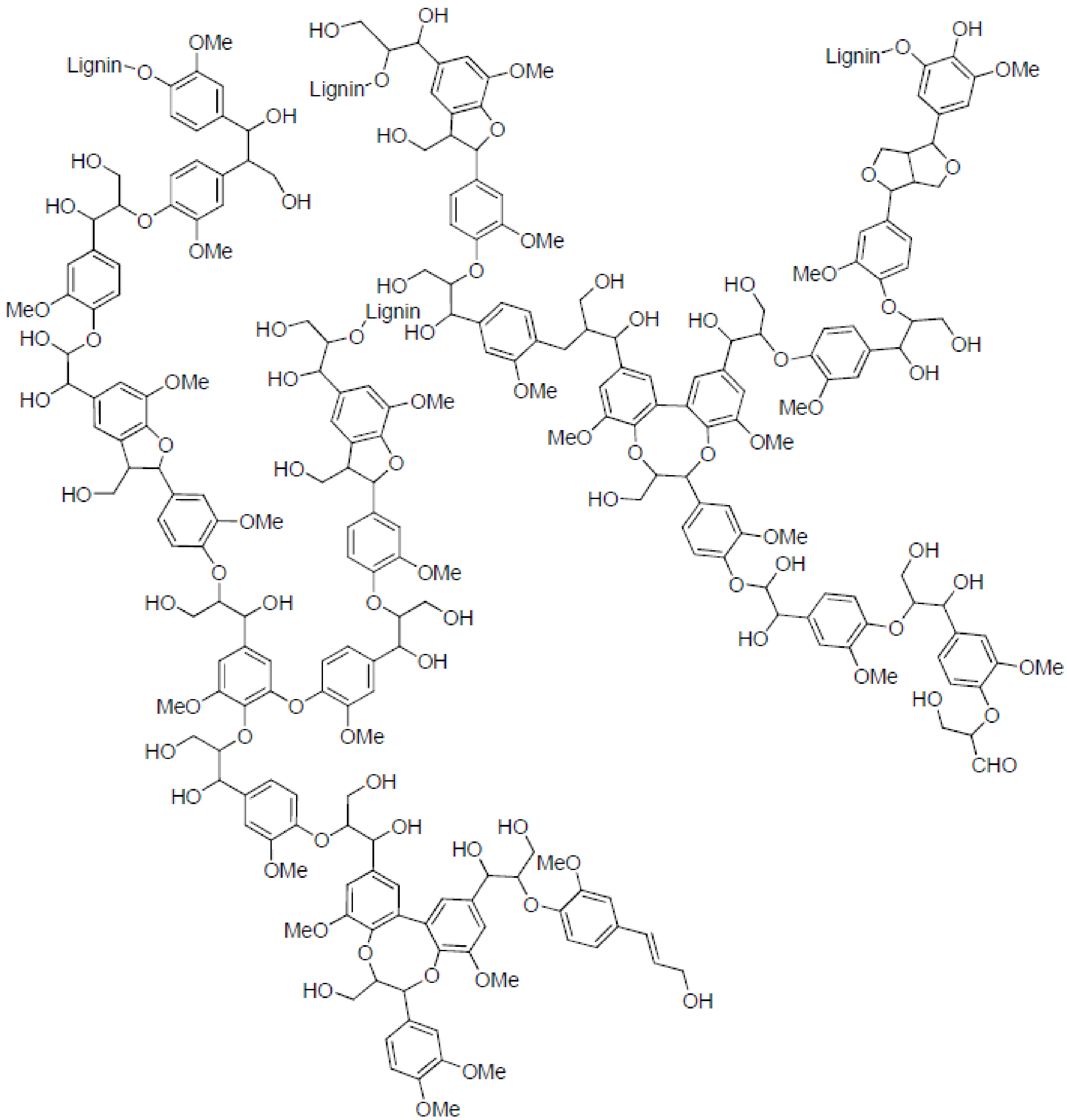

Figure 1-5. Proposed structure of the lignin (Ralph *et al.* 2004)

1.7. Recalcitrant Nature of the Biomass

Various biomasses contain different amounts of cellulose, hemicellulose and lignin. Generally biomass contains 35-50% cellulose, 20–40% hemicellulose and 20–30% lignin by weight (Chandra *et al.* 2007, McKendry 2002). Biomass recalcitrance is directly related to the inherent properties, such as lignin content, cellulose accessibility to cellulase (CAC) and crystallinity (CC). To overcome the recalcitrant nature of the biomass, it is generally subjected to pretreatment that can alter the cell wall structure. This provides the accessibility to cellulase enzymes to cellulose substrate during enzymatic saccharification.

1.8. Research Objectives

The following are the main objectives undertaken in this study:

1. To optimize the pretreatment conditions using dilute acid pretreatment on 4 different agricultural residues namely sorghum-BMR, sorghum-NBMR, kenaf, sunn hemp, and sunflower hulls.

2. To determine the rate coefficients and Arrhenius parameters for xylan hydrolysis and subsequent de-hydration of furfural for the above mentioned feedstocks by considering a pseudo fist order kinetic model.

3. To perform enzymatic hydrolysis on the pretreated substrates using cellulase enzymes and determine the efficacy of the conversion of fermentable sugars for each feedstock.

4. To explore the effect of pretreatment and enzymatic hydrolysis using Lewis acid agents as compared with dilute acids on corn stover biomass.

2. LITERATURE REVIEW

The major components of lignocellulosic feed stocks are polymers known as cellulose, hemicellulose and lignin. Apart from these groups, there are trace level components, mainly ash, proteins, nitrates and nitrites.

2.1. Bio- Refinery Process

One of the proposed processes to overcome the recalcitrant nature of the biomass is to perform pretreatment. Before discussing in detail each pretreatment process, a brief overview of the production of biofuel is presented.

There are four essential steps in the bio-conversion of biomass into biofuel, 1) pretreatment, 2) enzymatic saccharification, 3) fermentation, and 4) product purification as evident from Figure 2-1 (Wyman 1999b) .

Large bales of biomass are first sent to a feedstock handling area to alter the macroscopic structure of the biomass into microscopic so that higher yields of hemicellulose hydrolysis can be achieved during pretreatment (Zhu *et al.* 2005). Pretreatment is followed by enzymatic saccharification to convert crystalline cellulose into fermentable glucose by the action of cellulase enzymes. Next, solutions containing hemicellulose and glucose monomers are fermented into

biofuel through a genetically engineered strain, such as *Escherichia coli*. Biofuel is recovered from the fermentation through distillation and other separation steps to remove residual water (Mosier *et al.* 2005). The unreacted lignin, cellulose, hemicellulose and ash are recovered from the distillation column and burned as fuel to supply the power for the process, or converted to other co-products (Galbe, Zacchi 2007).

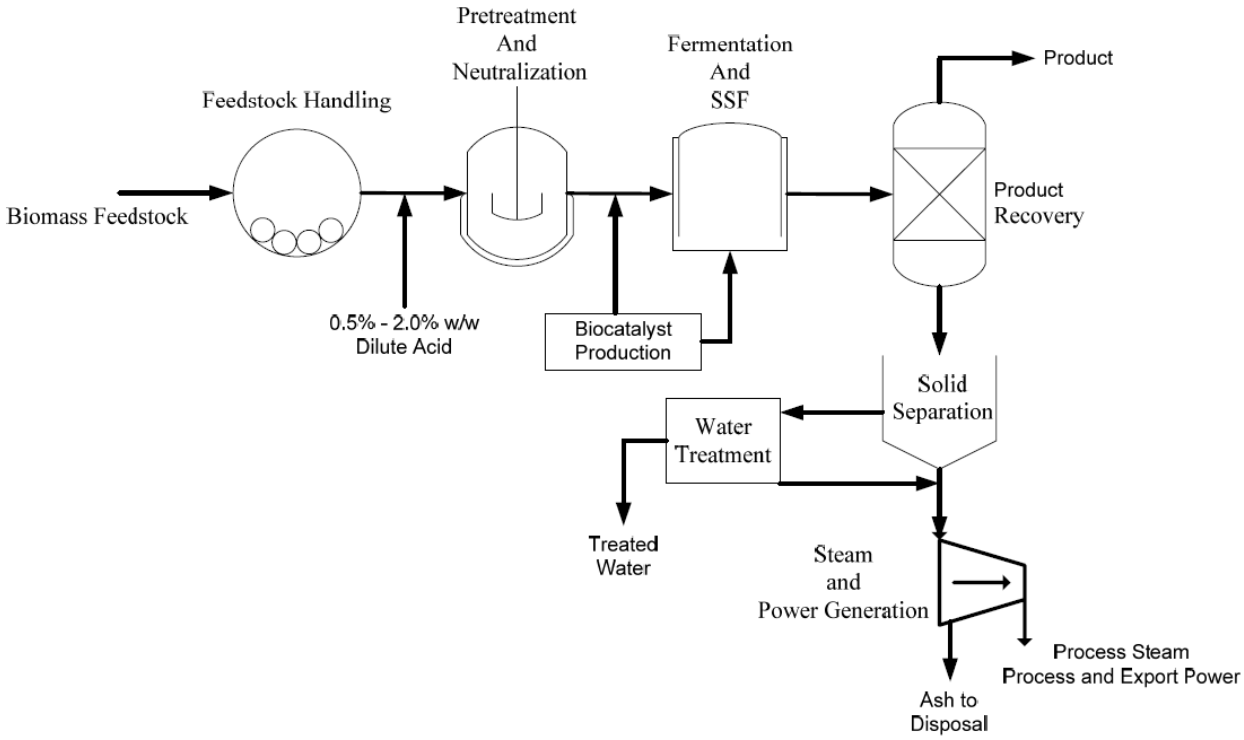

Figure 2-1. Schematic of biomass conversion into biofuels by NREL process configuration

2.2. Pretreatment Methods

They are generally classified into three different groups, physical, biological, and chemical. This literature review primarily focuses on chemical pretreatments.

2.2.1. Physical Pretreatment

Chipping, shredding, grinding and milling are amongst the different mechanical size reduction methods that have been used to enhance the digestibility of lignocellulosic biomass (Angelidaki *et al.* 2009). Physical pretreatment is generally associated with a change in macroscopic structure that can enhance the surface area and reduce the degree of polymerization (DP) (Sun, Cheng 2002). Chipping reduces the biomass size to 10-30 mm; grinding and milling reduce the size to 0.2-2mm. Further reduction in biomass size less than 0.4 mm has little effect on monomer carbohydrate yields (Chang *et al.* 1997). Moreover, grinding and milling can reduce heat and mass transfer limitations. The energy requirement for size reduction of the biomass depends on both biomass characteristics and final particle size. For hardwoods, this requirement is higher than for agricultural residues. This is one of the reasons for considering agricultural residues such as sorghum, kenaf, corn stover over hard woods such as aspen and balsam (Chang *et al.* 1997).

2.2.2. Biological Pretreatments

Biological pretreatment of cellulose, hemicellulose and lignin is done by the action of enzyme producing bacteria. White and brown-rot fungi have been reported to degrade lignocellulose materials, with white-rot being the most effeicient at biological pretreatment of biomass (Lee 1997, Sun, Cheng 2002). The brown rot fungi primarily degrade only cellulose, while white rot fungi degrade both lignin and cellulose by producing enzymes such as peroxidases, polyphenol oxidases, maganese-dependent peroxidases, and laccases that degrade lignin (Hatakka 1994, Vares *et al.* 1993).

The major drawback of biological pretreatment is that the rate is too slow for industrial purposes (Agbor *et al.* 2011). Moreover, the requirement of carefully maintained growth conditions, and the large amount of space to perform biological pretreatments are the disadvantages that make biological pretreatment less attractive on an industrial scale (Agbor *et al.* 2011). Microorganisms consume some of the cellulose and hemicellulose during pretreatment, which may be a major drawback for this kind of pretreatment. Biological pretreatment could be exploited as a first step default pretreatment in combination with another pretreatment method or on its own if the biomass has a low lignin content (Itoh *et al.* 2003, Magnusson *et al.* 2008). However for large scale production it is still in its infancy stage. Further research with genetically engineered fungi with high yields can help commercialize this process.

2.2.3. Pretreatment with Water

2.4.3.1. Liquid Hot Water Pretreatment (LHW)

LHW pretreatment results in hydrolysis of hemicellulose and removal of some portion of the lignin, thus making cellulose in the biomass more accessible to cellulases during enzymatic hydrolysis (Yang, Wyman 2004). This process focuses on the pretreatment of the biomass between 180-210°C under pressure for 20-40 min (Bobleter 1994). The flow through reactor configuration in which a stationary bed of lignocellulosic biomass impregnated by hot water was reported by Yang and Wang (2004) to be more effective for removing hemicellulose and lignin (Yang, Wyman 2004). Hot water cleaves hemiacetal linkages thus liberating acids during hydrolysis, which facilitates the breakage of ether linkages in biomass (Antal Jr 1996). Moreover, the cleavage of O-acetyl groups and uronic acid during hydrolysis could catalyze the

formation and removal of oligosaccharides, or further hydrolyze hemicellulose to monomeric sugars (Mosier *et al.* 2005). However, longer reaction times lead to the formation of fermentation degradation products such as furfural and hydroxy methyl furfural (HMF) (Palmqvist, Hahn-Hägerdal 2000).

The advantages of LHW treatment is that moderate temperatures between 180-210 °C are used, minimizing the formation of degradation products. This eliminates the need for a final washing step or neutralization because the pretreatment solvent here is water. The low cost of the solvent is also an advantage for large scale application (Agbor *et al.* 2011).

The disadvantage of LHW is that the amount of solubilized product is lower compared to other treatments. Downstream processing is also more energy demanding because of the large volumes of water involved.

2.4.3.2. Steam Pretreatment (SP)

Biomass is usually pretreated with high pressure saturated steam. The operating temperatures are around 180-280°C and pressure between 0.7 to 4.8 MPa (Saddler *et al.* 1993). The reaction time for this process is usually between 3-10 min to hydrolyze hemicellulose into monomers. The major fraction solubilized in the liquid phase is dominated by xylose while lignin is transformed as a result of high temperature. Acetic acid generated from acetyl groups associated with hemicellulose mediates the hydrolysis of hemicellulose hence this process is termed as auto hydrolysis (Weil *et al.* 1998). At high severity (270°C, 1 min), SP results in optimal hemicellulose solubilization but lower temperature and longer residence time (190°C, 10

min) have shown to be more favorable, since they help to avoid the formation of sugar degradation products that inhibit subsequent fermentation(Wright 1988).

The advantages of SP are that 1) It makes a limited use of chemicals; 2) It does not result in excessive dilution of the resulting sugars; 3) It requires a low energy input with no recycling or environmental costs (Wright 1988).

The disadvantages of SP are 1) Incomplete destruction of lignin-carbohydrate matrix, which results in the biomass less digestible during enzymatic hydrolysis; 2) Possible generation of fermentation inhibitors at higher temperatures; 3) The need to wash the hydrolyzate, which may decrease overall saccharification yields by 20–25% (Weil *et al.* 1998).

2.2.4. Chemical Pretreatment

Chemicals such as Bronsted acids, alkali, organic solvents, and ionic liquids are reported to have significant effects on the native structure of lignocellulosic biomass during pretreatment (Remsing *et al.* 2006). The pros and cons of each chemical pretreatment of biomass are further elucidated in the following sections.

2.4.4.1. Alkaline Pretreatment

Pretreatment with alkali, such as NaOH, KOH, $Ca(OH)_2$, causes swelling of biomass, which increases the internal surface area of the biomass, and decreases both the degree of polymerization, and cellulose crystallinity (Galbe, Zacchi 2007). It removes lignin and hemicellulose from the lignocellulosic biomass. Saponification of ester bonds in hemicellulose

and the cleavage of glycosyl bonds in hemicellulose/cellulose leads to a drastic loss of sugars in the pretreatment (Kumar *et al.* 2009).

The advantage of alkaline pretreatment is that it utilizes low reaction temperatures (100-180 °C) and pressures (1-3 atm). This leads to less sugar degradation as compared to acid pretreatment.

The major disadvantage of this pretreatment is that it has to be carried for hours and sometimes even days depending on the feedstocks. The major function alkali hydrolysis is to de-polymerize and hydrolyze lignin from the biomass. However, according to the latest study conducted by obtaining (Zhu, Pan 2010) hydrolysis of hemicellulose in the liquid phase is imperative for higher yields of fermentable sugars during enzymatic saccharification as compared to the removal of lignin. Hence biomass pretreatment with alkali leads to lower yields of fermentable sugars.

2.4.4.2. Ammonia Fiber Expansion (AFEX)

AFEX is a physic-chemical process; it is similar to SP operating at high pressure but is conducted at ambient temperatures less than 90°C. At ambient temperatures the pretreatment can take up to10–60 days while at higher temperatures (150–190°C) the effect of ammonia is rapid and the duration of pretreatment is reduced to minutes (Alizadeh *et al.* 2005). In the AFEX process the biomass is exposed to ammonia at a given temperature and high pressure which causes swelling and phase change in cellulose crystallinity of biomass in addition to the alteration and removal of lignin (Foster *et al.* 2001). This increases the reactivity of the

remaining carbohydrates after pretreatment (Vlasenko *et al.* 1997). The pretreated biomass is easily hydrolyzable with close to theoretical yields after enzymatic hydrolysis at low enzyme loadings compared to pretreated biomass from other pretreatment methods.

In AFEX pretreatment, the biomass is brought in contact with anhydrous liquid ammonia with a loading ratio of 1:1 to 1:2 (1–2 kg of ammonia/kg of dry biomass) for 10–60 min at 60–90°C and pressures above 3 MPa (Agbor *et al.* 2011). The biomass and ammonia mixture is heated for about 30 min to the desired temperature in a closed vessel. After reaching the desired temperature, the vent valve is opened rapidly to relieve the pressure. This rapid release causes evaporation of the ammonia that is volatile at atmospheric pressure and this in turn leads to drop in temperature (Dale, Moreira 1982). The chemical effect of ammonia under pressure causes the cellulosic biomass to swell, thus increasing the accessible surface area while de-crystallizing cellulose. This results in a phase change in the crystalline structure of cellulose (O'Sullivan Antoinette C 1997). A small amount of hemicellulose is solubilized in the AFEX treatment.

The advantages of AFEX treatment is 1) the liquid hydrolyzate is favored by fermentation organisms as no conditioning is required (Teymouri *et al.* 2005); 2) the ammonia from the process can be recovered and recycled back thus making this process continuous; 3) ammonia pretreatments have a high selectivity for the reaction with lignin; 4) it eliminates the water washing step since ammonia is volatile and superheated ammonia vapor at 200 °C is used to strip the residual ammonia in the pretreated biomass; and 5) ammonia could serve as a nitrogen source during the subsequent fermentation process.

The disadvantage of AFEX process is that it is ineffective when the biomass has high

lignin content (McMillan 1994). The stench of ammonia has a negative impact on pilot and industrial scale applications. The environmental concern about this process is debatable.

2.4.4.3. Lime Pretreatment (LP)

Lime pretreatment is a physicochemical low cost alkaline pretreatment to enhance the digestibility of biomass (Chang, Holtzapple 2000). The pretreatment utilizes aqueous $Ca(OH)_2$ at low temperatures and pressures as a pretreatment agent to solubilize hemicellulose and lignin (Chang *et al.* 1997). The LP reaction temperature ranged between 25–130°C. At ambient temperatures (25°C) the LP could take weeks whereas at high temperatures (120°C) only 2 h were required for the pretreatment of switch grass, solubilizing ≈ 26% xylan and 29–33% lignin (Chang *et al.* 1997). The LP cleaves acetyl linkages of the hemicellulose thus dissolving lignin in the liquid phase (Chang *et al.* 1998). Oxidative factors come into play when oxygen is introduced at high pressures to enhance the pretreatment.

The advantages of LP pretreatment are that it requires low reagent amounts as compared to sodium, potassium, and ammonium hydroxide. It is also easier to recover calcium carbonate (Sharma *et al.* 2002). Moreover, LP performed at temperatures below 100°C results in lower energy demands as compared to other processes. It also reduces the capital cost, since the use of expensive alloys in construction of pretreatment reactors is not required (Wyman *et al.* 2005a). Large piles of biomass could simply be pretreated without the need for high pressure reactors and using a simple design for pilot plant construction.

The disadvantage of LP is that it is not very effective for removing lignin efficiently in the cases of high lignin biomass (Chang *et al.* 2001). There is a significant loss of hemicellulose and cellulose as the LP is not very efficient reagent. LP requires large volumes of water for washing and also to reduction of the pH of the cellulose substrate. The oxidation of lignin to other soluble aromatic compounds is a risk due to the possible formation of inhibitors (Hendriks, Zeeman 2009a).

2.4.4.4. Organosolv Pretreatment (OP)

The purpose of organosolv pretreatment is to achieve a lower degree of delignification. OP can occur in a variety of organic or aqueous-organic solvent mixtures with or without a catalyst such as HCl or H_2SO_4. Organic acids including oxalic acid, acetylsalicylic acid, and salicylic acid can also be used as catalysts for the solubilization of hemicellulose and extraction of lignin with organic solvents or their aqueous solutions (Sun, Cheng 2002).

OP are conducted at high temperatures (100–250°C) using low boiling point solvents (methanol and ethanol), high boiling point alcohols (ethylene glycol, glycerol, tetrahydrofurfuryl alcohol) and other classes of organic compounds including ethers, ketones, phenols, organic acids, and dimethyl sulfoxide (Thring *et al.* 1990). OP with alcohol removes lignin extensively and results in a complete hemicellulose solubilization by: 1) Hydrolyzing the internal lignin bonds, as well as the ether and 4-O-methylglucuronic acids ester bonds between lignin and hemicellulose; and 2) Hydrolyzing glycosidic bonds in hemicellulose, and partially in cellulose depending on process conditions (Zhao *et al.* 2009).

The advantage of OP is that this method readily yields three separate fraction's lignin, hemicellulose stream, and relatively pure cellulose stream (Duff, Murray 1996). Organosolv lignin is sulfur free, with a high purity and low molecular weight. It can be used as fuel to power the pretreatment plant or be further purified to obtain high quality lignin which is used a substitute for polymeric materials such as phenolic powders resins, polyurethane, polyisocyanate foams and epoxy resins that are used for the manufacture of bioplastics (Zhang 2008). This is the only pretreatment method that can handle biomasses with high lignin content. Organic solvents from the process can be easily recovered through a distillation process (Pan *et al.* 2005). OP is one of the few pretreatment processes that does not require a significant size reduction of the biomass particles making it less energy intensive (Silverstein *et al.* 2007).

The disadvantages of OP pretreatment are 1) the cost of catalyst makes OP pretreatment expensive; 2) side reactions such as the acid catalyzed degradation of monosaccharides into furfural and 5-HMF that are inhibitory to fermentation microorganisms, and 3) the use of a volatile organic liquid at high temperature may lead to inherent fireand explosion hazards, environmental, health and safety concerns.

2.4.4.5. Carbon Dioxide Expansion

This pretreatment involves the use of supercritical CO_2 to enhance the digestibility of lignocellulosic biomass. In this process the biomass is placed in a high pressure vessel and the vessel is heated to a desired temperature. The supercritical CO_2 penetrates the wetted biomass (Hendriks, Zeeman 2009b) and aids in hydrolysis of hemicellulose and the release of the pressurized gas resulting in the disruption of the biomass native structure and increasing the

accessible surface area (Zheng *et al.* 1995).

The advantages are that CO_2 is of low cost as a pretreatment solvent, no generation of inhibitory products, the use of low temperatures and high solids capacity are attractive features. However, the disadvantage includes high equipment costs, since it requires high pressure (170-240 bar) conditions during the CO_2 pretreatment. This is a major limitation to the application of this process on a large scale (Agbor *et al.* 2011).

2.4.4.6. Ionic Liquids (IL)

A novel approach to physicochemical pretreatments of the biomass is the use of ionic liquids. IL are solvents that have high polarities, thermal stabilities, negligible vapor pressures and low melting points (< 100°C) consisting entirely of ions (Wasserscheid, Keim 2000). Most ILs used in biomass fractionation are imidazonium salts (Wasserscheid, Keim 2000). Studies show that 1-allyl-3-methylimidazonium chloride (AMIMCl) and 1-butyl-3-methylimidazonium chloride (BMIMCl) can be used efficiently as non-derivatizing solvents for the dissolution of cellulose at temperatures below 100°C (Zhang, Lynd 2006). The mechanism of IL action is tha they cleave the hydrogen bonds from the carbohydrate chain thus disrupting the cell wall structure (Moulthrop *et al.* 2005).

The advantages of IL are that they are generally environmentally friendly (Pu *et al.* 2007) and can be recovered and reused by using various methods such as pervaporation, reverse osmosis, salting out, and ionic exchange (Zavrel *et al.* 2009). However, the economic analysis suggests that this process is one of the most expensive ways to convert biomass into biofuels.

2.4.4.7. Dilute Acid (DA) Pretreatment

The pretreatment of biomass using DA has received much attention among all the pretreatment methods (Lee *et al.* 1999). The DA is a well-developed process that has high reaction rates and improved cellulose hydrolysis (Cara *et al.* 2008). Among the DA process agents used are hydrochloric acid, nitric acid, phosphoric acid, or sulfuric acid (Torget *et al.* 1992a). Dilute sulfuric acid is generally used as a reagent since it is the amount of inhibitor products formed during pretreatment are low as compared to other Bronsted acids (Nguyen, 2000). Moreover it is less corrosive than HCl. It is mixed with biomass to solubilize hemicellulose therefore increasing the accessibility of cellulose in the biomass (Kim *et al.* 2000).

In the DA process, dry biomass is added to a solution of sulfuric acid (< 4 wt%) and heated to a desired temperature in a batch or multiclave reactor (Torget *et al.* 1990). DA pretreatment is carried at temperatures ranging from 140 to 215°C. The residence time ranges from 5 to 90 minutes depending on the temperature of the pretreatment. The DA pretreatment promotes hemicellulose hydrolysis to shorter chain oligomers in the first step and later into monomeric forms (Nguyen 2000).

The advantages of DA are that high reaction rates lead to very economical process. According to the study conducted by (Lloyd and Wyman, 2005) it would cost around $1.25/G to produce bioethanol. It has a higher yield in hemicellulose hydrolysis as compared to any other process discussed in the above sections.

The disadvantages of the DA process include the production of fermentation inhibitors such as furfural and HMF. Neutralization of the liquid phase is necessary before fermentation. Expensive alloys have to be used in the construction of reactors as inexpensive metals are prone to corrosion in the acidic environment at high reaction temperatures.

2.4.4.8. Pretreatment with Lewis Acids

This pretreatment uses inorganic Lewis acids primarily composed of metal salts such as $FeCl_3$, $FeCl_2$, $CuCl_2$, $AlCl_3$. These Lewis acids have the ability to acidify the solution by forming ligand complexes with water molecules (Yu *et al.* 2011). They hydrolyze hemicellulose in the biomass into its monomeric constituents. Generally the reaction temperature used in the pretreatment is lower compared to dilute acid pretreatment. The reaction temperature usually ranges between 140-160°C. The reaction time ranges from a few seconds to minutes (Li *et al.* 2008).

The advantages of this pretreatment are that the reaction conditions are less severe. The rate of hemicellulose hydrolysis is generally higher than that of the dilute acid pretreatment (Kamireddy *et al.* 2013a).

The disadvantages of this pretreatment are that the Lewis acids are more expensive than Bronsted acids or alkali agents. Moreover, they have some adverse effects during fermentation as Lewis acid promotes complex reactions that result in formation of very viscous tar like substance called humins at high reaction temperatures (Li *et al.* 2008).

2.3. Pretreatment Process Economic Analysis

Pretreatment is one of the most costly steps in biofuel production, accounting for about 33% of the total processing costs in the base case of NREL design. This value has been underestimated real importance of pretreatment since it greatly affects the enzymatic hydrolysis and fermentation process. The 33% evaluation also takes into account the cost of de-toxification of several inhibitory through various processes.

The formation of inhibitory products during pretreatment is unavoidable since they originate from, 1) hydrolysis of extractive components, organic and sugar acids esterified to hemicellulose fraction (acetic, formic acids) and solubilized lignin derivatives; 2) degradation products of solubilized sugars (furfural, HMF); 3) degradation products of lignin (cinnmaldehyde, syringaldehyde); 4 corrosion products (metal ions from dissociation of acids).

(Eggeman, Elander 2005) has compared and published plant level cost for producing one gallon of biofuel for several pretreatment options. The plant level cash cost is also the same as the lowest ethanol price at which the plant will stay operational, even though the plant would be losing money at these market conditions. As such, it defines the competitive position of the proposed facility within the existing ethanol market. In the analysis, cash cost is comprised by six components: net stover, other variable costs, and fixed costs without depreciation as evident from Figure 2-2. Net stover, by analogy with the net corn concept used in corn processing, is defined as the cost of stover feedstock less the value of the electricity co-product. Other variable costs accounts for the cost of enzymes, chemicals, etc. in which the quantities required are tied to the plant production rate. Fixed costs include labor, maintenance, insurance, and other costs not

tied to production rate.

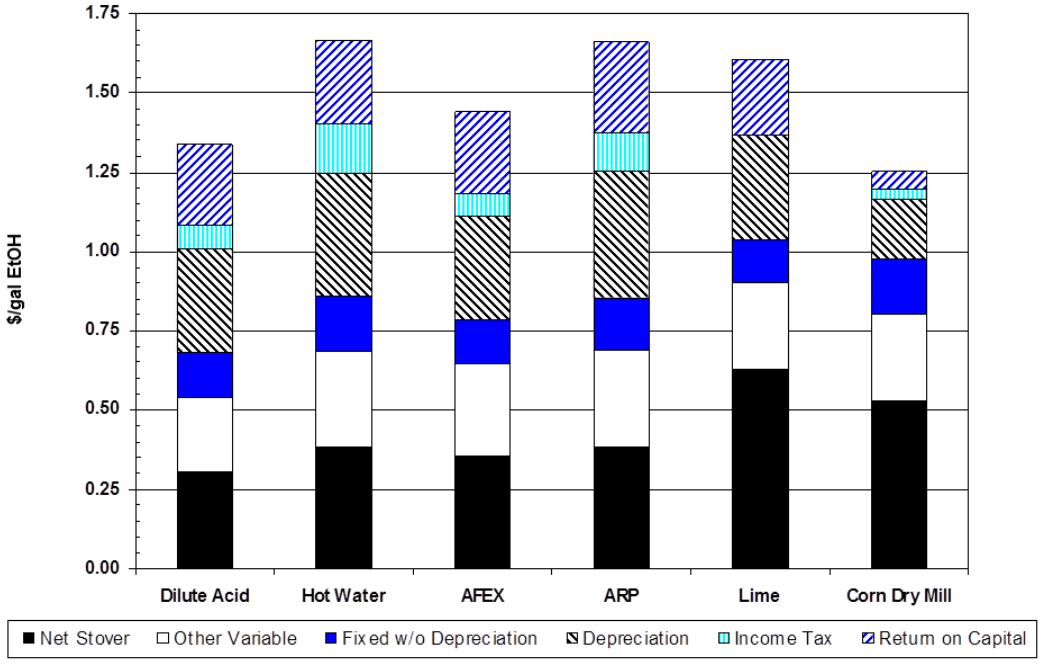

Figure 2-2. Comparison of the cost of various pretreatment technologies (Eggeman, Elander 2005)

2.4. Hydrolysis of Pretreated Biomass

The nature of the plant cell wall was evolved in such a manner that it is highly recalcitrant to degrade with individual enzyme. The complex and diverse structure of plant cell wall necessitates a variety of enzymes for hydrolysis of cellulose and hemicellulose (Foster *et al.* 2001). For efficient hydrolysis of polysaccharides in plant cell walls requires synergistic action of different proteins that meant to cleave different substrates (Kamireddy *et al.* 2013a). Cellulase is a complex mixture of diverse enzymes namely endo- glucanases, exo-glucanases, and β-glucosidases. These enzymes has been detected as intracellular and extracellular enzymes in fungi such as *T.reesei*.

