UNIVERSITY OF CALIFORNIA
RIVERSIDE

Dilute Acid Hydrolysis of Oligomers in Hydrothermal Pretreatment Hydrolyzate into Monomers with High Yields

A Thesis submitted in partial satisfaction
of the requirements for the degree of

Master of Science

in

Chemical and Environmental Engineering

by

Yueh-Du Tsai

September 2012

Thesis Committee:
Dr. Charles E. Wyman, Chairperson
Dr. Akua Asa-Awuku
Dr. Ian Wheeldon

The Thesis of Yueh-Du Tsai is approved:

_____

_____

_____

Committee Chairperson

University of California, Riverside

Acknowledgements

First, I would like to thank my advisor, Professor Charles E. Wyman for giving me this opportunity to work in his group and his patience, encouragement and support during this two years at University of California at Riverside. He always inspires me and giving a new aspect with his knowledge.

And second, I am grateful to my family. Without their love and support, I would not have the courage to follow this path.

In addition, I appreciate the staff and other professors of University of California, Riverside and Center for Environmental Research and Technology (CE-CERT) and the Chemical and Environmental Engineering Department for contributing in many ways to this work.

Special thanks to Dr. Taiying Zhang and Dr. Rajeev Kumar for always being willing to guide me and give me advice on this work. I would like to thank Hongjia Li for and helping me on experiments. I also appreciate other group members for helping me in these two years.

ABSTRACT OF THE THESIS

Dilute Acid Hydrolysis of Oligomers in Hydrothermal Pretreatment Hydrolyzate into Monomers with High Yields

by

Yueh-Du Tsai

Master of Science, Graduate Program in Chemical and Environmental Engineering
University of California, Riverside, September 2012
Dr. Charles E. Wyman, Chairperson

Biomass is one of the most important renewable energy which can reduce the energy consumption of fossil fuels and carbon dioxide emission. Ethanol converted from celluloses and hemicelluloses in lignocellulosic biomass by biochemical technology has great potential to replace gasoline for transportation fuels. Hemicelluloses content abundant of fermentable five carbon sugars which can be release by thermal/chemical pretreatment process. Dilute acid and hydrothermal pretreatment are two efficient ways to hydrolyze hemicellulose into xylose. However, most of hemicelluloses are hydrolyzed into its oligomers form which cannot be fermented to ethanol during hydrothermal pretreatment. Although, dilute acid pretreatment can easily hydrolyze hemicelluloses to monomeric xylose, the degradation products from xylose are dramatically increased when the pretreatment severity goes higher. This study focus on maximizing xylose

yields of pretreatment and minimizing degradation products during the process. Two steps pretreatment was applied to achieve this goal. The first step is hydrothermal pretreatment which convers hemicellulose into both oligomers and monomers form, and following the second step dilute sulfuric acid hydrolysis at low temperature to convert oligomers into monomers with minimum degradation products. Combine severity factor was used to estimate reaction time with certain temperature and acid concentration. The results show that high xylose yields and low degradation products were observed. The kinetics of oligomers hydrolysis was developed and the acid concentration and temperature effects were considered. Rate constants (k) based on first order homogeneous kinetic models and activation energy were also estimated. NREL biochemical ethanol conversion process was applied to estimate both capital and operating costs of the two step hemicellulose hydrolysis process. Consequently, the dilute acid hydrolysis process convers most of the xylooligomers into xylose without forming large degradation products. And both capital and operating costs are reduced by using this two-step hemicellulose hydrolysis.

Table of Contents

List of Figures

List of Tables

Chapter 1

Introduction

1.1 Background

Over the past one hundred years, energy demand increased significantly because of the industrial revolution and population growth. Around 80% of the energy supply now comes from fossil fuels such as oil, natural gas, and coal; and most of these energy resources are in the Mideast. Around the middle of the twentieth century, people started to notice that fossil fuels cannot be considered as the main source of energy. Furthermore, an essential issue related to consumption of fossil fuels is their impact on the environment and especially global climate change and air pollution(Goldemberg 2007).Carbon dioxide is the major greenhouse gas and has been increased in concentration by about 1.5 times during 60years. Total carbon dioxide emissions were about 5,633 million metric tons in 2009.Petroleum accounted for about 42% of this total with coal and natural gas contributing about 35% and 23%, respectively. The transportation sector produced about 33% of the total carbon dioxide emissions in the U.S. because petroleum is the main energy source for vehicles (U.S. Energy Information Administration, Annual Energy Review 2010).With declining fossil fuel resources and environmental issues, it is important to develop economical and efficient alternative energy sources to fossil fuels. Renewable/sustainable energy options include hydroelectric, biomass, wind, solar,

geothermal, and ocean tides and currently represent about 8% of total U.S. energy consumption (U.S. Energy Information Administration, Annual Energy Review 2010). Biomass was the major energy source for mankind before the discovery of fossil fuels, and declined from meeting over 70% of the world's total energy demand in 1860 to only about 7% in 1990 (Klass 1998). In 2010, approximately 48% of U.S. renewable energy was from biomass, but half of it was via traditional biomass that generates heat by combustion. Because of improvements in biotechnology, bioethanol produced from biomass has become a sustainable source of liquid fuels. In 2011, about 13.9 billion gallons of fuel ethanol was produced from corn starch in the United States (RFA, 2012 Ethanol Industry Outlook). However, because corn and other grains are vital for food and feed, the cost of feedstock could be dramatically influenced by food price fluctuations. Lignocellulosic biomass such as forestry and agriculture residues, woody and grassy crops, and solid waste (paper and wood) have potential to be bioethanol feedstock that are inexpensive and abundant(Wyman 1999). In addition, as illustrated in Figure 1.1, cellulosic ethanol is an efficient way to reduce carbon dioxide emissions because carbon dioxide produced from consumption of ethanol is captured when growing new energy crops to replace those harvested as feedstock (Farrell 2006).Another advantage is that fuel ethanol can be blended directly into gasoline and used by vehicles without modifying their engines. Currently, more than95% of the gasoline consumed in the U.S. contains 10% ethanol (E10), and E15 is expected to be used within a few years (RFA, 2012 Ethanol Industry Outlook).

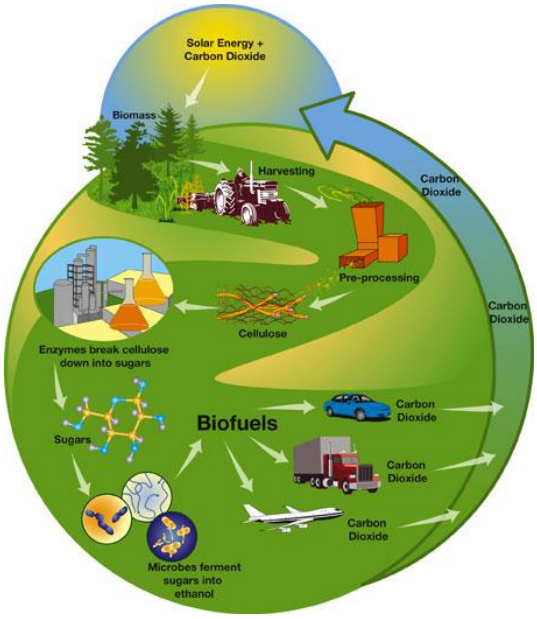


Figure 1.1 Biofuels and carbon dioxide cycle. (http://www.cert.ucr.edu/research/ses/CellulosicBiomassLab.html)

1.2 Conversion process of cellulosic biomass to ethanol

Unlike ethanol production from sugar cane or corn starch, making ethanol from cellulosic biomass is more difficult due to the complex and recalcitrant structure of cellulosic materials. Cellulosic biomass contains three main components: 1) cellulose which is made up of glucose units joined together by β-1,4 glucosidic linkages in a crystalline structure, 2) hemicellulose that contains hexsosans (glucose, galactose, and mannose) and pentosans (xylose and arabinose) in an amorphous structure, and 3) lignin which is a three dimentional aromatic polymer (Nishiyama et al. 2002, Saha 2003). To release the five fermentable sugars in cellulose and hemicellulose and ferment them to

ethanol, a series of steps are needed, with the major operations outlined in Figure 1.2(Wyman 1999, 2008).

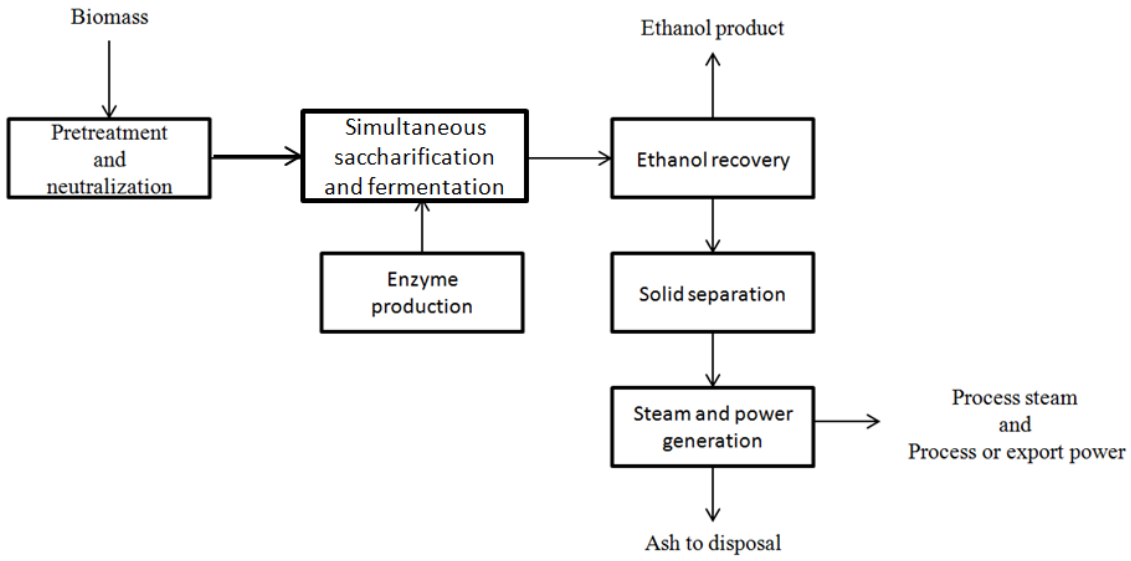


Figure 1.2 Thermochemical/biological conversion process for ethanol production from cellulosic biomass.

The roll of pretreatment in this sequence isto disrupt the biomass structure and increase the accessibility of enzymes to cellulose. Furthermore, hemicellulose could be broken down to release its component sugars during pretreatment(McMillan 1994, Wyman et al. 2005). Figure 1.3 illustrates the effect of pretreatment on lignocellulosic materials. Several pretreatment technologies have been developed, however, the choice of pretreatment depends on the combination of feedstocks, enzymes, and organisms used (Wooley 1999, Wyman 2008). Enzyme and yeast are added in the second step of simultaneous saccharification and fermentation (SSF) to hydrolyze glucose by the enzymes and then ferment the five and six carbon sugars to ethanol (Ohgren et al. 2007). Next the product ethanol is separated from the other components in the SSF process by

distillation. The lignin solid residues can be burned to provide heat and electricity used by conversion process or exported to the grid.

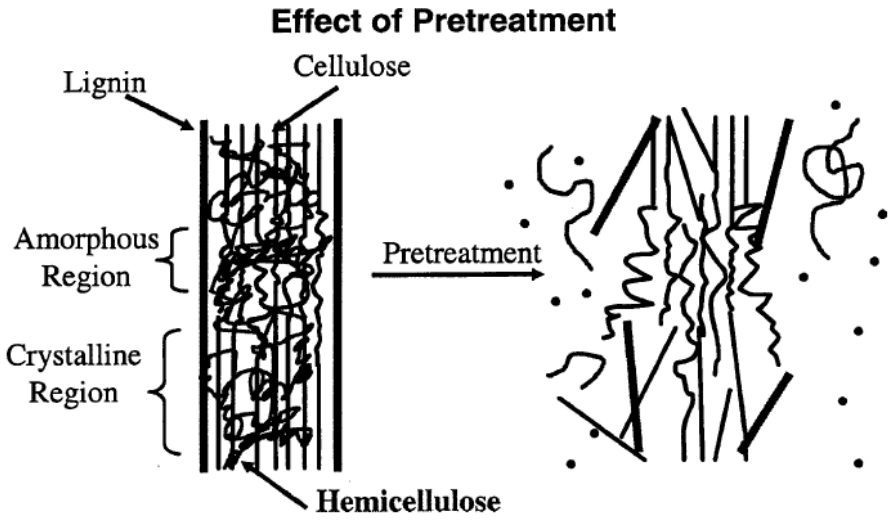


Figure1.3. Disruption of cellulosic biomass by pretreatment (Hsu et al., 1980).

1.3 Hemicellulose hydrolysis

Hemicellulose, an amorphouspolysaccharide with a 1, 4-β-D-xylan backbone is one of the most abundant materials in cellulosic biomass. It can be depolymerized into monomeric xylose or short chain xylooligomer fragments, depending on bond scission during hydrothermal and dilute acid pretreatment. Xylooligomers are important intermediates of hemicellulose breakdown and also are potentially important in many fields such as food, pharmaceutical, and agricultural applications (Vazquez et al. 2000). Over the past few decades, many kinetic models were developed to describe hemicellulose hydrolysis. A simple two step model is based on hemicellulose hydrolysis to xylose monomers which then

degrades to furfural and other degradation products (Saeman 1945). Although oligomers are formed during hemicellulose breakdown, a major assumption for this model is that the oligomer depolymerization rate is much faster than the hydrolysis of hemicellulose-to-oligomers so that their concentration is kept low (Bhandari et al. 1984, Maloney et al. 1986). The accuracy of model predictions could be improved for many substrates by representing hemicellulose as two fractions that hydrolyze to monomers at different rates(Kobayashi and Sakai 1956). In 2001, Garrote et al. introduced a new model which included xylooligomers as intermediates in the path from hemicellulose to xylose(Garrote et al. 2001).

1.4 Thesis objectives

Hydrothermal pretreatment of cellulosic biomass can produce highly digestible cellulose by predominately breaking down hemicellulose in biomass with just hot water or steam. This approach has many important advantages compared to dilute acid pretreatment that is often favoredfor biological conversion including lower cost materials of construction, lower costs for chemicals, possible elimination of hydrolyzate conditioning prior to SSF, and avoidance of fouling of heat exchangers in distillation. However, about 85% of the xylose released during pretreatment is as xylooligomers that many organisms cannot ferment to ethanol or other products, and a subsequent post hydrolysis step with enzymes or dilute acid is needed to produce fermentable sugars from the oligomers. Post hydrolysis with dilute acid is the most promising near term option,

but it is important to carry out this operation at low temperatures while realizing high yields and low xylose degradation in order for this option to be better than dilute acid pretreatment alone. Thus, the objective of this work is to determine post hydrolysis conditions that will realize high xylose yields from the hydrolyzate from hydrothermal pretreatment at low temperatures. In addition, the kinetics of xylooligomers decomposition will be modeled to help optimize the post hydrolysis approach, and the cost of the coupled hydrothermal pretreatment and post hydrolysis system will be estimated and compared to that for conventional dilute acid pretreatment.

1.5 References

Bhandari N, Macdonald D, Bakhshi N. 1984. Kinetic-studies of corn stover saccharification using sulfuric-acid. Biotechnology and Bioengineering 26: 320-327.

Farrell A. 2006. Ethanol can contribute to energy and environmental goals (vol 311, pg 506, 2006). Science 312: 1748-1748.

Garrote G, Dominguez H, Parajo J. 2001. Kinetic modelling of corncob autohydrolysis. Process Biochemistry 36: 571-578.

Goldemberg J. 2007. Ethanol for a sustainable energy future. Science 315: 808-810.

Klass DL. 1998. Biomass for renewable energy, fuels, and chemicals. San Diego: Academic Press.

Kobayashi T, Sakai Y. 1956. Hydrolysis rate of pentosan of hardwood in dilute sulfuric acid. Bulletin of the Agricultural Chemical Society of Japan 20: 1-7.

Maloney M, Chapman T, Baker A. 1986. An engineering analysis of the production of xylose by dilute acid-hydrolysis of hardwood hemicellulose. Biotechnology Progress 2: 192-202.

McMillan J. 1994. Pretreatment of lignocellulosic biomass, in Enzymatic

Conversion of Biomass for Fuels Production. American Chemical Society: 292–324.

Nishiyama Y, Langan P, Chanzy H. 2002. Crystal structure and hydrogen-bonding system in cellulose 1 beta from synchrotron X-ray and neutron fiber diffraction. Journal of the American Chemical Society 124: 9074-9082.

Ohgren K, Bura R, Lesnicki G, Saddler J, Zacchi G. 2007. A comparison between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using steam-pretreated corn stover. Process Biochemistry 42: 834-839.

Saeman J. 1945. Kinetics of wood saccharification - hydrolysis of cellulose and decomposition of sugars in dilute acid at high temperature. Industrial and Engineering Chemistry 37: 43-52.

Saha B. 2003. Hemicellulose bioconversion. Journal of Industrial Microbiology & Biotechnology 30: 279-291.

Vazquez M, Alonso J, Dominguez H, Parajo J. 2000. Xylooligosaccharides: manufacture and applications. Trends in Food Science & Technology 11: 387-393.

Wooley R, Rith M, Glassner D and Sheehan J. 1999. Process design and costing of bioethanol technology: a tool for determining the status and direction of research and development. BiotechnolProg: 794–803.

Wyman C.E. 1999. Biomass ethanol: Technical progress, opportunities, and commercial challenges. Annual Review of Energy and the Environment 24: 189-226.

. 2008. Cellulosic ethanol: A unique sustainable liquid transportation fuel. Mrs Bulletin 33: 381-383.

Wyman C.E., Dale B, Elander R, Holtzapple M, Ladisch M, Lee Y. 2005. Coordinated development of leading biomass pretreatment technologies. Bioresource Technology 96: 1959-1966.

Chapter 2

Literature Review

2.1Bioethanol Feedstock

Biomass is one of the most important resources for production of renewable energy on the earth. Liquid fuels produced from biomass can reduce consumption of fossil fuels which will become scarcer in coming decades and also lower carbon dioxide emission. Biomass converts solar energy, carbon dioxide, and water into carbohydrates and oxygen by photosynthesis, as follows (Klass 1998):

$$nH_2O + nCO_2 + \text{light} \xrightarrow{\text{Chlorophyll}} (CH_2O) + O_2$$

Plants capture around 0.1~1% of the incident solar energy. To produce liquid fuels or other valuable products from biomass, an inexpensive and abundant feedstock is needed because it dramatically influences product economics, location of facilities, process development, environmental benefits, and impacts (Lynd et al. 1999). The energy requirement for growing feedstock should include irrigation, fertilizer manufacture, and transportation related to biomass growth. Another important matter is growth rate of plants that varies with different species over a range of 6 ~ 90 metric tons/ ha-year (Klass 1998).Biomass which has a faster growth rate and low energy requirement can be developed through genetic engineering and breeding technologies. Biofuels feedstocks include agricultural wastes and residues; woody crops; urban wastes; starch, sugar,

herbaceous, and woody energy crops; oil-rich plants; and aquatic plants such as algae, kelp and water hyacinth. Table 2.1summarized the composition of different feedstocks.

Table 2.1 Composition of selectived crops by weight persentage and dry basis (Towler et al. 2004)

Component	Crops										
	Corn grain	Corn stover	Cane	Cane bagasse	Sweet Sorghum	Eucalyptus	Pine	Algae	Kelp	Water hyacinth
Extractives	17.24	5.60	43.00	1.81	23.26	2.18	2.88	18.02	12.92	18.51
Uronic acid	0.00	2.98	0.00	1.25	1.13	4.61	2.67	0.00	0.00	0.00
Saccharides	71.26	62.84	37.00	68.38	53.31	62.92	66.45	54.68	48.71	62.50
Hemicellulose	25.29	23.23	15.00	25.88	17.42	14.63	21.90	44.90	16.90	33.20
Cellulose/algin	45.98	37.60	22.00	42.50	35.90	48.29	44.55	9.78	31.81	29.30
Lignin	9.20	18.55	11.00	24.28	16.98	29.16	27.67	0.00	0.00	4.79
Ash	2.30	10.04	9.00	4.29	5.32	1.13	0.32	27.30	38.37	14.20
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
References	Schroeder, 1997	NREL[a], 2003	Shleser, 2002	NREL[a], 2003	NREL[a], 2003	NREL[a], 2003	NREL[a], 2003	Cruz, 1997	Klass, 1973	Van Thu, 1999

[a]The National Renewable Energy Laboratory (2003). Biomass feedstock composition and properties database

Sugar from crops such as sugar cane has been used as a feedstock for bioethanol production for many years. The juices from sugar cane contain sugars which can be directly fermented to ethanol by microorganism after conditioning. In addition, the residue after juices production (bagasse) are combusted to provide heat and power to the production facility with excess left to export to the power grid. However, there are some disadvantages of sugar crops. One is potential higher price than other carbohydrate sources, and a second is seasonal availability (Lynd et al. 1999).Starch-rich crops such as corn and wheat are commonly used for making bioethanol commercially in the U.S. Unlike sugar, production of ethanol from starch crops needs additional steps of cooking, liquefaction, scarification to break the α- 1,4 and α- 1,6 glycoside linkages of starch to form fermentable sugars such as glucose and sucrose (Klass 1998).The no carbohydrate fraction of corn and wheat are high in protein and oil which can be recovered as valuable coproducts such as food oil and animal feed (Lynd et al. 1999). In general, starch-rich crops can rely on established feedstock production and processing facilities, and the alpha glucose bonds in starch are more easily hydrolyzed to sugar than lignocellulose. However, competition with food can make corn more expensive and cause price instabilities. Lignocellulosic biomass offers lower price, lower chemical and energy inputs for production, a larger supply due to the abundance and diversity of lignocellulosic biomass (forestry and agricultural residues, municipal solid waste, and woody and grassy crops) and less competition with food or feed crops. Lignocellulosic biomass is considered a second generation feedstock because commercial plants have not yet been built to convert it to fuels economically. As pretreatment, enzyme and genetic

technologies have been improved to overcome the recalcitrance of cellulosic biomass and more efficiently breakdown biomass with lower energy requirement, it could be commercialized in a few years (Wyman 1999).

2.2 Composition and structure of lignocellulosic biomass

Lignocellulosic is one of the most abundant resources in the world. Cellulosic ethanol processes mostly focus on solubilizing carbohydrates and converting the carbohydrates to ethanol with high yields, but cellulosic biomass is a complex structural material that is difficult to breakdown into sugars. Therefore, understanding the physical structure of lignocellulosic biomass is very important. The main components which make up plant cell walls are cellulose (35-50%), hemicellulose (15-35%), lignin (5-30%), and usually much smaller amount of pectin, protein, extractives, and ash (Chandra et al. 2007, Kumar P et al. 2009). The extractives can include carbohydrates such as sucrose and amylose which can be dissolved in water. Ash is defined as material that does not burn (Department of Energy, Feedstock Composition Gallery 2005). Cellulose, hemicellulose, and lignin form a three dimensional cross linked structure that supports plants but make the sugars more difficult to release. The amount of these components can vary with different species, growing regions, weather conditions, soil type and conditions, fertilization practices, harvesting and storage situation, and storage time.

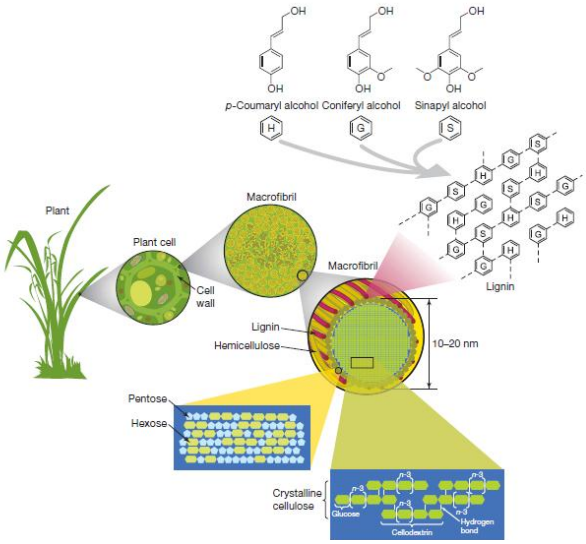
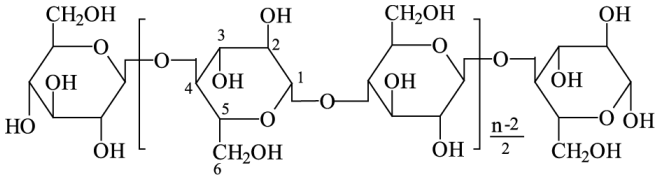


Figure 2.1 Structure of lignocellulosic biomass (Rubin 2008).

2.2.1Cellulose

Cellulose is long chain polysaccharide of D-glucopyranose monomers with β-1, 4 linkages (Van Wyk 2001). The average of degree of polymerization of cellulose is about 10,000 to 15,000 glucose units, and each unit is attached in reverse of the one next to it (180°) to form the repetitive unit called cellobiose, as shown in Figure 2.2a (Gao Z.H. 2005). This chain structure has two terminal groups which have a closed-ring and aliphatic structures and a carbonyl group. Cellulose chains are parallel and aligned together to form a crystalline mircofibrils by intra- and inter-hydrogen bonding. Then, these mircofibrils associate with hemicellulose and lignin as a cross-linked polysaccharides. Moreover, because hydroxyl groups and aliphatic hydrogen atoms are in the middle and axial position of cellulose chains, respectively, the top and bottom of the chain are hydrophobic, and its sides are hydrophilic (Cowling and Kirk 1976).

A



B

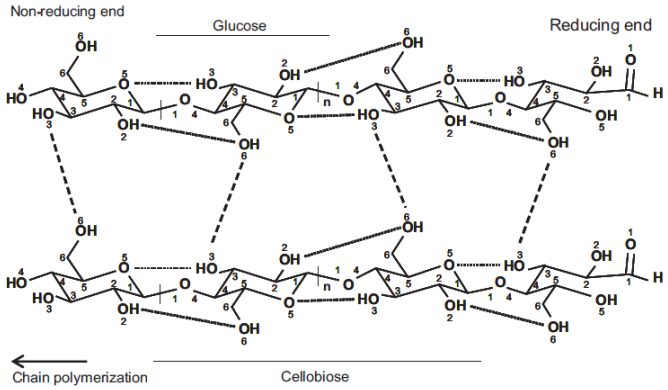


Figure 2.2 Structure of linear cellulose chain and repetitive dimer unit (A) hydrogen bonding pattern of cellulose (B). Dash lines: inter-chain hydrogen bonding; Dotted lines: intra-chain hydrogen bonding(Gao Z.H. et al., 2005).

However, some amorphous celluloses exist as microfibrils which are more easily hydrolyzed compared to crystalline cellulose (Newman and Davidson 2004).Breaking the crystalline structure of cellulose by thermochemical treatment increases accessibility of enzymes. Yet, the intrinsic structure is not the only factor restricting enzymes attaching to cellulose. The network between cellulose microfibrils, hemicellulose, and lignin also limits enzyme accessibility.

2.2.2Hemicellulose

Hemicelluloses are heterogeneous polysaccharides representing about 15–35% of lignocellulose biomass and can be extracted from the cell wall by alkali aqueous

solutions. Unlike cellulose which has a crystalline structure, hemicelluloses are not and consequently more easily hydrolyzed into monomeric sugars by thermochemical process (McCann et al. 2000). The components of hemicelluloses are five carbon sugars (xylose, arabinose), six carbon sugars (glucose, galactose, mannose), and sugar acids, typically with β-1, 4 linked backbones. Based on the polymer structure, hemicelluloses can be classified as xylan, mannan, xyloglucan, glucomannan, and arabinoxylan and have lower degrees of polymerization than cellulose of around 70~200 (Puls 1997).Glucomannans are the main polysaccharides in softwood hemicellulose, and xylan are the main polysaccharide in hardwood hemicellulose. Xylan consist of xylopyranose units with β-1, 4 linkages as a backbone, and most of xylose units are substituted with arabinose, glucuronic acid, 4-O-methyl glucuronic acid, and combinations of acidic and natural sugars at O-2 and/or O-3 of xylose unit (Saulnier et al. 1995).