Endo-glucanases randomly cleave the amorphous regions of cellulose substrates into shorter cellulose oligomers (Chandra *et al.* 2007). The average size of endo-glucanase is around 35-75 KDa (Dalton).

Exo-glucanases acts on the cellulose chain from reducing and non-reducing ends of shorter chain oligomers and release cellobiose as the main product. Cellobiohydrolases are larger molecules than the endo-glucanases with average of 41-85 KDa and are also glycosylated (Chen *et al.* 2012).

β-glucosidases hydrolyze the cellobiose and cello-oligosaccharides from the non-reducing ends and form glucose as the end product of cellulose hydrolysis. It also helps in competitive inhibition of cellobiose (Leu, Zhu 2012). β-glucosidases is the largest of all three cellulolytic enzymes and has a molecular weight from 41-170KDa. Like endo and exo glucanases most of the β-glucosidases are also glycosylated.

In order to effectively hydrolyze hemicellulose that are rich in pentose polymer carbohydrates are hydrolyzed into monomers using xylanases. They are known to catalyze the hydrolysis reaction of β-(1-4) bonds between xylan chain including xylo-oligosaccharide (XOS). The reactivity of XOS with xylanases reduces with decrease in the degree of polymerization (Wright 1988). Few xylanases are specific towards the xylan molecules, although some are able hydrolyze cellulose as well (Foster *et al.* 2001). The overall reactivity of the xylanases are effected by acetylation and also the presence of auxiliary debranching enzymes such as α-glucurunidase, α-arbinofuranosidase, and acetylxylan esterase. The xylanases have molecular weight of 10-85 KDa. Xylanases below 30 KDa or basic proteins and above 30 KDa are acidic

proteins (Kim *et al.* 2000).

2.5. Concluding Remarks on Pretreatment Processes

The pros and cons of each chemical agent have been discussed in the above sections. The primary goal of pretreatment is to overcome the recalcitrant nature of the biomass and to convert it into biofuels and value-added chemicals. In order to achieve this goal, chemical agents are added during the pretreatment. This improves the fermentable sugar yields during the pretreatment and enzymatic hydrolysis.

Based on the literature review, it can be concluded that the pretreatment using dilute acid is one of best viable options for pretreatment. Moreover, a recent study conducted by (Leu, Zhu 2012) concluded that hydrolysis of hemicellulose is more important as compared to the removal of lignin for higher fermentable sugar yields during pretreatment. This results in higher yields during enzymatic saccharification for all agricultural feedstocks (Kamireddy *et al.* 2013b).

To make biofuel competitive from the economical point of view as compared to conventional fossil fuels it is necessary to improve the fermentable sugar yields during pretreatment. The improvements should lead to lower energy utilization and also minimum amount of degradation products during pretreatment.

3. CONVERSION OF FORAGE SORGHUM, SUNN HEMP AND KENAF INTO BIOFUELS THROUH DILUTE ACID PRETREATMENT

3.1. Abstract

Forage Sorghum (*Sorghum bicolor* (L.) Moench), brown mid-rib (SBMR) and sorghum non brown mid rib (SNBMR) species; sunn hemp (*Crotalaria juncea* L.) and kenaf (*Hibiscus cannabinus* L) are primarily used as forage and fiber crops, respectively. In this study, these crops were evaluated as feedstocks for biofuels and value added chemicals. This was achieved using dilute acid pretreatment and enzymatic hydrolysis using commercial cellulase enzymes. The highest hemicellulose yield was observed for SNBMR 95 wt%, followed by SBMR with 91 wt% at combined severity factor (CSF) 1.56 and 1.44 respectively, similarly for sunn hemp and the kenaf yield was observed at 72 and 80 wt% at CSF 1.48, 1.72. At harsher pretreatment conditions, the hemicellulose yield decreased in all the biomasses due to degradation. In similar fashion, the overall glucan saccharification yield after enzymatic hydrolysis for SNBMR was found to be 90 wt% followed by kenaf 88wt%, SBMR 84 wt% at CSF 1.47, 1.72 and 1.24. For sunn hemp it was observed to be 68 wt% at CSF 2.06. This was mainly due to the high crystallinity index of sunn hemp as compared with that of sorghum. Overall, from the results it

can be concluded that SBMR and SNBMR have a greater potential for biofuel production as compared with sunn hemp biomass.

3.2. Introduction

In U.S, it is proposed that biofuels should supply up to 30% of the US transportation fuel requirement, which is currently 140 BGY (Billion Gallons per Year) (Singh 2012). The current U.S. Renewable Fuels Standards (RFS2) goal is to produce 36 BGY of transportation fuel from renewable sources, as indicated originally in the Energy Independence and Security Act of 2007 (EISA), which became effective on July 1, 2010. The North Central states contribution to the RFS2 goal for 2022 is expected to be approximately 43% (Singh 2012). The US Department of Agriculture (USDA) estimates 13.4 BGY of the 2022 total goal will be provided by dedicated energy crops (Kamireddy *et al.* 2013b). To achieve this goal a new source of lignocellulose feedstocks that can have a higher yield of dry matter per hectare is necessary. In addition pretreatment, and conversion efficiencies have to be improved.

Lignocellulosic biomass generally consists of cellulose, hemicellulose, lignin, and ash in varying amounts. The conversion of lignocellulosic biomass usually follows three steps 1) Pretreatment; 2) Enzymatic hydrolysis; 3) Fermentation (Christakopoulos *et al.* 1993). Although there are different ways to pretreat biomass, such as dilute acid, alkali, and steam pretreatment. Pretreatment with dilute acid is still considered an effecient and relatively inexpensive method for several types of biomasses (Saha *et al.* 2005). Dilute acid pretreatment cleaves the hemicellulose linkages into monomeric pentose sugars so that the crystalline cellulose is accessible to cellulase enzymes during enzymatic hydrolysis. According to the study conducted by (Leu, Zhu 2012),

hemicellulose removal is more important than lignin removal for efficient pretreated substrate digestibility by cellulases.

Grain sorghum is known as an energy crop which can be easily converted to ethanol. Sorghum bagasse has a great potential as a lignocellulosic feedstock, since its greater adaptability to various climatic conditions and drought tolerance, low water and high nutrient use efficiency, and high biomass yields per hectare (Rooney *et al.* 2007). This is primarily because they are photoperiod sensitive (Meki *et al.* 2013). Forage sorghum has high above ground dry matter (ADM) compared with other dedicated energy crops such as switchgrass (*Panicum virgatum*) and miscanthus (*Miscanthus x giganteus*). Forage sorghum can grow in areas that are completely unfavorable to corn (*Zea mays* L.) production. It can grow to height of 1.8 to 4.5 m (Dien *et al.* 2009). According to the study conducted by (Rocateli *et al.* 2012), sorghum biomass can yield up to 26.04 to 30.3 tons/ha. There are three different types of SBMR mutants frequently grown in U.S (*bmr-6*, *bmr-12*, and *bmr-18*) (Sarath *et al.* 2008). These *bmr*-mutants are primarily preferred due to 2-5 wt% lower lignin content. Generally, lignin is highly resistant to chemical cleavage and higher content and may physically act as a barrier for the cellulase enzymes. Lower lignin in the cell walls and stem structure found to be less impervious to cellulolytic fermentation in the rumen for cattle. Hence, SBMR silage has a significant potential to serve as warm season forage for lactating dairy cattle (Oliver *et al.* 2005). Similarly, it was assumed that a higher lignin content could have inverse correlation during enzymatic hydrolysis of the pretreated substrates. However, recent studies suggest that for herbaceous biomasses no such correlation is valid (Zhu *et al.* 2012).

Kenaf is a warm-seasonal annual plant in the *Malvaceae* family closely related to cotton (*Gossypiumhirsutum L.*). It is native to tropical Africa and was introduced into the United States in the 1940s as a substitute for jute (*Crochorus olitorious L.)*. Kenaf is used in a number of applications such as papermaking, production of twine, liquid absorbents and composites. It is a woody herb that requires less water, fertilizers and pesticides than most of the other crops while producing one of the highest biomass yields of 15-19 tons/ha (Murphy *et al.* 2007).

Sunn hemp is a tropical legume crop that can grow between 1.2 to 1.8 m in height for 9 to 12 weeks (Mansoer *et al.* 1997). It has a rapid growth rate, and low nutrient requirements. Sunn hemp dry matter biomass yield can fluctuate between 7.6 and 12.2 tons/ha (Cantrell *et al.* 2010) . Sunn hemp has been evaluated as a source of forage crop for cattle. It was evaluated as a bioenergy feedstock using the pyrolysis method (Cantrell *et al.* 2010). However, it has never been evaluated as a source for biofuel generation via biochemical conversion process.

The primary objective of this study was to pretreat SBMR, SNBMR, sunn hemp, kenaf feedstocks under a set of similar conditions and compare which biomass results in higher fermentable sugar yields (hemicellulose and glucan saccharification yields).

3.3. Experimental Methods

3.3.1. Biomass Harvest

All the four biomasses were grown and harvested from the North Dakota State University experimental site in Fargo and Prosper, ND. All the crops were harvested at the end of August of 2011. As it was proved from the studies conducted by (Mansoer *et al.* 1997) that harvesting

between these periods may lead to high biomass yields. They were harvested between 50-60 days after plantation.

The biomasses were washed to remove soil residues that were present during harvesting as they can be a source of error during compositional analysis. In addition, this process removes the extractable ash from the biomasses. All the biomasses were air dried so that the final moisture content in the biomass was less than 5 wt%. These dried biomasses were pulverized in a Wiley mill from the NDSU Plant Science department. The particle size distribution of the biomasses ranged between 50 to100 µm. All the biomasses were stored in zip-lock bags at room temperature.

3.3.2. Compositional Analysis

It was necessary to remove the inorganic structural material from the biomass prior to the analysis to prevent interference with the downstream process of the biomass sample. Failure to remove these extractives may result in an error in structural sugars concentration values. They tend interfere with un-structural free carbohydrates present in the biomass. It also may result in falsely high lignin values when unhydrolyzed carbohydrates condense with acid insoluble lignin. Composition of the raw SBMR, SNBMR and sunn hemp was measured according to the National Renewable Energy Laboratory (NREL) LAP protocols. A two-stage extraction process (24 h of water extraction followed by 18 h of ethanol extraction) was performed to remove extractives such as nitrites/nitrates, proteins, chlorophyll, and waxes. The extraction was performed using Soxhlet apparatus. The working of Soxhelt extraction procedure is well documented in NREL LAP TP- 510-42619. The biomass loading was 5 g and the volume of

water and ethanol solvents used were 190 ml to remove extractives. The water and ethanol solvents were oven dried and weighed to account for the overall extractives weight. The source and individual components of these extractives were not verified. After extraction, biomasses were oven dried for 12 h at 105 °C. Then the extractive free biomasses were analyzed for structural carbohydrates using HPLC and lignin based on a NREL LAP protocol (NREL/ TP-510-42618).

3.3.3. Pretreatment in a Batch Reactor

Batch reactor system is based upon the 300 mL EZE-Seal reactor made by Autoclave Engineers (Erie, PA). This reactor was equipped with a heating/cooling jacket. In order to mitigate the effect of dissolved ions upon the pretreatment reactions, as well as reduce corrosion, the wetted parts of this reactor were made from Hastelloy-C 276. This reactor was equipped with a magnetic drive system for agitation of the solid slurry as shown in Figure 3-1. To generate the steam used in this process a 3 kW Sussman (Long Island City, NY) saturated steam generator was used in tandem with a custom-built steam accumulation drum. The steam generator was rated for a maximum operating pressure of 689.4 kPa which corresponds to a maximum steam temperature of 166 °C. Although the steam accumulation drum in discussion here was custom built, there were many other options available commercially. The steam accumulation drum was necessary for the system to provide operable system dynamics, given the relatively small internal volume of the steam generator itself. The volume of the steam accumulation drum was 30 L. The steam accumulation drum was well insulated and equipped with a bottoms reboiler to aid in maintaining the steam temperature. The average heating kinetics of the reactor was around 35

°C/min. The agitation was performed by a magnetic motor and was maintained constant at 60 rpm throughout the reaction period. Steam was injected into the reactor from the boiler by operating a 3-way valve manually. Once the desired temperature was reached, the reaction time was commenced. After the desired reaction time, steam was shut off and cooling water was pumped into the external jacket of the reactor. Once the reactor was cooled down below 40 °C, slurry samples were withdrawn from the reactor into polyethylene bottles.

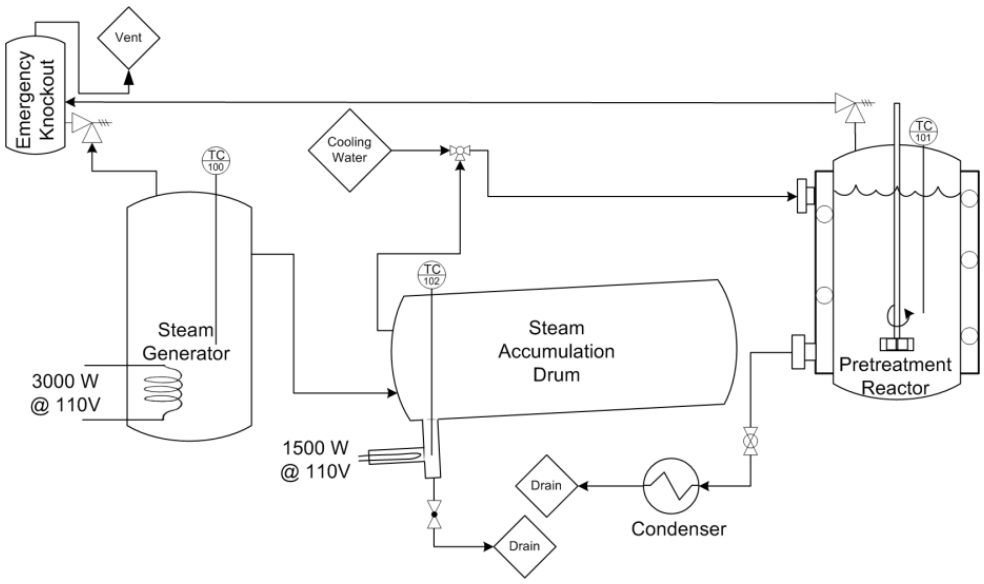

Figure 3-1 Schematic of the batch reactor system used in the pretreatment of lignocellulose.

Dry biomass was added to an appropriate amount of the sulfuric acid solution (deionized water and sulfuric acid) so that the solid to liquid ratio was 1:10. The ratio and the reaction parameters were chosen from our previous pretreatment studies performed on cornstover and sugarbeet.

3.3.4. Fractional Factorial Design

The three independent factors considered for the evaluation of each biomass are acid concentration, reaction temperature, reaction time. The parameters for the three factors are summarized in Table 3-1. Generally a full factorial design with 2^k design that consists of two levels and k being number of factors employed to evaluate effect of different factors on the pretreatment. This led to a total of 54 pretreatments for all the three biomasses combined (18 for each biomass) including two center points and replicates. It turns out, that when the number of pretreatments for a full factorial design is relatively large the desired information can often be obtained by performing only a fraction of the full factorial design (Taguchi 1987), which is often referred to as a fractional factorial design. In other words, fractional factorial design provides an alternative when the number of pretreatments for a full factorial design is too large to be practical (Taguchi 1987). With a fractional factorial design, the effect of independent parameters on a response can be studied in a very economical and practical way (Taguchi 1987). This resulted in a total of 40 pretreatments (10 for each biomass) including replicates and two-center points.

Table 3-1 The coded levels and actual values for the 2^k fractional factorial design.

Run	Coded levels			Actual values		
	Temperature (°C)	Acid concentration (wt%)	Time (min)	Temperature (°C)	Acid conc†(wt%)	Time (min)
1	−1	1	−1	150	2.0	10
2	−1	1	−1	150	2.0	10
3	−1	−1	1	150	1.0	20
4	−1	−1	1	150	1.0	20
5	1	−1	−1	160	1.0	10
6	1	−1	−1	160	1.0	10
7	1	1	1	160	2.0	20
8	1	1	1	160	2.0	20
9	0	0	0	155	1.5	15
10	0	0	0	155	1.5	15

† conc= concentration

3.3.5. Combined Severity Factor

Combined severity factor (CSF) combines the experimental effects of temperature, reaction time and pH to enable an easy comparison of results and to facilitate a process control as given in (Equation 3-1). CSF is derived from the observation that reaction rates double for every 10 °C increase in temperature. The denominator value of 14.75 is the conventional activation energy assuming the overall reaction is hydrolytic and the overall conversion is a first order. The reference temperature is taken as 100 °C since it is assumed that biomass hydrolysis starts above this reference temperature (Lloyd, Wyman 2005). However, in our studies it was evident that pH was a significant factor affecting the pretreatment.

$$CSF = Log_{10}\left[t \times \exp\left(\frac{T_H - T_R}{14.75}\right)\right] - pH \qquad (3\text{-}1)$$

Where t is the reaction time in minutes, T_H is reaction temperature in °C, T_R is a reference temperature generally used as 100 °C and pH is the acidity of the aqueous solution.

3.3.6. Analytical Procedures

Pretreated slurry samples were vacuum-filtered and collected as liquid hydrolyzates and solid substrates. The liquid hydrolyzate samples were analyzed for pentose and hexose saccharides and inhibitor products. This analysis was performed based on the NREL analytical procedures (NREL/ TP- 510-42623). A quantitative analysis for determining monosaccharides present in liquid hydrolyzates was performed by Agilent 1200 HPLC (High Pressure Liquid Chromatography) with Transgenomic CHO-Pb column length 300.0 x7.8 mm (Omaha, NE). All samples were analyzed by HPLC. The mobile phase used for analysis was Milli-Q deionized water with a flow rate of 0.6 mL/min. Prior to analyzing pretreated hydrolyzate samples, a set of calibration standards were run to validate the HPLC RID (Refractive Index Detector). The concentrations of the standards ranged from 0.5 to 18 g/L. In addition, one concentration of 4 g/L was analyzed (for every 8 injections of the samples) to test for analytical system performance. The standard solutions and sugar recovery standard solution consisted of D-(+) glucose, D-(+) xylose, D-(+) galactose, L-(+) arabinose, and D-(+) mannose.

Fermentation inhibitor products such as acetic acid, furfural and 5-hydroxymethyl furfural (HMF) were analyzed using an Agilent 1200 HPLC with Phenomenex Rezex RFQ 100.0 x 7.8 mm column (Torrance, CA). The 0.01 N sulfuric acid mobile phase with a flow rate of 1.0 mL/min was used for analysis (Scarlata, Hyman 2010). The standards for fermentation inhibitor products were obtained from Absolute Standards, Inc (Hamden, CT).

The solid substrates were washed with Milli Q DI water until the pH 7.0 was reached. Then these washed solid substrate was analyzed for cellulose, hemicellulose, and lignin contents. This analysis was based on the NREL analytical procedure (NREL/TP-510-42618).

3.3.7. Enzymatic Hydrolysis

The enzymatic hydrolysis on cellulose rich solid substrate was performed in a thermal incubator (Thermo Scientific, MaxQ 4000) at 50 °C and 250 rpm for 72 h. Hydrolysis was performed with a sodium citrate buffer of a 50 mM/L concentration (pH of 4.8 based on NREL TP/LAP 510-42629) and sodium azide with a concentration of 20 mg/mL. The cellulose loading for enzymatic hydrolysis was 1wt% . A cellulase enzyme commercially available known as Accellerase 1500 (Genencor, Palo Alto CA) was used to perform the enzymatic hydrolysis. The actual amount of protein content in 1 mL of pure enzyme solution was found to be 84.1 mg of protein. Hence, 20 mg of protein /g of cellulose enzyme loading were used to perform the enzymatic hydrolysis. This enzyme loading was based on our previous studies conducted on sunflower (*Helianthus annuus* L.) hulls and sugarbeet (Donkoh *et al.* 2012). After hydrolysis, samples were filtered and analyzed using HPLC for glucan saccharification yield. This analysis was similar to the analysis mentioned in section 2.6 (NREL/ TP- 510-42623). The yield of the

pretreated substrate was calculated by using (Equation. 4-2). The value of 0.9 was used in the equation as a correction factor for hydration.

$$\%\,\mathrm{GSY} = \frac{\text{Conc of glucose after hydrolysis} \times \text{total volume of assay} \times 0.9 \times 100}{\text{Grams of glucan added}} \qquad (4\text{-}2)$$

GSY= glucan saccharification yield

3.4. Chapter Results and Discussion

3.4.1. Gravimetric Analysis

All the four biomass compositions were compared with corn stover, as it is most widely used biomass for the production biofuel through biochemical conversion. The data was obtained by procedures described in section 3.3.2. The SBMR and SNBMR glucan content was lower than the kenaf and sunn hemp. The maximum hemicellulose content was found for SNBMR and it ranged from 17.3 to 23.7%. The lignin content ranged from 13.8 to 17.2 wt%. The amount of extractives was higher for all the biomasses harvested from Fargo as evident from Table 3-2. The source of these extractives was unknown and beyond the scope of this study. The amount of structural ash present in all the biomasses ranged from 0.3 to 6 wt%. This ash content is reasonable for acid pretreatment, since there is a probability that the ash content above 10 wt% may neutralize some of the acid that is used in the pretreatment (Lloyd, Wyman 2003).

Table 3-2 Compositional analysis of different biomasses with two standard deviation

Feedstock	Glucan	Hemicellulose	Lignin	Extractives	Ash
	----------% dry weight----------				
SNBMR	33.9±0.5	23.7±0.4	15.8±0.4	26.0±0.1	3.3±0.3
SBMR	33.7±0.8	21.9±1.1	13.9±0.4	25.4±0.6	4.2±0.1
Sunn hemp	37.1±1.1	21.7±0.5	13.8±1.1	22.6±0.2	5.2±0.3
Kenaf	42.5±4.2	17.3±2.0	17.2±2.1	21.0±1.0	0.3±0.1

3.4.2. Hemicellulose Hydrolysis in the Liquid Fraction Samples

The liquid hydrolyzate samples were analyzed for pentose sugars (xylose, arabinose) and hexose sugars (glucose, galactose, and mannose). The amount of hemicellulose was found to be predominant in all the feedstocks. The amount of hemicellulose concentration increased with severity of the pretreatment, in all the biomasses. However, at high severity pretreatments, there was a decrease in the yields primarily due to degradation. It was mainly due to xylose degradation into furfural (Zhao *et al.* 2007). Moreover, hemicellulose yields in SNBMR were found to be slightly higher than in SBMR, this was due to a higher amount structural ash in SBMR Table 3-2, since higher structural ash content may lead to acid neutralization during pretreatment (Lloyd, Wyman 2004). The highest hemicellulose yield observed for SNBMR was around 93 wt%, followed by SBMR with 91 wt% at a combined severity factors (CSF) 1.56 and 1.44 respectively. However, for sunn hemp and kenaf the maximum hemicellulose yield was observed at 80 and 72 wt% at CSF 1.48 and 1.74 respectively as evident from Figure 3-2. In addition, the pretreated liquid hydrolyzate pH was observed to be different for each biomass.

This explains the different CSF values even though the starting pH were same (based on the acid wt%). This is primarily due to the fact that conversion of hemicellulose to fermentable sugar monomers requires two steps, 1) cleavage of the xylosidic bonds, and 2) cleavage of covalently-bonded acetyl ester groups, as the latter step may be a rate limiting. This explains that the concentration of acetic acid for each biomass was observed to be different as evident from Figure 3-5. This could be the reason for the observed phenomenon that the pH of the pretreated liquid hydrolyzate samples were unique for each biomass (Chen *et al.* 2012).

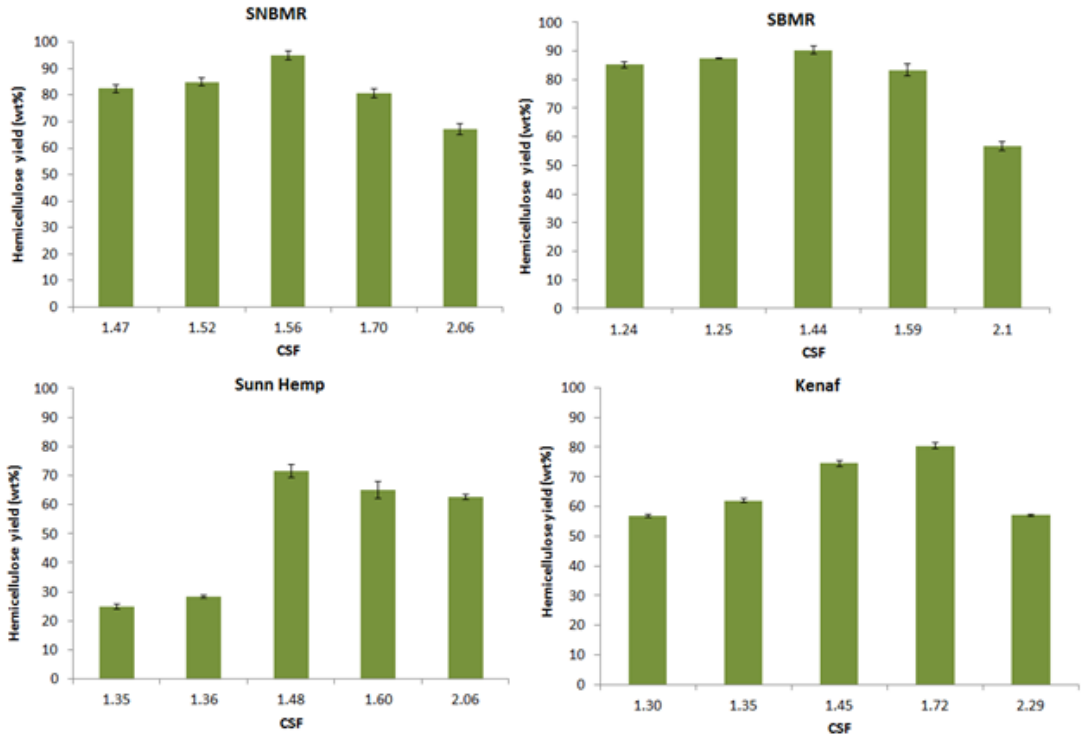

Figure 3-2 Hemicellulose yields of liquid hydrolyzate samples after the pretreatment. SBMR; SNBMR; sunn hemp; kenaf

3.4.3. Composition of Solid Fraction of the Pretreated Substrates

Dilute acid pretreatment is a hydrolytic process that solubilizes the major part of hemicellulose (Torget *et al.* 1990). However, crystalline cellulose and lignin mostly stay intact as the solid substrate after the pretreatment (Torget *et al.* 1990). Dilute acid pretreatment was observed to be more sensitive towards SBMR and SNBMR biomasses as the amount of hemicellulose retained in the solid substrate was relatively low as compared with sunn hemp and kenaf as evident from Figure 3-3. The amount of hemicellulose in SBMR and SNBMR ranged from 1 to 4 wt% in sunn hemp it ranged between 2 and 7 wt% and in kenaf it ranged from 2 to 9 wt%. Nevertheless, the ash content was higher in SBMR and SNBMR as compared to sunn hemp and kenaf. This was primarily due to fact that the amount of extractable ash was relatively higher, since soil particles during the harvest in the SBMR and SNBMR biomass was greater as compared with the other two biomasses. The amount of acid soluble and acid insoluble lignin was found to be lower in SBMR as compared with SNBMR and sunn hemp. The average amount of lignin retained in the pretreated substrate samples ranged from 27-33 wt% for SBMR, 31-37 wt% for SNBMR, 30-36 wt% for sunn hemp and 26 to 31 wt% for kenaf. The results were in agreement with the data of raw composition analysis Table 3-2, as the initial SBMR lignin content was less compared to SNBMR, sunn hemp and kenaf. From Figure 3-3 it is evident that cellulose degraded at harsher pretreatment conditions for SBMR and SNBMR as cellulose yields were observed to be lower. The results suggest that sorghum biomass has a higher amorphous cellulose content as compared to sunn hemp and kenaf (O'Sullivan Antoinette C 1997). The average cellulose retained ranged from 48-55 wt% for SBMR, 50-53 wt% for SNBMR, 48-56 wt% for sunn hemp, and 60 to 63 wt% for kenaf.

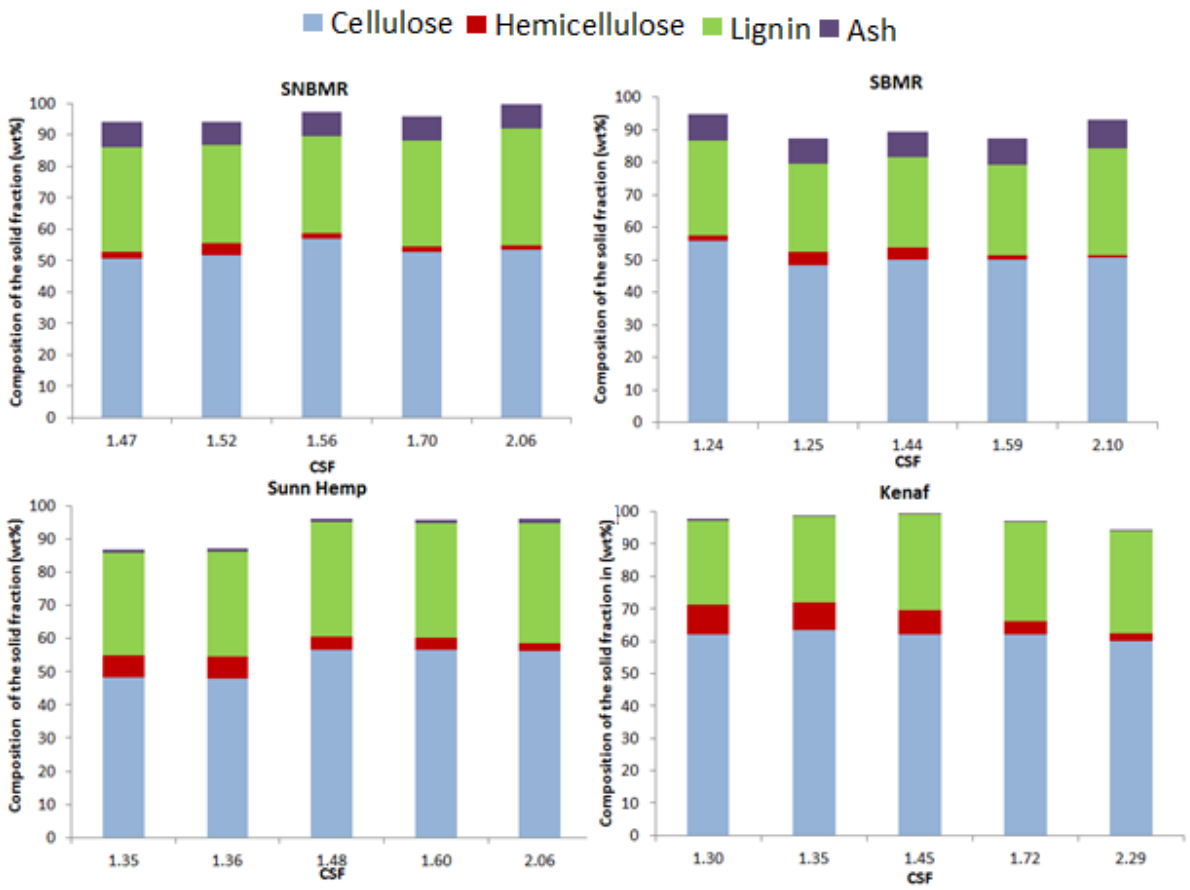

Figure 3-3 Composition of different compounds present in the solid fraction after the pretreatment. SBMR; SNBMR; sunn hemp; kenaf.