Figure 2.3 shows several types of xylan. Arabinoxylansare the predominant hemicellulose of grasses with L-arabinofuranose attached 1α→2 and/or 1α→3 linkages to the xylose units throughout the chain. Glucuronoxylans, major components of the secondary cell walls of dicots, contain α(1,2)-linked D-glucuronyl, 4-O-methyl glucuronic acid (every 10 xylose units), and O-acetyl groups. Glucuronoarabinoxylans present in secondary wall of softwoods have arabinose units on O-3 position and 4-O-methyl glucuronic acid added to O-2 position (York and O'Neill 2008).

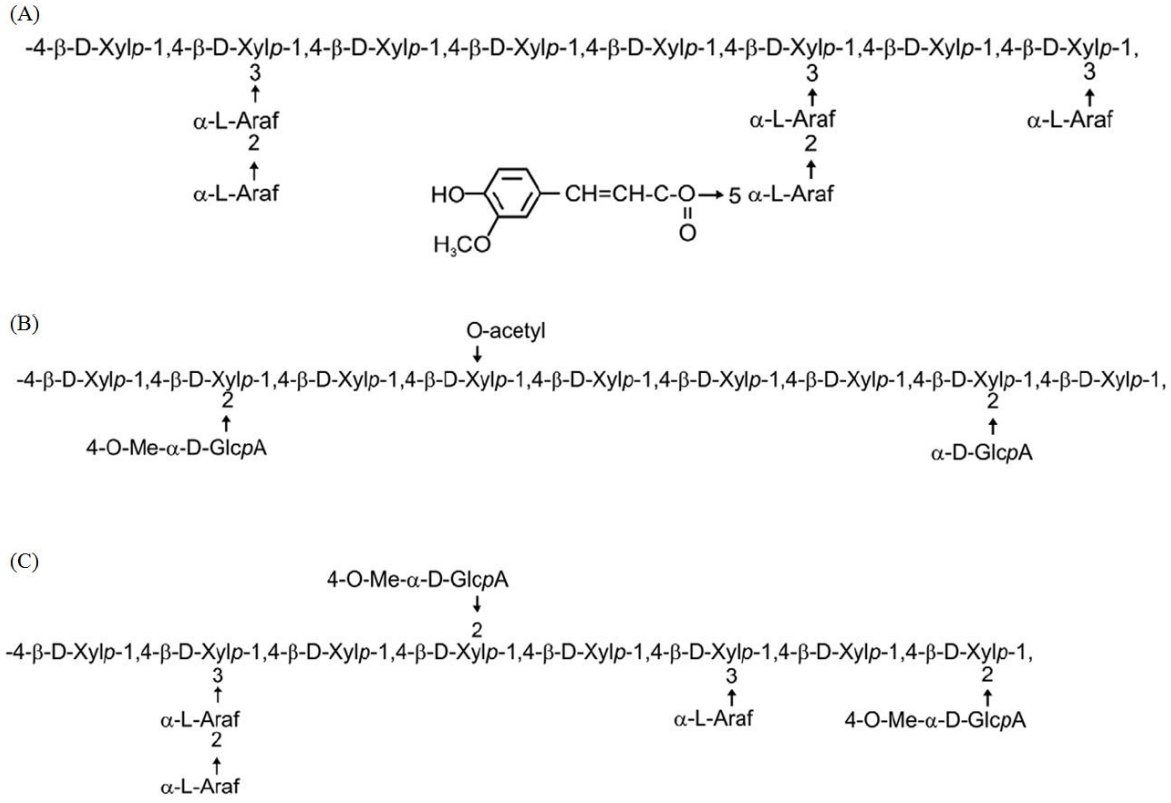


Figure 2.3 Different xylan structures: (A) Feruloylatedarabinoxylans, (B) Glucuronoxylans, and (C) Glucuronoarabinoxylans.

2.2.3Lignin

Lignin is a complex, high molecular weight, and cross-linked aromatic polymer which joins cellulose, hemicellulose, and pectin to form lignocellulosic compounds and provides structural support, facilitates water transport, and resists external stresses such as diseases, insects, and low temperatures. The polysaccharide components of plant cell walls are highly hydrophilic and thus permeable to water, whereas lignin is

more hydrophobic (Theander and Aman 1984). Figure 2.4 is an example of lignin structure (Adler 1977).

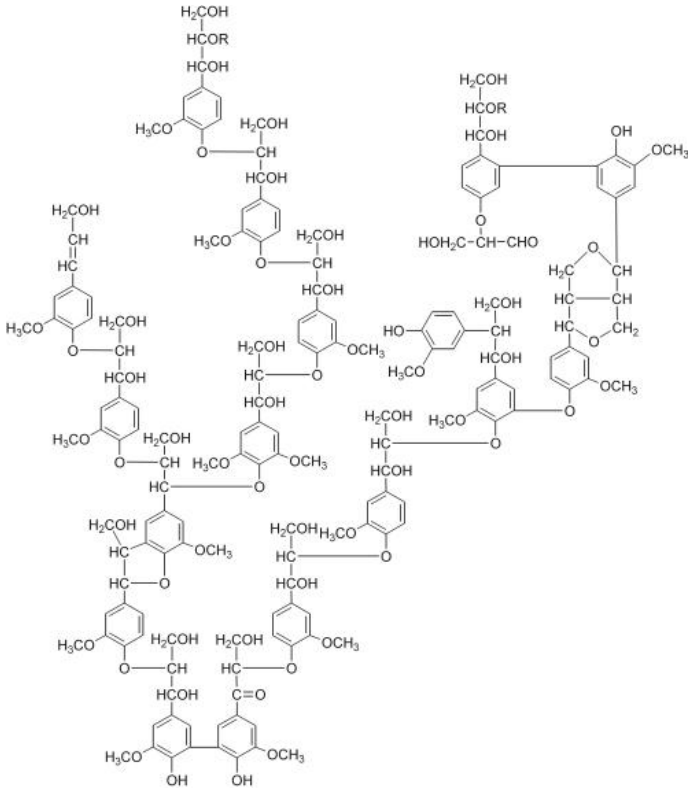


Figure 2.4 Structure and composition of lignin (Adler, 1977).

The three types of phenylpropanoid units incorporated into lignin are *p*-hydroxyphenyl, guaiacyl, and syringyl. These phenylpropanoid units are produced from the alcohols, *p*-coumaryl, coniferyl, and sinapyl alcohols shown in Figure 2.5, respectively. The amount and composition of lignin depends on the plant species and environmental factors (Boerjan et al. 2003). Hardwood lignins contain guaiacyl and syringyllignin, but softwood lignins mostly consist of guaiacyl units. Lignins from grasses are the most

complex and incorporate *p*-hydroxyphenyl, guaiacyl, and syringyllignins that are associated with other chemicals such as phenolic, *p*-coumaric, ferulic, and amino acids (Van Soest 1982). A small fraction of lignin is soluble in dilute acid (<10%),with the amount dissolved influenced by temperature and acid concentration.(Lavarack et al. 2002).

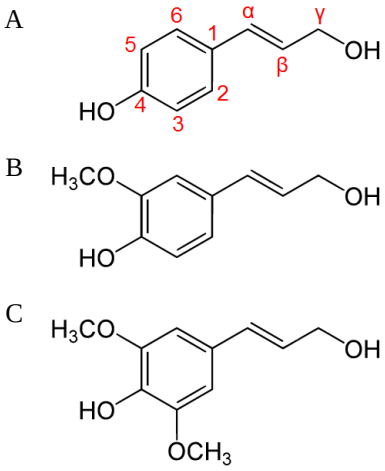


Figure 2.5 Chemical structure of (A) *p*-coumaryl, (B) coniferyl, and (C) sinapyl alcohols (Boerjan et al., 2003).

2.3Effects of pretreatment

Ethanol produced from biomass has the potential to reduce conventional fossil fuel use and net carbon dioxide emissions. However, converting lignocellulosic biomass to ethanol is more difficult than corn, wheat, or sugarcane due to the complex structure of cellulose, hemicellulose, and lignin, in addition to the crystalline structure of cellulose itself (Hsu 1996).As a result, operations are needed for pretreatment, enzyme production, enzymatic hydrolysis, fermentation, and ethanol purification. Pretreatment is one of the

most expensive steps (about 30 ¢ /gallon ethanol) in this process (Mosier et al. 2005) and can be important to:

1). Disrupt the complex structure of cellulose, hemicellulose, and lignin, and disturb the crystalline structure of cellulose to provide more available sites for enzymes.

2). Hydrolyze hemicellulose to fermentable sugar monomers.

3). Remove lignin from biomass.

Pretreatment reduces the cost of ethanol production by reducing enzyme demands and achieving high total glucose and xylose yields (Mosier et al. 2005, Wyman et al. 2009). Several pretreatment technologies have been studied over a few decades including physical pretreatments such as ball milling, compression milling, and comminution; pretreatments based on such chemicals as acids, bases, hydrogen peroxide, ozone, dioxane, and phenol; thermal pretreatments; and combination of these three. However, thermochemical pretreatments are favored, and some of them are considered for use in large scale ethanol conversion processes. The effect of various pretreatments are summarized in Table 2.2(Mosier et al. 2005).

Table 2.2 Effect of various pretreatment methods on the chemical composition and chemical/physical structure of lignocellulosic biomass (Mosier et al., 2005)

Pretreatment type	Increases accessible	Decrystalizes cellulose	Removes hemicellulose	Removes lignin	Alters lignin structure
steam explosion	●	-	●	-	○
Liquid hot water	●	ND	●	-	○
pH controlled hot water	●	ND	●	-	ND
Flow-through liquid hot water	●	ND	●	○	○
Dilute acid	●	-	●	-	●
Flow-through acid	●	-	●	○	●
AFEX	●	●	○	●	●
ARP	●	●	○	●	●
Lime	●	ND	○	●	●

● Major effect ○ Minor effect ND: Not Determined
AFEX: Ammonia Fiber Expansion ARP: Ammonia Recycle Percolation

Steam explosion pretreatment is a hydrothermal technology that heats biomass in few minutes with high pressure steam without added chemicals. Hemicelluloses are hydrolyzed during this process because of the acetic and others acid released from the acetyl group in hemicelluloses, and water also acts as an acid at high temperatures. The surface area of biomass is increased due to the rapidly changing pressure and temperature, but the crystalline structure of cellulose changes only slightly (Brownell et al. 1986, Mosier et al. 2005). Hot water pretreatment is another hydrothermal pretreatment operated at 160~ 240℃ for 10 to 30 minutes at elevated pressure. During this process, cellulose accessibility is improved, and hemicelluloses are hydrolyzed to oligomers and monomers by acetic and others acid from hemicellulose. Although hot water can recover almost 80% of hemicelluloses at favored conditions, reaction for longer times would hurt yields by degrading sugars and also form inhibitors to downstream enzymatic hydrolysis and fermentation processes. To reduce formation of degradation products, bases are added to control pH between 4 and 7, at which conditions most of the hemicellulose is hydrolyzed to oligomers. Lignin structure is slightly altered, and a small amount of lignin is removed into liquid phase during hot water pretreatment. Typically, flowthrough reactor systems remove much more lignin than batch devices and enhance hemicellulose removal (Mosier et al. 2005, Wyman et al. 2005), it would be challenging to apply to commercial scale operations, and the large amount of water used would result in high energy costs.

Pretreatment with bases such as lime, ammonia (ammonia fiber expansion (AFEX) and ammonia recycle percolation (ARP)) can breakdown linkages between lignin and

polysaccharides and remove lignin. Furthermore, during the AFEX or ARP process, the structure of cellulose is changed because of ammonolysis of glucuronic crosslinks. Thus, base pretreatment not only increases accessibility of micro- structure but macro-structure of lignocellulosic biomass, reducing enzyme loadings needed to realize high yields (Kim et al. 2002, Lin et al. 1984, Mosier et al. 2005). However, only some of the hemicellulose is hydrolyzed to sugar oligomers or monomers during base pretreatment, while acetyl and uronic acid groups in hemicellulose are removed. Lime pretreatment also removes a large fraction of the lignin and some hemicellulose from biomass, at relatively low temperatures and pressures. Yet, the reaction time for lime pretreatment usually takes at least several hours up to several days. Another limitation is salts formation during the pretreatment increase the cost due to precipitation.

Acid pretreatment has been developed for many years, and dilute sulfuric acid pretreatment has received the most attention and has been used at the commercial scale. Hemicellulose is hydrolyzed to fermentable sugars that dissolve in the liquid phase, and over 90% of hemicellulose can be removed by acid pretreatment by selection of the proper operating conditions. Although only a small part of glucose is hydrolyzed from cellulose and little lignin is removed, enzyme accessibility still increases significantly because most of hemicellulose is removed and the lignin structure is disrupted. The result is high glucose yields by enzymatic hydrolysis for moderate to high enzyme loadings (Hsu 1996, Wyman et al. 2005, Yang and Wyman 2004).However, dilute acid pretreatment has some disadvantages such as higher capital costs due to acid and pressure resistant vessels, high pressure, and the need for neutralization and conditioning of

pretreated hydrolyzate (Hsu 1996, Wyman 1999). In addition, some other effects must also be considered including the need for additional acid to neutralize the minerals in biomass and the influence of temperature on hydrogen ion concentration (Lloyd and Wyman 2004)

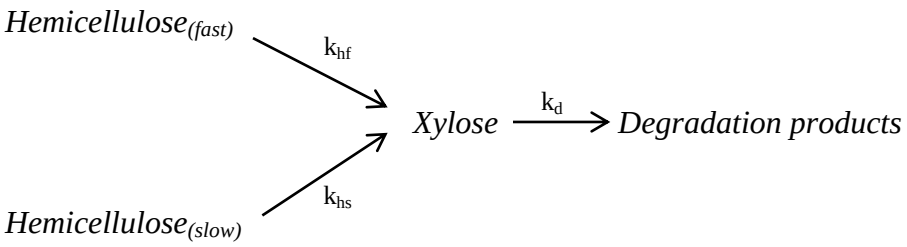
2.4 Hemicellulose hydrolysis

Hemicellulose consists about 15% to 35% of plant tissue and contains valuable sugars which can be fermented to ethanol or other products. Removing hemicellulose has been shown to increase enzyme accessibility and result in higher glucose yields from cellulose (Yang and Wyman 2004).In addition, oligomers from pretreatment are strong inhibitors to enzymatic hydrolysis (Kumar R and Wyman 2009). Dilute acid and hydrothermal pretreatment can break down hemicellulose in biomass to form sugars and oligomers dissolved in the aqueous liquid phase. The kinetics of hemicellulose hydrolysis has been studied for many years, with most studies based on pseudo-first order kinetic models. The simplest kinetic models to describe hemicellulose hydrolysis are based on models developed in 1945 by Saeman to describe cellulose hydrolysis and treat hemicellulose hydrolysis as a two-step reaction described as follows(Saeman 1945).

$$Hemicellulose \xrightarrow{k_h} Xylose \xrightarrow{k_d} Degradation\ products$$

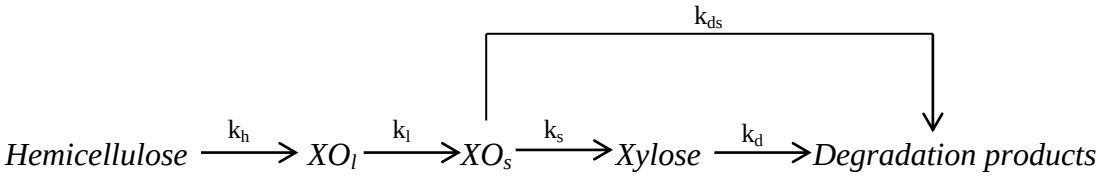
where k_h and k_d are rate constants for hemicellulose hydrolysis and xylose degradation, respectively. This approach assumes that hemicellulose hydrolyzes to monomeric xylose which in turn continues to degrade to furfural and other products. A few years later,

Kobayashi and Sakai divided hemicellulose into two fractions that each has a different hydrolysis rate (fast and slow) in dilute acid pretreatment. This biphasic kinetic model better fit experimental data, with the reaction pathway expressed as (Kobayashi and Sakai 1956):

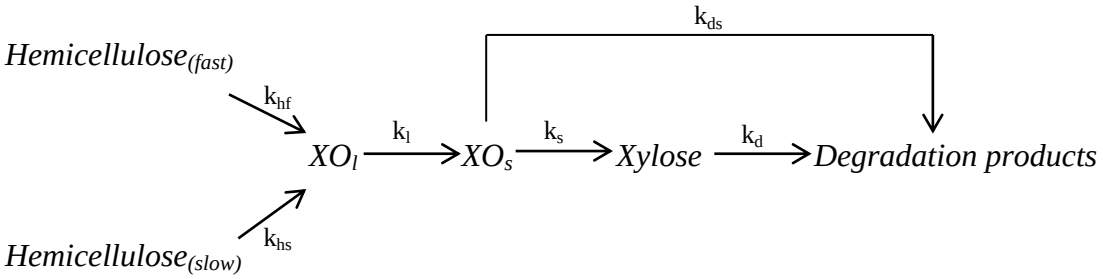


where k_{hf} and k_{hs} are rate constants for fast and slow hydrolysis, respectively.

The kinetic models above did not include a role for oligomers. Nevertheless, oligomers are important intermediates during hemicellulose hydrolysis, and their concentration tends to be greater at moderate pretreatment severity. The following hydrolysis pathway has been developed to include oligomers (Garrote G et al. 1999, 2001b):



where XO_l and XO_s are long chain and short chain soluble oligomers, respectively, and k_l, k_s and k_{ds} are rate constants for decomposition of long chain oligomers, conversion of short chain oligomers to xylose, and direct degradation of short chain oligomers to non-sugar products, respectively. In 2001, Garrote et al. modified their model to incorporate biphasic hydrolysis kinetics as follows (Garrote G. et al. 2001a):



In National Renewable Energy Laboratory (NREL) pretreatment resrarch, 75% xylan-to-xylose yields were achieved by dilute acid pretreatment at the bench scale in 2007 (Weiss et al. 2007). However, only ~60% xylan-to-xylose yields were achieved by continuous pilot-scale operation since the process conditions cannot be well controlled. Therefore, the secondary oligomer conversion step was added to convert xylooligomer to xylose with low degradation products formation, and achieved 75% xylan-to-xylose yields. After modification of horizontal reactor, 79.6% xylan-to-xylose conversion was achieved with 6.4% degradation, 5% unreacted xylan, and 9% xylooligomers that did not convert to monomeric xylose (Nagle et al. 2009).

2.5 Conclusions

Although lignocellulosic biomass has a good potentail as a feedstock of ethanol conversion process. The cost of ethanol produced from lignocellulosic biomass still not economical enough to compete with gasoline due to the high cost of pretreatment and enzymatic hydrolysis process. To lower the cost of these process, more economical pretreament pathway, operating conditions and low-cost feedstocks should be studied.

Hemicellulose is one of most abundant saccharides in the plant tissue and can be converted into fermentable sugars. Moreover, the removal of hemicellulose decreases enzyme usage resulting lower cost. Diluted acid pretreatment is a efficient process to convert hemicellulose to monomeric xylose with low pH and high temperature. However, the degradation products were formed at the same time. Therefore, two step pretreatment is needed to convert as much as possible sugars. Hydrothermal pretreatment can remove hemicellulose without producing large amount of degradation products, but most of the removed hemicellulose are in oligomer form which is a strong inhibitor for enzymatic hydrolysis. So, it is important to convert xylooligomers into xylose without forming too much degradation products. The kinetics of xylooligomers hydrolysis in dilute acid need to be established that is better able to predict pretreatment performance.

2.6 References

Adler E. 1977. Lignin chemistry - past, present and future. Wood Science and Technology 11: 169-218.

Boerjan W, Ralph J, Baucher M. 2003. Lignin biosynthesis. Annual Review of Plant Biology 54: 519-546.

Brownell HH, Yu EKC, Saddler JN. 1986. steam-explosion pretreatment of wood - effect of chip size, acid, moisture-content and pressure-drop. Biotechnology and Bioengineering 28: 792-801.

Chandra R, Bura R, Mabee W, Berlin A, Pan X, Saddler J, Olsson L. 2007. Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics? Biofuels 108: 67-93.

Cowling EB, Kirk TK. 1976. Properties of Cellulose and lignocellulosic materials as substrates for enzymatic conversion processes. Biotechnology and Bioengineering: 95-123.

Gao Z.H. GJY, Wang X.M., Li Z.G., Bai X.D. 2005. FTIR and XPS study of the reaction of phenyl isocyanate and cellulose with different moisture contents. Pigment & Resin Technology 34: 282 – 289.

Garrote G, Dominguez H, Parajo J. 1999. Hydrothermal processing of lignocellulosic materials. Holz Als Roh-Und Werkstoff 57: 191-202.

Garrote G, Dominguez H, Parajo JC . 2001a. Autohydrolysis of corncob:study of non-isothermal operation for xylooligosaccharide production. Journal of Food Engineering: 211-218.

Garrote G, Dominguez H, Parajo J. 2001b. Kinetic modelling of corncob autohydrolysis. Process Biochemistry 36: 571-578.

Hsu T. 1996. Bioethanol: Production and Utilization. Taylor and Francis. Washington D.C.

Kim TH, Kim JS, Sunwoo C, Lee YY. 2002. Delignification aspect of enzymatic hydrolysis in the ARP process. Paper presented at 24th Symposium on Biotechnology for Fuels and Chemicals.

Klass DL. 1998. Biomass for Renewable Energy, Fuels and Chemicals. San Diego: Academic Press.

Kobayashi T, Sakai Y. 1956. Hydrolysis rate of pentosan of hardwood in dilute sulfuric acid. Bulletin of the Agricultural Chemical Society of Japan 20: 1-7.

Kumar P, Barrett D, Delwiche M, Stroeve P. 2009. methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Industrial & Engineering Chemistry Research 48: 3713-3729.

Kumar R, Wyman C. 2009. cellulase adsorption and relationship to features of corn stover solids produced by leading pretreatments. Biotechnology and Bioengineering 103: 252-267.

Lavarack BP, Griffin GJ, Rodman D. 2002. The acid hydrolysis of sugarcane bagasse hemicellulose to produce xylose, arabinose, glucose and other products. Biomass and Bioenergy 23: 367-380.

Lin KW, Ladisch MR, Schaefer D, Noller CH, Lechtenberg V, Tsao GT. 1984. Review on effect of pretreatment on digestibility of cellulosic materials. AIChE Symposium Series 102–106.

Lloyd T.A., Wyman C.E. 2004. Predicted effects of mineral neutralization and bisulfate formation on hydrogen ion concentration for dilute sulfuric acid pretreatment. Applied Biochemistry and Biotechnology 113: 1013-1022.

Lynd L, Wyman C.E., Gerngross T. 1999. Biocommodity engineering. Biotechnology Progress 15: 777-793.

McCann M, Milioni D, Stacey N, Carpita N, Hofte H, Reiter W, Roberts K. 2000. Molecular architecture of the plant cell wall. Abstracts of Papers of the American Chemical Society 219: U259-U259.

Mosier N, Hendrickson R, Ho N, Sedlak M, Ladisch M. 2005. Optimization of pH controlled liquid hot water pretreatment of corn stover. Bioresource Technology 96: 1986-1993.

Nagle, N.; Kuhn, E.; Sievers, D.; Tucker, M.; Weiss, N.; Elander, R. Parametric study using the continuous horizontal pretreatment reactor using corn stover to determine operating conditions that achieve 80% yield of monomeric xylose. D Milestone Report, Document #10575 Golden, CO: National Renewable Energy Laboratory, September 2009.

National Renewable Energy Laboratory, process design and economics for biochemical conversion of lignocellulosic biomass to ethanol dilute-acid pretreatment and enzymatic hydrolysis of corn stover, Technical Report NREL/TP-5100-47764 May 2011

Newman R, Davidson T. 2004. Crystalline forms and cross-sectional dimensions of cellulose microfibrils in the Florideophyceae (Rhodophyta). Botanica Marina 47: 490-495.

Puls J. 1997. Chemistry and biochemistry of hemicelluloses: Relationship between hemicellulose structure and enzymes required for hydrolysis. Macromolecular Symposia 120: 183-196.

Rubin E. 2008. Genomics of cellulosic biofuels. Nature 454: 841-845.

Saeman J. 1945. Kinetics of wood saccharification - hydrolysis of cellulose and decomposition of sugars in dilute acid at high temperature. Industrial and Engineering Chemistry 37: 43-52.

Saulnier L, Marot C, Chanliaud E, Thibault J. 1995. Cell-wall polysaccharide interactions in maize bran. Carbohydrate Polymers 26: 279-287.

Schroeder, J.W. (1997). Corn gluten feed: Composition, storage, handling, feeding and value. North Dakota State University Extension Service and U.S. Department of Agriculture cooperating Report AS-1127 (p. 1).

Theander, Aman P. 1984. Anatomical and Chemical Characteristics. Pages 45-78 in Sundstol F, Owen E, eds. Straw and other fibrous by-products as feed. Amsterdam, Holland: Elsevier.

The National Renewable Energy Laboratory.2003, Biomass feedstock composition and properties database, http://www.ott.doe.gov/biofuels/properties_database.html.

Towler G, Oroskar A, Smith S. 2004. Development of a sustainable liquid fuels infrastructure based on biomass. Environmental Progress 23: 334-341.

U.S. Department of Energy, Feedstock Composition Gallery, U.S.
Department of Energy, Washington, DC

Van Soest PJ. 1982. Nutritional Ecology of the Ruminant. Corvallis, OR.: O&B Books.

Van Wyk JP. 2001. Biotechnology and the utilization of biowaste as a resource for bioproduct development. Trends Biotechnol. 19: 172–177

Weiss, N.; Nagle, N.; Elander, R.; Tucker, M. Achieve 75% xylose yield in laboratory scale high solids pretreatment reactor on corn stem internode. D Milestone Report, Document #9926. Golden, CO: National Renewable Energy Laboratory, 2007.

Wyman C.E. 1999. Biomass ethanol: Technical progress, opportunities, and commercial challenges. Annual Review of Energy and the Environment 24: 189-226.

Wyman C.E., Dale B, Elander R, Holtzapple M, Ladisch M, Lee Y. 2005. Comparative sugar recovery data from laboratory scale application of leading pretreatment technologies to corn stover. Bioresource Technology 96: 2026-2032.

Wyman C.E., Dale B, Elander R, Holtzapple M, Ladisch M, Lee Y, Mitchinson C, Saddler J. 2009. Comparative Sugar Recovery and Fermentation Data Following

Pretreatment of Poplar Wood by Leading Technologies. Biotechnology Progress 25: 333-339.

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 86: 88-95.

York W, O'Neill M. 2008. Biochemical control of xylan biosynthesis - which end is up? Current Opinion in Plant Biology 11: 258-265.

CHAPTER 3

Dilute Acid Hydrolysis of Xylose Oligomers from Hydrothermal Pretreatment into Monomers

3.1 Abstract

Many studies have determined xylose yields from hydrothermal or dilute acid pretreatment, but a large fraction of the xylose released into solution, particularly in the case of hydrothermal pretreatment, is in the form of oligomers that many biological and solid catalysts cannot effectively utilize. Thus, the oligomers must be broken down into more compatible monomers, and high yields at moderate conditions are important to low costs. Thus, in this study, xylooligomers released by batch hydrothermal pretreatment at 190℃ for 15 minute were hydrolyzed with 0.25%, 0.50%, 0.75% and 1.00% w/w sulfuric acid concentrations at 101℃, 110℃, 120℃, and 130℃ to determine yields of xylose. Monomeric xylose concentrations were measured directly before and after hydrolysis by high performance liquid chromatography (HPLC),while post-hydrolysis was needed to determine how oligomer concentrations changed over the course of the process. Xylose yields from treatment with 0.50%, 0.75% and 1.00% w/w acid concentrations were 90+% at all temperatures and 95+% with 0.75% and 1.00% w/w acid concentrations at 110℃, 120℃, and 130℃ but dropped slightly to 81% at 130℃with 0.25% acid. The results

shows high yields and low degradation of xylose monomers in dilute acid hydrolysis process.