3.4.4. Enzymatic Hydrolysis of the Solid Fraction

In the native state, cellulose exists as a semi-crystalline polymer (O'Sullivan Antoinette C 1997). In Figure 3-4 we can observe that the overall glucan saccharification yield after enzymatic hydrolysis for SNBMR was found to be 90 wt% followed by SBMR 84 wt% at CSF 1.47 and 1.24 respectively. In the case of sunn hemp and kenaf it was found to be 68 and 88 wt% at CSF 2.06 and 2.29 respectively. This difference was attributed to a lower amount of hemicellulose retained in the biomass due to higher de-acetylation during pretreatment because there is an inverse correlation between the acetylation and glucan saccharification yields (Chen *et al.* 2012). It was interesting to note that for SBMR and SNBMR at CSF (1.25, 1.52) the yield was observed to be lower from the initial CSF values (1.24, 1.47). This was attributed to a high hemicellulose content in the pretreated solid substrate as evident from Figure 3-3. This conclusion was validated by similar studies conducted by (Leu, Zhu 2012, Zhu *et al.* 2012). Moreover, the results suggest that a higher acid concentration, low reaction temperatures and time are vital for good saccharification yields after enzymatic hydrolysis for SBMR and SNBMR. In addition at higher CSF (2.10, 2.06) for SBMR and SNBMR, the glucan sacchariifcation yield decreased to 74 and 65 wt%. This was primarily due to the presence of some hemicellulose in the solid substrates which led to a decrease in the activity of the enzymes (Zhu *et al.* 2012).

Overall, the yield of sunn hemp biomass was less as compared to SNBMR, SBMR and kenaf. These results are in agreement with the study conducted by (Torget *et al.* 1990) who concluded that cellulose in legume species such as sunn hemp is found to be more recalcitrant than that from dedicated energy crops such as SBMR, SNBMR and kenaf (Torget *et al.* 1990, Torget *et al.* 1992b). Moreover, legume crops are also rich in pectins and these compound can

also act as recalcitrant to enzymes (Leu, Zhu 2012). Moreover, the difference in degradability is also related to differences in plant cell wall structures between SNBMR, SBMR, and kenaf, as compared to sunn hemp. In addition, it can also be referred to a high crystallinity index of raw sunn hemp biomass as compared to kenaf, SBMR and SNBMR (Joonobi *et al.* 2010, Theerarattananoon *et al.* 2011) as evident from Table 3-3. These factors could be the reasons for low yields.

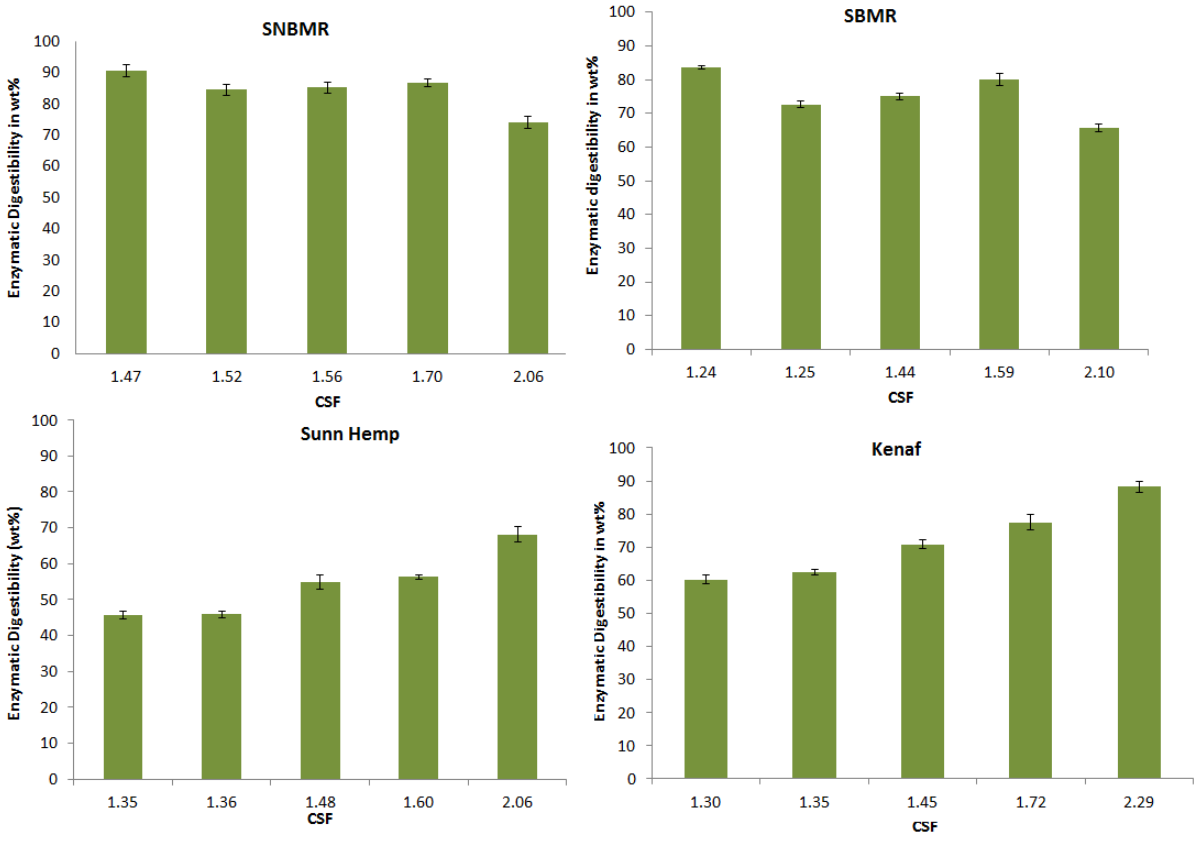

Figure 3-4 Glucan saccharification yield of pretreated solid substrate biomass samples. SBMR; SNBMR; sunn hemp; kenaf.

Table 3-3 Crystallinity index of sunn hemp and sorghum biomass before pretreatment.

Biomass	Crystallinity index	References
Sunn Hemp	81.26	(Kalia *et al.* 2011)
Kenaf	48.24	(Jonoobi *et al.* 2009)
Sorghum BMR	37.04	(Theerarattananoon *et al.* 2011)
Sorghum NBMR	32.58	(Theerarattananoon *et al.* 2011)

3.4.5. Concentration of Inhibitor Products

The three major inhibitor products analyzed in hydrolyzate samples were acetic acid, furfural, and HMF. From Figure 3-5 it was evident that the concentration of furfural increased with CSF for all four feedstocks. This was due to decrease in xylose concentration as evident from Figure 3-2 as de-hydration of xylose leads to furfural. The concentration of furfural ranged from 0.75 to 3.40 g/L for SBMR, 0.68 to 3.81 g/L for SNBMR, 0.0 to 2.81 g/L for sunn hemp and 2.4 to 3.9 g/L for kenaf. The absence of furfural at low severity for sunn hemp biomass was primarily due to the limitation in the RID detector of HPLC as any compound concentration less than 0.1 g/L cannot be detected. (Weil *et al.* 2002) studied the concentration of furfural concentration toxicity level for different bacteria. They observed that the furfural toxicity level for *Saccharomyces cerevisiae* yeast is 3-4 g/L (Weil *et al.* 2002). *E. coli* bacteria can ferment pentose sugars into ethanol and they can withstand the maximum furfural concentration level around 3 g/L. In addition, they can tolerate different kinds of aliphatic and aromatic acids present in the liquid hydrolyzate solution(Klinke *et al.* 2004). From these results conducted by other researchers, it can be concluded that the temperature region between 150 and160 °C, with acid

concentration of 1 to 2 wt% works well for all the four feedstocks. Moreover, removal of inhibitors (de-toxification) of the liquid hydrolyzate samples can be avoided thus increasing the economic viability of the process.

Similarly, dehydration of three water molecules from glucose results in HMF. It was interesting to note that the concentration of HMF for SBMR and SNBMR at harsher pretreatment conditions decreased marginally since HMF was further degraded into levulinic acid (Zhao *et al.* 2009). The concentration of HMF in SBMR ranged from 0.46-0.92 g/L, in SNBMR it ranged from 0.59-1.75 g/L, in sunn hemp the amount of HMF variation was minimal; it ranged from 0.20-0.31 g/L and for kenaf it ranged between 1.5 to 4.9 g/L. The reason for high concentration of HMF for kenaf was due to the presence of high amounts of unstructured sugars in the biomass. The acetic acid concentration was higher for biomasses treated at harsher pretreatment conditions (especially with 2% acid concentration). This was mainly due to cleaving acetyl linkages at higher acid pretreatment. The acetic acid concentration ranged from 0.16-2.31 g/L for SBMR, 0.68-5.01 g/L for SNBMR, 1.09-2.90 g/L for sunn hemp and for kenaf it was around 5.2 to 7.0 g/L. From the acetic acid results it can be concluded that acetyl linkages between hemicellulose and lignin could be more abundant in kenaf and SBMR as compared to other feedstocks (Theerarattananoon *et al.* 2011).

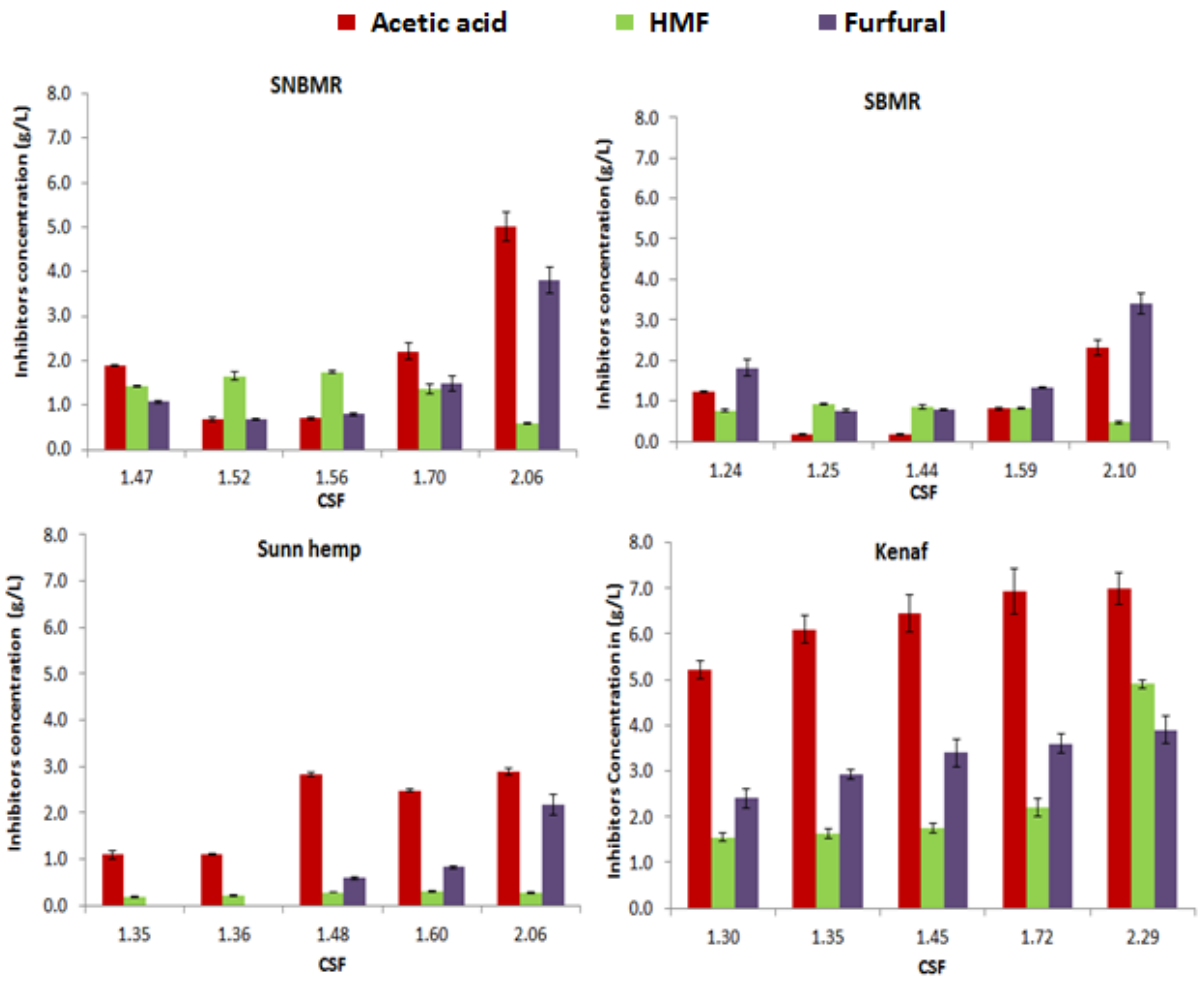

Figure 3-5 Inhibitors concentration in the hydrolyzate samples after the pretreatment. SBMR; SNBMR; sunn hemp; kenaf.

3.5. Conclusion

The hemicellulose yields and glucan saccharification yields for all the four biomasses were studied under same pretreatment conditions. The difference between cell wall linkages of the legume sunn hemp and non-legume (SBMR, SNBMR, kenaf) were different. Moreover, the carbohydrate yields were heavily dominated by the crystallinity index of individual raw biomasses. The hemicellulose yields for all the biomasses increased with CSF. However, at

higher CSF the yield decreased primarily due to sugar degradation. Moreover, the hemicellulose yields of SNBMR, SBMR, kenaf were higher compared to sunn hemp. From the glucan saccharification yield it can be concluded that both SBMR SNBMR and kenaf behaved similarly. However, sunn hemp had lower glucan saccharification yields as higher amount hemicellulose was still retained in the pretreated substrates. The inhibitor products concentration has increased with the increase in severity of the pretreatment for all the biomasses.

4. DETERMINATION OF REACTION RATES COEFFICIENTS IN THE PRODUCTION OF XYLOSE AND FUFURAL FROM FOUR SPECIES OF LIGNOCELLULOSIC BIOMASS

4.1. Abstract

A kinetic study of acid pretreatment was conducted for sorghum non-brown mid rib (SNBMR), sorghum-brown mid rib (SBMR), sunn hemp and kenaf, focusing on rates of xylose monomer and furfural formation. The kinetics was investigated using two independent variables, reaction temperature (150 & 160 °C) and acid concentration (1 & 2 wt%), with a constant dry biomass loading of 10 wt% and a treatment time up to 20 min. The experimental data were fitted using a two-step kinetic model based on irreversible pseudo first order kinetics at each step. Varied kinetic orders on the acid concentration, ranging from near-zero to >3, were observed for both xylose and furfural formation, the values depending heavily on the feedstock used and the product. The crystallinity index of raw biomass was shown to be a major factor influencing the rate of both xylose and furfural formation. A positive correlation was observed between the activation energy and biomass crystallinity index for both xylose and furfural formation whereas the reaction order on the acid concentration increased with the crystallinity index only for xylose formation. Reflecting the observed kinetic features, the pretreatment parameters for maximum yields varied for each biomass. The maximum xylose yields for kenaf, SNBMR, SBMR and

sunn hemp were 80.2±1.1, 77.1±0.5, 78.5±1.9 and 72.1±0.3 wt% of the initial xylan, respectively; whereas the corresponding maximum furfural yields (for 20 min treatments) were 33.4±0.4, 46.2±0.3, 36.5±0.1 and 10.1±0.2 wt%. The practical significance of using this kinetic model for selective formation of the target product was demonstrated.

4.2. Experimental Method

4.2.1. Chemical Characterization

Composition of the raw kenaf, SBMR, SNBMR and sunn hemp was assessed according to the National Renewable Energy Laboratory (NREL) Laboratory Analytical Procedure (LAP) protocol (NREL/TP-510-42619). The inorganic structural material (extractives), such as nitrites/nitrates, proteins, chlorophyll and waxes, had to be removed from the biomass prior to analysis to avoid an error in the structural sugar content according to a NREL LAP protocol (NREL/TP-510-42619). High amounts of extractives may also result in an overestimation of the lignin amount when unhydrolyzed carbohydrates condense with the acid insoluble lignin. A two-stage extraction process (24 h of water extraction followed by 18 h of ethanol extraction) was performed to remove extractives. Water and ethanol were removed from the resulting extract by oven drying; the remaining solid was weighed to account for the overall extractives weight. After extraction, the biomass was oven dried for 12 h at 105 °C and analyzed for both structural carbohydrates and lignin based on a specific NREL LAP protocol (NREL/ TP-510-42618). The feedstock carbohydrate composition is summarized in Table 4-1.

Table 4-1 Feedstock composition analysis

	Dry wt%							
Species	Glucan	Xylan	Galactan	Mannan	Arabinan	Lignin	Ash	Extractives
SNBMR	33.9±0.5	15.2±0.2	4.2±0.1	3.8±0.1	0.5±0.1	15.8±0.4	3.3±0.3	26.0±0.1
SBMR	33.7±0.8	13.0±0.6	4.5±0.1	3.8±0.2	0.6±0.1	13.9±0.4	4.2±0.1	25.4±0.6
Sunn hemp	37.1±0.8	9.9±0.5	6.1±0.1	5.4±0.1	0.3±0.1	13.8±1.1	5.2±0.3	22.6±0.2
Kenaf	42.5±4.2	14.0±1.2	2.2±0.4	0.4±0.1	0.7±0.3	17.2±2.1	0.3±0.1	21.0±1.0

4.2.2. Pretreatment

The biomass pretreatment was conducted in a jacketed batch reactor with a 300-mL internal volume manufactured by Auto Clave Engineers, Erie, PA. The reactor was made of Hastelloy C-276 to mitigate the acidic corrosion at high temperatures. Dry biomass (21 g (10 wt%)) was added to an appropriate amount of 1.0% or 2.0% sulfuric acid solution (prepared by mixing deionized water and sulfuric acid) to make a 10% solid content mixture. The heating source used for the reactor was saturated steam drawn into the reactor's jacket by a three-way valve. More detailed information regarding the reactor schematic and setup was published elsewhere (Degenstein *et al.* 2011). The agitation rate in the reactor was maintained constant at 60 rpm throughout the reaction. The reactor heating rate was (35±3) °C/min. Once the desired temperature was reached, it was maintained constant and the reaction time commenced. At the allotted times, the reactor was cooled by passing tap water into the external jacket. Once the reactor was cooled below 40 °C, the reaction slurry was discharged and collected in a polyethylene bottle for further analysis. The temperature data were recorded with the aid of Picolog software throughout the reaction time. All experiments were duplicated.

Table 4-2 Pretreatment conditions employed for each biomass.

1 wt% Acid Concentration	2 wt% Acid Concentration
150 °C	150 °C
160 °C	160 °C

The varied operational conditions are listed in Table 4-2. Each pretreatment experiment was performed up to a maximum reaction time of 20 min. The liquid hydrolyzate samples of each biomass were withdrawn every 2 minutes.

4.2.3. Model

A pseudo first order irreversible reaction model was used proposed earlier (Jensen *et al.* 2008), which follows the Arrhenius-type kinetics with the mechanism including the hydrolysis of xylan in hemicellulose into xylose monomer and its subsequent degradation into furfural, see (Equation 4-1). During pretreatment, acetic acid is derived from the hydrolysis of acetyl linkages that are bind to hemicellulose (Danalatos, Archontoulis 2010). This acid could be an inhibitor during the fermentation process of the pretreated liquid hydrolyztes as it tends to effects cell metabolism by lowering its pH. Studies indicate that generation of acetic acid does not follow the Equation 4-1 (Kalia *et al.* 2011).In addition, the amount of acetyl groups were lower in the backbone for agricultural residues such as SBMR,SNBMR, kenaf and switch grass as compared to hard woods such as Aspen, Balsam (vom Stein *et al.* 2011). Hence in the kinetic study of xylan the formation of acetic acid is ignored.

$$X \xrightarrow{k_1} X_m \xrightarrow{k_2} F \qquad (4\text{-}1)$$

where X stands for initial xylan, Xm is the xylose monomer and F stands for furfural.

The kinetic coefficients, ki, are pseudo-first order constants of the corresponding reactions,

Rate of xylose formation= k1 [X]

Rate of xylose degradation = k2 [Xm],

where the brackets designate the concentration, mol/L, of the corresponding chemical.

The mass balance on [X],[Xm] and [F] can be described by the following equations.

$$\frac{d[X]}{dt} = -k_1\,[X] \;\; with \;\; [X](0) = [X_o] \qquad (4-2)$$

where [X]o is the initial xylan concentration;

$$\frac{d[X]_m}{dt} = k_1[X] - k_2[X_m] \quad with\ [X]_m(0) = 0 \qquad (4-3)$$

$$\frac{d[F]}{dt} = -k_2[X_m] \quad with \quad [F](0) = 0 \qquad (4-4)$$

By solving linear differential (Equations 4-2 to 4-4) with their corresponding initial conditions, the time dependent expressions below are readily obtained.

$$[X](t) = [X_o] \times e^{(-k_1 t)} \qquad (4-5)$$

$$[X]_m(t) = \frac{k_1}{k_2 - k_1} \times \left(e^{(-k_1 t)} - e^{(-k_2 t)}\right) \times [X_o] \qquad (4-6)$$

Since $$[F] = [X_o] - [X_m] - [X] \qquad (4-7)$$

$$[F](t) = \left(1 + \frac{k_1 \times e^{(-k_2 t)} - k_2 \times e^{(-k_1 t)}}{k_2 - k_1}\right) \times [X_o] \quad (4-8)$$

(Equation 4-8) is obtained as an analytical solution of (Equations 4-5 and 4-6).

Xylose oligomer formation was not explicitly included in the model used, for two reasons. First, the previous studies showed that more detailed models tend to overestimate the amounts of xylose oligomers. Our pretreatment acid concentration was high; under such conditions the oligomer concentration was observed to be insignificant (Lloyd, Wyman 2003, Morinelly et al. 2009). Second, as will be shown in this study, the consideration of just two

logistically relevant kinetic constants within the model may streamline the results' interpretation thus improving its practical applicability.

The amount of xylose yield was calculated as a mole equivalent of xylan, by applying the ratio of the xylan unit and xylose molecular weights (0.88) as shown in (Equation 4-9) where [Xm] is the concentration of xylose monomer.

$$Xylose\ \% = \frac{[X_m] \times volume\ of\ solution\ for\ pretreatment\ \times 0.88}{weight\ of\ starting\ \ xylan} \times 100 \quad (4-9)$$

The furfural yield was calculated as a mole equivalent of xylose, by applying the ratio of its molecular weights of furfural and xylose to express it as xylose equivalent (0.64) as shown in (Equation 4-10) where [F] is the furfural concentration in the liquid hydrolyzate after the pretreatment.

$$Furfural\ \ \% = \frac{[F] \times volume\ of\ solution\ used\ for\ pretreatment \times 0.64}{weight\ of\ starting\ xylose} \times 100\ (4-10)$$

A fraction of the furfural present in the liquid hydrolyzates may have originated from the degradation of the other aldopentose occurring in hemicellulose, arabinose (Nabarlatz et al. 2004). However, arabinan, the essential arabinose precursor, was present only in trace amounts (≤ 1 wt%) in all feedstocks as evident from Table 4-1. Hence, the contribution of arabinose degradation was ignored.

The data sets for each of the four severity conditions studied for each species were fitted using the Lavenberg-Marquardt non-linear curve fitting method in Mathcad15 (Needham, MA). The kinetic coefficients obtained are functions of absolute temperature, acid concentration and inherent factors according to the Arrhenius equation, (Equation 4-11) (Nabarlatz et al. 2004).

$$k_i = A\, e^{\left(-\frac{Ea}{RT}\right)} \qquad (4-11)$$

$$A = A_o[C]^{n_i} \qquad (4-12)$$

where T is the absolute temperature (K), C is the acid concentration in wt %, A is the effective pre-exponential factor (1/min), ni is the reaction rate order (dimensionless), Ea is the Arrhenius activation energy (kJ/mol), and R = 8.3143 × 10-3 (universal gas constant, kJ/mol-K), A_o is the inherent (concentration-independent) pre-exponential factor. Model parameters A_o, ni, and Ei for both xylose formation and xylose degradation were fitted for each species. Since the acid concentration is traditionally measured in wt% as opposed to molar concentrations, the numerical values and units of A and A0 differ from those used in chemical kinetics. However, the values of two most important parameters, ni and Ea, maintain their physical significance. This feature will be used henceforth to provide valuable mechanistic information and practical recommendations.

4.3. Chapter Results and Discussion

4.3.1. Determination of kinetic parameters

The rate coefficients obtained according to (Equation 4-1) for all feedstocks are listed in Table 4-3. These rate constant values follow a similar pattern to that reported in the earlier studies conducted on aspen, corn stover, balsam and switch grass; namely, k_1 is greater than k_2 for any given feedstock, both constants increasing with the increase of either acid concentration or reaction temperature (Shen, Wyman 2011a).

Table 4-3 Kinetic coefficients obtained using the model described by Equations 4-5to 4-8

Acid concentration wt%	k_i, s^{-1}	SNBMR		SBMR		Sunn hemp		Kenaf	
		150 °C	160 °C	150 °C	160 °C	150 °C	160 °C	150 °C	160 °C
1	k_1	1.32×10^{-1}	1.37×10^{-1}	8.39×10^{-2}	1.01×10^{-1}	1.11×10^{-2}	2.50×10^{-2}	6.35×10^{-2}	9.32×10^{-2}
	k_2	1.55×10^{-2}	1.75×10^{-2}	3.30×10^{-3}	8.90×10^{-3}	1.00×10^{-3}	5.00×10^{-3}	2.90×10^{-3}	3.10×10^{-3}
2	k_1	1.51×10^{-1}	1.67×10^{-1}	1.35×10^{-1}	1.58×10^{-1}	1.01×10^{-1}	1.04×10^{-1}	1.19×10^{-1}	1.41×10^{-1}
	k_2	2.74×10^{-2}	3.09×10^{-2}	3.03×10^{-2}	4.60×10^{-2}	7.80×10^{-3}	1.05×10^{-2}	1.80×10^{-2}	3.05×10^{-2}

The obtained numerical values of rate coefficients were also similar to those reported in the earlier studies (Jensen *et al.* 2008, Morinelly *et al.* 2009) on various other biomasses. The observed differences between the rate coefficients for various feedstocks suggest a significant variation in the component distribution and lignocellulosic structure arrangement as suggested earlier (Morinelly *et al.* 2009).

The maximum xylose yields for kenaf, SNBMR, SBMR and sunn hemp are tabulated in Table 4-4. The observed product yields correlated with the obtained kinetic coefficients, i.e., specific reaction rates Table 4-3. Since the first-order kinetic constant, at a given time, reflects the natural logarithm of the ratio of the initial and final reactant concentrations as expressed in (Equation 4-5), i.e., the product yield, the observed correlation of these two parameters is expected. The significance of this correlation is that it shows that the process occurs under kinetic, as opposed to thermodynamic, control, thus justifying the use of irreversible kinetics in the proposed model.

Table 4-4 Maximum yields of xylose and furfural for four feedstocks obtained under the listed reaction parameters.

Biomass	Acid Concentration in wt%	Reaction Temperature (°C)	Reaction Time (min)	Maximum Xylose Yield (wt%)
SNBMR	1	150	18	76.9±0.5
SBMR	1	150	20	77.9±1.9
Sunn Hemp	2	160	20	72.1±0.3
Kenaf	1	160	20	80.2±1.1

The only exception from this trend was kenaf, for which the highest xylose yield was obtained yet the values of k_1 were smaller than those of SNBMR and SBMR. However, this exception can be explained by a rather slow furfural formation at the lowest acid concentration considered, as further in the sections on the acid concentration and temperature. Note that the maximum xylose yield upon kenaf hydrolysis was obtained at a higher temperature than that of SNBMR and SBMR; the xylose yields obtained correlated with the corresponding values of k_1. The maximum furfural yields obtained experimentally were 46.2±0.3, 36.5±0.1, 33.4±0.4, 10.1±0.2 wt% for SNBMR, SBMR, kenaf and sunn hemp. The conditions for obtaining these

yields were 160 °C, and 2 wt% acid concentrations for all feedstocks, i.e., the maximum severity treatment conditions.

4.3.2. Model Justification

Figures 4-1 & 4-2 depict the experimental data for xylose formation and degradation, respectively, as well as their match with the kinetic curves obtained upon using the model parameters. In case of SBMR the model tends to slightly underpredict the xylose and overpredict the furfural formation at the highest acid concentration for intermediate times. Apart from this slight discrepancy, the model was in good agreement with the experimental data for both xylan hydrolysis into xylose monomer and its subsequent de-hydration to furfural.

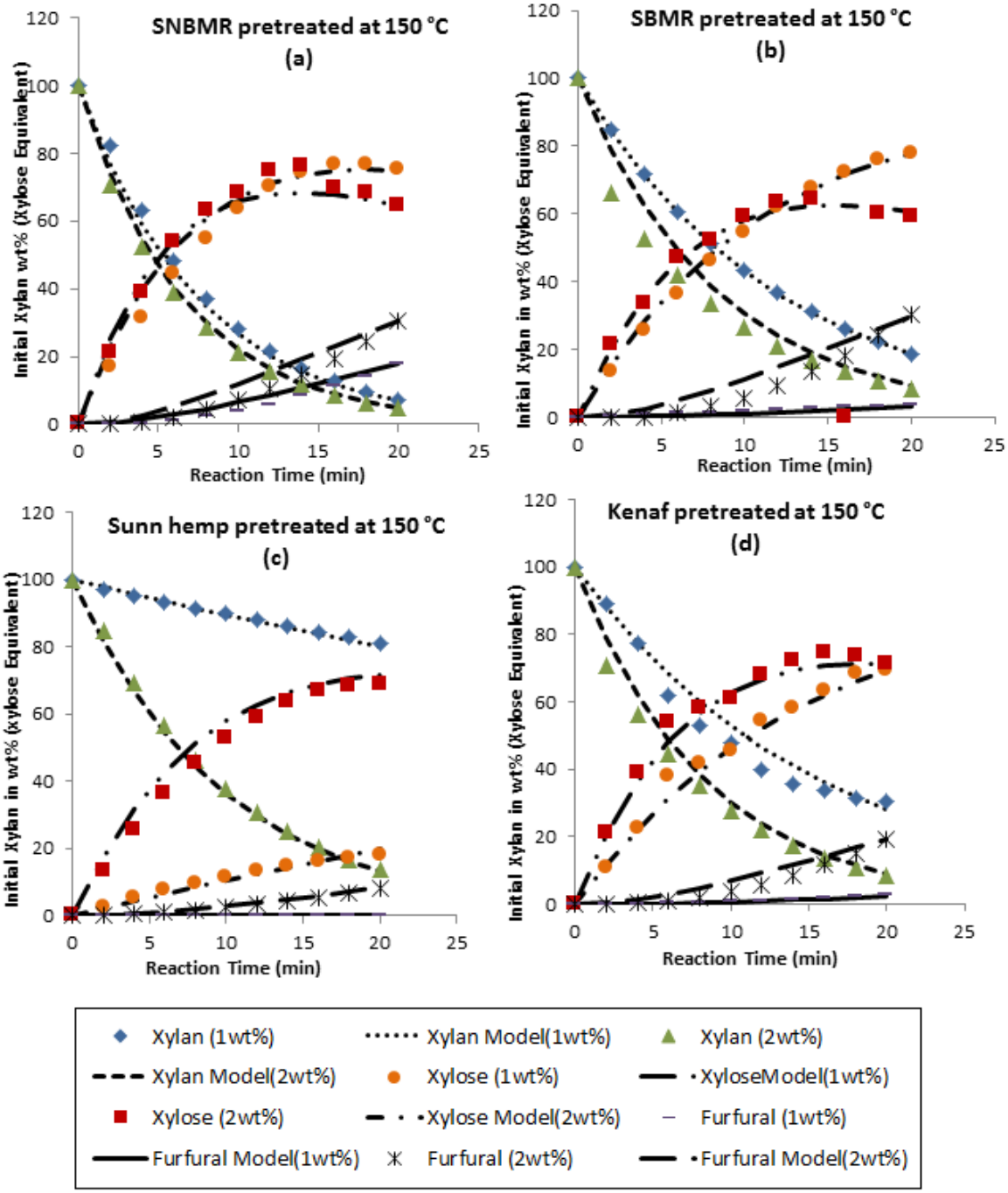

Figure 4-1 Model prediction and experimental data for xylan, xylose and furfural concentration profiles at 150 °C at 1wt% and 2wt% acid concentrations for a) SNBMR, b) SBMR, c) sunn hemp, d) kenaf.