3.2 Introduction

Production of liquid fuels from lignocellulosic biomass such as agriculture and forest residues, municipal solid waste, and herbaceous and woody energy crops has great potential as a sustainable energy source due to the low cost and abundance of the feedstock (Wyman 2003). Lignocellulosic biomass is composed of cellulose, hemicellulose and lignin. About 35% ~ 50% of cellulosic biomass consists of cellulose, a ß-1, 4 linked glucose units in a crystalline structure' (Cowling and Kirk 1976, Wada et al. 2008). Unlike cellulose, hemicellulose is a heterogeneous polymer of xylose, arabinose, mannose, glucose, galactose and sugar acids (Saha 2003). Lignin and cellulose are connected by hemicellulose covalent and non-covalent bonds (Thomson 1993), and this association is much easier to decompose than cellulose by thermochemical treatment (Jacobsen and Wyman 2000).

Pretreatment is one of the most important and expensive steps in conversion of biomass into fuels, and pretreatment is essential to achieving high yields in enzymatic hydrolysis through increasing cellulose accessibility to enzymes. Many pretreatment processes have been researched and could be classified as biological, physical, chemical and physico-chemical pretreatments. Hydrothermal pretreatment with just hot water or steam has the advantage of not requiring addition of chemicals that contribute costs and

create complications in downstream processes. This approach is effective in achieving high yields of sugars from enzymatic hydrolysis of cellulose while recovering much of the hemicellulose sugars into solution. However, in this case, hemicellulose is broken down to produce a xylooligomer enriched solution with much lower amounts of monomeric xylose which can in turn degrade to various aldehydes (Mosier et al. 2005). Organisms have been genetically modified to ferment xylose and other hemicellulose sugars to ethanol with high yields. However, although xylooligomers are important intermediates in hemicellulose hydrolysis into xylose, many organisms cannot readily ferment oligomers to ethanol or other valuable products (Vazquez et al. 2000). Thus, low cost technologies must be developed to hydrolyze hemicellulose oligomers from hydrothermal pretreatment into fermentable monomers with high yields.

Sulfuric acid has been widely recognized as an inexpensive catalyst for hydrolysis of hemicellulose to sugars with high yields, with temperatures of about 160C generally found to produce the highest yields. However, less information is available on application of sulfuric acid to hydrolyze oligomers. Furthermore, it would be highly desirable to employ lower temperatures to keep vessel containment costs low while achieving high yields and also to not sacrifice many of the advantages of hydrothermal compared to dilute acid pretreatment. Thus, in this study, different concentrations of dilute sulfuric acid were applied to hydrolyze xylooligomers from hydrothermal pretreatment into xylose at 101℃, 110℃, 120℃ and 130℃. The combined severity factor, CSF, was then applied to relate the effects of temperature, acid concentration and reaction time on xylose yields.

3.3 Material and Methods

3.3.1Materials

The corn stover used in this study was provided by National renewable Energy Laboratory (NREL) and stored in plastic Ziploc bags placed in a freezer (- 20 ^{o}C) Its composition was determined according to standard NREL analytical procedure (LAP 001, 002 and 003) and was found to contain about 24.0% of xylan, 31.6% of glucan, 3.2 % of arabinan18.7% Klason-lignin, and 1.74% ash.

3.3.2 Experimental system

Figure 3.1 illustrates the experimental procedure used. Corn stover was impregnated with DI water for at least 4 hours at room temperature prior to hydrothermal pretreatment. After hydrothermal pretreatment, the slurry was poured onto a filter to separate the liquid and solid fractions. One portion of the filtrate was analyzed by high performance liquid chromatography (HPLC) to directly measure monomeric xylose; another portion was subjected to a post-hydrolysis to determine the overall concentration of xylooligomers and monomers, and a third portion was employed for processing to monomers with dilute acid. A part of liquid after dilute acid hydrolysis was subjected to post hydrolysis, and the rest was directly analyzed by HPLC to determine the amount of oligomers left and the amount of monomers produced, respectively.

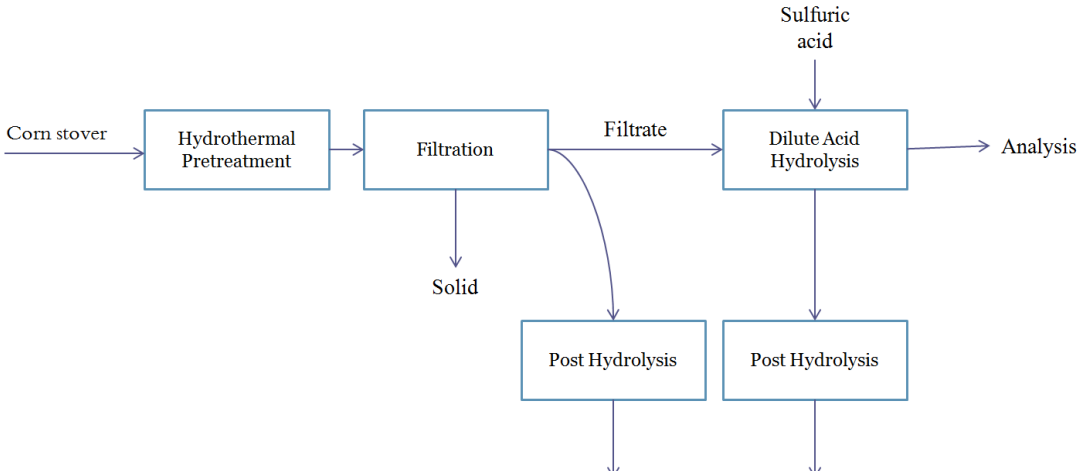


Figure 3.1 Experimental procedure of dilute acid hydrolysis

3.3.3 Measurement of xylose monomers on HPLC

Liquid samples were transferred into microcentrifuge tubes and centrifuged at 2,400 rpm for 10 minutes. After centrifuging, the filtrate was pipetted into 500µl polyethylene HPLC vials (Alltech Associates Inc., Deerfield, IL) and were run along with calibration standards on a Waters Alliance HPLC separation module 2695 (Waters Corporation, Milford, MA) equipped with an Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA) and a refractive index model 2414detector. The flow rate was set at 0.6 ml/min, and the HPLC heater was set at 85℃. The xylose monomer concentration was determined based on calibration sugar standards.

3.3.4 Determination of xylooligomers concentration (post-hydrolysis)

To determine the amount of xylooligomers in the liquid from hydrothermal pretreatment prior to hydrolysis experiments, 72% sulfuric acid was added to each sample to bring the total acid concentration to 4%.These samples were then autoclaved at 121℃ for 1 hour to breakdown oligomers into monomers (NREL, 2004). Sugar recovery standards were run along with the samples in the autoclave to allow for correction for sugar losses by degradation. Total xylose concentrations in the samples were measured by HPLC equipped with an Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, and CA) and a model 2414refractive index detector. From this information, the total amount of xylooligomers in the hydrolyzate was calculated as (Yang and Wyman 2008):

Oligomers (g) = Total xylose (g) in the hydrolyzate corrected for degradation – Monomeric xylose (g) in the hydrolyzate liquid before autoclaving. (3.1)

3.3.5 Preparation of xylooligomer solutions by hydrothermal pretreatment

A 1 liter Parr reactor(Parr Instruments, Moline, IL) was used to prepare large quantities of xylooligomers enriched solution. In this case, a 10% (w/w) solid loading of corn stover based on dry weight (moisture content was determined by NREL LAP-001 procedure) was impregnated with DI water at room temperature for at least 4 hours. Then the presoaked slurry was transferred into 1-L Hastily C Parr reactor fitted with a 3.5 in. diameter helical impeller on a two-piece shaft. The impeller was driven by speed DC motor (A1750HC, Parr Instruments, Moline, IL) set to about 100 RPM. The reactor was sealed and transferred to a 4kW sand bath (model SBL-2D TechNet, Princeton, NJ) set at

360 ℃ for fast heat up. Once the Parr reactor temperature reached200 ℃ (monitoring by K-type thermo-couple), it was raised out of the sand bath to keep the bottom about 1 to 2 cm above the sand surface. The temperature was controlled within ±2 ℃ of target temperature of 190 ℃ by raising or dropping the reactor slightly as needed (Lloyd and Wyman 2005) and held at that value for 15 minutes. Figure 3.2 shows the Parr reactor and 4kW sand bath employed.

Figure 3.2 (A) Parr reactor and (B) Sand bath

At the end of reaction period, the reactor was transferred into a bucket filled with room temperature water. After the contents temperature dropped below 60 ℃, the reactor was opened, and the pretreated slurry was filtered through 125mm diameter Whatmanfilter paper. A portion of the filtrate was treated according to the post-hydrolysis procedure to determine concentrations of xylooligomers and xylose. The rest was stored in 1 L glass bottles at room temperature to avoid substantial precipitation of xylooligomers at lower temperatures.

3.3.6 Dilute acid hydrolysis of hydrolyzate

The hydrolyzate from hydrothermal pretreatment was hydrolyzed with dilute sulfuric acid to convert the xylooligomers to monomeric xylose in a high throughput pretreatment and enzymatic hydrolysis system (HTPH) (Studer et al. 2010). The hydrolyzate was divided into 4 sets, and sulfuric acid was added to each one to bring the concentration to 0.25%, 0.5%, 0.75% and 1% w/w. These samples with four different acid concentration were transferred into a custom made well-plate consisting of 96 Hastelloy round cups (i.d 6.9 mm x 10.7 mm inside length) with reaction volumes of 300µLeach resting on a 3mm thick bottom plate made of Aluminum 7075 (127.8 mm in length, 85.5 mm in width). All 96wellson the plate were sealed during hydrolysis by covering the entire assembly with a silicone gasket (thickness 1.5875 mm, durometer hardness A40) that was sandwiched between top and bottom stainless steel plates that were clamped together with four 1/4 inch-20 threaded bolts (6.35 mm-20) with spring washers (flat load 1,500N) at each corner of the plate, as shown in Figure 3.3.

Figure 3.3 High throughput pretreatment and hydrolysis system used for dilute sulfuric acid hydrolysis of liquid from hydrothermal pretreatment (M. H. Studer and C. E. Wyman et al., Development of a Novel High Throughput Pretreatment System, 2010).

The assembled plate was placed horizontally and lengthwise inside a custom-made steam chamber (shown in Figure 3.4) made of readily available steam rated (to 1 MPa steam pressure) 316 stainless steel 4 inch (0.102 m) nipples and fittings (McMaster, Santa Fe Springs, CA). A ball valve at one end provided loading access, and a cooling water inlet at the opposite end allowed rapid cool down in less than 30 seconds. Steam generated by a high pressure steam boiler (FB-075-L, Fulton Companies, Pulaski, NY) was used to rapidly heat up the plate to 101℃, 110℃, 120℃, or 130℃ for 3 minutes to 500 minutes. After the plate was cooled down, half of each sample was centrifuged, and the liquid portion was then analyzed by HPLC to measure the xylose monomer concentration. Additional sulfuric acid was added into the other half of the samples to bring the acid concentration to 4% for post-hydrolysis directly to determine the oligomers concentration.

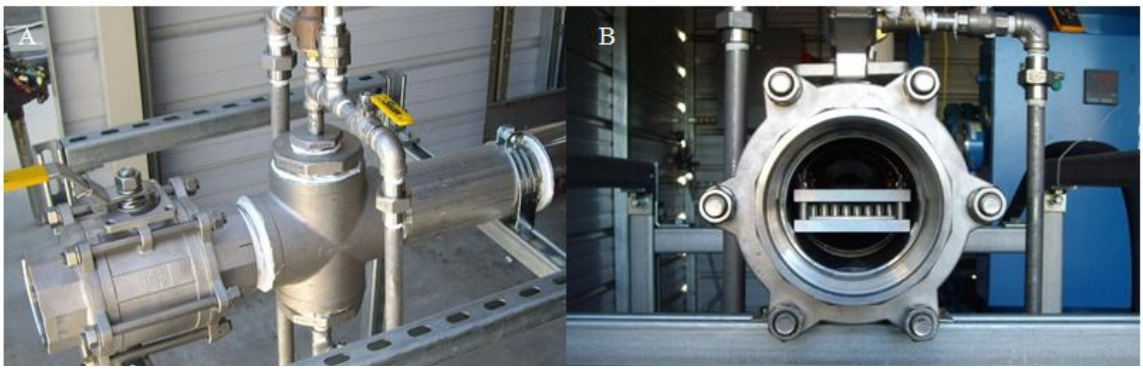


Figure 3.4 (A) Steam chamber and (B) access through ball valve to load the chamber with the HTPH assembly.

The xylose yield was calculation by the following equation:

$$\text{Xylose yield (\%)} = \frac{\text{Xylose conc. (g/L) after dilute acid pretreatment} - \text{Xylose conc.(g/L) after hydrothermal pretreatment}}{\text{Xylooligomers conc.(g/L) after hydrothermal pretreatment}} \times 100\%$$

3.3.7 Combined severity parameter calculation

The combined severity parameter was used to integrate temperature, reaction time, and acid concentration into one single variable (Lloyd and Wyman 2005). This builds from the severity parameter defined as:

$$R_0 = t \cdot \exp[(T_H - T_R)/14.75] \tag{3.3}$$

where t is reaction time in minutes, T_H is the reaction temperature in ℃, and T_R is the reference temperature, usually 100℃. Because acid concentration also affects yields, it taken into account by adjusting the severity parameter for the reaction pH to give the combined severity parameter (Lloyd and Wyman 2005):

$$\log \text{CSF} = \log R_0 - \text{pH} \tag{3.4}$$

The hydronium ions activity of the sulfuric acid solution is influenced by temperature, and it was very difficult to measure pH above 100℃. Thus, the pH was calculated by the following equation (Lloyd and Wyman 2004):

$$pH = -\log\left[\left[-\left(\frac{K_2^o}{\gamma_{SO_4^=}} - M + N\right) + \left(\left(\frac{K_2^o}{\gamma_{SO_4^=}} - M + N\right)^2 + 8\frac{K_2^o}{\gamma_{SO_4^=}}M\right)^{1/2}\right]\frac{\gamma_{H^+}}{2}\right] \tag{3.5}$$

where M is the acid concentration in mol/L and N is the neutralizing capacity of the substrate in mol/L.

Because dilute acid hydrolysis only used the liquid portion of the hydrothermal pretreatment slurry, the effect of neutralization should be very small and was not accounted for in this work. Therefore, equation 3.5 could be simplified to:

$$pH = -\log\left[\left[-\left(\frac{K_2^o}{\gamma_{SO_4^=}} - M\right) + \left(\left(\frac{K_2^o}{\gamma_{SO_4^=}} - M\right)^2 + 8\frac{K_2^o}{\gamma_{SO_4^=}}M\right)^{1/2}\right]\frac{\gamma_{H^+}}{2}\right] \quad (3.6)$$

in which K_2^o is the equilibrium constant for dissociation of the second proton that can be calculated by following equation:

$$\log K_2^o = 56.889 - 19.8858 \ \log T - 2307.9/T - .006473 \ T \quad (3.7)$$

where T is the absolute temperature in K and the expression for γ_i is:

$$-\log\gamma_i = Az^2\left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.2I\right) \quad (3.8)$$

in which

$I = \text{Ionic Strength} = \frac{1}{2}\sum n_i z_i^2$

$\gamma_i = \text{Ionic Activity Coefficient}$

$A = \text{Debye} - \text{Huckel Constant} = 1.825x10^6(\varepsilon T)^{-1.5}$

$\varepsilon = \text{Dielectric Constant of Water} = 132.88 - .208T$

$z = \text{Ionic Charge}$

$n = \text{Ion Molarity}$

$T = \text{Temperature, K}$

3.4 Results and discussion

3.4.1 Verification of post-hydrolysis at different initial acid concentrations

Post-hydrolysis was used to determine the total amount of xylose present as monomer and oligomers in solution by bringing the sulfuric acid concentration of liquid samples up to 4% followed by heating at 121℃ for 1 hour. Application of this method was very straightforward for liquid samples from hydrothermal pretreatment because there was no acid in the solution. However, the addition of acid to samples following dilute acid hydrolysis of the hydrothermal hydrolyzate had to be adjusted to take into accounts the acid concentration in these samples. Thus, the amounts of additional 72% sulfuric acid were calculated based on the samples initial acid concentration.

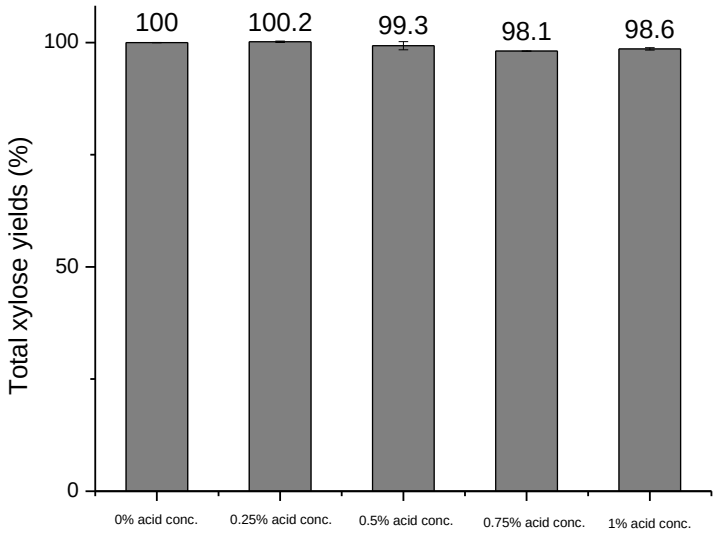


Figure 3.5 Total xylose yields after post-hydrolysis for different initial acid concentration with corresponding supplemental acid

The percent recovery of xylose in samples following post hydrolysis of the liquid from hydrothermal pretreatment are shown in Figure 3.5 for different acid concentrations. The bars from left to right represent different initial acid concentrations. Additional 72% sulfuric acid was added to each sample to bring the final acid concentration to 4%, and the adding amounts were according to their initial acid concentrations. After post hydrolysis, the xylose yields were measured and used the 0% initial acid concentration sample as a base line. The values on the top of each bar represent the percent difference in xylose yields measured between those that had acid in the samples at the beginning and those without acid. The results show that the total xylose yields differences are less than 2%, verifying that adding acid could effectively post-hydrolyze oligomers without

significant losses. In addition, this approach simplifies the procedure of determination of xylooligomers concentration because no need to measure the pH value of samples again.

3.4.2 Xylose and xylooligomers content in hydrolyzate from hydrothermal pretreatment prepared at different conditions

As noted earlier, the initial xylooligomer enriched liquid sample was prepared by hydrothermal pretreatment of corn stover in a 1L Parr reactor heated in a sand bath. In this work, the following conditions were applied to hydrothermal pretreatment to identify conditions that maximized the total xylose yields in the liquid hydrolyzate: reaction temperatures of 160℃, 180℃, and 200℃for reaction times of 2 to 271 minute for a10% w/w solid loading. The results show that the highest total xylose yield was observed at 200^{o}C for 14.3 minutes, corresponding to a yield of 77.3% of the total possible xylose (Zhang unpublished data).

3.4.3 The effect of temperature and acid concentration on xylose yields from hydrolysis of hydrothermal hydrolyzate

Liquid hydrolyzate from hydrothermal pretreatment of corn stover at 200℃ for 14.3 minutes was treated with 0.25%, 0.50%, 0.75%, and 1% w/w sulfuric acid concentrations at temperatures of 101, 110, 120, and 130℃. The plots of xylose yields at each temperature versus time in Figure 3.6 show that yields always increased with acid concentration, as expected. Except 0.25% acid loading, xylose yields reached over 90% before the end of the allowed reaction time. For a 0.25% w/w acid concentration, xylose yields were 56% at 101℃, 78% at 110℃, 75% at 120℃ and 81% at 130℃ and were still

increasing at the end of the reaction time. Consistent with the combined severity parameter, the reaction time to reach the same yield doubled when the acid concentration was cut in half. Moreover, xylose yields increased exponentially because xylooligomers primarily formed monomeric xylose and degradation was very limited at the temperatures run.

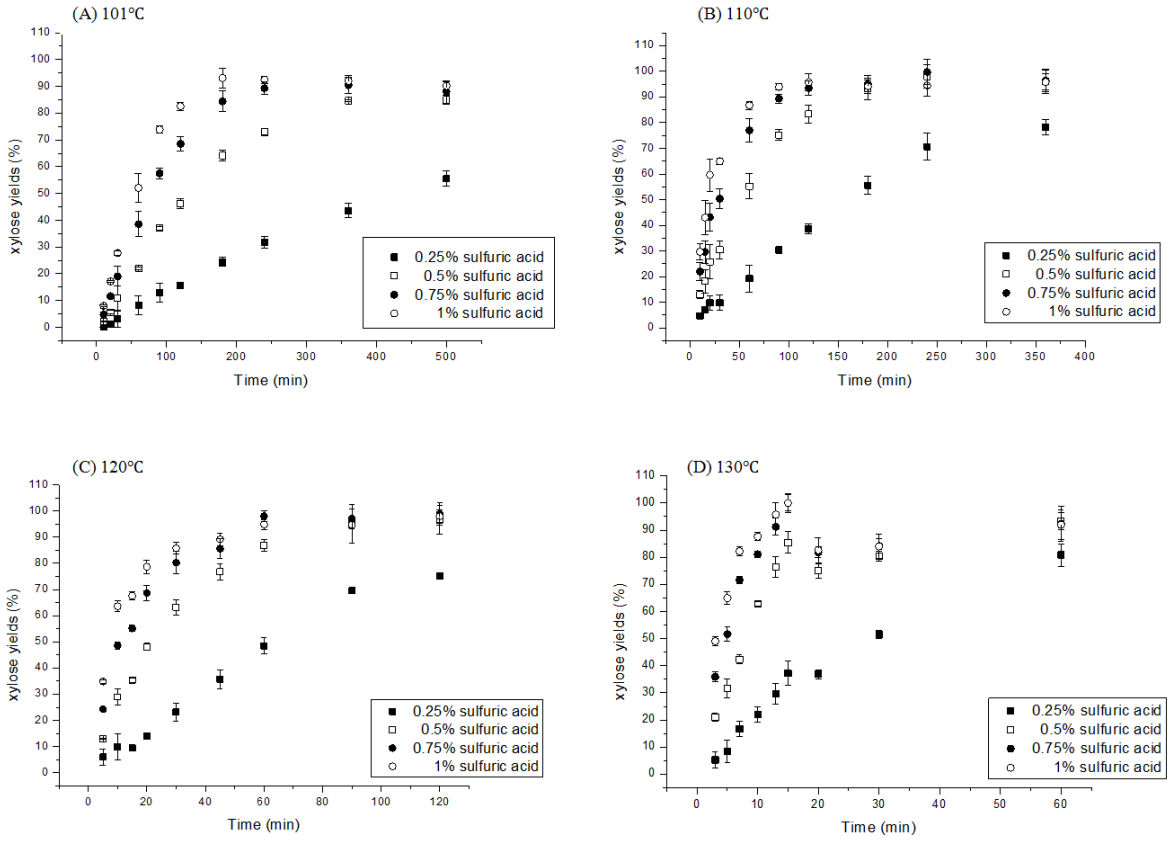


Figure 3.6 Xylose yields vs. time following treatment of liquid from hydrothermal pretreatment with 0.25, 0.50, 0.75, and 1% w/w sulfuric acid concentrations at (A) 101°C, (B) 110°C, (C) 120°C, and (D) 130°C.

Figure3.7 shows how xylose yields change with temperature at the each acid concentration. Consistent with expectations, higher temperatures resulted in much higher

rates. For example, it took around 200 minutes to reach a 90% yield at 101℃ but only about 10 minutes at 130℃ for the same acid concentration. The total xylose yields were greater than 85%, with most above 90%, for all temperatures and acid concentrations, except 0.25% w/w acid. Typically, the reaction time increased 3 times when temperature dropped from 130℃ to 120℃; 2 times when temperature dropped from 120℃ to 110℃; 3 times when temperature dropped from 110℃ to 101℃ for every 10℃ decrease in temperature, consistent with the exponential dependence on temperature in the combined severity parameter expression.

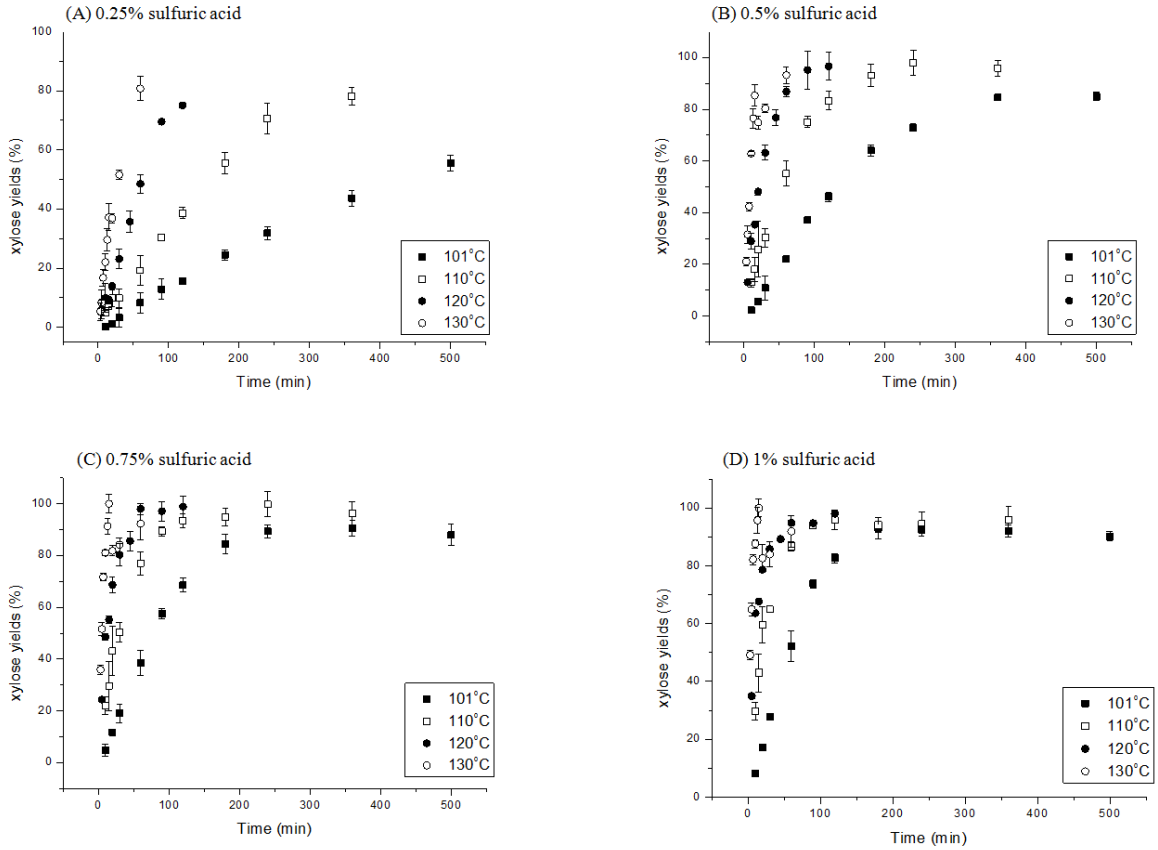


Figure 3.7 Xylose yields vs. time for liquid from hydrothermal pretreatment treated at 101, 110, 120, and 130℃with sulfuric acid concentrations of (w/w acid) (A) 0.25% (B) 0.5% (C) 0.75% and(D) 1% w/w.