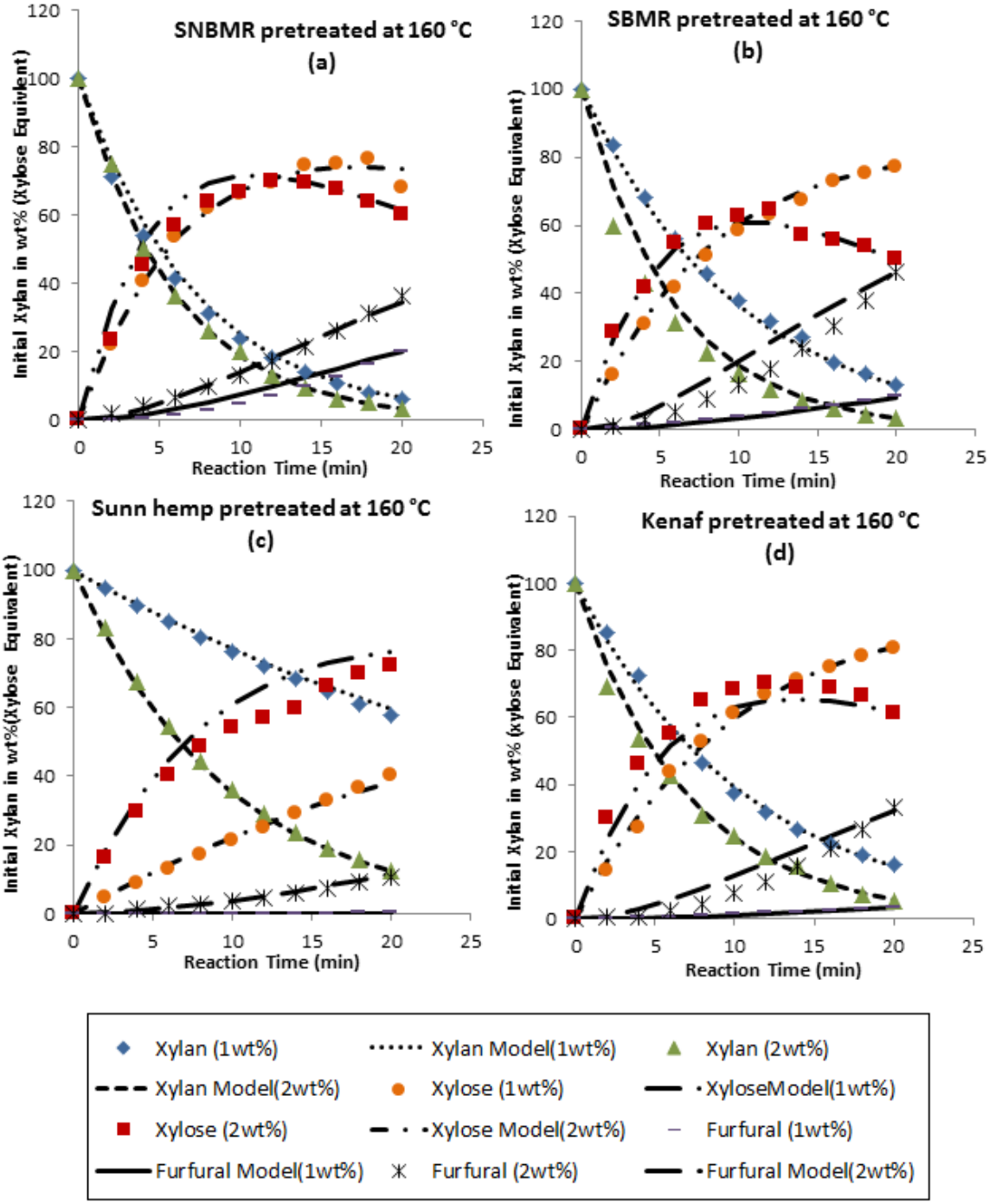

Figure 4-2 Model prediction and experimental data for xylan, xylose and furfural concentration profiles at 160 °C at 1wt% and 2wt% acid concentrations for a) SNBMR, b) SBMR, c) sunn hemp, d) kenaf.

The next question in model validation was whether the obtained kinetic parameters listed in Table 4-5 could be varied without significantly altering the match with experimental data. Two approaches were used to address this issue. First, the F-test was conducted, i.e., minimizing the sum of squared errors (SSE) between the theoretical model and the experimental data by varying the pre-exponential factor, activation energy and reaction order on the acid concentration (Yat *et al.* 2008a). Table 4-6 lists the SSE corresponding to the best-fit values described by (Equation 4-11). The difference in variance between the experimental rate coefficient and model was low as evident from Table 4-6. The data sets either passed the F-test ($F<F_{critical}$) or nearly passed it. One of the instances when $F>F_{critical}$ was the xylose formation from SBMR mentioned in the previous paragraph. The other two cases were the furfural formation from sunn hemp and xylose formation from kenaf; however, the corresponding panels of Figure 4-1& Figure 4-2 show that these deviations resulted from a small bias observed only at intermediate time values. It is of note that an alternative model based on parallel rather than sequential reactions led to an order of magnitude higher variance, with a poor fit of experimental data. Thus, the applied model used can be deemed adequate, given the inherent homogeneity of the system used.

Table 4-5 Fitted Arrhenius parameters obtained from Equation 4-11 from the kinetic

coefficients listed in Table 4-3

	Xylose Formation (k_1)				Xylose Degradation (k_2)			
Biomass	n_1	A_{01} (min^{-1})	E_1 (kJ/mol)	R^2	n_2	A_{02} (min^{-1})	E_2 (kJ/mol)	R^2
SNBMR	0.2	11.5	15.7	0.91	0.5	4.4×10^{4}	52.3	0.99
SBMR	0.6	76.4	24.0	0.96	3.2	2.3×10^{5}	63.6	0.94
Sunn hemp	2.8	622	38.0	0.98	1.6	5.0×10^{7}	84.3	0.91
Kenaf	0.9	108	26.2	0.94	2.6	2.4×10^{7}	80.4	0.99

Table 4-6 Sum of squared errors and F values for the experimental and model parameters.

Biomass		SSE	Variance for Experimental Parameters	Variance for Model	F value	$F_{Critical}$
SNBMR	k_1	4.4×10^{-4}	2.4×10^{-4}	1.8×10^{-4}	1.36	9.27
	k_2	4.5×10^{-5}	5.6×10^{-5}	2.7×10^{-5}	2.01	9.27
SBMR	k_1	1.0×10^{-5}	1.1×10^{-3}	1.1×10^{-3}	0.96	0.10
	k_2	4.9×10^{-5}	3.8×10^{-4}	3.4×10^{-4}	1.12	9.27
Sunn Hemp	k_1	4.9×10^{-5}	2.4×10^{-3}	2.1×10^{-3}	1.12	9.27
	k_2	1.5×10^{-5}	1.6×10^{-5}	2.4×10^{-5}	0.66	0.10
Kenaf	k_1	2.9×10^{-4}	1.1×10^{-3}	1.5×10^{-3}	0.76	0.10
	k_2	4.0×10^{-6}	1.7×10^{-4}	1.7×10^{-4}	1.03	9.27

Second, the observed reaction orders were verified by replacing the obtained numerical values of n_i with the kinetically relevant integers (0, 1, 2) in (Equation 4-11) and running the model with these artificially set values. This led to poor predictions of the rate coefficients, leading to a significant failure of the F-test; furthermore, in most of the cases the activation energies obtained with such set values of parameter *n* turned out to be *negative*. This, in turn, would suggest that the reaction rate decreases with an increase in temperature, which is just opposite to what was observed Table 4-3. Hence, it could be concluded that the kinetic parameters, including the effective rate orders predicted by the model and listed in Table 4-5 are

significant and accurate. The following sections analyze, one by one, the main factors affecting the reaction rates, i.e., the rate order on the acid concentration and activation energies, as well as their correlation to the biomass inherent parameters.

4.3.3. Influence of Reaction Order

The most characteristic and unusual kinetic feature observed was the occurrence of high kinetic orders on the acid concentration suggesting a simultaneous action of several proton donors on the functional groups near the bond to be broken at the rate-limiting step. Due to the inherent sample heterogeneity, the observed numerical values Table 4-5 reflect *effective* mean values, so they are not necessarily integers. The observed significant variation of this kinetic parameter indicates that the reaction mechanisms of various crops pre-treatment differ in details. For instance, the n_i values for SNBMR were found to be lower than 1 for both xylose formation and xylose degradation; they deviated considerably from the rest of crops. This difference suggests that hemicellulose in SNBMR does not require a concerted attack of several acid molecules, i.e., occurs readily. As shown previously in the literature, native xylan is not homogeneous and could be represented as a combination of fast and slow reacting polysaccharide (Shen, Wyman 2011b). Thus it appears that the fast reacting xylan is more abundant in SNBMR as compared to the other crops considered.

The values of n_i for the rest of the crops considered were found to be larger than those observed in the earlier studies (Jensen *et al.* 2008, Morinelly *et al.* 2009) conducted on aspen, balsam, bass wood, red maple, switch grass, even though most of these feedstocks consisted of

woody biomass, which is supposed to be more resistant to pre-treatment. The apparent reason is that those studies used lower acid concentrations (< 0.8 wt%). Perhaps, a new mechanistic path is enabled at higher acid concentrations (apparently above a certain threshold acid concentration value), allowing for a more efficient treatment of the slow-reacting xylan fraction.

To confirm this hypothesis, the same kinetic parameters as those used in this study are listed in Table 4-7 for the earlier studies conducted at lower acid concentrations (< 0.8 wt%). It can be seen from Table 4-7 that the lower xylan hydrolysis rates observed under such conditions result from not only lower kinetic orders on acids but also from significantly higher Arrhenius activation energies than those observed in the current study. Thus, increasing the acid concentration appears to enable the otherwise inaccessible path with a lower activation energy barrier, just as suggested.

Table 4-7 Kinetic parameters reported in literature obtained at lower acid concentrations (< 0.8 wt%) for activation energy and reaction order (Morinelly *et al.* 2009).

Biomass	CrI	Ea for Xylose Yield (kJ/mol)	Reaction Order for Xylose Yield	Ea for Furfural Yield (kJ/mol)	Reaction Order for Furfural Yield
Aspen	47%	69	1.22	132	1.2
Balsam	49%	84	1.33	125	1.55
Switch Grass	69%	89	2.47	106	0.06

Table 4-8 Optimum xylose yield conditions based on <5 wt% furfural yield for four feedstocks

Biomass	Acid Concentration in wt%	Reaction Temperature (°C)	Reaction Time (min)	Maximum xylose yield (wt%)
SNBMR	1	150	10	63.4±0.2
SBMR	1	150	20	77.9±1.9
Sunn Hemp	2	150	14	63.6±0.7
Kenaf	1	150	10	69.3±0.4

The observed difference in reaction orders on the acid concentration between xylose and furfural formation presents an opportunity for achieving higher yields of the intermediate, xylose, at the expense of furfural. Such "optimum" xylose yields, i.e., those with a reasonable xylan conversion yet with less than 5% furfural yield, are listed in Table 4-8 along with the reaction conditions leading to such yields. The resulting low furfural concentrations, less than 3-4 g/L, would not lead to any adverse effects on *Saccharomyces cerevisiae* strains, as they were shown to perform efficient fermentation into bio-ethanol under such conditions for liquid hydrolyzate samples (Klinke *et al.* 2004).

As evident from Table 4-8, SBMR and kenaf, featuring higher values of n_2 for furfural formation compared to n_1, should be treated not only for shorter time but also with a relatively lower acid concentration. Conversely, sunn hemp requires a higher acid concentration to be converted to xylose as evident from the values of n_1 shown in Table 4-5; a significant accumulation of xylose would occur even at a higher acid concentration. This suggestion corroborates the conditions under which the maximum xylose yield was achieved for sunn hemp. By contrast, for SNBMR the similarity of n_1 and n_2 values significantly hinders the separation of two sequential steps, which leads to lower xylose yields under any conditions; this feature explains the low optimum xylose yield for this feedstock.

The other factor that may lead to a preferred xylose accumulation is temperature; as shown in the next section, the model applied allows for the decomposition of the commonly used single lumped severity factor into its components.

4.3.4. Effect of Temperature

For any given feedstock, the values of Arrhenius activation energy were higher for xylose formation than for its subsequent hydrolysis, indicating that furfural should be formed at greater amounts at higher temperature. This conclusion corroborates the trends in product yields observed in the current study Table 4-4 as well as the published information (Jensen *et al.* 2008). The E_a values for xylose formation were found to be significantly lower than those of xylose degradation, with the difference exceeding 35 kcal/mol. Given such a large ΔE_a value, even a small increase in temperature would be expected to significantly increase the yield of furfural.

The ΔE_a value is particularly large for kenaf, being 54 kJ/mol, explaining the observed largest yield of xylose before it converted to furfural Table 4-5. Perhaps, crops with the maximum ΔE_a value may be most applicable for this scenario.

However, if only the temperatures were varied and the acid concentrations were a less significant factor, the yields of xylose and furfural would exhibit similar trends for all feedstocks. The observation that, countering this assumption, the maximum yield of xylose was still obtained at a higher temperature for sunn hemp, further emphasizes the importance of acid concentration as a separate parameter, as shown in the previous section. This observation also led us to the consideration of dependence of reaction kinetic parameters on the inherent biomass parameters covered in the next section.

4.3.5. Effect of Biomass Crystallinity Index

Attempts to correlate the obtained kinetic constants with any features of feedstock composition listed in Table 4-1 were unsuccessful. However, the values of both k_1 and k_2 consistently increased with a decrease of the raw biomass crystallinity index, which is 81.26, 48.20, 37.02 and 32.58 % for sunn hemp, kenaf, SBMR and SNBMR, respectively (Jonoobi *et al.* 2009, Kalia *et al.* 2011)

In an attempt to separate the influence of acid concentration and temperature on the rate of xylose and furfural formation, both n_i and E_a were plotted versus the biomass crystallinity in Figure 4-3a,b, respectively. Figure 4-3a shows that the activation energies of both reactions increase along with the biomass crystallinity index. This result was expected for the first reaction

since most of the hemicellulose that contains xylan is bonded to crystalline cellulose. It is less intuitive for the furfural formation because one might assume that once xylose is released into the solution, the crystallinity index should not play a major role. The obtained results indicate that the xylose formed remains encased in water-insoluble cellulose, which appear to hinder the access of hydronium ions to this essential precursor of furfural. The alternative explanation assuming the parallel rather than sequential furfural formation directly from xylene failed to describe the experimental data as mentioned earlier.

As for the reaction order on the acid, a positive correlation with the biomass crystallinity index was observed for xylan to xylose hydrolysis, n_1 Figure 4-3b. The initial hydrolysis of xylan to xylose is indeed expected to be hindered by a higher biomass crystallinity as the simultaneous action of multiple hydronium ions becomes essential to hydrolyze a more stable xylan fraction embedded into crystalline clusters. By contrast, the rate order for xylose to furfural conversion, n_2, showed no correlation with the crystallinity index. Thus, the remaining cellulose appears to be detached from the xylose formed, acting more like a mechanical rather than chemical barrier in more crystalline structures; so just higher temperature but no extra hydronium ions are required to produce furfural.

The kinetic parameters obtained in other works at lower acid concentrations Table 4-7 show direct correlations with the crystallinity index only for E_{a1} and n_1, i.e., xylan hydrolysis, but not for furfural formation. This difference may be interpreted as that the low-acid treatment leaves a fraction of xylose being trapped within the cellulose sheath, thus rendering it inaccessible to further conversion, unlike the high acid concentration process.

Apart from the crystallinity index, other factors can influence the xylan hydrolysis, such as 1) diffusional limitations of hydronium ions' mobility; 2) non-homogenous reactions at the xylan- water interface (Jacobsen, Wyman 2000). However, both of these factors may be linked to biomass crystallinity. For example, the activation energies for the first reaction, i.e., xylan hydrolysis, are so low in the least crystalline biomasses, SBMR and, in part, SNBMR and kenaf, that this process may be diffusion-limited thus explaining the observed low values of n_1 for these feedstocks, as opposed to more crystalline sunn hemp Figure 4-3a.

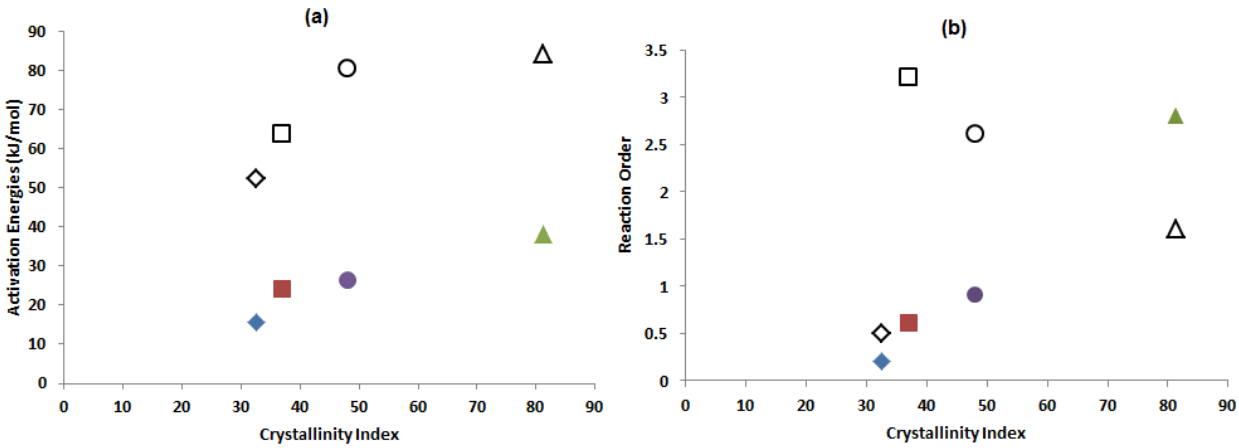

Figure 4-3 The effect of crystallinity index on a) activation energy for both E_1 (closed symbols) and E_2 (open symbols); b) reaction order on the acid concentration, n_1 (closed) and n_2 (open) for four feedstocks.

4.3.6. Practical Implications for Pretreatment

Unlike the earlier proposed detailed models accounting for the formation of xylose oligomers, the simplified model used allows for making practical recommendations because the indexes "1" and "2" in all kinetic parameters are directly related to the first and second reactions

of (Equation 4-1). The oligomer formation as well as the availability of several paths of xylan hydrolysis are still reflected in the effective values of kinetic parameters, n_i and E_a. The model also separates the influence of temperature and acid concentration on the rates of these two reactions.

The following recommendations directly based on the model can be made for optimizing the xylose formation: a) lower acid concentrations and lower reaction temperatures are required for SNBMR hydrolysis; b) for SBMR and kenaf, higher acid concentration and low temperature are recommended; c) sunn hemp treatment would benefit from higher reaction temperatures and higher acid concentrations. If, conversely, the bio-refinery goal is to produce furfural rather than xylose, a) SNBMR treatment should be conducted at acid concentration (≥ 2 wt%) and relatively low reaction temperatures (150 -160 °C); b) SBMR, kenaf, sunn hemp treatment requires acid concentrations (≥ 2 wt%) and higher reaction temperatures (≥ 160 °C) , with the reaction time being as long as it would not lead to the degradation of pentose sugar backbone in all cases.

4.4. Conclusion

A simplified two-step kinetic model adequately describes the hemicellulose hydrolysis of four crops. Though temperature and acid concentration exhibited a qualitatively similar influence on the rates of xylose formation and hydrolysis, the quantitative effects were different, thus affecting the trends in obtaining maximum xylose and furfural yield under varied reaction conditions. The Arrhenius activation energy values consistently increased with the biomass crystallinity index or both reactions. Effective reaction rate orders on acids of both xylose and

furfural formation vary significantly for different crops increasing when the acid concentration exceeds 1 wt%. However, this increase occurs selectively for high-crystallinity biomasses and only for xylose formation, thus creating crop-specific scenarios if the yield of xylose is to be optimized.

5. PRETREATMENT AND ENZYMATIC HYDROLYSIS OF SUNFLOWERHULLS FOR FERMENTABLE SUGAR PRODUCTION

5.1. Abstract

Sunflower is a widely adapted crop and can be grown in every temperature region. In the U.S., 2 million acres were cultivated with sunflowers in 2009. During industrial processing, large quantities of hulls are obtained as a waste product from the dehulling process. This study focused on converting the sunflower hulls into fermentable sugars by dilute acid pretreatment and enzymatic hydrolysis. Raw sunflower hulls are composed of cellulose (34±1.1 wt%), lignin (25±0.9 wt%), xylan and arabinan (27±1.5 wt%), extractives (13±2.5 wt%) and traces of ash. Sunflower hulls were first subjected to pretreatment by varying three independent factors: 1) acid concentration (0.5-2.0 wt%); 2) reaction temperatures (140-160 °C); and 3) reaction times (10-30 min). Slurry samples obtained after pretreatment were separated into liquid and solid fractions. Liquid fractions were analyzed for monomeric and oligomeric sugars and inhibitor products by HPLC. Enzymatic saccharification was then performed on pretreated solid fractions to convert remaining cellulose into glucose. The results showed an increase in acid concentration and reaction temperature gave high hemicellulose yield in the liquid fraction. However, an increase in reaction time resulted in degradation of pentose carbohydrates into furfural. The maximum

hemicellulose yield predicted by the model was 60% at 150 °C for 30 min at 1.25% acid concentration. The maximum cellulose digestibility of the enzymatic saccharification was 53.5% at 160 °C for 30 min at 2% acid concentration.

5.2. Introduction

Lignocellulosic biomass, such as forest residue, agricultural residue, yard waste and wood products, are a great source of energy that may be used for biofuel generation. They store energy from sunlight in their chemical bonds (McKendry 2002). Lignocellulose material is the most abundant and one of the cheapest materials available in the world for renewable energy production (Sassner *et al.* 2006).

Lignocellulosic material mainly consists of cellulose, hemicelluloses, and lignin. Cellulose is a homo polymer composed of six-carbon sugars. Hemicellulose is a heteropolymer of five-carbon and six-carbon sugars including xylose, arabinose, galactose, and mannose. These carbohydrates can be converted into fermentable sugars through pretreatment followed by enzymatic Saccharification (Zheng *et al.* 2009). Efficient pretreatment methods must be developed to maximize the fermentable sugar yield and to minimize degradation products (Jørgensen *et al.* 2007). Currently, dilute acid pretreatment of lignocellulosic biomass followed by enzymatic saccharification is proven to be one of the most promising and economical processes to obtain fermentable sugars for production of biofuels (Hendriks, Zeeman 2009c). Extensive research has been carried out to convert waste products obtained from industrial processing such as bagasse and pulp into lignocellulosic ethanol (Binod *et al.* 2012, Shi *et al.*

2011). However, little published data are available about converting sunflower hulls into bioethanol using dilute acid pretreatment and enzymatic saccharification.

The production of sunflower seeds in the United States was approximately 1.5MMT in 2009. Sunflower hulls are obtained as a waste product from the de-hulling process. Sunflower hulls have little commercial value and become a disposal problem because of their low bulk density (Sharma *et al.* 2004). The effect of alkali pretreatment on sunflower hulls and stalks has been studied to some extent by researchers, but the effect of dilute acid pretreatment and its outcome on the enzymatic saccharification have yet to be evaluated (Sharma *et al.* 2004). The present study was carried out to evaluate these waste hulls as a raw material for lignocellulosic ethanol production.

The primary goal of this study was to evaluate the effectiveness of the dilute acid pretreatment through the removal of xylan from the sunflower hulls to enhance the enzymatic digestibility of cellulose. The pretreatment of sunflower hulls was performed by taking three different factors into consideration: reaction time, reaction temperature, and acid concentration. Based on the experimental results, a model was formulated on the hemicellulose conversion yield. The criteria of optimization were high hemicellulose yield and low inhibitors such as acetic acid, Hydroxymethylfurfural (HMF) and furfural production in the hydrolyzate. Enzymatic saccharification was performed on pretreated solid substrate to evaluate the resulting sugar production.

5.3. Experimental Method

5.3.1. Raw Sunflower Hulls

The raw sunflower hulls were obtained from Dahlgren & Company, Inc. (Crookston, MN). The sunflower seeds were passed through the seed mill where seeds open up. To separate the mixture of seeds and hulls were dropped in water. The hulls will float on the water and removed easily. The separated hulls were air dried. The size of sunflower hulls was approximately 6-8 mm. Moisture content of the raw sunflower hulls was determined by oven drying at 105 °C for 12 h.

5.3.2. Compositional Analysis

It is necessary to remove the inorganic structural material from the biomass prior to analysis to prevent incorrect values of the cell wall components. Failure to remove these extractives may result in error in structural sugars values. It may also result in falsely high lignin values when unhydrolyzed carbohydrates condense with acid insoluble lignin. Composition of the original sunflower hulls was measured according to the National Renewable Energy Laboratory (NREL) LAP protocol (NREL/ TP-510-42619). Two-stage extraction processes (24 h of water extraction and 18 h of ethanol extraction) were performed to remove extractives such as nitrites/nitrates, proteins, chlorophyll, and waxes. The water and ethanol solvents were oven dried and weighed to account for the overall extractives weight. The source and individual components of these extractives were not verified. After extraction, hulls were oven dried for 12 h at 105 °C. Then the extractive free hulls were analyzed for structural carbohydrates and lignin based on the NREL LAP protocol (NREL/ TP-510-42618).

5.3.3. Box Behnken Design (BBD)

BBD gives an efficient estimation of quadratic terms and their interactions for 3 factors (Singh, Bishnoi 2012). Moreover, the numberof pretreatment runs are reduced in BBD as compared Central Composite Design (CCD). For 4 or more factors the advantage BBD of fewer runs disappears. Pretreatment of sunflower hulls was performed and analyzed using 15 experiments and additional 5 more runs (total including eight factorial points; six axial points and six replicates at the center points). These 20 experiments were generated using Minitab 15 software (Minitab, State College, PA) by taking high and low values of the three independent variables. The values for these factors were chosen based on the previous experimental results as summarized in Table 5-1. The twenty design matrix of the pretreatment conditions including all the three factors were summarized in Table 5-3. Analysis of variance (ANOVA) was conducted with a Minitab 15 software by using BBD. The significance of hemicellulose yield in the hydrolyzate (Y_1) was studied by considering three factors variables reaction temperature (X_1), reaction time (X_2), acid concentration (X_3) and the interactions between the factors. The significance of the model was evaluated by the value of R^2. The interval of R^2 is between zero and one. The closer the value of R^2 is to (1) implies the better model fits the sample data. The experimental data was analyzed by the Minitab 15 software.

Table 5-1 Pretreatment factors considered in CCD

Factors	Units	Levels		
		-1	0	1
Reaction Temperature (X_1)	°C	140	150	160
Reaction Time (X_2)	min	10	20	30
Acid Concentration (X_3)	wt%	0.50%	1.25%	2%

The mathematical design equation for each response was a second order quadratic equation given by (Equation 5-1). The coefficients and response surface were determined by the Minitab 15 software.

$$Yi = \beta o + \sum \beta i\, Xi + \sum \beta ii\, Xi^2 + + \sum \beta ij\, Xi\, Xj \qquad (5\text{-}1)$$

Where Yi is the predicted response variable, $\beta o, \beta i, \beta ii, \beta ij$ are constant regression coefficients of the model and $XiXj\ (i=1,2,3;\ j=1,2,3\ i \neq j)$ are the independent variables.

5.3.4. Analytical Procedures

5.3.4.1. Determination of Monomeric Sugars in the Liquid Fraction (Hydrolyzate)

After pretreatment, the slurry samples were vacuum filtered and separated into liquid and solid fractions. The hydrolyzate was then analyzed for monomeric and oligomeric sugars. Prior to analysis, hydrolyzate samples were neutralized by adding calcium carbonate until a pH range of 5.0-6.0 was obtained. The neutralized samples were filtered in order to remove contaminants using a 0.2µm filter (Millipore, Billerica, MA) into glass vials. The sugar analysis was performed in an Agilent 1200 HPLC (Palo Alto, CA) fitted with a Transgenomic CHO-Pb column (300 mm x 7.8 mm). The column temperature was maintained at 80 °C. The Refractive Index Detector (RID) temperature was maintained at 55 °C during the analysis. The mobile phase used was DI water. The flow rate was maintained at 0.6 mL/min. The analysis time for each sample was 35 min (Decker *et al.* 2009). Calibration verification standards with concentration of 4 gm/L were analyzed prior to analysis of liquid hydrolyzate solution to test for HPLC system performance.

The standard solution consisted of D-(+)glucose, D-(+)xylose, D-(+)galactose, L-(+) arabinose, and D-(+)mannose.

5.3.4.2. Determination of Oligomeric Sugars in the Liquid Fraction (Hydrolyzate)

It was performed to account for the amount of heterogeneous oligomers that are liberated into the liquid fraction in addition to monomeric sugars during pretreatment because these sugars are of little commercial value. It is imperative to hydrolyze them into homogenous monomers through a secondary hydrolysis process. The process includes autoclaving the liquid fraction samples at 121 °C for 60 min. The samples were analyzed in the HPLC system similarly as described in Section 2.5.1. This method is based on the NREL LAP protocol of determination of sugar by products and degradation products in liquid fraction process samples (NREL/ TP - 510-42623).

5.3.4.3. Structural Carbohydrates and Lignin in the Pretreated Solid Residue

This analysis was performed to determine the amount of cellulose, xylan and lignin retained in the solid fraction after the pretreatment. The solid samples were air dried for 4-5 days at room temperature and milled into 100-mesh particle size. Three hundred milligrams (mg) of milled solid biomass were loaded in the pressure tubes manufactured by (Ace Glass Incorporated, Vineland NJ) and three mL of 72 wt% sulfuric acid was added to the biomass. The tubes were placed in a water bath at 30 °C for 1 h. Then the acid concentration was reduced to 4% by adding 84 mL of DI water to each pressure tube. These pressure tubes were placed in an autoclave oven at 121°C for 60 min. The resultant slurry was vacuum filtered by pouring the

mixture into porous ceramic crucibles (Coorstek, Oakridge, TN). The liquid fraction was analyzed for the amount of acid soluble lignin (ASL) using a UV-Vis spectrometer (manufactured by Thermo Scientific, Waltham, MA), and carbohydrates using HPLC. The solid residue retained in the crucibles was oven dried at 105°C for 12 h to determine the acid insoluble lignin content (AIL). Then the crucibles were placed in a muffle furnace at 575 °C for 24 h to and then weighed to determine the ash content. This method is based on the NREL LAP protocol (NREL/ TP-510-42618).

5.3.4.4. Determination of Inhibitor Products

Liquid fractions of pretreated samples that were rich in 5-carbon sugars can be fermented into bio-fuel using a *Pichia stiptis*. In order to effectively convert sugars into biofuels, the inhibitor products in the liquid fraction such as (acetic acid, HMF and Furfurals) should be monitored. The analysis was performed using Agilent 1200 series HPLC system with a Phenomenex Rezex RFQ column at 80 °C. The mobile phase was a 0.01 N sulfuric acid solution. The flow rate was maintained at 1.0 mL/min (Sluiter *et al.* 2010). The verification standards for inhibitor products were obtained from Absolute Standards, Inc (Hamden, CT).