3.4.4 Xylose yields vs. combined severity parameter

Figure 3.8 is a plot of xylose yields for all 4 temperatures and 4 acid concentrations run for dilute acid hydrolysis of the hydrothermal hydrolyzates versus the log of the combined severity parameter (equation 3.4)based on the adjusted pH value (equation 3.5). The data shows the xylose yields reached about 90% when the log CSF was around 1.3 at 101℃, around 1.0 at 110℃ and 120℃, around 0.7 at 130℃. Thus, the combined severity parameter did not perfectly account for the effects of temperature. This outcome suggests that the simple exponential function used in the severity parameter calculation does not fit hydrolysis data as well at these lower temperatures as it does at higher temperatures for which it was originally developed. Such an outcome is to be expected since the change in rate will change with temperature for a given activation energy. As a result, a different rule of thumb/correlation is needed to more tightly group how temperature

impacts yields.

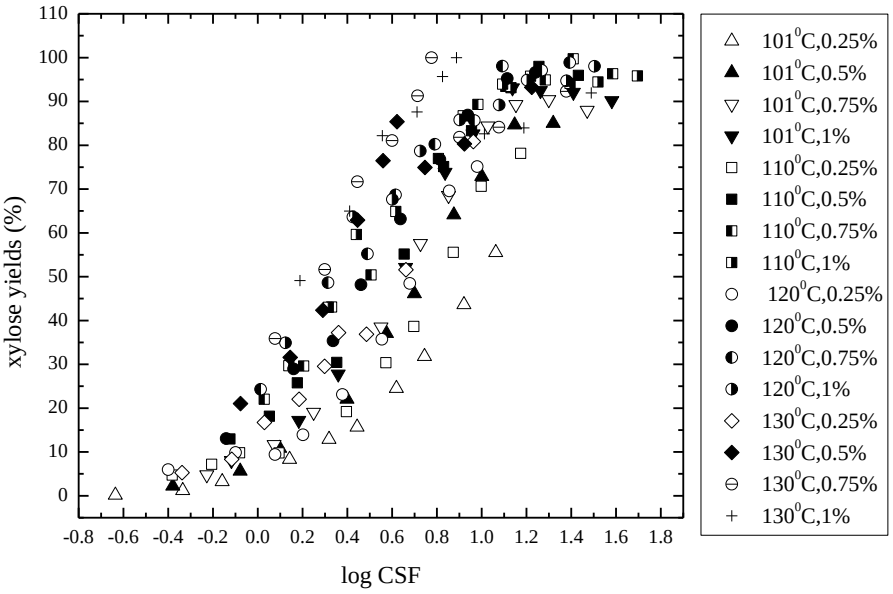


Figure 3.8 Xylose yields vs. combined severity factor for all temperature and acid concentration

In Figure 3.9 are the xylose yields versus combine severity factor at different temperature for all acid concentration. Typically, higher acid concentration has higher xylose yield at the same log CSF and the differences of xylose yields at the same log CSF are more significant when temperature is increased but eventually reach the maximum xylose yields (90%~95% for 101℃, 95%~99% for 110, 120 and 130℃) except 0.25% wt. Figure 3.10 shows xylose yields vs. combined severity factor at each acid concentration for all temperatures. Compare to Figure 3.9, the data are more scatter and bigger difference of xylose yields at the same log CSF. It reveals that xylooligomers hydrolysis is more dependent on temperature than acid concentration because

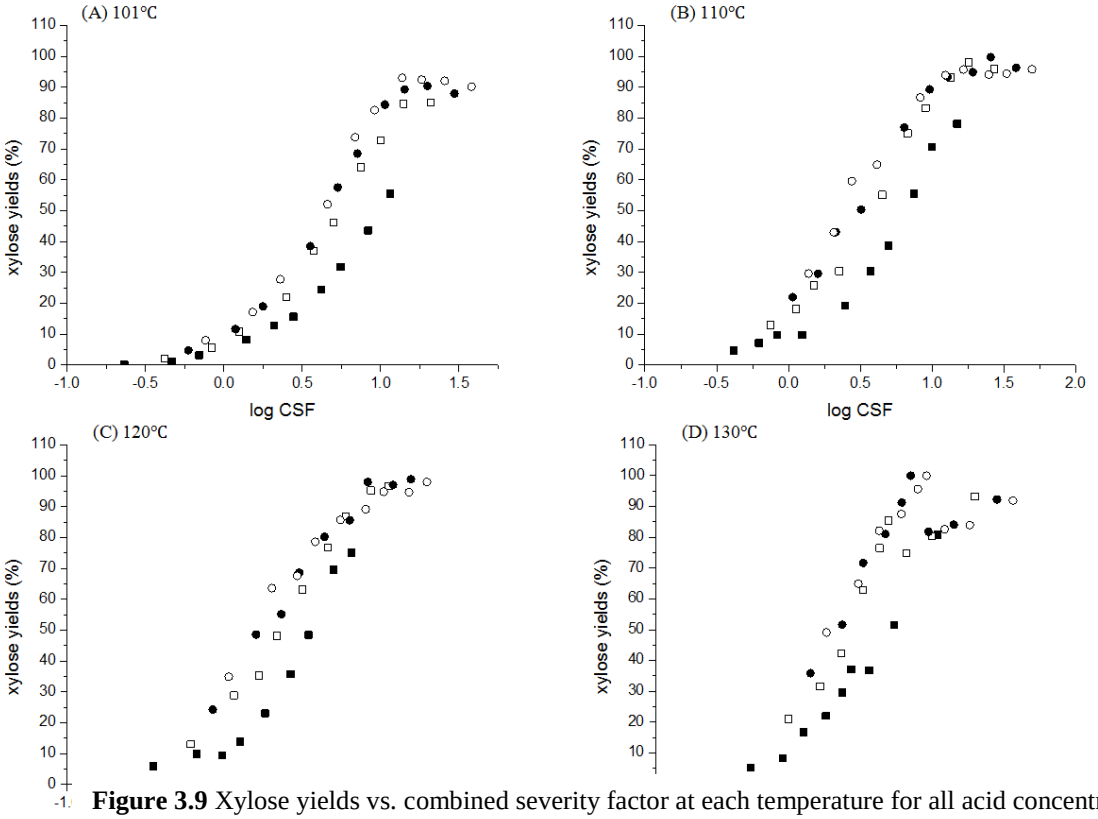


Figure 3.9 Xylose yields vs. combined severity factor at each temperature for all acid concentration (A) 101℃, (B) 110℃, (C) 120℃, and (D) 130℃. (■) 0.25% wt. (□) 0.5% wt. (●) 0.75% wt. (○) 1% wt. acid concentration

temperature provides the energy to overcome the activity energy and complete the reaction. Acid acts as a catalyst to lower the activity energy and reduce the reaction time for the reaction.

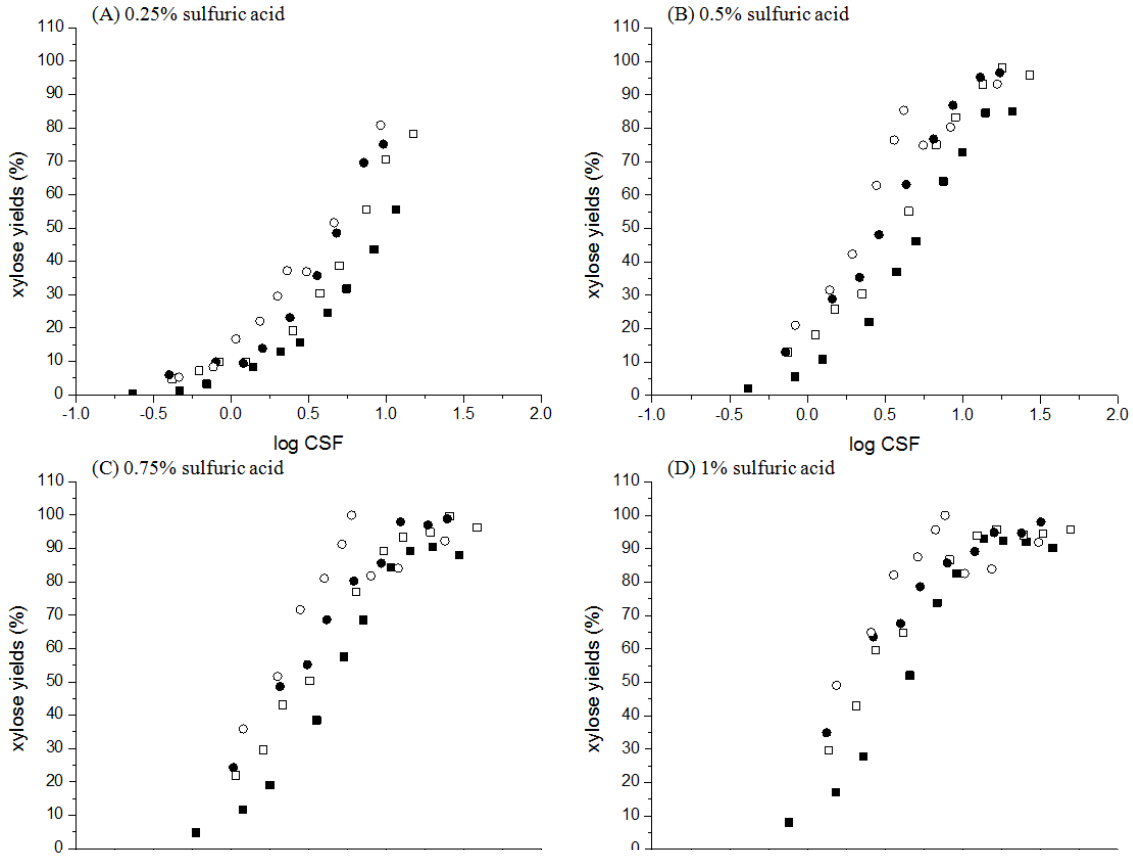


Figure 3.10 Xylose yields vs. combined severity factor at each acid concentration for all temperatures (A) 0.25% wt., (B) 0.5% wt., (C) 0.75% wt., and (D) 1%. (■) 101℃ (□) 110℃ (●) 120℃ (○) 130℃

Overall, these results show that the combined severity parameter can serve as a valuable tool with which to make rapid calculations of tradeoffs in the impacts of time, temperature, and acid concentration on xylose yields for hydrolysis of oligomers in hydrolyzate produced by hydrothermal pretreatment. For instance, equations 3.3 and 3.4 can be combined and rearranged to estimate the time to get a particular yield for a given acid concentration and temperature:

$$t = \frac{10^{(CSF+pH)}}{Exp[(T-100)/14.75]} \quad (3.9)$$

where t is reaction time in minute and T is temperature in ℃.

3.5 Conclusions

These experiments showed that very high yields of monomeric xylose could be realized from xylooligomers in the hydrolyzate from hydrothermal pretreatment (90+%)without much xylose degradation over a temperature range of 101 to130℃ for 0.50, 0.75, and 1.00% w/w sulfuric acid concentrations. However, xylooligomers broke down very slowly to xylose for an acid concentration of 0.25% w/w. The results showed that the log of the combined severity parameter (CSF) corresponding to the maximum xylose yield changed with temperature, with values for 90% yields are 1.3 at 130℃, 1.0 at 120℃, and 0.7 at 130℃. Thus, a different correlation is needed at the lower temperatures applied here to better group the data and improve the accuracy in predicting the reaction time to maximum yields at given temperatures and acid concentrations.

3.5 References

Cowling E, Kirk T. 1976. Properties of cellulose and lignocellulosic materials as substrates for enzymatic conversion processes. Biotechnology and Bioengineering: 95-123.

Jacobsen S, Wyman C.E. 2000. Cellulose and hemicellulose hydrolysis models for application to current and novel pretreatment processes. Applied Biochemistry and Biotechnology 84-6: 81-96.

Lloyd T.A, Wyman C.E. 2004. Predicted effects of mineral neutralization and bisulfate formation on hydrogen ion concentration for dilute sulfuric acid pretreatment. Applied Biochemistry and Biotechnology 113: 1013-1022.

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 96: 1967-1977.

Mosier N, Hendrickson R, Ho N, Sedlak M, Ladisch M. 2005. Optimization of pH controlled liquid hot water pretreatment of corn stover. Bioresource Technology 96: 1986-1993.

NREL, Standerd biomass analytical procedures - NREL Laboratory Analytical Procedures.
< http://www.nrel.gov/biomass/analytical_procedures.html>, Golden, CO.

Saha B. 2003. Hemicellulose bioconversion. journal of industrial microbiology & biotechnology 30: 279-291.

Studer M, DeMartini J, Brethauer S, McKenzie H, Wyman C.E. 2010. Engineering of a high-throughput screening system to identify cellulosic biomass, pretreatments, and enzyme formulations that enhance sugar release. Biotechnology and Bioengineering 105: 231-238.

Thomson J. 1993. Molecular-biology of xylan degradation. Fems Microbiology Reviews 10: 65-82.

Vazquez M, Alonso J, Dominguez H, Parajo J. 2000. Xylooligosaccharides: manufacture and applications. Trends in Food Science & Technology 11: 387-393.

Wada M, Nishiyama Y, Chanzy H, Forsyth T, Langan P. 2008. The structure of celluloses. Powder Diffraction 23: 92-95.

Wyman C.E. 2003. Potential synergies and challenges in refining cellulosic biomass to fuels, chemicals, and power. Biotechnology Progress 19: 254-262.

Yang B, Wyman C.E. 2008. Characterization of the degree of polyperization of xylooligomers produced by flowthrough hydrolysis of pure xylan and corn stover with water. Bioresource Technology: 5756-5762.