5.3.5. Enzymatic Hydrolysis

The enzymatic saccharification was performed on a washed pretreated solid substrate in a thermal incubator at 50 °C and 250 rpm for 72 h. Compositional analysis of the pretreated solid substrate was performed and the amount of cellulose retained in the substrate was measured by HPLC analysis. Then the biomass was accurately measured so that 0.1 g (1%) of dry β-glucan

was available for enzymatic saccharification. The solid substrate was loaded in a 50 mL centrifuge tube and 5 mL of a sodium citrate buffer with pH 4.8 and approximately 4.5 mL of DI water were added to the tube. The total volume of the reagents and solid substrate was approximately 10 ml. Accellerase 1500 enzyme was supplied by Genencor International (Palo Alto, CA). The reagents and enzyme loading concentration considered to perform enzymatic sacachrification were summarized in Table 5-2. This procedure and equation mentioned below were from the NREL LAP protocol (NREL/TP 510-42629). After 72 h the liquid hydrolyzate samples were filtered into glass vials and the analysis of cellulose digestibility was performed by Agilent 1200 HPLC system with a Transgenomic CHO-782 Pb column. Since enzymes convert cellulose glucose the cellulose digestibility was measured by integrating the glucose retention peak from the HPLC data. The yield of the pretreated substrate was calculated by using the Equation 5-2. The value of 0.9 was used in the equation as a correction factor for hydration.

$$\%\ Digestion = \frac{Grams\ of\ \ glucan\ digested \times 0.9 \times 100}{Grams\ of\ \ glucan\ added} \qquad (5\text{-}2)$$

Table 5-2 Enzymatic saccharification conditions

Conditions	Set points
Cellulose loading	1%
Temperature	50 °C
Time	72 h
Enzyme loading	40 mg/g of β-glucan
Sodium azide	20 mg/mL

5.4. Results and Discussions

5.4.1. Gravimetric Analysis

The major hull components measured as dry wt% were cellulose (34.1±1.1), followed by lignin (25.3±0.9), xylan (21.2±1.5), galactan (3.5±0.6), arabinan (0.5±0.1) and extractives (13.2±2.5), which were primarily composed of waxes, proteins, nitrates and nitrites. The amount of ash present in sunflower hulls was approximately (0.4±0.1) wt%. The initial xylan percentage corresponds to 4.2 ±0.8 g for 20 g of hulls loading for pretreatment.

5.4.2. Effect of Pretreatment Conditions on Hydrolyzate Samples

The influence of three factors on sunflower hull biomass pretreatment has been studied as shown in the Table 5-3. A quadratic model was formulated for xylose yield in the hydrolyzate as a response variable (Y_1). Table 5-4 summarizes the model coefficients obtained from ANOVA table for different measured responses together with a statistical significance R^2 (Lu *et al.* 2007). The p value was used as a tool to check the significance of each coefficient. The larger the magnitude of Students t value and smaller the p value, the more significant is the corresponding coefficients and their interactions (Lu *et al.* 2007). The model (Equation 5-3) included only the significant coefficients ($P < 0.05$) as summarized from Table 5-4 (Antony 2003, Singh 2001). In addition, the R^2 value for the model was approximately 0.986 implying that only 0.014 of the variance in the data was not predicted by the model due to noise. Figure 5-1 shows a good agreement of the predicted and experimental values for the percentage hemicellulose yield.

$$Y_1 = 51.02 + 6.20\,X_1 + 3.92\,X_2 + 12.36\,X_3 - 3.60\,X_1^2 - 13.48\,X_3^2 + 5.47\,X_1X_3 \qquad (5\text{-}3)$$

Table 5-3 Liquid hydrolyzate hemicellulose yield determined experimentally and theoretically predicted by the model

Reaction temperature (°C)	Reaction time(min)	Acid conc†%	pH	CSF‡	Hemicellulose yield (observed)%	Hemicellulose yield (predicted)%
140	10	0.5	1.67	0.50	4.2±0.2	3.3±0.0
160	10	0.5	1.89	0.87	27.8±0.7	29.3±0.0
140	10	2.0	1.26	0.91	41.5±0.4	43.0±0.0
140	30	0.5	1.71	0.94	15.7±1.1	17.9±0.0
150	20	0.5	1.67	1.10	23.9±0.5	25.1±0.0
140	20	1.25	1.35	1.12	39.9±0.6	41.2±0.0
150	10	1.25	1.28	1.19	44.1±0.6	47.8±0.0
160	30	0.5	1.87	1.37	36.8±2.1	38.6±0.0
150	20	1.25	1.37	1.40	51.3±0.3	51.0±0.0
150	20	1.25	1.35	1.42	49.8±0.4	51.0±0.0
150	20	1.25	1.35	1.42	49.4±1.4	51.1±0.0
150	20	1.25	1.33	1.44	52.0±1.2	51.0±0.0
140	30	2.0	1.21	1.44	47.7±0.1	49.5±0.0
150	20	1.25	1.31	1.46	48.9±0.3	51.0±0.0
150	20	1.25	1.23	1.54	50.6±1.9	51.0±0.0
150	30	1.25	1.35	1.59	58.6±3.5	59.6±0.0
160	10	1.25	1.06	1.70	56.4±1.4	56.6±0.0
150	20	2.0	1.03	1.74	52.6±0.8	49.9±0.0
160	20	2.0	1.22	1.84	47.6±0.3	47.0±0.0
160	30	2.0	1.10	2.14	43.7±0.1	48.3±0.0

† Acid concentration; ‡ Combined severity factor

Table 5-4 Analysis of variance table of the coefficients and corresponding P values

Term	Coefficients	Standard Error Coefficient	T	P
Constant	51.02	0.914	55.78	<0.001
X_1	6.20	0.892	6.946	<0.001
X_2	3.92	0.965	4.058	<0.001
X_3	12.36	0.892	13.85	<0.001
X_1*X_1	-3.60	1.551	-2.32	0.04
X_2*X_2	-0.73	1.568	-0.46	NS
X_3*X_3	-13.48	1.551	-8.69	<0.001
X_1*X_2	-1.32	1.112	-1.19	NS
X_1*X_3	5.47	1.012	-5.4	<0.001
X_2*X_3	-2.03	1.112	-1.83	NS

R^2 = 0.98, R^2(predicted) = 0.86, R^2(adjusted) = 0.97, NS= Not significant

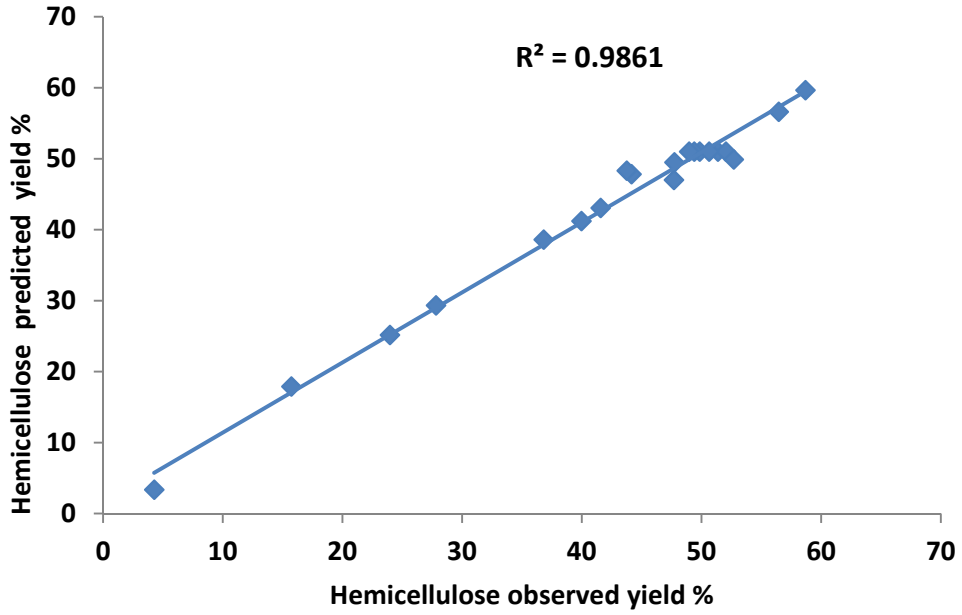

Figure 5-1 Experimental versus predicted values of hemicellulose yield in the hydrolyzate

5.4.1. Analysis of Liquid Fraction

During the pretreatment, polymeric hemicellulose is easily hydrolyzed into monomers by dilute acids under moderate conditions. However, more extreme conditions are required to hydrolyze crystalline cellulose. The success of the pretreatment is commonly evaluated by the hemicellulose yield. The yield was analyzed by accounting for monomeric and oligomeric sugars in the liquid hydrolyzate using HPLC. The hemicellulose yield in the hydrolyzate increased from 0.5 CSF to 1.59 CSF as summarized in Table 5-3. At a higher severity factor, the hemicellulose yield decreased significantly. This can be explained by the formation of furfural due to the degradation of pentose carbohydrates in the liquid fraction (Sharma et al. 2004). However, it is interesting to note that at 0.91 CSF the yield was higher compared to 0.94 CSF implying that the presence of a higher acid concentration plays a vital role in hydrolysis of hemicellulose. The maximum hemicellulose yield observed experimentally was 59 wt% at 1.59 CSF. The low hemicellulose yield can be explained by the presence of longer chain oligomers, which may be predominant in the hulls. The dissolution and diffusion rates of longer chain oligomers in the solution take longer times compared to shorter oligomers (Yan et al. 2009).

5.4.2. Evaluation of Pretreated Solid Residue

The composition of solid substrates in terms of cellulose, xylan and lignin was expressed in Figure 5-2. Solid recovery varied from 94 wt% at low CSF to 74 wt% at high CSF. Figure 5-2 shows that the xylan content in the solid fraction decreased as the severity of a pretreatment increased. The minimum amount of xylan retained was less than one percent at CSF of 2.14, the cellulose content increased up to a severity factor of 1.54 but declined slightly at higher severity

factors. This can be explained by the degradation of hexose carbohydrates into HMF through dehydration, which is attributed to a stronger interaction of protons with water than with the OH functional group of the pyranose ring of glucose. This is the critical step in the proposed mechanism for the formation of 5-HMF at high severity factors (Eva, James 1998). Lignin consists of phenolic monolignols such as p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. The amount of lignin ranged from 33- 48 wt% in the solid substrate.

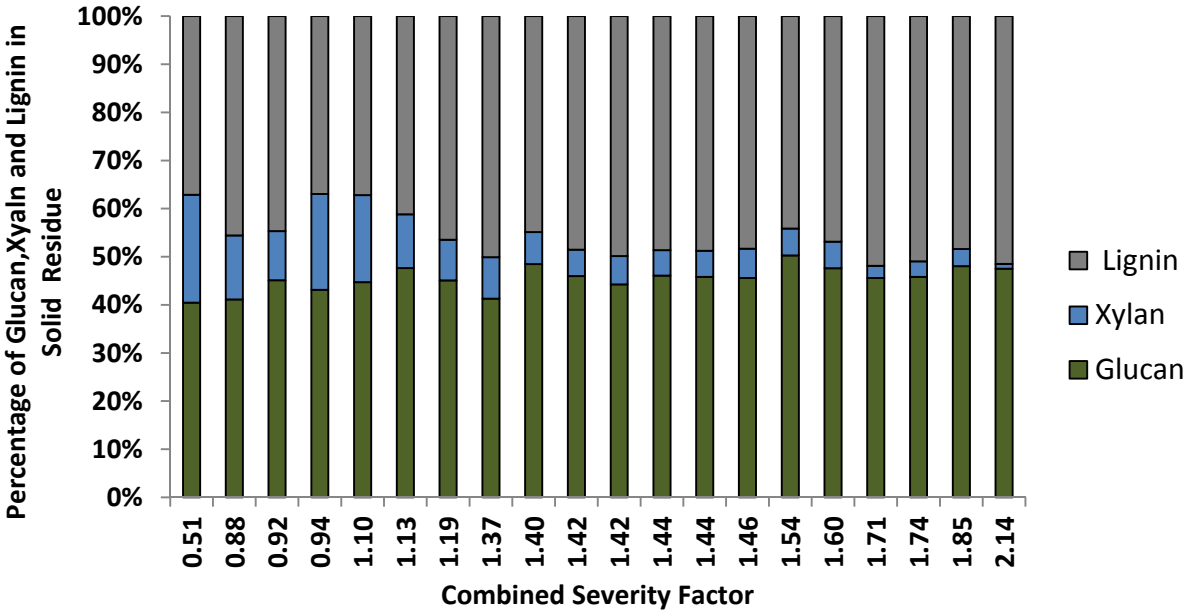

Figure 5-2 Percentage of cellulose, xylan and lignin retained in the solid substrate after the pretreatment

5.4.3. Enzymatic Saccharification

The optimum temperature and pH for sunflower hulls enzymatic saccharification was found by other researchers to be at 50 °C and at pH 4.8 (Sharma *et al.* 2004). The maximum β-glucan digestibility observed was 53.5 wt% at a 2 wt% acid concentration as evident from Figure

5-3. The digestibility yield of pretreated sunflower hulls was lower compared to the corn stover biomass. The maximum digestibility observed for corn stover was between 80-87 wt% when treated at 1.4 wt% acid concentration (Schell *et al.* 2003). A possible explanation is the presence of high lignin content in the solid substrate as evident from Figure 5-3. It leads to lignin sites competing against cellulose sites for enzymes. The enzymes that were adsorbed by the lignin sites became ineffective by forming lignin enzyme complexes. Other researchers proved that there is a quantitative inverse correlation between the lignin content and enzymatic digestibility (Guo *et al.* 2009).

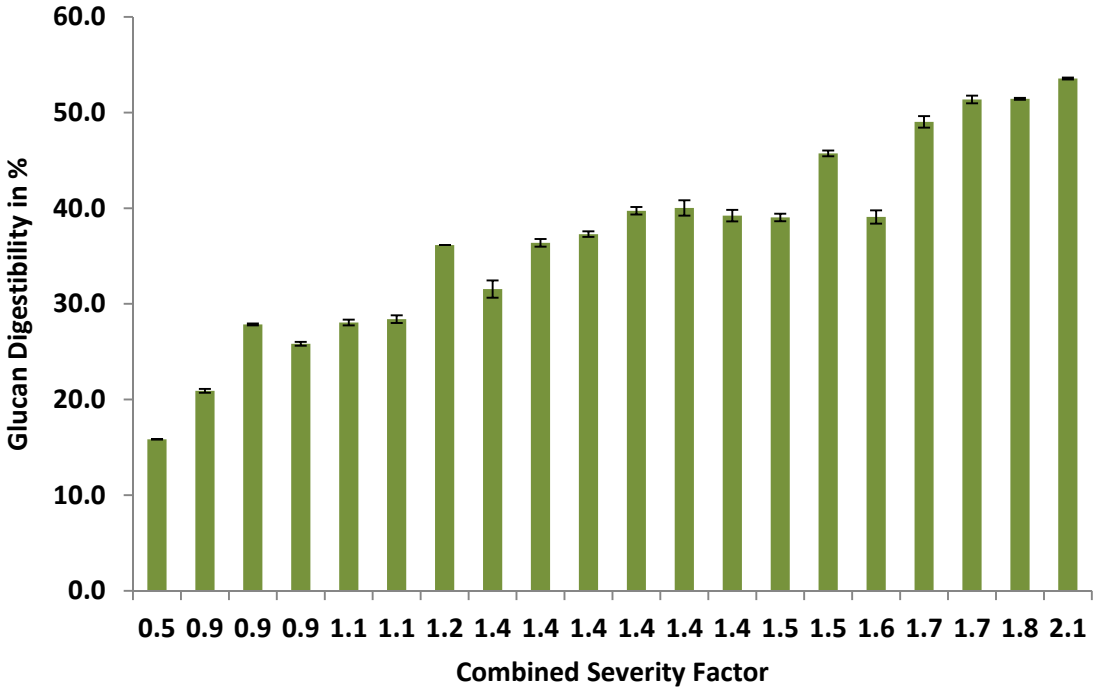

Figure 5-3 Enzymatic hydrolysis yield of pretreated cellulose substrate

5.4.4. Degradation Products

All the degradation products (acetic acid, furfural, HMF) are known to act as inhibitors for enzyme activity under selected conditions during the fermentation process. Acetic acid is liberated from acetyl linkages present in the hemicellulose fraction; furfural and HMF are products of pentose and hexose degradation respectively (Helle *et al.* 2003). HMF was present in trace amount in the liquid hydrolyzate and there was not much variation in the yields of HMF as shown in Table 5-5. It implies that hexose sugars degrade at much higher temperatures and at high acid concentrations. However, the amount of pentose sugar degradation product ranged from zero mg/mL at low CSF to 5.6 mg/mL at high CSF. The mechanism for conversion of pentose into furfural involves the conversion into xylulose through an isomerization reaction and

then the dehydration of xylulose leads into furfural (O'Neill *et al.* 2009). A possible explanation for high yields of furfural is that the pentose carbohydrate degradation is favored by high reaction temperature and long reaction time. The results obtained on the degradation products were in agreement with the study conducted by (QI et al., 2008). According to their study, pentose sugars decompose more rapidly compared to glucose.

Table 5-5 Concentration of inhibitor products present in the liquid hydrolyzate at different CSF

CSF	Acetic acid (mg/mL)	HMF (mg/mL)	Furfural (mg/mL)
0.5	0.4±0.0	0.0	0
0.9	1.4±0.2	0.1±0.0	0.2±0.0
0.9	3.9±0.9	0.1±0.0	0.1±0.0
0.9	1.0±0.3	0.0	0.0
1.1	1.1±0.0	0.0	0.0
1.1	3.8±0.1	0.2±0.0	0.2±0.0
1.2	3.9±0.6	0.2±0.0	0.3±0.1
1.4	2.9±0.5	0.1±0.0	0.6±0.1
1.4	4.9±0.4	0.3±0.1	0.8±0.1
1.4	5.1±0.3	0.2±0.1	0.9±0.1
1.4	4.8±0.7	0.2±0.1	0.8±0.3
1.4	5.2±0.1	0.2±0.0	0.9±0.3
1.4	4.9±0.3	0.2±0.0	0.9±0.1
1.5	4.7±0.9	0.2±0.1	0.8±0.0
1.5	5.3±0.3	0.2±0.1	0.9±0.2
1.6	5.6±0.6	0.2±0.1	1.5±0.4
1.7	5.2±0.2	0.1±0.0	1.4±0.3
1.7	5.4±0.7	0.2±0.0	2.5±0.5
1.8	5.2±1.4	0.2±0.0	2.9±0.4
2.1	5.5±1.1	0.2±0.0	5.6±0.9

5.5. Conclusion

The effects of reaction time, reaction temperature, and acid concentration on the sunflower hulls biomass pretreatment process were studied using a Central Composite Design methodology. These three factors and their interactions were statistically analyzed for the hemicellulose yield. The maximum hemicellulose yield predicted by the model was 62 wt% at 158 °C for 20 min at 1.75 wt% acid concentration. The amount of fermentable sugars formed after the enzymatic

hydrolysis showed a linear increase with the severity of the pretreatment. The maximum cellulose digestibility was observed to be 53.5 wt% at 2.14 CSF. The low digestibility implies that high lignin content in the biomass may be inhibiting the complete hydrolysis of cellulose during the enzymatic hydrolysis. It implies that irreversible adsorption of lignin on crystalline cellulose structure was occurring. In order to convert cellulose and hemicellulose effectively into fermentable sugars during enzymatic saccharification, sunflower hulls may need to undergo de-lignification prior to acid pretreatment. Degradation products were studied in the liquid fraction of pretreated samples. Increase in the severity of pretreatment led to augmentation of inhibitor products such as acetic acid and pentose carbohydrates degradation into furfural. However, the amount of glucose degradation to HMF was relatively low compared with acetic acid and furfural. Other factors worth investigating during the sunflower hulls pretreatment in the future are the effect of particle size, pore volume, and the surface area available. Those factors may play a role in effectively converting cellulose into fermentable sugars for renewable fuels and chemicals production.

6. DETERMINING THE KINETICS OF SUNFLOWER HULLS USING DILUTE ACID PRETREATMENT IN THE PRODUCTION OF XYLOSE AND FURFURAL

6.1. Abstract

Pretreatment of sunflower hulls was conducted under varied dilute acid concentrations (0.5-2.0 wt%), reaction temperatures ranging between 140-160 °C and the reaction time up to 30 min. The conversion of xylan into xylose and furfural was investigated. The maximum xylose and furfural recoveries were 54.5±0.7 and 24.0±1.1 wt%, respectively were obtained at different reaction times with 2.0 wt% acid concentration at 160 °C. The experimental data were fitted into a two-step kinetic model based on irreversible pseudo-first order kinetics at each step. The model was successfully validated using the F-test. Sunflower hulls showed a greater recalcitrance to acid pretreatment than other agricultural crops, such as kenaf, sorghum and sunn hemp. This feature was ascribed to the occurrence of a wax layer on the cell wall surface with a high lignin content, which may act as a barrier hindering the acid access to acetyl linkages in xylan.

6.2. Experimental Method

6.2.1. Pretreatment

The pretreatment operating procedure for hulls is similar to that explained in **Chapter 3**.The pretreatment reaction parameters were selected to approximate those that the current National Renewable Energy Laboratory (NREL) process design conditions for biochemical conversion of lignocellulosic biomass to ethanol (Humbird et al., 2011). According to the report, the described NREL process design uses a temperature of 158°C in a dilute-acid pretreatment batch reactor. Large amounts of poorly fermentable oligomers are formed at lower reaction temperatures and acid concentrations whereas significant further degradation of C_5 products usually occurs under higher severity conditions. Hence we have chosen to perform this study between 140-160 °C; the operational conditions are listed in Table 6-1. The acid concentration was varied between 0.5-2.0 wt %. Each pretreatment experiment was performed up to a maximum reaction time of 30 min. The liquid hydrolyzate samples were withdrawn every five minutes.

Table 6-1 Pretreatment conditions

0.5 wt% Acid Concentration	1.25 wt% Acid Concentration	2 wt% Acid Concentration
140 °C	140 °C	140 °C
150 °C	150 °C	150 °C
160 °C	160 °C	160 °C

6.2.2. Model

The model used to determine the rate coefficients and Arrhenius parameters is already explained in **Chapter 4**. The amount of oligomers present in the liquid hydrolyzate samples were ignored in the modeling since the concentration ranged from 0.21 g/L at 140 °C, 0.5 wt% acid concentration to 0.0 g/L at 160 °C, 2 wt%.

6.3. Results and Discussion

6.3.1. Effect of Acid Concentration and Reaction Temperature on Xylose and Furfural Yields

Table 6-2 lists the concentration of xylose produced from xylan as a function of time. The dynamics of xylan hydrolysis is similar to that observed with other feedstocks; namely, the xylose concentration increases and then declines, with a concomitant increase of the furfural concentration (Hosseini, Shah 2009).

From the Table 6-2 data it is evident that the amount of xylose observed was rather low at lower reaction temperatures and acid concentrations, even at longer reaction times. Higher xylose yields were obtained at higher acid concentrations, even at lower temperatures. The effect of acid concentration was thus found to be more pronounced than that of reaction temperature.

Table 6-2 Concentration of xylose in the liquid hydrolyzate samples, g/L

Acid concentration (%wt)	Reaction temp†(°C)	Reaction time (min)						
		0	5	10	15	20	25	30
0.5	140	0	0.5±0.1	1.0±0.3	1.5±0.1	2.2±0.0	2.8±0.4	3.6±0.3
1.25	140	0	3.2±0.1	5.8±0.7	7.8±0.6	9.1±0.1	9.9±0.3	10.1±0.1
2.0	140	0	4.9±0.2	8.2±0.5	10.4±0.7	11.5±0.3	11.5±0.2	10.5±0.1
0.5	150	0	1.9±0.0	3.4±1.1	4.6±0.9	5.5±0.5	6.0±0.2	6.2±0.6
1.25	150	0	5.1±0.4	8.7±1.3	11.3±0.6	12.8±0.3	13.4±1.2	13.0±1.1
2.0	150	0	5.0±0.1	8.7±0.3	11.0±0.5	12.1±0.7	11.8±0.1	10.1±0.3
0.5	160	0	3.3±0.3	5.7±0.1	7.4±0.2	8.4±0.3	8.6±0.1	8.2±0.1
1.25	160	0	4.0±0.3	7.2±0.3	9.6±0.5	11.2±2.1	12.1±1.8	12.2±0.7
2.0	160	0	6.7±0.9	11.2±0.6	13.6±0.6	13.7±1.1	11.7±0.9	7.5±0.1

†temp = Temperature

Table 6-3 lists the furfural concentrations recovered as a result of xylose chemical de-hydration. Low furfural yields were obtained at lower acid concentrations, even at higher reaction temperatures. However, using higher acid concentrations led to higher furfural yields. Two other observations concerning threshold xylose concentrations can be made based on Table 6-3 data. First, furfural formation was observed only when the xylose concentration exceeded 5.8±0.7 g/L that corresponds to 24.0±1.1 wt%. Second, the xylose concentration never exceeded 13.7±1.1 g/L corresponding to a 57.1±0.7 wt% yield, followed by a decline with a concomitant furfural formation. The analysis of trends observed in xylose and furfural formation is provided in the next section.

Table 6-3 Concentration of furfural in the liquid hydrolyzate samples, g/L.

Acid Concentration (%wt)	Reaction Temperature (°C)	Reaction Time (min)						
		0	5	10	15	20	25	30
0.5	140	0	0	0	0	0	0	0
1.25	140	0	0	0	0.1±0.1	0.2±0.1	0.3±0.1	0.5±0.1
2.0	140	0	0.1±0.1	0.2±0.1	0.3±0.1	0.5±0.1	0.7±0.3	0.9±0.1
0.5	150	0	0	0	0	0	0.01±0	0.02±0
1.25	150	0	0.3±0.1	0.5±0.1	0.8±0.1	1.1±0.4	1.4±0.3	1.6±0.1
2.0	150	0	0.4±0.1	0.9±0.4	1.4±0.3	1.9±0.7	2.5±0.3	3.2±0.4
0.5	160	0	0.1±0.1	0.2±0.1	0.3±0.1	0.4±0.1	0.5±0.1	0.6±0.2
1.25	160	0	0.8±0.3	1.5±0.7	2.0±1.0	2.5±0.6	2.9±0.4	3.1±0.3
2.0	160	0	1.6±0.3	2.9±0.2	3.9±0.6	4.7±1.1	5.3±0.7	5.6±0.6

6.3.2. Model Justification

Figure 6-1 shows the experimental data along with the simulation produced using the time dependent expressions as derived in **Chapter 4** (Equations 4-2 to 4-5). The best fitted kinetic constants k_1 and k_2 for the proposed model are listed in Table 6-4. The kinetic constants were higher for xylose monomer than for furfural formation as expected, because a similar pattern (faster xylose formation followed by its slower hydrolysis to furfural) was observed earlier for any other crop considered (Morinelly *et al.* 2009). The rate increased for both xylose and furfural formation with the increase of reaction temperature and acid concentration, which was also expected based on the literature analysis (Kamireddy *et al.* 2013c).

From Figure 6-1 it is evident that the fitted parameters predicted the experimental data reasonably well. The only exception was the furfural formation at 160 °C. As can be seen in Figure 6-1c, the model under-predicted the furfural formation at this highest temperature used, particularly for the highest acid concentration, 2.0 wt%. These effects can be explained by subsequent reactions of furfural decomposition, which are more pronounced at the highest severity conditions (Bensah, Mensah 2013, Sun, Cheng 2005, vom Stein *et al.* 2011).

To further justify the model used, the observed reaction orders, n_i (Table 6-5) were replaced with the kinetically relevant integers (0, 1, 2) and plugged in (Equation 4-11) that was described in **Chapter 4**; then, the model was run with these artificially set values. As a result of this treatment, the model lost its predictive power; furthermore, in most of the cases the activation energies obtained in such a way turned out to be *negative*, thus contradicting the experimentally observed trend (Table 6-4).

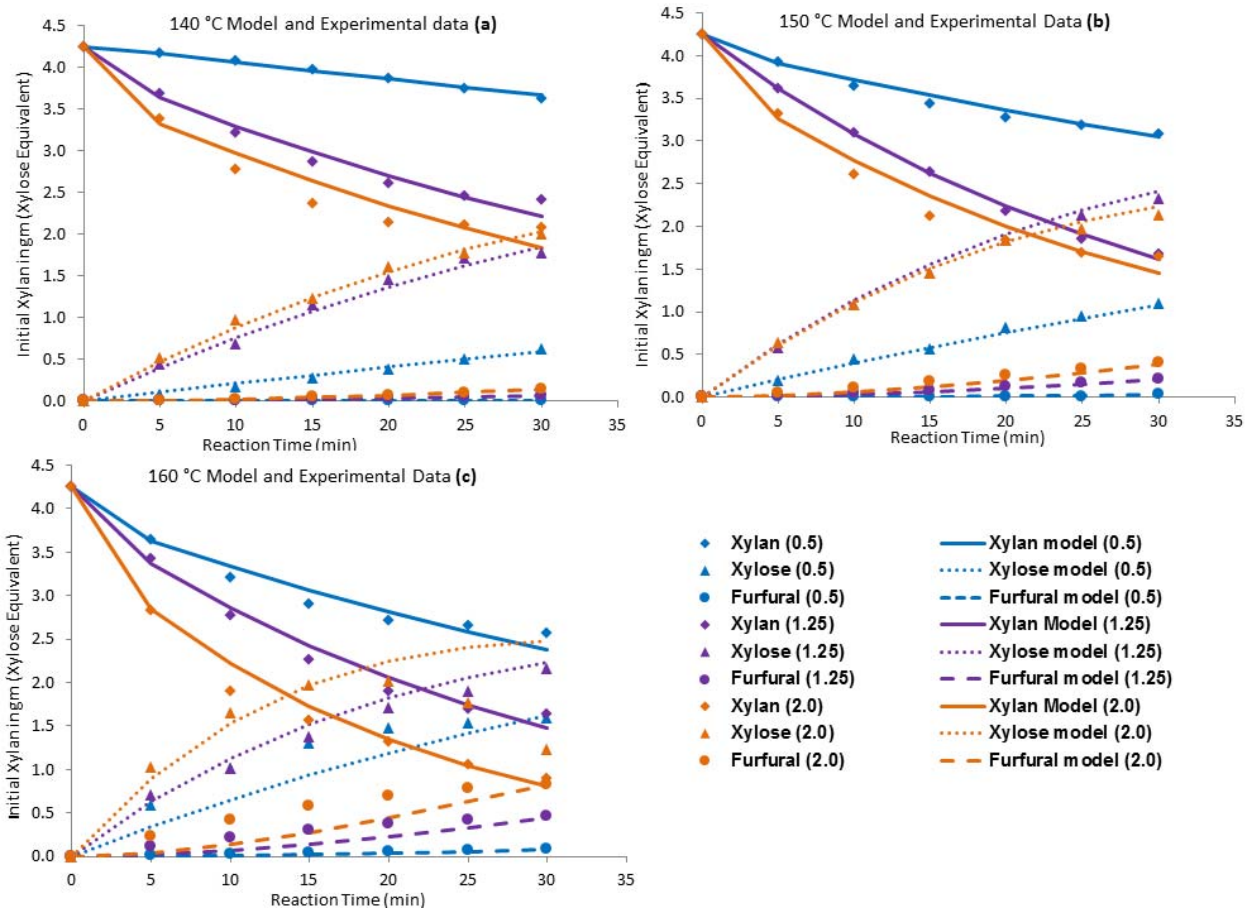

Figure 6-1 Model and experimental data for xylan, xylose and furfural for sun hulls a) pretreated at 140 °C at 0.5, 1.25 and 2.0 wt% acid concentrations for hulls; b) pretreated at 150 °C at 0.5, 1.25 and 2.0 wt% ; c) pretreated 160 °C at 0.5, 1.25 and 2.0 wt%.

Table 6-4 Best-fitted rate constants of Sunflower Hulls

Reaction Temperature (°C)	Rate Constant (min^{-1})	Acid Concentration in (wt%) 0.5	1.25	2.0
140	$\boldsymbol{k_1}$	5.0×10^{-3}	2.0×10^{-2}	2.4×10^{-2}
150		1.1×10^{-2}	3.2×10^{-2}	3.2×10^{-2}
160		1.7×10^{-2}	3.3×10^{-2}	5.0×10^{-2}
140	$\boldsymbol{k_2}$	0.00	2.0×10^{-3}	4.0×10^{-3}
150		1.5×10^{-3}	4.8×10^{-3}	9.5×10^{-3}
160		2.8×10^{-3}	1.1×10^{-2}	1.6×10^{-2}

Table 6-5 Fitted Arrhenius parameters (Equation 4-11) obtained using the kinetic constants of Table 6-4

Biomass		acid (wt%) exponenent, n_i (unit less)	pre-exponential factor, A (min^{-1})	activation energy, E_i (kJ/mol)	R^2
Sunflower Hulls	$\boldsymbol{k_1}$	0.99	1.67×10^{5}	56.58	0.89
	$\boldsymbol{k_2}$	1.38	4.53×10^{9}	98.03	0.92

Model testing is often performed by comparing the R^2 values obtained by least square fitting; however, exponential kinetic data may be skewed as a result of linearization. Hence an F-test was performed instead, comparing the experimental data with those generated by the theoretical model by varying the pre-exponential factor, activation energy and dimensionless reaction order (Yat *et al.* 2008b). Table 6-6 suggests that the experimental data fitted the model accurately as the sums of squared errors (SSE) values were low. The differences between the experimental rate coefficients and those generated by the model were low as the sets passed the F-test ($F>F_{critical}$). Thus, the model applied can be deemed adequate, despite the inherent heterogeneity of the system used.

Table 6-6 F- test of the two sample variance for k_1 and k_2 rate coefficient for both the experiment and model.

	k_1 observed	k_1 predicted	k_2 observed	k_2 predicted
Mean	2.4×10^{-2}	2.2×10^{-2}	5.6×10^{-3}	5.9×10^{-3}
Variance	1.8×10^{-4}	2.0×10^{-4}	2.7×10^{-5}	2.9×10^{-5}
SSE	1.7×10^{-5}		6.4×10^{-6}	
Observations	9	9	9	9
Df	8	8	8	8
F	9.0×10^{-1}		9.0×10^{-1}	
P(F<=f) one-tail	4.4×10^{-1}		4.5×10^{-1}	
$F_{critical}$ one-tail	2.9×10^{-1}		2.9×10^{-1}	

SSE= Sum of squared errors; Df= Degrees of freedom

6.3.3. Reasons for a Relative Recalcitrance of Sunflower Hulls

The amount of initial xylan present in hulls is high as compared to other crops as evident from Table 6-7. The comparison of runs conducted under varied conditions shows that the amount of xylan hydrolyzed was lower whenever the acid concentrations were lower. More than

50 wt% of the initial xylan was still retained in hulls at a 0.5wt% acid concentration at 140 °C. By contrast, almost 80 wt% of the initial xylan was hydrolyzed at 160 °C with a 2 wt% acid concentration. Compared to other lignocellulosic biomasses such as forage sorghum, kenaf and sunn hemp pretreated under similar conditions, xylan hydrolysis was significantly less pronounced for hulls (Kamireddy *et al.* 2013c).

This difference could be due to a unique cell wall structure specific for sunflower hulls. It consists of a black pigmented layer with a high wax content. The presence of a wax layer at the surface of hulls is to protect the seeds against mold by repelling water (Carelli *et al.* 2002). This wax/lignin barrier may hinder the access of hydronium ions to xylan resulting in both a lower effective reaction order on the acid, n_1, and higher activation energy for xylan hydrolysis. As a result, the process occurs under lower *effective* acid concentrations than set by the bulk acid concentration. Corroborating this assumption, the values of n_i and E_i obtained in this study are similar to those obtained for other crops such as aspen, balsam, switch grass, at lower acid concentrations (Morinelly *et al.* 2009).

To obtain higher xylose yields during acid pretreatment, one practical recommendation would thus be subjecting hulls to a prior ethanol or other organic solvent extraction to dissolve the waxes. An alternative would be a de-lignification prior to xylan hydrolysis, e.g., an alkaline pre-treatment.

Table 6-7 Content of xylan for various biomass species as compared to hulls

Biomass species	Xylan content	References
Corn stover	18.1±2.1	(Kamireddy *et al.* 2013c)
Sorghum NBMR†	15.2±0.2	(Kamireddy *et al.* 2013c)
Sorghum BMR‡	13.0±0.6	(Kamireddy *et al.* 2013c)
Sunn hemp	9.9±0.5	(Kamireddy *et al.* 2013c)
Kenaf	14.0±1.2	(Kamireddy *et al.* 2013b)
Wheat straw	6.4±0.1	(Ahmed *et al.* 2010)
Sunflower hulls	21.2±1.5	(Kamireddy *et al.* 2012a)

†= Non Brown Mid Rib; ‡ Brown Mid Rib

6.4. Conclusion

In this study the effect of dilute acid concentration, reaction temperature was studied on sunflower hulls. The maximum xylose and furfural recoveries were 54.5±0.7 and 24.0±1.1 wt%, respectively were obtained at different reaction times with 2.0 wt% acid concentration at 160 °C. The experimental data were fitted into a two-step kinetic model based on irreversible pseudo-first order kinetics at each step. The model was successfully validated using the F-test. Sunflower hulls showed a higher recalcitrance to acid pretreatment as compared to many other agricultural residues. This difference was explained by a high lignin and wax content of the cell walls, which could act as a barrier to the hydronium ions resulting in an increase of the activation energy and lowering the effective reaction rate order on the acid. To obtain higher xylose yields, either prior de-lignification or de-waxing by ethanol extraction may be recommended.

7. EFFECTS AND MECHANISM OF LEWIS ACID ACTION ON PRETREATMENT AND ENZYMATIC DIGESTIBILITY OF CORN STOVER

7.1. Abstract

The effects of three Lewis acids including $FeCl_3$, $CuCl_2$ and $AlCl_3$ on corn stover pretreatment and enzymatic hydrolysis were studied under lower severity conditions (reaction temperature of 150-160 °C, salt concentration of 0.075-0.125M, reaction time of 10 min). The results were compared with dilute sulfuric acid pretreatment under the same conditions. The maximum monomeric xylose yield was observed to be 93 and 94wt% when $CuCl_2$ and $FeCl_3$ were used as salts in the pretreatment at 160 °C for 10 min at 0.125M concentrations, which were higher than the sulfuric acid pretreatment yields at the same reaction conditions. However, the monomeric xylose yield for corn stover pretreated with $AlCl_3$ was observed to be merely 8 wt% at the same condition. This could be explained by isomerization of xylose to xylulose and subsequent dehydration into furfural. However, enzymatic digestibility yields for the three Lewis acids were greater than 92 wt% for all the samples pretreated at 160 °C. These yields were higher than those of sulfuric acid pretreated samples at the same reaction conditions. The overall formation of fermentation inhibitor products for samples pretreated with $CuCl_2$ and $FeCl_3$ was observed to be almost similar to the control samples (pretreated with sulfuric acid).

7.2. Introduction

The heterogeneous nature of lignocellulosic biomass which consists of cellulose, hemicellulose and lignin acts as a recalcitrance to enzymatic hydrolysis in conversion of cellulose into fermentable sugars. It is imperative to disrupt hemicellulose and lignin bonds for accessing the cellulose sites by the enzymes. The pretreatment is generally performed using various inorganic acids and bases such as dilute acid, alkali, and liquid hot water. Each pretreatment technique has its own merits and demerits. However, development of new pretreatment technologies that are highly efficient, economically feasible, and environmentally friendly is vital for large scale commercialization of biofuels and value added chemicals (Liu *et al.* 2009).

According to Consortium of Applied Fundamentals and Innovation (CAFI), the most effective and economical way to convert biomass to fermentable sugars is to hydrolyze hemicellulose using dilute acid pretreatment and followed by enzymatic hydrolysis (Wyman *et al.* 2005b). However, pretreatment of biomass with dilute acids may have lower xylose yields. In addition, corrosive properties of dilute acid pretreatment agent require the use of expensive materials that may increase the capital cost (Kumar, Wyman 2008).

Lewis acids are expected to show a higher catalytic activity than Bronsted acids, with the possibility of being easily separated from the reaction products by supporting them on a carrier (Yu *et al.* 2011). Several studies have reported that some Lewis acids can catalytically hydrolyze carbohydrates into useful feedstock chemicals (Liu *et al.* 2009, Seri *et al.* 2002, Zhao *et al.* 2007). Lewis acids can also increase the decomposition rate of cellulose and hemicellulose. Liu

et al., 2009 pretreated corn stover with inorganic chlorides including NaCl, KCl, $CaCl_2$, $MgCl_2$, $FeSO_4$, $FeCl_3$, and $Fe_2(SO_4)_3$, and found that $FeCl_3$ significantly increased the hemicellulose hydrolysis in aqueous solutions at reaction temperatures from 140 to 200 °C with high hemicellulose recovery. Hemicellulose removal increased 11-fold when the corn stover was pretreated with 0.1 M $FeCl_3$ compared to a pretreatment with hot water under the same conditions (Liu *et al.* 2009) Other studies revealed that acid-ferrous ion-assisted pretreatment increases the solubilization and enzymatic digestion of both cellulose and hemicellulose in the form of monosaccharides and this pretreatment likely targets multiple chemistries in plant cell wall polymer networks, including those represented by the C-O-C and C-H bonds in cellulose (Wei *et al.* 2011). In addition, the Lewis acids can be recovered as metal hydroxides after the pretreatment by using a process called ultrafiltration (Bernata *et al.* 2008). These hydroxides can be treated with acids (in our case, hydrochloric acid) to convert back to Lewis acids and reuse in the process.

Though some work has been done on the effects of inorganic salts on corn stover pretreatment, there is little information available on the reaction mechanism. The purpose of this study is to propose the chemical mechanism on hemicellulose hydrolysis with the Lewis acids during pretreatment. In order to reduce the energy consumption and the degradation products formation, the pretreatments were performed at low severity conditions (reaction temperature, salt concentration, and time). Moreover, this study aims to provide an insight on selectivity of Lewis acids that can yield high fermentable sugars. In this study pretreatment of corn stover was performed using two earlier untested Lewis acids ($CuCl_2$, $AlCl_3$) and results were compared with

$FeCl_3$ and dilute sulfuric acid pretreatments. The alkali and alkaline earth metals such as KCl, NaCl, $CaCl_2$, and $MgCl_2$ were ignored in the study since the addition of these Lewis acids led to low hemicellulose hydrolysis (Liu *et al.* 2009).

7.3. Experimental Methods

7.3.1. Biomass Gravimetric Analysis

The feed stock material was provided by the National Renewable Energy Laboratory (NREL) (Golden, CO). Corn stover was harvested from Wray, CO and milled to a ¼" size. The composition of corn stover was analyzed at NREL. It contains 33.4 wt% cellulose, 21.8 wt% hemicellulose, 11.2 wt% lignin, 3.7 wt% ash, and 9.3 wt% extractives by dry weight (Weiss *et al.* 2010).

7.3.2. Pretreatment in a Batch Reactor

The biomass pretreatment was performed in a 300-mL internal volume, jacketed batch reactor (Auto Clave Engineers, Erie, PA). The reactor was made of Hastelloy C-276 to mitigate the effects of acid corrosion at high temperatures. Twenty-one grams of dry biomass was added to 200 mL of a Lewis acid solution (10 wt% dry biomass). The reaction temperature and Lewis acid concentration evaluated for the study were 150 and 160 °C and 0.075-0.125 M, respectively. The reaction time maintained was around 10 min. These conditions were chosen to compare the results obtained by pretreatment of corn stover with sulfuric acid that was performed by previous researchers. The heating source used for the reactor was saturated steam for a fast heating ramp. Saturated steam was drawn into the external jacket of the reactor by operating a three-way valve.

The agitation in the reactor was maintained constant at 60 rpm throughout the reaction. After the desired temperature was achieved, the reaction time commenced and the temperature in the reactor was maintained constant by operating the 3-way valve manually (Degenstein *et al.* 2011). When the reaction was over, the reactor was then cooled by passing the cooling water into the external jacket. Once the reactor was cooled below 40 °C, the slurry in the reactor was discharged and collected in a polyethylene bottle for further analysis. The temperature data from the reactor were recorded with the aid of picolog software throughout the reaction time. All the pretreatment experiments were duplicated.

The Lewis acids used in the study are prone to yield high amounts of inhibitor products in liquid hydrolyzates (Su *et al.* 2009). Moreover, the increase in reaction temperature and time will result in a greater degradation of hemicellulose and cellulose (Liu *et al.* 2009). In order to mitigate these effects we have chosen to study the effect of these Lewis acids at lower severity conditions. From our previous experimental data on corn stover pretreatment with dilute acid we found that reaction temperatures between 150 and 160 °C, reaction time of 10 min and acid concentration of 0.075 M were sufficient to obtain significant hemicellulose yields after pretreatment and enzymatic digestibility of cellulose from enzymatic hydrolysis. Glucose and xylose monomers can react with their individual degradation products such as furfural and HMF to form undesired solid humins, which are highly polymerized insoluble carbonaceous species (Dutta *et al.* 2012). This is the primary reason for conducting all the pretreatments under low severity conditions, so that the formation of this undesired product (humin) can be avoided.

7.3.3. Determination of Monomeric Sugars in the Liquid Fraction (Hydrolyzate)

After pretreatment, the slurry samples were vacuum filtered and separated into liquid and solid fractions. The hydrolyzates were then analyzed for monomeric and oligomeric carbohydrates. Prior to analysis, hydrolyzate samples were neutralized by adding calcium carbonate until a pH range of 5.0-6.0 was obtained. Neutralization was necessary according to the HPLC column manufacturer (Transgenomic, Omha NE) guidelines. The neutralized samples were filtered in order to purify the hydrolyzates using a 0.2 μm filter (Millipore, Billerica, MA) into glass vials. The sugar analysis was performed in an Agilent 1200 High Pressure Liquid Chromatography (HPLC) (Palo Alto, CA) equipped with Transgenomic CHO-Pb column (300 mm×7.8 mm). The column temperature was maintained at 80 °C and the Refractive Index Detector (RID) temperature was maintained at 55 °C during the analysis. DI water with 0.6 mL/min flow rate was used as the mobile phase for sugar analysis. The analysis time for each sample was 35 min (Silverstein *et al.* 2007). Calibration standards were run prior to the analysis of the samples. The concentrations of standards ranged between 0.5 to 18 g/L. The known concentration of the sugar standard (4 g/L) was run frequently (every 8 samples) to test the accuracy of the column and RID. The standard solution and sugar recovery standard solution consist of D-(+) glucose, D-(+) xylose, D-(+) galactose, L-(+) arabinose, and D-(+) mannose. Xylose yield was calculated from (Equation 7-1).

$$\text{Xylose Yield} = \frac{\text{Concentration of xylose } \left(\frac{\text{g}}{\text{L}}\right) * \text{Volume of the pretreated liquid (L)}}{\text{Weight of the xylose in the raw biomass (g)}} \qquad (7\text{-}1)$$

7.3.4. Determination of Structural Carbohydrates and Lignin in the Pretreated Solid Residue

The analyses were performed to determine the amount of cellulose, hemicellulose and lignin retained in the solid fraction after pretreatment. The solid samples were air dried for 4-5 days at room temperature and milled into 100-mesh particle size. Three hundred milligrams of milled solid biomass was loaded in the pressure tubes (Ace Glass Incorporated, Vineland NJ) and 3.0 mL of 72% sulfuric acid were added to the biomass. The tubes were placed in a 30°C water bath for 1 h. Then the acid concentration was reduced to 4% by adding 84 mL of DI water to each pressure tube. These pressure tubes were placed in an autoclave oven at 121 °C for 30 min. The resultant slurry was vacuum filtered using porous ceramic crucibles (Coorstek, Oakridge, TN). The liquid fraction was analyzed for the amount of acid soluble lignin (ASL) using a UV-Vis spectrometer (Thermo Scientific, Waltham, MA), and carbohydrates using HPLC. Solid residue retained in the crucibles was oven dried at 105 °C for 12 h to determine the acid insoluble lignin content (AIL). Then the crucibles were placed in a muffle furnace at 575 °C for 24 h and then weighed to determine the ash content. This method is based on the NREL LAP protocol (NREL/ TP-510-42618).

7.3.5. Determination of Fermentation Inhibitors

The liquid fraction of the pretreated samples was rich in pentose sugars, which can be fermented into biofuel using *Pichia stiptis*. However, the side products formed during pretreatment such as acetic acid, HMF and furfural act as fermentation inhibitors during the fermentation process. In order to effectively convert the sugars into biofuels, fermentation

inhibitor products in the liquid fraction should be analyzed and controlled. The analysis was performed using an Agilent 1200 series HPLC system with a Phenomenex Rezex RFQ column. The column temperature was maintained at 80°C and the mobile phase contained 0.01 N sulfuric acid solution. The flow rate for analysis was maintained at 1 mL/min (Sluiter *et al.* 2010). The verification standards for inhibitor products were obtained from Absolute Standards, Inc (Hamden, CT). The time taken for analysis of each sample was 13 min.

7.3.6. Enzymatic Saccharification

The enzymatic saccharification was performed on washed pretreated solid substrate in a thermal incubator at 50 °C and 250 rpm for 72 h. Compositional analysis of the pretreated solid substrate was measured by HPLC. Then the biomass was accurately weighed so that 0.2 g of glucan was available for enzymatic saccharification. The solid substrate was loaded in a centrifuge tube with 10 mL of 0.1M sodium citrate buffer (pH 4.8) and DI water was added to acquire a total volume of 20 mL. Sodium azide with a concentration of 20 mg/mL was added to the solution in order to inhibit the bacterial growth during the 72 h enzymatic hydrolysis. Cellulase enzyme commercially known as (Accellerase 1500) was used to perform enzymatic hydrolysis. It has been provided by Genencor International (Palo Alto, CA). This procedure is based on the NREL LAP protocol (NREL/TP 510-42629). After 72 h, the liquid hydrolyzate samples were filtered into glass vials and the sugar concentrations were determined by an Agilent 1200 HPLC system with Transgenomic CHO-782 Pb column.

7.4. Results and Discussion

7.4.1. Reaction Mechanism of Lewis Acid Action

Lewis acids such as $CuCl_2$, $FeCl_3$ and $AlCl_3$ dissociate into complex ions in aqueous media. Their Lewis acid character emerges from the ability to attract electron pairs. They form coordinate bonds with six water molecules. The general nomenclature of metals ions ligand complexes is given as $[M(H_2O)_n]^{z+}$ (where M is the metal ion, z is the cation oxidation state and n is the solvation number typically ranging between 4 to 9) (Román-Leshkov, Davis 2011).Water molecules bond as mono-dentate ligands with the central metal cation. The central metal cation polarizes (withdraws electron density) from the water molecules. It can be inferred that by adding Lewis acids to water results in complex cation formation. Water molecules that are near to the metal cation form a primary hydration sphere. A secondary hydration sphere is also formed when water molecules bond to the primary hydration sphere. The formation of the secondary hydration sphere is a consequence of the fact that water molecules directly bonded to hydrated cations are of more acidic nature and they can form relatively strong hydrogen bonds (Grzybkowski 2006). These metal cations thus formed acts as Lewis acids with the coordinated water molecules from the hydrated cation participating as nucleophiles (Cotton *et al.* 1988, Peng *et al.* 2010). It can be proposed that $FeCl_3$ and $AlCl_3$ follow a similar reaction path by forming six coordinate bonds with water molecules. In a similar way it can be assumed as copper cation forms stable complexes with six water molecules. However, the copper cation has a distorted octahedral structure with an elongated axis in water. It results in copper cation forming coordinate bonds with eight water molecules to form a stable complex (four from primary

hydration sphere and four from secondary hydration sphere) (Grzybkowski 2006).

(Peng *et al.* 2010) proved that product yields depend mainly on the type of Lewis acids used rather than the acidity of the solution. They studied different Lewis acids under the same initial pH values of the reaction system and found out that even at the same initial pH, the yields of levulinic acid formed from rehydration of 5- HMF were different with various Lewis acids. The results further demonstrated that the type of Lewis acids played a major role in the hemicellulose hydrolysis into monosaccharaides (Peng *et al.* 2010). Therefore, we decided to conduct our pretreatments using the same Lewis acids concentrations rather than the same pH values.

These complex cations then follow the seven step mechanisms to de-polymerize hemicellulose into monosaccharides as shown schematically in Figure 7-1.

1) Diffusion of complex cation through the wet lignocellulosic matrix;

2) Protonation of the oxygen of heterocyclic hemiacetal between the sugar monomers;

3) Breaking of the ether bond;

4) Generation of carbocation as intermediate;

5) Solvation of the carbocation with water;

6) Regeneration of the complex cation with cogeneration of the sugar monomer, hemicellulose, oligomer, or polymer depending on the position of the hemiacetal bond;

7) Diffusion of the reaction products in the liquid phase and re-initiation of the second step (Mamman *et al.* 2008).

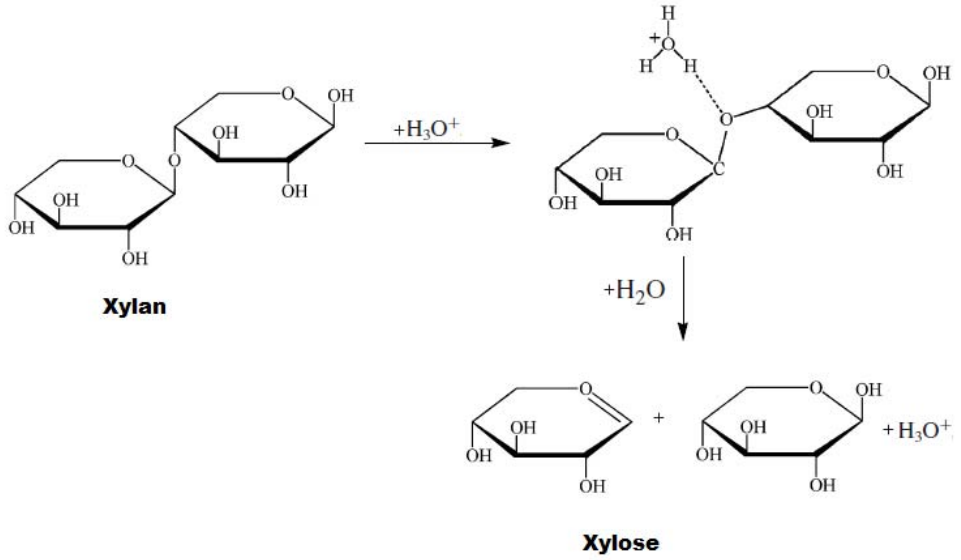

Figure 7-1 Mechanism of conversion of xylan into xylose through the formation of a hydronium ion (Dong *et al.* 2009).

7.4.2. Interaction of Lewis Acids with Water Solvents

The interaction of Lewis acids with the solvent molecules influences their catalytic activity. It is measured by the electron pair acceptor capacity number AN. Water has one of the highest AN number (54.8) compared to other solvents such as benzene (8.2) (Román-Leshkov, Davis 2011). Once formed, aqua ions in water will invariably undergo hydrolysis reactions to produce hydroxide species, consequently losing most of their Lewis acid character. Cations that do not lose their character for their size and charge are hard transition metal ions or lanthanide ions and $AlCl_3$ (Román-Leshkov, Davis 2011). The acid strength of a Lewis acid in a solution is determined by the pK_a value. Table 7-1 summarizes the pK_a values of different metal cations. The lower pK_a indicates the stronger acidic character of the metal cations in the solution. The data in Table 7-1 shows that Fe(III), Al(III), and Cu(II) form stronger acids in the aqueous solution than

other cations listed in Table 7-1. These values also explain the low xylose yields when Lewis acids such as NaCl, KCl, $FeCl_2$, $MgCl_2$ and $CaCl_2$ were used in studies conducted by Liu et al., 2009 on corn stover and Yu et al., 2011 on sorghum biomass. Since, alkali and alkaline earth Lewis acids dissociate into ions in water.

Table 7-1 pK_a values of various metal cations (Román-Leshkov, Davis 2011)

Cation	pK_a
Fe(III)	2.46
Fe(II)	9.49
Cu(II)	6.50
Al(III)	4.85
Na(I)	14.1
Sr(II)	13.2
Mg(II)	11.4
Ca(II)	12.7

7.4.3. Effect of pH on the Extraction Liquors

The pH values were measured for all the solutions (DI water mixed with Lewis acids) prior to pretreatment and after pretreatment. The pH values were decreased for all the solutions after pretreatment as shown in Table 7-2. It indicates that the cleavage of acetyl linkages of hemicellulose results in formation of acetic acid in the pretreated hydrolyzates. The pH values of the pretreated liquor at 160 °C were lower than those of pretreated liquor at 150 °C. This can be

explained since an increase in temperature decreases the viscosity of the solution and increases the ionic mobility and also leads to dissociation of molecules in the solution. These results were in agreement with the study conducted by (Pedersen *et al.* 2011).

Table 7-2 pH values of Lewis acids before and after the pretreatment

	Reaction temperature (°C)	Lewis acids concentration (Molarity)	pH of the solutions prior to pretreatment	pH of the solutions after pretreatment
$CuCl_2$	150	0.075	4.0	1.8
$CuCl_2$	150	0.125	3.7	1.7
$FeCl_3$	150	0.075	1.8	1.6
$FeCl_3$	150	0.125	1.7	1.5
$AlCl_3$	150	0.075	3.9	2.8
$AlCl_3$	150	0.125	3.6	2.6
$CuCl_2$	160	0.075	4.0	1.7
$CuCl_2$	160	0.125	3.7	1.5
$FeCl_3$	160	0.075	1.8	1.6
$FeCl_3$	160	0.125	1.7	1.3
$AlCl_3$	160	0.075	3.9	2.5
$AlCl_3$	160	0.125	3.6	2.4

7.4.4. Effect of Lewis Acid Concentrations and Temperature on Hemicellulose

The three Lewis acids were used in the pretreatment experiments and the pretreated samples were analyzed for hemicellulose hydrolysis. Table 7-3 shows the mass balance of xylose, in various fractions in terms of yield %. Corn stover pretreated with 0.075 M concentration of $CuCl_2$ at 150 °C has 95% hemicellulose hydrolyzed in the form of both monomer and oligomeric sugars. The increase in salt concentrations reduced the amount of oligomers in the liquid hydrolyzate from 24% to 8% at the same reaction temperature. Reduction

in oligomers suggests that the higher amount of hydronium ions generated can lead to rapid hydrolysis of hemicellulose. There was approximately a 10% increase in monomeric sugar yields when the $FeCl_3$ concentration increased from 0.075 to 0.125 M at 150 °C. Overall the yields of pentose sugars were higher compared to the control samples pretreated with sulfuric acid. The amount of xylose loss ranged between 0-2 wt% for $CuCl_2$ and $FeCl_3$ salts. Xylose loss ranged between 7-9% for control samples pretreated with only sulfuric acid and 8-17% in samples treated with $AlCl_3$. It implies that Lewis acids ($FeCl_3$ and $CuCl_2$) can reduce the loss by 4 times therefore it may provide good yields in fermentation of liquid hydrolyzates. Overall, $CuCl_2$ and $FeCl_3$ Lewis acids catalytic activity were found to be almost similar. The samples treated with $AlCl_3$ produced unexpected results. The hemicellulose conversion yields decreased from 71 to 59% in the liquid hydrolyzate with an increase in Lewis acid concentration. This phenomenon could be explained as follows. First, Al^{3+} aids in conversion of xylose to xylulose through a 1,2-hydride shift (aldose to ketose isomerization). Second, Al^{3+} converts the resulting xylulose into a hydronium ion. Deprotonation of this species produces an enol, which loses three molecules of water to form a furfural. Glucose forms HMF through this isomerization mechanism, implying that xylose might do so under similar conditions (Binder *et al.* 2010). Aluminum promotes rapid dehydrocylization of pentose sugars from xylose as shown by Figure 7-2. Moreover, the Al^{3+} cation undergoes faster condensation reaction thus forming more furfural from xylose (Mansilla *et al.* 1998).

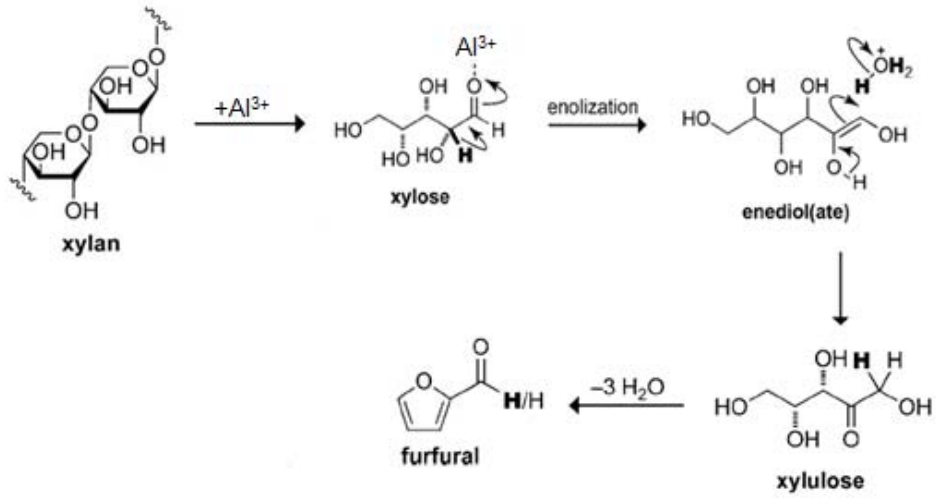

Figure 7-2 Mechanism of $AlCl_3$ salt conversion of xylose into furfural through hydride shift mechanism (Binder *et al.* 2010).

The increase in temperature increased the yield of monomeric sugars in the liquid hydrolyzate samples. From Table 7-4, it was clear that the oligomers yield was almost 0 wt% for samples pretreated with $CuCl_2$ and $FeCl_3$ at 0.125 M at 160 °C. The amount of hemicellulose retained in the solid fraction was between 2-3%. An interesting fact to note was that the amount of furfural formed with $CuCl_2$ and $FeCl_3$ pretreatment was between 4-6%. The apparent reason is that Lewis acid interactions need to be disrupted for successful coordination between the Lewis acid and the Lewis base site. Once formed, complex metal cations will invariably undergo hydrolysis reactions to produce hydroxide or oxide species, consequently losing most of their Lewis acid character (Román-Leshkov, Davis 2011).

However, studies have proved that hard Lewis acid cations such as Al^{3+} are active in the aqueous phase due to the presence of carboxylic acids such as acetic acid. This explains high (18-22 %) furfural yields when samples were pretreated with $AlCl_3$ (Peng *et al.* 2010).

Table 7-3 Mass balance of xylose in various fractions of pretreated corn stover at 150 °C

	Monomeric xylose	Xylulose	Oligomeric xylose	Solid fraction	Furfural yield	Loss
$CuCl_2$ (0.075)	71.0±1.3	0	23.6±1.2	5.2±0.0	1.0±0.0	0
$FeCl_3$ (0.075)	75.2±1.9	0	17.8±0.7	5.0±0.5	0.9±0.3	1.3±0.0
$AlCl_3$ (0.075)	25.5±0.9	12.3±1.1	44.6±3.4	7.9±0.4	1.5±0.2	8.6±1.1
H_2SO_4 (0.075)	60.7±3.4	0	24.4±2.4	6.7±0.5	0	7.0±2.1
$CuCl_2$ (0.125)	85.3±2.3	0	8.3±1.6	3.1±0.0	0.8±0.0	2.6±1.3
$FeCl_3$ (0.125)	85.7±1.2	0	9.1±0.6	2.9±0.2	2.2±0.4	0
$AlCl_3$ (0.125)	19.4±5.6	15.2±0.8	39.7±5.7	5.0±0.3	4.4±0.7	17.5±3.4
H_2SO_4 (0.125)	71.1±2.8	0	16.2±1.6	4.0±0.9	0	9.1±1.7

Overall, the effect of Lewis acids on hemicellulose hydrolysis followed the order: $FeCl_3$>$CuCl_2$>H_2SO_4>$AlCl_3$. The effects of transition metals are high on the xylose yields as compared to $AlCl_3$.

Table 7-4 Mass balance of xylose in various fractions of pretreated corn stover at 160 °C

	Monomeric xylose	Xylulose	Oligomeric xylose	Solid fraction	Furfural yield	Loss
$CuCl_2$ (0.075)	88.1±2.6	0	2.7±0.2	5.0±0.4	1.6±0.1	2.1±0.3
$FeCl_3$ (0.075)	89.8±3.1	0	2.1±0.5	2.3±0.1	4.1±0.5	0.8±0.0
$AlCl_3$ (0.075)	12.1±0.4	38.3±7.4	19.4±2.1	3.1±0.6	18.4±3.5	9.9±0.4
H_2SO_4 (0.075)	81.9±1.2	0	4.1±0.9	8.2±0.3	4.1±1.2	1.0±0.0
$CuCl_2$ (0.125)	93.3±2.4	0	0	2.1±0.0	5.3±0.3	0
$FeCl_3$ (0.125)	93.7±3.9	0	0	3.3±0.0	4.3±0.7	0
$AlCl_3$ (0.125)	8.0±0.0	31.4±4.1	16.6±1.4	3.9±0.6	21.7±4.2	18.2±1.6
H_2SO_4 (0.125)	81.6±1.6	0	1.9±0.3	4.7±0.3	6.1±0.3	5.4±1.2

7.4.5. Cellulose Hydrolysis in the Liquid Fraction

The hydrolysis of cellulose in liquid hydrolyzates was measured by monitoring glucose formation by HPLC. The samples pretreated with $CuCl_2$ had higher glucose concentrations than $FeCl_3$ and $AlCl_3$ samples as summarized in Table 7-5. The results were found to be in agreement with the study conducted (Peng *et al.* 2010).The glucose yields were found to be similar for control samples (pretreated with sulfuric acid) as compared to $FeCl_3$. Fructose was not found in samples pretreated with $CuCl_2$ and $FeCl_3$ implying that these Lewis acids do not contribute to the isomerization of glucose (This may be due to low severity conditions). However, increase in temperature and salts concentration decreased the glucose yield and increased the fructose yield

for $AlCl_3$ pretreated samples. This can be explained schematically from Figure 7-3. In the cellulose hydrolysis step, the 1,4-glucosidic bonds could be weakened partially because of coordination with Al^{3+} could be more prone to water attack to form glucose and oligomers. Then, the complex promotes rapid mutarotation of the α-anomer of glucose to the β-form through hydrogen bonds of chloride anions with the hydroxyl groups similar to what was proposed for $CrCl_2$ by (Li *et al.* 2009). The hemiacetal portion of β-glucopyranose then forms an enolate anion complex leading to isomerization of glucose to fructose, which would be dehydrated to HMF (Li *et al.* 2009). This study also confirmed that the presence of $AlCl_3$ can promote aldose to ketose isomerization.

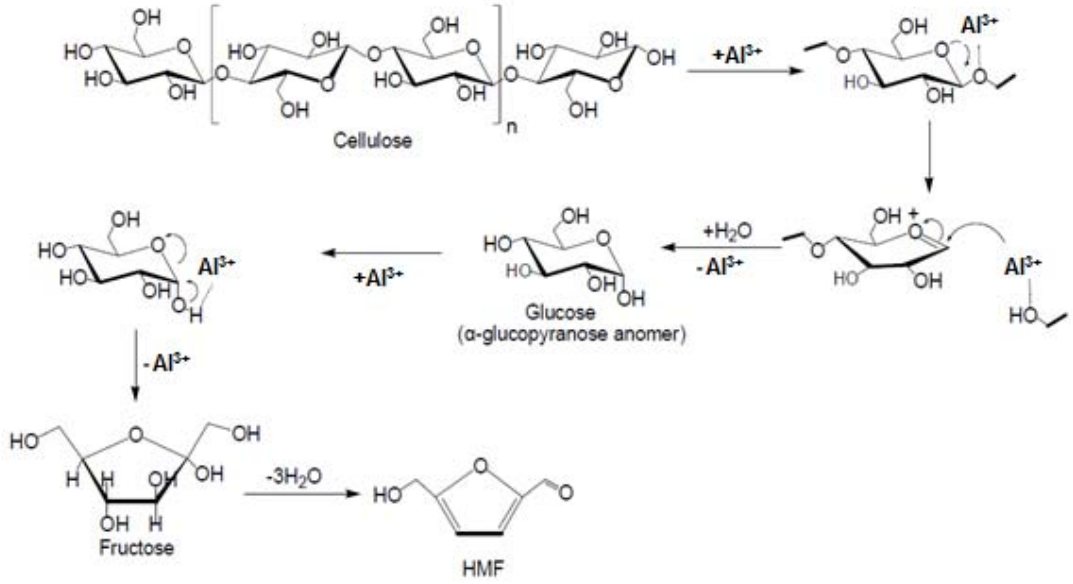

Figure 7-3 Proposed mechanism of $AlCl_3$ salt conversion of amorphous cellulose into fructose and HMF of xylose into furfural through a hydride shift mechanism (Peng *et al.* 2010).

Table 7-5 Glucose and fructose concentrations in liquid hydrolyzate samples at 150 and 160 °C at 0.075 and 0.125 M catalyst concentration for 10 min.

		150 °C		160 °C	
Catalyst	Concentration (M)	Glucose (gL^{-1})	Fructose (gL^{-1})	Glucose (gL^{-1})	Fructose (gL^{-1})
$CuCl_2$	0.075	4.1±0.1	0.0	4.8±0.4	0.0
$CuCl_2$	0.125	4.2±0.1	0.0	5.0±0.3	0.0
$FeCl_3$	0.075	3.6±0.3	0.0	4.2±0.4	0.0
$FeCl_3$	0.125	3.8±0.2	0.0	4.6±0.3	0.0
$AlCl_3$	0.075	2.1±0.4	1.3±0.2	1.5±0.1	2.9
$AlCl_3$	0.125	2.3±0.2	1.6	1.2	3.2±0.1
H_2SO_4	0.075	3.5±0.5	0.0	4.5±0.5	0.0
H_2SO_4	0.125	3.5±0.4	0.0	4.7±0.4	0.0

7.4.6. Fermentation Inhibitors in Liquid Hydrolyzates

There are several well-known fermentation inhibitors that are formed during the dilute acid pretreatment of lignocellulosic biomass (Zhao *et al.* 2007).The three major inhibitor products that were studied are 5-hydroxymethylfurfural (HMF), furfural and acetic acid.

Formation of acetic acid is mainly due to two functional groups in plant biomass: ester and acetyl linkages. The acid pretreatment cleaves these acetyl linkages forming acetic acid in the liquid hydrolyzates during pretreatment. The concentration of acetic acid was higher for biomass pretreated with 0.125 M of $FeCl_3$ and $CuCl_2$ at 160 °C. These results can be validated by the high yields of xylose in the liquid hydrolyzates shown in Table 7-3 & Table 7-4. However, the data in Table 7-6 showed that the acetic acid concentration for biomass pretreated with $AlCl_3$ was lower compared with other Lewis acids. The mechanism of HMF formation from lignocellulose pretreated was studied earlier (Yang *et al.* 2012).They claimed that cellulose converts into glucose, followed by isomerization of the glucose monomers into fructose and dehydration of fructose to HMF. However, the presence of water solvent may lead to rehydration of HMF into levulinic acid (Yang *et al.* 2012). This could be a possible reason for lower yields of HMF during pretreatment. The amount of furfural was found to be higher in samples pretreated with $AlCl_3$ as compared to samples pretreated with $CuCl_2$ and $FeCl_3$ and sulfuric acid concentration. The reason for a higher yield of furfural was primarily due to Al^{3+} cation remaining active in the presence of acetic acid in the aqueous solution as compared to Fe^{3+} and Cu^{2+} cations that lost their Lewis acid character during hydrolysis of hemicellulose.

Table 7-6 Concentration of inhibitor products in liquid hydrolyzates pretreated at 150 and 160 °C at 0.075 and 0.125 M catalyst concentration for 10 min.

Catalyst	Reaction temperature °C	Lewis acids concentration (M)	Acetic acid (gL^{-1})	HMF(gL^{-1})	Furfural (gL^{-1})
$CuCl_2$	150	0.075	1.9±0.5	0.3±0.0	0
$CuCl_2$	150	0.125	2.3±0.4	0.2±0.0	0
$FeCl_3$	150	0.075	2.5±0.5	0.3±0.0	0
$FeCl_3$	150	0.125	2.5±0.4	0.3±0.0	0
$AlCl_3$	150	0.075	1.4±0.5	0.3±0.0	0.3±0.0
$AlCl_3$	150	0.125	1.2±0.2	0.4±0.0	1.1±0.2
H_2SO_4	150	0.075	2.0±0.3	0.3±0.0	0
H_2SO_4	150	0.125	2.7±0.2	0.9±0.1	1.8±0.2
$CuCl_2$	160	0.075	2.8±0.1	0.4±0.0	0.6±0.1
$CuCl_2$	160	0.125	3.4±0.3	0.4±0.0	1.5±0.3
$FeCl_3$	160	0.075	2.9±0.5	0.4±0.0	1.2±0.0
$FeCl_3$	160	0.125	3.3±0.6	0.5±0.0	1.2±0.2
$AlCl_3$	160	0.075	2.2±0.2	0.7±0.0	5.3±0.1
$AlCl_3$	160	0.125	2.2±0.4	0.8±0.0	6.5±0.7
H_2SO_4	160	0.075	2.7±0.4	0.3±0.0	0.5±0.1
H_2SO_4	160	0.125	2.3±0.5	1.1±0.1	2.4±0.1

7.4.7. Composition of Pretreated Solid Fractions

Solid fraction samples were analyzed for the amounts of cellulose, hemicellulose, lignin and ash after pretreatment. Figure 7-4 & Figure 7-5 showed that different Lewis acids had no effect on the lignin content of the biomass as lignin contents remained unchanged and ranged from 24-27 wt% for all the samples. The ash contents also remained constant within 4 to 5 wt% for all the samples. Corn stover pretreated with $CuCl_2$ had 3 wt% lower cellulose than other samples pretreated with other Lewis acids; it indicates that $CuCl_2$ had a stronger effect on cellulose degradation than other Lewis acids. Overall, the amount of hemicellulose retained in solid fraction was at least 2-3 wt% lower for Lewis acids as compared to sulfuric acid.

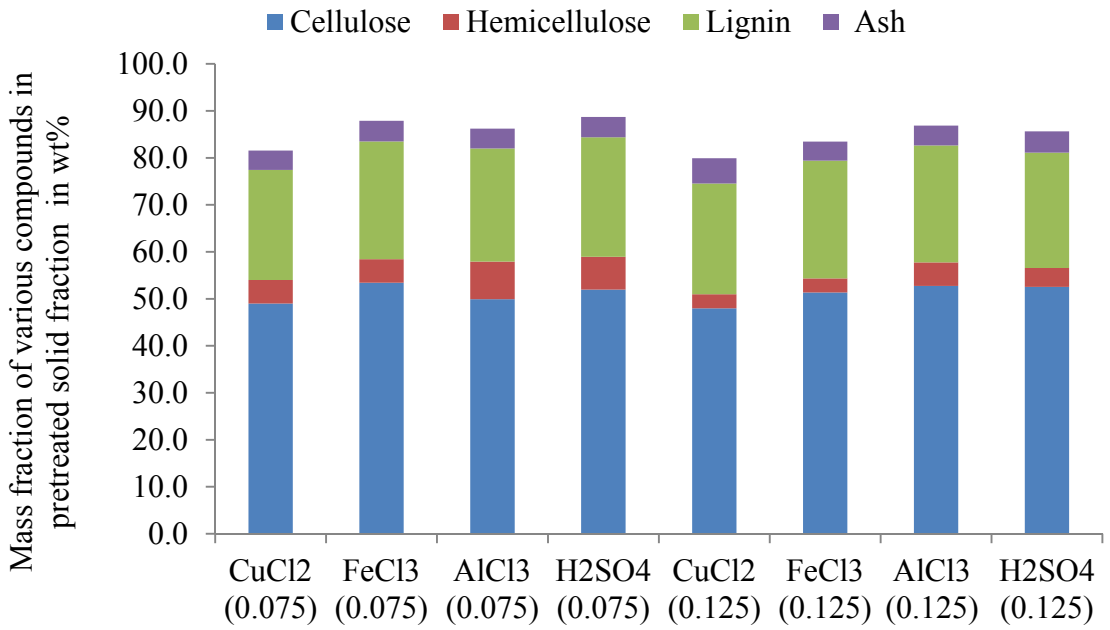

Figure 7-4 Mass balance of pretreated corn stover using Lewis acids at 150 °C

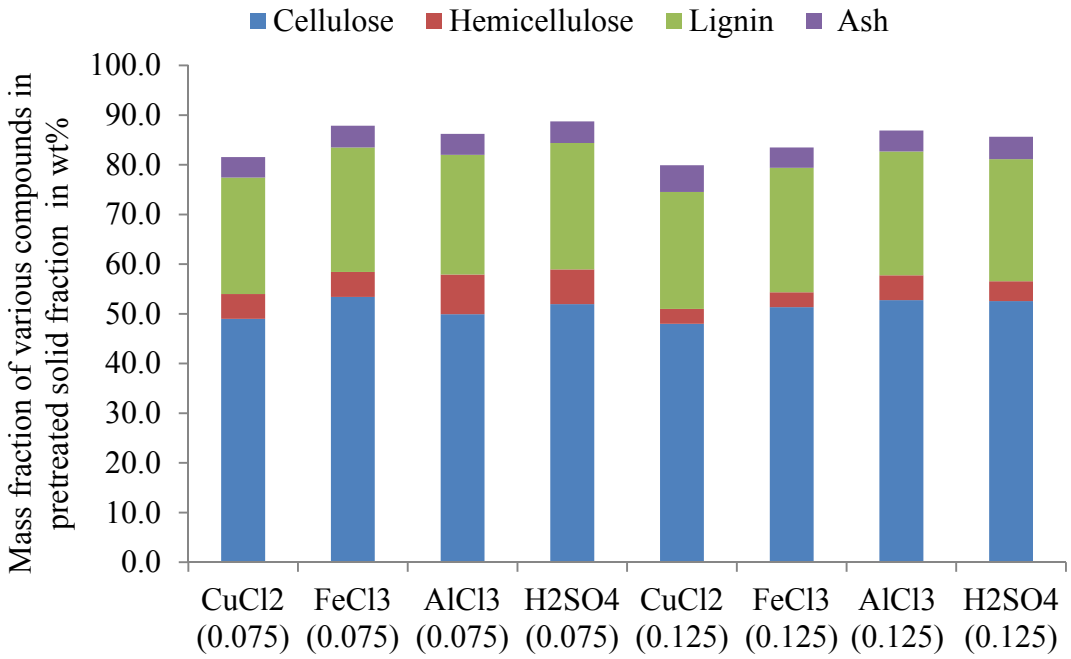

Figure 7-5 Mass balance of pretreated corn stover using Lewis acids at 160 °C

7.4.8. Enzymatic Digestibility of Solid Substrates

Enzymatic digestibility of all the washed pretreated samples was analyzed in order to investigate the overall effects of these Lewis acids on corn stover pretreatment and enzymatic hydrolysis. The pretreated solid substrate contains mostly cellulose, lignin with a trace of hemicellulose. The presence of lignin generally has a negative effect on the enzymatic hydrolysis yields, since enzymes that are adsorbed by lignin sites form lignin-enzyme complexes and considered as ineffective (Kamireddy *et al.* 2012b).This explains the lower yields in control samples (pretreated with sulfuric acid) as compared to Lewis acids. The presence of metal complexes had in fact enhanced the yields of enzymatic digestibility compared to sulfuric acid as summarized by Table 7-7. This was due to the presence of metal cations, which can eliminate the

inhibition of enzymes by lignin through formation of lignin-metal complexes. Thus more cellulose sites were accessible by the enzymes for hydrolysis. These results were in agreement with studies conducted by (Liu, Zhu 2010). Enzymatic digestibility was observed to be greater than 92 wt% for all the samples treated with Lewis acid salts compared to control samples at 160 °C at different concentrations. However, corn stover pretreated with $AlCl_3$ pretreated at 150 °C had a lower enzymatic digestibility as compared to control samples. This was due to the presence of a higher amount of hemicellulose still retained in the pretreated corn stover at 150 °C as compared to 160 °C. The presence of hemicellulose limits the enzyme accessibility to cellulose sites. Moreover, the cellulase enzymes do not have the ability to convert hemicellulose retained in the pretreated samples into fermentable sugars.

Table 7-7 Enzymatic digestibility of solid substrate of corn stover pretreated at150 and 160°C at 0.075 and 0.125 M catalyst concentration for 10 min.

Catalyst	Reaction temperature (°C)	Concentration (M)	Enzymatic digestibility (wt%)
$CuCl_2$	150	0.075	82.7±1.6
$CuCl_2$	150	0.125	92.5±1.0
$FeCl_3$	150	0.075	71.9±2.3
$FeCl_3$	150	0.125	96.6±1.6
$AlCl_3$	150	0.075	54.1±0.7
$AlCl_3$	150	0.125	76.8±2.2
H_2SO_4	150	0.075	55.6±1.3
H_2SO_4	150	0.125	84.0±0.7
$CuCl_2$	160	0.075	92.7±0.7
$CuCl_2$	160	0.125	97.3±1.5
$FeCl_3$	160	0.075	96.0±1.4
$FeCl_3$	160	0.125	99.6±0.5
$AlCl_3$	160	0.075	97.8±0.5
$AlCl_3$	160	0.125	100.0±1.0
H_2SO_4	160	0.075	72.0±1.4
H_2SO_4	160	0.125	88.7±0.8

7.5. Conclusion

This study has shown the effect of different types of Lewis acids on pretreatment and enzymatic hydrolysis of corn stover. Transition metal Lewis acids (such as $FeCl_3$ and $CuCl_2$) significantly improved the xylose yield during pretreatment and enzymatic digestibility of cellulose compared with dilute sulfuric acid. By contrast $AlCl_3$ showed a higher catalytic activity in the formation xylulose and furfural from xylose at both 150 and 160 °C and at different Lewis acids concentrations. The presence of $AlCl_3$ could also isomerize glucose into fructose. The catalytic performance depends on the acidity of the solution due to the addition of the Lewis acids. The selectivity of Lewis acids for biomass pretreatment can be based on both lower pK_a and chemical hardness values in eV, since higher chemical hardness leads to faster degradation

of hemicellulose hydrolysis as observed from the results even though value of $AlCl_3$ has lower pK_a value than $CuCl_2$. $FeCl_3$ and $CuCl_2$ salts were superior if the purpose of the pretreatment is to extract hemicellulose. $AlCl_3$ salt can be an excellent choice if purpose of the pretreatment is to produce bio-based chemicals such as furfural. Overall all the three Lewis acids are found to be valuable in converting biomass into useful substrates for producing renewable fuels or green chemicals compared with Bronsted acid such as sulfuric acid. These findings will improve the methods used for the production of fermentable sugars and value-added chemicals from lignocellulosic biomass using Lewis acids at low severity, thus making the production of biofuels more affordable.

8. CONCLUSION AND FUTURE WORK

8.1. Conclusions

8.1.1. Acid Pretreatment of Agricultural Feedstocks

This study showed the effect of acid pretreatment on four feedstocks. They are considered as low energy input crops since their growth rates are between 100-180 $kgNha^{-1}$. The pretreatment conditions showed high selectivity for hemicellulose hydrolysis and low inhibitor product formation in the liquid hydrolyzate samples for all the lignocellulosic feedstocks. The highest hemicellulose yield was observed for SNBMR 95 wt%, followed by SBMR with 91 wt% at combined severity factor (CSF) 1.56 and 1.44 for sunn hemp yield was observed at 72 wt% at CSF 1.48 and for kenaf it was around 80 wt% at CSF 1.72. At harsher pretreatment conditions the hemicellulose yield decreased in all the biomasses due to the degradation of pentose carbohydrates into furfural. The solid fraction that is rich in cellulose is subjected to enzymatic hydrolysis with cellulase enzymes yielded high amounts of fermentable sugars. The overall glucan saccharification yield after enzymatic hydrolysis for SNBMR was found to be 90 wt% followed by kenaf 88 wt%, SBMR 84 wt% at CSF 1.47, 1.72 and 1.24 respectively. For sunn hemp the maximum amount of hemicellulose yields were observed to be 68 wt% at CSF 2.06. The general trend observed in the hemicellulose hydrolysis was higher that a crystallinity index

of the raw biomass led to lower hemicellulose hydrolysis rates, pretreated under similar conditions.

8.1.2. Acid Pretreatment of Industrial Waste (Sunflower Hulls)

Sunflower hulls showed a higher recalcitrance to acid pretreatment compared to many other agricultural residues. This difference was explained by its high lignin and wax contents of the cell walls, which could act as a barrier to the hydronium ions resulting in an increase of the activation energy and lowering the effective reaction rate order on the acid. The maximum hemicellulose yield was observed to be 60% at 150 °C for 30 min at 1.25% acid concentration. The maximum cellulose digestibility of the enzymatic saccharification was 53.5% at 160 °C for a 30 min pretreatment at 2% acid concentration.

8.1.3. Lewis Acids Pretreatment of Corn Stover

This study has shown the effect of different types of Lewis acids on pretreatment and enzymatic hydrolysis of corn stover. Transition Lewis acids (such as $FeCl_3$ and $CuCl_2$) significantly improved the xylose yield during pretreatment and enzymatic digestibility of cellulose compared with dilute sulfuric acid. The maximum monomeric xylose yield was observed to be 93 and 94wt% when $CuCl_2$ and $FeCl_3$ were used as salts in the pretreatment at 160 °C for 10 min at 0.125M concentrations, which were 12-14 wt% higher than the sulfuric acid pretreatment yields at the same reaction conditions. However, monomeric xylose yield for corn stover pretreated with $AlCl_3$ was observed to be merely 8 wt% at the same conditions. This could be explained by the isomerization of xylose to xylulose and subsequent dehydration into

furfural. However, enzymatic digestibility yields for the three Lewis acids were greater than 92 wt% for all the samples pretreated at 160 °C. These yields were approximately equal to those obtained in 4wt% sulfuric acid pretreated samples at the same reaction conditions. Group IIIA Lewis acid ($AlCl_3$) showed a higher catalytic activity in the formation of furfural from xylose. The presence of $AlCl_3$ could also isomerize glucose into fructose.

Recently, pretreatment with acids (either Bronsted or Lewis acids) is found to be important step to produce biofuels (Wyman *et al.,*2005). Significant work has been carried out on efficient production biofuels at lab scale. Parameters such as solid to liquid ratio, reaction temperature, residence time and acid concentration have extensively studied on various Lignocellulosic feedstocks in a batch reactor. Recently, reactor modifications such as percolation, plug-flow, flow through reactor, counter-current and shrinking bed have been successfully tested and validated by NREL have showed improved results (Lloyd *et al.,*2005). Better understanding of the kinetics and multiple reactions paths during pretreatment in these improved reactors may further enhance hemicellulose hydrolysis. Economics and environmental impact are two important considerations for the selection of dilute acid hydrolysis based pretreatment technology. In future, technologies based on robust optimization tools for dilute acid hydrolysis, finding the ideal acid or mixed dilute acids considering the basic parameters and suitable reactors would be the ultimate choice.

During hemicellulose hydrolysis a number of inhibitors are produced that can impede the rate of enzymatic hydrolysis and fermentation processes. Application of nano-particles-based membrane systems and their implication in hemicellulose hydrolysis may also provide better

results in short times with fewer by-products.

8.2. Future Work

The following research directions are suggested for the future study to improve the yields of fermentable sugars and also be further extension of my findings

8.2.1. Extension of Lewis Acids Pretreatment with Other Feedstocks

Although there is some literature available for the pretreatment of Lewis acids with wheat straw and rice hulls (Liu, Zhu 2010, Wei *et al.* 2011). Most of the literature on Lewis acids agents showed an increase in yields of fermentable carbohydrates as compared to dilute acid pretreatment. Hence, further extension of this study to energy crops such as sorghum, sunn hemp, miscanthus, switchgrass is necessary to know the efficacy of these agents with respect to these feedstocks.

8.2.2. Extension of This Study for Larger Biomass Loading

Due to limitations with small reactor specifications, the feedstock loading considered was 10 wt%. It is based on the previous researcher data. The effects on efficiency on increase in biomass loading between 15–40 wt% require further investigation. This can be performed using batch reactors that have 10-15 L of internal volume or continuous process using CSTR (Continuous Stirred Tank Reactors). They have several advantages; 1) Increase in the yields for fermentable sugars; 2) Lower energy input for the same efficiency.

8.2.3. Deconstruction of Lignin after the Pretreatment

The acid pretreated solid substrates mainly consist of cellulose and lignin. In order for a biofuel plant to be profitable, efficient use of lignin is vital since lignin is source for various fuels and green polymers that can act as a substitute for fossil based derived chemicals. Moreover, removing lignin after pretreatment can increase the efficiency after enzymatic hydrolysis (Kamireddy *et al.* 2012b). Hence, an extension of the study that deals with the base catalyzed de-polymerization of lignin and hydrodeoxygenation is suggested.

REFERENCES

Agbor, V.B., Cicek, N., Sparling, R., Berlin, A. & Levin, D.B. 2011, "Biomass pretreatment: fundamentals toward application", *Biotechnology Advances,* vol. 29, no. 6, pp. 675-685.

Ahmed, I., Zia, M.A. & Iqbal, H.M.N. 2010, "Bioprocessing of proximally analyzed wheat straw for enhanced cellulase production through process optimization with Trichoderma viride under SSF", *Cellulose,* vol. 2, no. W3, pp. 100.

Akella, A.K., Saini, R.P. & Sharma, M.P. 2009, "Social, economical and environmental impacts of renewable energy systems", *Renewable Energy,* vol. 34, no. 2, pp. 390-396.

Alizadeh, H., Teymouri, F., Gilbert, T.I. & Dale, B.E. 2005, "Pretreatment of switchgrass by ammonia fiber explosion (AFEX)", *Applied Biochemistry and Biotechnology,* vol. 124, no. 1-3, pp. 1133-1141.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J., Guwy, A., Kalyuzhnyi, S., Jenicek, P. & Lier, J.v. 2009, "Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays", .

Antal Jr, M.J. 1996, "Water: A traditional solvent pregnant with new applications", *White Jr., HJ (Hrsg.),* .

Antony, J. 2003, *Design of experiments for engineers and scientists,* Butterworth-Heinemann.

Baños, R., Manzano-Agugliaro, F., Montoya, F.G., Gil, C., Alcayde, A. & Gómez, J. 2011, "Optimization methods applied to renewable and sustainable energy: A review", *Renewable and Sustainable Energy Reviews,* vol. 15, no. 4, pp. 1753-1766.

Bensah, E.C. & Mensah, M. 2013, "Chemical Pretreatment Methods for the Production of Cellulosic Ethanol: Technologies and Innovations", *International Journal of Chemical Engineering,* vol. 2013.

Bernata, X., Fortuny, A., Stüber, F., Bengoa, C., Fabregat, A. & Font, J. 2008, "Recovery of iron (III) from aqueous streams by ultrafiltration", *Desalination,* vol. 221, no. 1, pp. 413-418.

Binder, J.B., Blank, J.J., Cefali, A.V. & Raines, R.T. 2010, "Synthesis of furfural from xylose and xylan", *ChemSusChem,* vol. 3, no. 11, pp. 1268-1272.

Binod, P., Kuttiraja, M., Archana, M., Janu, K.U., Sindhu, R., Sukumaran, R.K. & Pandey, A. 2012, "High temperature pretreatment and hydrolysis of cotton stalk for producing sugars for bioethanol production", *Fuel,* vol. 92, no. 1, pp. 340-345.

Bobleter, O. 1994, "Hydrothermal degradation of polymers derived from plants", *Progress in Polymer Science,* vol. 19, no. 5, pp. 797-841.

Bowyer, J.L., Shmulsky, R., Haygreen, J.G. & Lilley, K. 2003, *Forest products and wood science: an introduction,* Iowa State Press Iowa.

Cantrell, K.B., Bauer, P.J. & Ro, K.S. 2010, "Utilization of summer legumes as bioenergy feedstocks", *Biomass and Bioenergy,* vol. 34, no. 12, pp. 1961-1967.

Cara, C., Ruiz, E., Oliva, J.M., Sáez, F. & Castro, E. 2008, "Conversion of olive tree biomass into fermentable sugars by dilute acid pretreatment and enzymatic saccharification", *Bioresource technology,* vol. 99, no. 6, pp. 1869-1876.

Carelli, A.A., Frizzera, L.M., Forbito, P.R. & Crapiste, G.H. 2002, "Wax composition of sunflower seed oils", *Journal of the American Oil Chemists' Society,* vol. 79, no. 8, pp. 763-768.

Chandra, R.P., Bura, R., Mabee, W., Berlin, A., Pan, X. & Saddler, J. 2007, "Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics?" in *Biofuels* Springer, , pp. 67-93.

Chang, V.S., Burr, B. & Holtzapple, M.T. 1997, "Lime pretreatment of switchgrass" in *Biotechnology for Fuels and Chemicals* Springer, , pp. 3-19.

Chang, V.S. & Holtzapple, M.T. 2000, "Fundamental factors affecting biomass enzymatic reactivity", *Twenty-First Symposium on Biotechnology for Fuels and Chemicals*Springer, , pp. 5.

Chang, V.S., Kaar, W.E., Burr, B. & Holtzapple, M.T. 2001, "Simultaneous saccharification and fermentation of lime-treated biomass", *Biotechnology Letters,* vol. 23, no. 16, pp. 1327-1333.

Chang, V.S., Nagwani, M. & Holtzapple, M.T. 1998, "Lime pretreatment of crop residues bagasse and wheat straw", *Applied Biochemistry and Biotechnology,* vol. 74, no. 3, pp. 135-159.

Chen, X., Shekiro, J., Franden, M.A., Wang, W., Zhang, M., Kuhn, E., Johnson, D.K. & Tucker, M.P. 2012, "The impacts of deacetylation prior to dilute acid pretreatment on the bioethanol process", *Biotechnology for Biofuels,* vol. 5, no. 8.

Chèze, B., Chevallier, J. & Gastineau, P. 2013, "Will technological progress be sufficient to stabilize CO2 emissions from air transport in the mid-term?", *Transportation Research Part D: Transport and Environment,* vol. 18, no. 0, pp. 91-96.

Christakopoulos, P., Li, L., Kekos, D. & Macris, B.J. 1993, "Direct conversion of sorghum carbohydrates to ethanol by a mixed microbial culture", *Bioresource technology,* vol. 45, no. 2, pp. 89-92.

Cotton, F.A., Wilkinson, G., Murillo, C.A. & Bochmann, M. 1988, *Advanced inorganic chemistry,* Wiley New York.

Dale, B.E. & Moreira, M.J. 1982, "Freeze-explosion technique for increasing cellulose hydrolysis", *Biotechnol. Bioeng. Symp.;(United States)*Colorado State Univ., Fort Collins, .

Danalatos, N. & Archontoulis, S. 2010, "Growth and biomass productivity of kenaf (< i> Hibiscus cannabinus, L.) under different agricultural inputs and management practices in central Greece", *Industrial Crops and Products,* vol. 32, no. 3, pp. 231-240.

Decker, S.R., Brunecky, R., Tucker, M.P., Himmel, M.E. & Selig, M.J. 2009, "High-throughput screening techniques for biomass conversion", *BioEnergy Research,* vol. 2, no. 4, pp. 179-192.

Degenstein, J.C., Kamireddy, S., Tucker, P. & Ji, Y. 2011, "Novel batch reactor for the dilute acid pretreatment of lignocellulosic feedstocks with improved heating and cooling kinetics", *Int.J.Chem.React.Eng,* vol. 9, pp. A95.

Dien, B.S., Sarath, G., Pedersen, J.F., Sattler, S.E., Chen, H., Funnell-Harris, D.L., Nichols, N.N. & Cotta, M.A. 2009, "Improved sugar conversion and ethanol yield for forage sorghum (Sorghum bicolor L. Moench) lines with reduced lignin contents", *BioEnergy Research,* vol. 2, no. 3, pp. 153-164.

Dong, H., Nimlos, M.R., Himmel, M.E., Johnson, D.K. & Qian, X. 2009, "The effects of water on β-d-xylose condensation reactions", *The Journal of Physical Chemistry A,* vol. 113, no. 30, pp. 8577-8585.

Donkoh, E., Degenstein, J., Tucker, M. & Ji, Y. 2012, "Optimization of enzymatic hydrolysis of dilute acid pretreated sugar beet pulp using response surface design", *Journal of Sugar Beet Research,* vol. 49, no. 1, pp. 26.

Duff, S.J. & Murray, W.D. 1996, "Bioconversion of forest products industry waste cellulosics to fuel ethanol: a review", *Bioresource technology,* vol. 55, no. 1, pp. 1-33.

Dutta, S., De, S., Saha, B. & Alam, M.I. 2012, "Advances in conversion of hemicellulosic biomass to furfural and upgrading to biofuels", *Catalysis Science & Technology,* vol. 2, no. 10, pp. 2025-2036.

Eggeman, T. & Elander, R.T. 2005, "Process and economic analysis of pretreatment technologies", *Bioresource technology,* vol. 96, no. 18, pp. 2019-2025.

Fargione, J.E., Plevin, R.J. & Hill, J.D. 2010, "The ecological impact of biofuels", *Annual Review of Ecology, Evolution, and Systematics,* vol. 41, pp. 351-377.

Fengel, D. & Wegener, G. 1983, *Wood: chemistry, ultrastructure, reactions,* Walter de Gruyter.

Foster, B.L., Dale, B.E. & Doran-Peterson, J.B. 2001, "Enzymatic hydrolysis of ammonia-treated sugar beet pulp", *Applied Biochemistry and Biotechnology,* vol. 91, no. 1-9, pp. 269-282.

Galbe, M. & Zacchi, G. 2007, "Pretreatment of lignocellulosic materials for efficient bioethanol production" in *Biofuels* Springer, , pp. 41-65.

Grzybkowski, W. 2006, "Nature and properties of metal cations in aqueous solutions", *Polish Journal of Environmental Studies,* vol. 15, no. 4, pp. 655.

Guo, G., Hsu, D., Chen, W., Chen, W. & Hwang, W. 2009, "Characterization of enzymatic saccharification for acid-pretreated lignocellulosic materials with different lignin composition", *Enzyme and microbial technology,* vol. 45, no. 2, pp. 80-87.

Hatakka, A. 1994, "Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation", *FEMS microbiology reviews,* vol. 13, no. 2, pp. 125-135.

Helle, S., Cameron, D., Lam, J., White, B. & Duff, S. 2003, "Effect of inhibitory compounds found in biomass hydrolysates on growth and xylose fermentation by a genetically engineered strain of< i> S. cerevisiae", *Enzyme and microbial technology,* vol. 33, no. 6, pp. 786-792.

Hendriks, A. & Zeeman, G. 2009a, "Pretreatments to enhance the digestibility of lignocellulosic biomass", *Bioresource technology,* vol. 100, no. 1, pp. 10-18.

Hendriks, A. & Zeeman, G. 2009b, "Pretreatments to enhance the digestibility of lignocellulosic biomass", *Bioresource technology,* vol. 100, no. 1, pp. 10-18.

Hendriks, A. & Zeeman, G. 2009c, "Pretreatments to enhance the digestibility of lignocellulosic biomass", *Bioresource technology,* vol. 100, no. 1, pp. 10-18.

Hon, D.N. & Shiraishi, N. 2000, *Wood and Cellulosic Chemistry, Revised, and Expanded,* CRC Press.

Hosseini, S.A. & Shah, N. 2009, "Multiscale modelling of hydrothermal biomass pretreatment for chip size optimization", *Bioresource technology,* vol. 100, no. 9, pp. 2621-2628.

Itoh, H., Wada, M., Honda, Y., Kuwahara, M. & Watanabe, T. 2003, "Bioorganosolve pretreatments for simultaneous saccharification and fermentation of beech wood by ethanolysis and white rot fungi", *Journal of Biotechnology,* vol. 103, no. 3, pp. 273-280.

Jacobsen, S.E. & Wyman, C.E. 2000, "Cellulose and hemicellulose hydrolysis models for application to current and novel pretreatment processes", *Twenty-First Symposium on Biotechnology for Fuels and Chemicals*Springer, , pp. 81.

Jensen, J., Morinelly, J., Aglan, A., Mix, A. & Shonnard, D.R. 2008, "Kinetic characterization of biomass dilute sulfuric acid hydrolysis: Mixtures of hardwoods, softwood, and switchgrass", *AIChE Journal,* vol. 54, no. 6, pp. 1637-1645.

Jonoobi, M., Niska, K.O., Harun, J. & Misra, M. 2009, "Chemical composition, crystallinity, and thermal degradation of bleached and unbleached kenaf bast (Hibiscus cannabinus) pulp and nanofibers", *BioResources,* vol. 4, no. 2, pp. 626-639.

Joonobi, M., Harun, J., Tahir, P.M., Zaini, L.H., SaifulAzry, S. & Makinejad, M.D. 2010, "CHARACTERISTIC OF NANOFIBERS EXTRACTED FROM KENAF CORE", *BioResources,* vol. 5, no. 4, pp. 2556-2566.

Jørgensen, H., Kristensen, J.B. & Felby, C. 2007, "Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities", *Biofuels, Bioproducts and Biorefining,* vol. 1, no. 2, pp. 119-134.

Kalia, S., Kumar, A. & Kaith, B. 2011, "Sunn hemp cellulose graft copolymers polyhydroxybutyrate composites: morphological and mechanical studies", *Adv Mat Lett,* vol. 2, pp. 17-25.

Kamireddy, S.R., Schaefer, C., Defrese, M., Degenstein, J. & Ji, Y. 2012a, "Pretreatment and enzymatic hydrolysis of sunflower hulls for fermentable sugar production", *International Journal of Agricultural and Biological Engineering,* vol. 5, no. 1, pp. 62-70.

Kamireddy, S.R., Schaefer, C., Defrese, M., Degenstein, J. & Ji, Y. 2012b, "Pretreatment and enzymatic hydrolysis of sunflower hulls for fermentable sugar production", *International Journal of Agricultural and Biological Engineering,* vol. 5, no. 1, pp. 62-70.

Kamireddy, S.R., Li, J., Tucker, M., Degenstein, J. & Ji, Y. 2013a, "Effects and Mechanism of Metal Chloride Salts on Pretreatment and Enzymatic Digestibility of Corn Stover", *Industrial & Engineering Chemistry Research,* vol. 52, no. 5, pp. 1775-1782.

Kamireddy, S.R., Li, J., Abbina, S., Berti, M., Tucker, M. & Ji, Y. 2013b, "Converting forage sorghum and sunn hemp into biofuels through dilute acid pretreatment", *Industrial Crops and Products,* vol. 49, pp. 598-609.

Kamireddy, S.R., Li, J., Abbina, S., Berti, M., Tucker, M. & Ji, Y. 2013c, "Converting forage sorghum and sunn hemp into biofuels through dilute acid pretreatment", *Industrial Crops and Products,* vol. 49, pp. 598-609.

Kim, S.B., Yum, D.M. & Park, S.C. 2000, "Step-change variation of acid concentration in a percolation reactor for hydrolysis of hardwood hemicellulose", *Bioresource technology,* vol. 72, no. 3, pp. 289-294.

Klinke, H.B., Thomsen, A. & Ahring, B.K. 2004, "Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass", *Applied Microbiology and Biotechnology,* vol. 66, no. 1, pp. 10-26.

Kumar, P., Barrett, D.M., Delwiche, M.J. & Stroeve, P. 2009, "Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production", *Industrial & Engineering Chemistry Research,* vol. 48, no. 8, pp. 3713-3729.

Kumar, R. & Wyman, C.E. 2008, "The impact of dilute sulfuric acid on the selectivity of xylooligomer depolymerization to monomers", *Carbohydrate research,* vol. 343, no. 2, pp. 290-300.

Lee, J. 1997, "Biological conversion of lignocellulosic biomass to ethanol", *Journal of Biotechnology,* vol. 56, no. 1, pp. 1-24.

Lee, Y., Iyer, P. & Torget, R.W. 1999, "Dilute-acid hydrolysis of lignocellulosic biomass" in *Recent Progress in Bioconversion of Lignocellulosics* Springer, , pp. 93-115.

Lee, T.-. & Chen, C.-. 2009, "Wind-photovoltaic capacity coordination for a time-of-use rate industrial user", *IET Renewable Power Generation,* vol. 3, no. 2, pp. 152-167.

Leu, S. & Zhu, J. 2012, "Substrate-related factors affecting enzymatic saccharification of lignocelluloses: our recent understanding", *BioEnergy Research,* , pp. 1-11.

Li, C., Zhang, Z. & Zhao, Z.K. 2009, "Direct conversion of glucose and cellulose to 5-hydroxymethylfurfural in ionic liquid under microwave irradiation", *Tetrahedron letters,* vol. 50, no. 38, pp. 5403-5405.

Li, W., Xu, J., Wang, J., Yan, Y., Zhu, X., Chen, M. & Tan, Z. 2008, "Studies of monosaccharide production through lignocellulosic waste hydrolysis using double acids", *Energy & Fuels,* vol. 22, no. 3, pp. 2015-2021.

Liu, H. & Zhu, J. 2010, "Eliminating inhibition of enzymatic hydrolysis by lignosulfonate in unwashed sulfite-pretreated aspen using metal salts", *Bioresource technology,* vol. 101, no. 23, pp. 9120-9127.

Liu, L., Sun, J., Cai, C., Wang, S., Pei, H. & Zhang, J. 2009, "Corn stover pretreatment by inorganic salts and its effects on hemicellulose and cellulose degradation", *Bioresource technology,* vol. 100, no. 23, pp. 5865-5871.

Lloyd, T.A. & Wyman, C.E. 2005, "Combined sugar yields for dilute sulfuric acid pretreatment of corn stover followed by enzymatic hydrolysis of the remaining solids", *Bioresource technology,* vol. 96, no. 18, pp. 1967-1977.

Lloyd, T.A. & Wyman, C.E. 2004, "Predicted effects of mineral neutralization and bisulfate formation on hydrogen ion concentration for dilute sulfuric acid pretreatment", *Proceedings of the Twenty-Fifth Symposium on Biotechnology for Fuels and Chemicals Held May 4–7, 2003, in Breckenridge, CO*Springer, , pp. 1013.

Lloyd, T. & Wyman, C.E. 2003, "Application of a depolymerization model for predicting thermochemical hydrolysis of hemicellulose" in *Biotechnology for Fuels and Chemicals* Springer, , pp. 53-67.

Lu, X., Zhang, Y., Yang, J. & Liang, Y. 2007, "Enzymatic hydrolysis of corn stover after pretreatment with dilute sulfuric acid", *Chemical Engineering & Technology,* vol. 30, no. 7, pp. 938-944.

Magnusson, L., Islam, R., Sparling, R., Levin, D. & Cicek, N. 2008, "Direct hydrogen production from cellulosic waste materials with a single-step dark fermentation process", *International Journal of Hydrogen Energy,* vol. 33, no. 20, pp. 5398-5403.

Mamman, A.S., Lee, J., Kim, Y., Hwang, I.T., Park, N., Hwang, Y.K., Chang, J. & Hwang, J. 2008, "Furfural: hemicellulose/xylosederived biochemical", *Biofuels, Bioproducts and Biorefining,* vol. 2, no. 5, pp. 438-454.

Mansilla, H.D., Baeza, J., Urzúa, S., Maturana, G., Villaseñor, J. & Durán, N. 1998, "Acid-catalysed hydrolysis of rice hull: evaluation of furfural production", *Bioresource technology,* vol. 66, no. 3, pp. 189-193.

Mansoer, Z., Reeves, D.W. & Wood, C. 1997, "Suitability of sunn hemp as an alternative late-summer legume cover crop", *Soil Science Society of America Journal,* vol. 61, no. 1, pp. 246-253.

McKendry, P. 2002, "Energy production from biomass (part 1): overview of biomass", *Bioresource technology,* vol. 83, no. 1, pp. 37-46.

McMillan, J.D. 1994, "Pretreatment of lignocellulosic biomass", *ACS symposium series*ACS Publications, , pp. 292.

Meki, M.N., Snider, J.L., Kiniry, J.R., Raper, R.L. & Rocateli, A.C. 2013, "Energy sorghum biomass harvest thresholds and tillage effects on soil organic carbon and bulk density", *Industrial Crops and Products,* vol. 43, pp. 172-182.

Morinelly, J.E., Jensen, J.R., Browne, M., Co, T.B. & Shonnard, D.R. 2009, "Kinetic characterization of xylose monomer and oligomer concentrations during dilute acid pretreatment of lignocellulosic biomass from forests and switchgrass", *Industrial & Engineering Chemistry Research,* vol. 48, no. 22, pp. 9877-9884.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y., Holtzapple, M. & Ladisch, M. 2005, "Features of promising technologies for pretreatment of lignocellulosic biomass", *Bioresource technology,* vol. 96, no. 6, pp. 673-686.

Moulthrop, J.S., Swatloski, R.P., Moyna, G. & Rogers, R.D. 2005, "High-resolution 13C NMR studies of cellulose and cellulose oligomers in ionic liquid solutions", *Chemical communications,* , no. 12, pp. 1557-1559.

Murphy, P.T., Moore, K.J., Richard, T.L. & Bern, C. 2007, "Enzyme enhanced solid-state fermentation of kenaf core fiber for storage and pretreatment", *Bioresource technology,* vol. 98, no. 16, pp. 3106-3111.

Nabarlatz, D., Farriol, X. & Montané, D. 2004, "Kinetic modeling of the autohydrolysis of lignocellulosic biomass for the production of hemicellulose-derived oligosaccharides", *Industrial & Engineering Chemistry Research,* vol. 43, no. 15, pp. 4124-4131.

Nguyen, L.M. 2000, "Organic matter composition, microbial biomass and microbial activity in gravel-bed constructed wetlands treating farm dairy wastewaters", *Ecological Engineering,* vol. 16, no. 2, pp. 199-221.

O'Neill, R., Ahmad, M.N., Vanoye, L. & Aiouache, F. 2009, "Kinetics of aqueous phase dehydration of xylose into furfural catalyzed by ZSM-5 zeolite", *Industrial & Engineering Chemistry Research,* vol. 48, no. 9, pp. 4300-4306.

Oliver, A., Pedersen, J., Grant, R. & Klopfenstein, T. 2005, "Comparative Effects of the Sorghum-6 and-12 Genes", *Crop Science,* vol. 45, no. 6, pp. 2234-2239.

O'Sullivan Antoinette C 1997, "Cellulose: the structure slowly unravels", *Cellulose,* vol. 4, no. 3, pp. 173-207.

Palmqvist, E. & Hahn-Hägerdal, B. 2000, "Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification", *Bioresource technology,* vol. 74, no. 1, pp. 17-24.

Pan, X., Xie, D., Gilkes, N., Gregg, D.J. & Saddler, J.N. 2005, "Strategies to enhance the enzymatic hydrolysis of pretreated softwood with high residual lignin content", *Twenty-Sixth Symposium on Biotechnology for Fuels and Chemicals*Springer, , pp. 1069.

Pedersen, M., Johansen, K.S. & Meyer, A.S. 2011, "Low temperature lignocellulose pretreatment: effects and interactions of pretreatment pH are critical for maximizing enzymatic monosaccharide yields from wheat straw", *Biotechnology for biofuels,* vol. 4, no. 11, pp. 19-27.

Peng, L., Lin, L., Zhang, J., Zhuang, J., Zhang, B. & Gong, Y. 2010, "Catalytic conversion of cellulose to levulinic acid by metal chlorides", *Molecules,* vol. 15, no. 8, pp. 5258-5272.

Perlack, R.D., Wright, L.L., Turhollow, A.F., Graham, R.L., Stokes, B.J. & Erbach, D.C. 2005, *Biomass as feedstock for a bioenergy and bioproducts industry: the technical feasibility of a billion-ton annual supply,* .

Pu, Y., Jiang, N. & Ragauskas, A.J. 2007, "Ionic liquid as a green solvent for lignin", *Journal of Wood Chemistry and Technology,* vol. 27, no. 1, pp. 23-33.

QI, W., ZHANG, S., XU, Q., REN, Z. & YAN, Y. 2008, "Degradation kinetics of xylose and glucose in hydrolysate containing dilute sulfuric acid", *过程工程学报,* vol. 8, no. 6.

Ralph, J., Lundquist, K., Brunow, G., Lu, F., Kim, H., Schatz, P.F., Marita, J.M., Hatfield, R.D., Ralph, S.A. & Christensen, J.H. 2004, "Lignins: natural polymers from oxidative coupling of 4-hydroxyphenyl-propanoids", *Phytochemistry Reviews,* vol. 3, no. 1-2, pp. 29-60.

Remsing, R.C., Swatloski, R.P., Rogers, R.D. & Moyna, G. 2006, "Mechanism of cellulose dissolution in the ionic liquid 1-n-butyl-3-methylimidazolium chloride: a 13C and 35/37Cl

NMR relaxation study on model systems", *Chemical Communications,* , no. 12, pp. 1271-1273.

RFA 2011 "RFA (Renewable Fuels Association). 2011. Ethanol Facts. <http://www.ethanolrfa.org/resource/facts>. Accessed June 2013.", .

Rocateli, A., Raper, R., Balkcom, K., Arriaga, F. & Bransby, D. 2012, "Biomass sorghum production and components under different irrigation/tillage systems for the southeastern US", *Industrial Crops and Products,* vol. 36, no. 1, pp. 589-598.

Román-Leshkov, Y. & Davis, M.E. 2011, "Activation of carbonyl-containing molecules with solid Lewis acids in aqueous media", *ACS Catalysis,* vol. 1, no. 11, pp. 1566-1580.

Rooney, W.L., Blumenthal, J., Bean, B. & Mullet, J.E. 2007, "Designing sorghum as a dedicated bioenergy feedstock", *Biofuels, Bioproducts and Biorefining,* vol. 1, no. 2, pp. 147-157.

Saddler, J., Ramos, L. & Breuil, C. 1993, "Steam pretreatment of lignocellulosic residues", *BIOTECHNOLOGY IN AGRICULTURE,* , pp. 73-73.

Saha, B.C. 2003, "Hemicellulose bioconversion", *Journal of Industrial Microbiology and Biotechnology,* vol. 30, no. 5, pp. 279-291.

Saha, B.C., Iten, L.B., Cotta, M.A. & Wu, Y.V. 2005, "Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol", *Process Biochemistry,* vol. 40, no. 12, pp. 3693-3700.

Sarath, G., Mitchell, R.B., Sattler, S.E., Funnell, D., Pedersen, J.F., Graybosch, R.A. & Vogel, K.P. 2008, "Opportunities and roadblocks in utilizing forages and small grains for liquid fuels", *Journal of industrial microbiology & biotechnology,* vol. 35, no. 5, pp. 343-354.

Sassner, P., Galbe, M. & Zacchi, G. 2006, "Bioethanol production based on simultaneous saccharification and fermentation of steam-pretreated< i> Salix at high dry-matter content", *Enzyme and microbial technology,* vol. 39, no. 4, pp. 756-762.

Scarlata, C.J. & Hyman, D.A. 2010, "Development and validation of a fast high pressure liquid chromatography method for the analysis of lignocellulosic biomass hydrolysis and fermentation products", *Journal of Chromatography A,* vol. 1217, no. 14, pp. 2082-2087.

Schell, D.J., Farmer, J., Newman, M. & McMILLAN, J.D. 2003, "Dilute-sulfuric acid pretreatment of corn stover in pilot-scale reactor" in *Biotechnology for Fuels and Chemicals* Springer, , pp. 69-85.

Seri, K., Sakaki, T., Shibata, M., Inoue, Y. & Ishida, H. 2002, "Lanthanum (III)-catalyzed degradation of cellulose at 250 C", *Bioresource technology,* vol. 81, no. 3, pp. 257-260.

Sharma, S.K., Kalra, K.L. & Grewal, H.S. 2002, "Enzymatic saccharification of pretreated sunflower stalks", *Biomass and Bioenergy,* vol. 23, no. 3, pp. 237-243.

Sharma, S.K., Kalra, K.L. & Kocher, G.S. 2004, "Fermentation of enzymatic hydrolysate of sunflower hulls for ethanol production and its scale-up", *Biomass and Bioenergy,* vol. 27, no. 4, pp. 399-402.

Shen, J. & Wyman, C.E. 2011a, "A novel mechanism and kinetic model to explain enhanced xylose yields from dilute sulfuric acid compared to hydrothermal pretreatment of corn stover", *Bioresource technology,* vol. 102, no. 19, pp. 9111-9120.

Shen, J. & Wyman, C.E. 2011b, "A novel mechanism and kinetic model to explain enhanced xylose yields from dilute sulfuric acid compared to hydrothermal pretreatment of corn stover", *Bioresource technology,* vol. 102, no. 19, pp. 9111-9120.

Shi, J., Ebrik, M.A. & Wyman, C.E. 2011, "Sugar yields from dilute sulfuric acid and sulfur dioxide pretreatments and subsequent enzymatic hydrolysis of switchgrass", *Bioresource technology,* vol. 102, no. 19, pp. 8930-8938.

Silverstein, R.A., Chen, Y., Sharma-Shivappa, R.R., Boyette, M.D. & Osborne, J. 2007, "A comparison of chemical pretreatment methods for improving saccharification of cotton stalks", *Bioresource technology,* vol. 98, no. 16, pp. 3000-3011.

Sims, R.E., Mabee, W., Saddler, J.N. & Taylor, M. 2010, "An overview of second generation biofuel technologies", *Bioresource technology,* vol. 101, no. 6, pp. 1570.

Singh, A. & Bishnoi, N.R. 2012, "Optimization of ethanol production from microwave alkali pretreated rice straw using statistical experimental designs by< i> Saccharomyces cerevisiae", *Industrial Crops and Products,* vol. 37, no. 1, pp. 334-341.

Singh, A.K. 2001, "The Process Evaluation Handbook, by Donald J.", *Practical Strategies for Experimenting,* vol. 43, no. 4, pp. 493.

Singh, R. 2012, "The National Bioeconomy Blueprint: Meeting grand challenges", *Industrial Biotechnology,* vol. 8, no. 3, pp. 94-96.

Sluiter, J.B., Ruiz, R.O., Scarlata, C.J., Sluiter, A.D. & Templeton, D.W. 2010, "Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods", *Journal of Agricultural and Food Chemistry,* vol. 58, no. 16, pp. 9043-9053.

Su, Y., Brown, H.M., Huang, X., Zhou, X., Amonette, J.E. & Zhang, Z.C. 2009, "Single-step conversion of cellulose to 5-hydroxymethylfurfural (HMF), a versatile platform chemical", *Applied Catalysis A: General,* vol. 361, no. 1, pp. 117-122.

Sun, Y. & Cheng, J.J. 2005, "Dilute acid pretreatment of rye straw and bermudagrass for ethanol production", *Bioresource technology,* vol. 96, no. 14, pp. 1599-1606.

Sun, Y. & Cheng, J. 2002, "Hydrolysis of lignocellulosic materials for ethanol production: a review", *Bioresource technology,* vol. 83, no. 1, pp. 1-11.

Taguchi, G. 1987, *System of experimental design: engineering methods to optimize quality and minimize costs,* UNIPUB/Kraus International Publications New York.

Teymouri, F., Laureano-Perez, L., Alizadeh, H. & Dale, B.E. 2005, "Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover", *Bioresource technology,* vol. 96, no. 18, pp. 2014-2018.

Theerarattananoon, K., Wu, X., Staggenborg, S., Propheter, R., Madl, R. & Wang, D. 2011, "Evaluation and characterization of sorghum biomass as feedstock for sugar production.", .

Thring, R.W., Chornet, E. & Overend, R.P. 1990, "Recovery of a solvolytic lignin: effects of spent liquor/acid volume ratio, acid concentration and temperature", *Biomass,* vol. 23, no. 4, pp. 289-305.

Torget, R., Himmel, M. & Grohmann, K. 1992a, "Dilute-acid pretreatment of two short-rotation herbaceous crops", *Applied Biochemistry and Biotechnology,* vol. 34, no. 1, pp. 115-123.

Torget, R., Himmel, M. & Grohmann, K. 1992b, "Dilute-acid pretreatment of two short-rotation herbaceous crops", *Applied Biochemistry and Biotechnology,* vol. 34, no. 1, pp. 115-123.

Torget, R., Werdene, P., Himmel, M. & Grohmann, K. 1990, "Dilute acid pretreatment of short rotation woody and herbaceous crops", *Applied Biochemistry and Biotechnology,* vol. 24, no. 1, pp. 115-126.

USDA Report 2011, "U.S. Department of Energy. 2011. Biomass multi-year program plan. U.S. Department of Energy, Energy Efficiency & Renewable Energy, Biomass Program, Washington, DC. (Accessed at http://www1.eere.energy.gov/biomass/pdfs/mypp_april_2011.pdf on June 25, 2013.)", .

Vares, T., Lundell, T.K. & Hatakka, A.I. 1993, "Production of multiple lignin peroxidases by the white-rot fungus< i> Phlebia ochraceofulva", *Enzyme and microbial technology,* vol. 15, no. 8, pp. 664-669.

Vine, E. 2008, "Breaking down the silos: The integration of energy efficiency, renewable energy, demand response and climate change", *Energy Efficiency,* vol. 1, no. 1, pp. 49-63.

Vlasenko, E.Y., Ding, H., Labavitch, J. & Shoemaker, S. 1997, "Enzymatic hydrolysis of pretreated rice straw", *Bioresource technology,* vol. 59, no. 2, pp. 109-119.

vom Stein, T., Grande, P.M., Kayser, H., Sibilla, F., Leitner, W. & de María, P.D. 2011, "From biomass to feedstock: one-step fractionation of lignocellulose components by the selective organic acid-catalyzed depolymerization of hemicellulose in a biphasic system", *Green Chemistry,* vol. 13, no. 7, pp. 1772-1777.

Wasserscheid, P. & Keim, W. 2000, "Ionic liquids-new" solutions" for transition metal catalysis", *Angewandte Chemie,* vol. 39, no. 21, pp. 3772-3789.

Wei, H., Donohoe, B.S., Vinzant, T.B., Ciesielski, P.N., Wang, W., Gedvilas, L.M., Zeng, Y., Johnson, D.K., Ding, S. & Himmel, M.E. 2011, "Elucidating the role of ferrous ion cocatalyst in enhancing dilute acid pretreatment of lignocellulosic biomass", *Biotechnology for biofuels,* vol. 4, no. 1, pp. 1-16.

Weil, J.R., Dien, B., Bothast, R., Hendrickson, R., Mosier, N.S. & Ladisch, M.R. 2002, "Removal of fermentation inhibitors formed during pretreatment of biomass by polymeric adsorbents", *Industrial & Engineering Chemistry Research,* vol. 41, no. 24, pp. 6132-6138.

Weil, J., Brewer, M., Hendrickson, R., Sarikaya, A. & Ladisch, M.R. 1998, "Continuous pH monitoring during pretreatment of yellow poplar wood sawdust by pressure cooking in water" in *Biotechnology for Fuels and Chemicals* Springer, , pp. 99-111.

Weiss, N.D., Farmer, J.D. & Schell, D.J. 2010, "Impact of corn stover composition on hemicellulose conversion during dilute acid pretreatment and enzymatic cellulose digestibility of the pretreated solids", *Bioresource technology,* vol. 101, no. 2, pp. 674-678.

Werther, J., Saenger, M., Hartge, E.-., Ogada, T. & Siagi, Z. 2000, "Combustion of agricultural residues", *Progress in Energy and Combustion Science,* vol. 26, no. 1, pp. 1-27.

Wright, J.D. 1988, "Ethanol from biomass by enzymatic hydrolysis", *Chem.Eng.Prog.;(United States),* vol. 84, no. 8.

Wyman, C.E. 1999a, "Biomass ethanol: technical progress, opportunities, and commercial challenges", *Annual Review of Energy and the Environment,* vol. 24, no. 1, pp. 189-226.

Wyman, C.E. 1999b, "Biomass ethanol: technical progress, opportunities, and commercial challenges", *Annual Review of Energy and the Environment,* vol. 24, no. 1, pp. 189-226.

Wyman, C.E., Dale, B.E., Elander, R.T., Holtzapple, M., Ladisch, M.R. & Lee, Y. 2005a, "Coordinated development of leading biomass pretreatment technologies", *Bioresource technology,* vol. 96, no. 18, pp. 1959-1966.

Wyman, C.E., Dale, B.E., Elander, R.T., Holtzapple, M., Ladisch, M.R. & Lee, Y. 2005b, "Coordinated development of leading biomass pretreatment technologies", *Bioresource technology,* vol. 96, no. 18, pp. 1959-1966.

Yang, B. & Wyman, C.E. 2004, "Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose", *Biotechnology and bioengineering,* vol. 86, no. 1, pp. 88-98.

Yang, Y., Hu, C. & Abu-Omar, M.M. 2012, "Conversion of glucose into furans in the presence of AlCl< sub> 3</sub> in an ethanol–water solvent system", *Bioresource technology,* vol. 116, pp. 190-194.

Yat, S.C., Berger, A. & Shonnard, D.R. 2008a, "Kinetic characterization for dilute sulfuric acid hydrolysis of timber varieties and switchgrass", *Bioresource technology,* vol. 99, no. 9, pp. 3855-3863.

Yat, S.C., Berger, A. & Shonnard, D.R. 2008b, "Kinetic characterization for dilute sulfuric acid hydrolysis of timber varieties and switchgrass", *Bioresource technology,* vol. 99, no. 9, pp. 3855-3863.

Yu, Q., Zhuang, X., Yuan, Z., Qi, W., Wang, Q. & Tan, X. 2011, "The effect of metal salts on the decomposition of sweet sorghum bagasse in flow-through liquid hot water", *Bioresource technology,* vol. 102, no. 3, pp. 3445-3450.

Zavrel, M., Bross, D., Funke, M., Büchs, J. & Spiess, A.C. 2009, "High-throughput screening for ionic liquids dissolving (ligno-) cellulose", *Bioresource technology,* vol. 100, no. 9, pp. 2580-2587.

Zhang, Y.P. & Lynd, L.R. 2006, "A functionally based model for hydrolysis of cellulose by fungal cellulase", *Biotechnology and bioengineering,* vol. 94, no. 5, pp. 888-898.

Zhang, Y.P. 2008, "Reviving the carbohydrate economy via multi-product lignocellulose biorefineries", *Journal of industrial microbiology & biotechnology,* vol. 35, no. 5, pp. 367-375.

Zhao, H., Holladay, J.E., Brown, H. & Zhang, Z.C. 2007, "Metal chlorides in ionic liquid solvents convert sugars to 5-hydroxymethylfurfural", *Science,* vol. 316, no. 5831, pp. 1597-1600.

Zhao, X., Cheng, K. & Liu, D. 2009, "Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis", *Applied Microbiology and Biotechnology,* vol. 82, no. 5, pp. 815-827.

Zheng, Y., Pan, Z. & Zhang, R. 2009, "Overview of biomass pretreatment for cellulosic ethanol production", *International Journal of Agricultural and Biological Engineering,* vol. 2, no. 3, pp. 51-68.

Zheng, Y., Lin, H., Wen, J., Cao, N., Yu, X. & Tsao, G.T. 1995, "Supercritical carbon dioxide explosion as a pretreatment for cellulose hydrolysis", *Biotechnology Letters,* vol. 17, no. 8, pp. 845-850.

Zhou, W., Lou, C., Li, Z., Lu, L. & Yang, H. 2010, "Current status of research on optimum sizing of stand-alone hybrid solar-wind power generation systems", *Applied Energy,* vol. 87, no. 2, pp. 380-389.

Zhu, J. & Pan, X. 2010, "Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation", *Bioresource technology,* vol. 101, no. 13, pp. 4992-5002.

Zhu, W., Houtman, C.J., Zhu, J., Gleisner, R. & Chen, K. 2012, "Quantitative predictions of bioconversion of aspen by dilute acid and SPORL pretreatments using a unified combined hydrolysis factor (< i> CHF)", *Process Biochemistry,* vol. 47, no. 5, pp. 785-791.

Zhu, Y., Lee, Y. & Elander, R.T. 2005, "Optimization of dilute-acid pretreatment of corn stover using a high-solids percolation reactor", *Twenty-Sixth Symposium on Biotechnology for Fuels and Chemicals*Springer, , pp. 1045.