Chapter 4

Kinetic Modeling of Dilute Acid Hydrolysis of Xylooligomers

4.1 Abstract

Kinetic models were developed to describe concentration profiles observed when a xylooligomer solution produced from hydrothermal pretreatment of corn stover was treated at 110℃ to 130℃with sulfuric acid concentrations of 0.25% to 1% w/w in a high throughput pretreatment and enzymatic hydrolysis system (HTPH).The kinetic model employed treated oligomers as a single compound and included the effects of both temperature and acid concentration on xylose formation from oligomers. Rate constants (k) based on first order homogeneous kinetic models of 0.140, 0.369, 1.032, and 2.741 min^{-1} at 101℃, 110℃, 120℃ and 130℃, respectively, provided a good fit to the data. These values are consistent with activation energy of 15.5kJ/min.

4.2 Introduction

Hemicelluloses typically the second most abundant component of those in lignocellulosic biomass and is associated with cellulose and lignin in the plant cell walls due to the branching structure (Puls 1997). Dilute acid or hydrothermal (just hot water or steam) pretreatments can easily hydrolyze hemicellulose into liquid phase catalyzed by hydronium. During either approach, hemicellulose is hydrolyzed to xylooligomers and

monomeric xylose which can in turn degrade into other products such as furfural. It is also important to maximize monomeric xylose yields because many organisms can only ferment xylose and not its oligomers into ethanol and other valuable products (Vazquez et al. 2000). In addition, it is important to minimize formation of furfural and other degradation products to realize high yield potential and avoid formation of compounds that inhibit fermentations. Kinetic models can be valuable assets in defining conditions that will meet these two objectives, and many studies have developed different kinetic models and pathways to describe hemicellulose hydrolysis in the past few decades. The simplest approach was to treat hemicellulose as directly hydrolyzing to monomeric xylose that then degrades to furfural (Saeman 1945).However, Kobayashi and Sakai in 1956 found that the drop in hydrolysis rate after about 70% conversion could be better described if they hypothesized that xylose was formed from fast and slow reacting hemicelluloses (Kobayashi and Sakai 1956). Later, in 2001, Garrote et al. introduced a new model to show that xylooligomers are important intermediates between hemicelluloses and xylose (Garrote G. et al. 2001a). Li, Lloyd and Kumar's approach is based on Garrote's model and further research on the decomposition kinetics of short chain xylooligomers which can directly degrade into degradation products without hydrolyze to xylose first (Li 2002, Kumar and Wyman 2008).

It can be advantageous to employ hydrothermal pretreatment to avoid the complications that use of dilute acids introduce to downstream operations, but the pretreatment hydrolyzate will be rich in hemicellulose oligomers. Because many fermentations cannot convert oligomers to ethanol or other products, it will be necessary

to employ enzymes or acids to hydrolyze the oligomers to sugars, and application of low temperatures that result in low pressures for oligomer hydrolysis could be beneficial to keep materials of construction cost reasonable. However, prior kinetic studies of hemicellulose and xyloolgomers breakdown in dilute acid and hydrothermal systems focused on overall hydrolysis of cellulosic biomass at higher temperatures typical for pretreatment, and it is important to understand whether high monomer yields can be obtained from oligomers at more modest conditions. Therefore, in this study, kinetic models were developed to describe dilute acid hydrolysis of xylooligomers in the hydrolyzate from hydrothermal pretreatment of corn stove rover a range of relatively low temperatures (101℃ to 130℃), and the effects of acid and oligomer concentrations on performance were determined.

4.3 Materials and Methods

4.3.1 Materials

Corn stover was obtained from National Renewable Energy Laboratory (NREL). Its composition as measured according to NREL analytical procedures was 24.0% xylan, 31.6% glucan, 3.2%arabinan, 18.7% Klason-lignin, and 1.74% ash.

4.3.2 Determination of xylose concentration in liquid streams

Samples of liquids from hydrothermal pretreatment dilute acid hydrolysis of that hydrolyzate, and post-hydrolysis to measure total xylose content were centrifuged at

2,400 rpm for 10 minute to separate liquids and solids. The liquid fraction was then analyzed by high performance liquid chromatography (HPLC, Model 2695, Waters Corporation, Milford, MA) using a model 2414 refractive index detector and an Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA). The HPLC flow rate was set at 0.6ml /min, with the heater at 85℃. Calibration sugar standards were used to calculate xylose concentrations (Sluiter et al. 2008).

4.3.3 Determination of xylooligomer concentrations

72% sulfuric acid was added to liquid samples from hydrothermal pretreatment to bring the solution to 4% acid concentration and then autoclaved at 121℃ for 60 minutes. Sugar recovery standards were also autoclaved with the samples at the same conditions to determine sugar losses during this post-hydrolysis procedure (Sluiter et al. 2006). Because samples from dilute acid hydrolysis of the hydrothermally prepared liquid contained sulfuric acid initially, the original acid concentration was accounted for in determination of the amounts of 72% sulfuric acid to add to bring the total to 4%. After post-hydrolysis, the samples were centrifuged and pipetted into 500µl polyethylene HPLC vials (Alltech Associates Inc., Deerfield, IL),and the contents were then analyzed using a Waters HPLC with calibration sugar standards and sugar recovery standards to determine xylose concentrations. The Waters HPLC was equipped with a refractive index detector (RI) and Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, and CA). The instrument conditions were 10µL sample volume, 0.6mL/min flow rate, 85℃ column temperature, and 15 minute run time for each sample. The overall xylooligomer

concentration in solution was calculated by the following equation (Yang and Wyman 2008):

Oligomers (g) = Total xylose (g) in the hydrolyzate after post-hydrolysis corrected for degradation – Monomeric xylose (g) in the hydorlyzate liquid before autoclaving. (4.1)

4.3.4 Preparation of enriched xylooligomer solution

The xylooligomer enriched solution was prepared by hydrothermal pretreatment of corn stover at 200℃ for 14.3 minutes in a 1-L stainless steel Parr reactor (Moline, IL) to maximize the concentration of oligomers in the hydrolyzate and minimize degradation to furfural (Zhang unpublished data). Prior to pretreatment, the corn stover was air-dried at room temperature for 24 hours, and the moisture content was measured based on NREL LAP-001 procedure. Then, the corn stover was soaked in DI water at a 10% w/w solids loading for at least 4 hour at room temperature. After impregnation, the slurry was transferred into a Parr reactor that was sealed and heated in a 4kW sand bath (model SBL-2D TechNet, Princeton, NJ) with the temperature set at 320℃ for fast heat up. The Parr reactor was raised about 1 cm to 2 cm above the sand surface when the target temperature was reached, with the height varied by raising or lowering the reactor to control the temperature within ±2 ℃ of the target value (Lloyd and Wyman 2005). At the end of the target reaction time, the reactor was lowered into a bucket of room temperature water to cool it to below 60℃. A turbine impeller driven by a DC motor

(A1750HC, Parr Instruments, Moline, IL) stirred the contents at about 100 rpm during heating and cooling to prevent settling of the solids and assure more uniform heat distribution. The cool slurry was poured into a filter to separate the liquid and solid. A small portion of the liquid was post hydrolyzed to determine the total concentration of xylose oligomers and monomers. The rest of the hydrolyzate was stored at room temperature until needed.

4.3.5 Dilute acid hydrolysis of xylooligomers

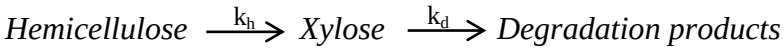
The xylooligomer enriched solution was hydrolyzed with dilute acid at temperatures of 101, 110, 120, and 130℃ with 0.25%, 0.5%, 0.75%, and 1.00% sulfuric acid for times from 3 to 500 minutes. This same solution was also diluted with an equal volume of DI water to test the effect of xylooligomer concentration on yields from dilute acid hydrolysis. To speed consideration of this wide range of conditions, all hydrolysis runs were made in a multi-well system that our group had developed previously for high throughput pretreatment and enzymatic hydrolysis (HTPH); however, no enzymatic hydrolysis was performed (Studer et al. 2010). To use this system, samples were transferred into the custom-made well-plate consisting of 96 Hastelloy cylindrical cups (i.d.6.9 mm x 10.7 mm inside length), each with reaction volumes of 300μL. The 96-wellswere placed on a dimpled plate to hold them in place, the resulting assembly was covered with a silicone gasket, and the entire system was sandwiched between top and bottom stainless steel plates that were held in place by four 1/4 inch-20 threaded bolts with spring washers to prevent leaking during hydrolysis. The assembled plate was then

inserted through a large ball valve into a custom-made steam chamber (McMaster, Santa Fe Springs, CA) with steam from a steam boiler (FB-075-L, Fulton Companies, Pulaski, NY) introduced through a valve at one end for rapid heating and cooling water introduced at the other end for rapid cool down at the conclusion of a run. Steam heating provided more stable and rapid heating and cooling profiles that possible with the conventional sand bath. After hydrolysis was completed, the samples were subjected to post-hydrolysis to determine xylose and xylooligomer concentrations.

4.4 Results and Discussion

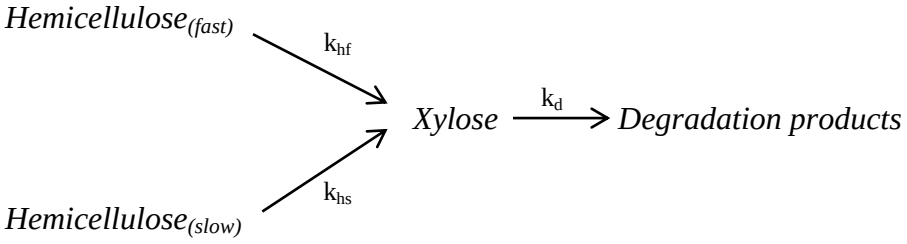
4.4.1 Reaction pathway

A number of reaction pathways have been applied to describe hemicellulose hydrolysis, with most based on assumed homogeneous first order rate laws to describe the kinetics. For those that include oligomers, the first step in hydrolysis is based on breaking down hemicellulose into different chain length solubilized oligomers that then break down to form xylose which in turn degrades to furfural and other degradation products. However, some studies assume that the reaction of oligomers to xylose is so much faster than the rate of oligomers formation that oligomers can be ignored; such an approach is particularly appropriate for dilute acid hydrolysis in which oligomer concentrations are small compared to monomers. This approach gives the simplistic hemicellulose hydrolysis kinetic model that was developed as an extension of prior models applied to cellulose hydrolysis for many years (Saeman 1945):



where k_h and k_d are rate constants for the hydrolysis and degradation reactions, respectively.

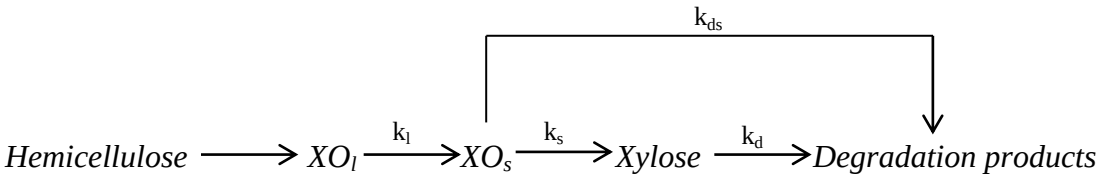
In 1956, Kobayashi and Sakai introduced a kinetics model based on dividing hemicellulose into fast-hydrolysis-hemicellulose and slow-hydrolysis-hemicellulose, and several studies base their kinetic expressions on this model (Jacobsen and Wyman 2000, Kobayashi and Sakai 1956, Liu et al. 2012).



In which k_{hf} is the kinetic rate constant for the hemicellulose fraction that hydrolyzes rapidly, k_{hs} is the rate constant for the hemicellulose fraction that hydrolyzes more slowly, and k_d is the rate constant for breakdown of xylose to furfural and other degradation products.

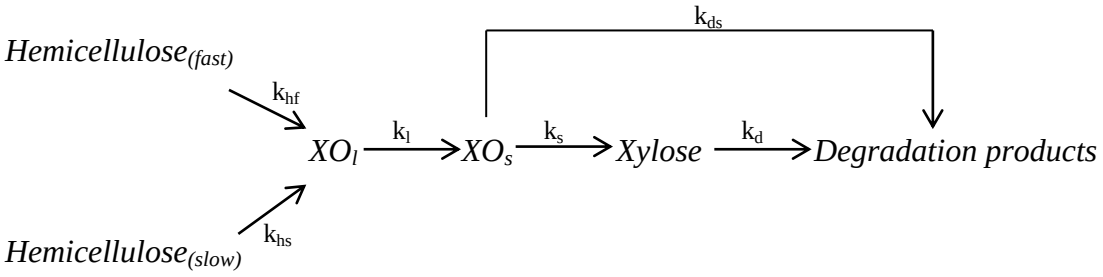
Although ignored in these simple models, some xylooligomers are present in the liquid phase during hemicellulose hydrolysis by dilute acids (Jacobsen and Wyman 2000) and are in particularly high concentrations at shorter reaction times and lower acid concentrations for dilute acid pretreatment. Furthermore, because oligomers are far more prevalent than monomers in the hydrolyzate from hydrothermal pretreatment, they must be accounted to reasonably describe hydrothermal pretreatment reactions. In 2001,

Garrote et al. introduced a kinetic model that included two groups of dissolved in hydrothermal pretreatments(Garrote G et al. 2001b):



where XO_l and XO_s are long chain and short chain soluble oligomers and k_l, k_s, k_{ds}, and k_d are rate constants for decomposition of long chain oligomers to short chain oligomers, conversion of short chain oligomers to xylose, degradation of short chained oligomers to non-sugar products, and degradation of xylose to furfural and other non-sugar products.

In 2001, Garrote et al. modified their model and incorporating the biphasic hydrolysis model (Garrote G. et al. 2001a).



The focus of this study was on hydrolysis of xylooligomers in the solution from hydrothermal pretreatment into xylose as catalyzed by dilute sulfuric acid at 101℃ to 130℃. To model this system, all oligomers were grouped together as a single species

XOs that was hydrolyzed to xylose monomer that could further breakdown to form furfural and other degradation products according to the following pathway:

$$XOs \xrightarrow{k'} Xylose \xrightarrow{k_a} Degradation\ products$$

The rate equation corresponding to this mechanism was expressed as:

$$\frac{d[XOs]}{dt} = -k'[XOs]^n \tag{4.2}$$

where [XOs] is the total concentration of all xyloologomers dissolved in the hydrolyzate, k' is the rate constant, and n is order of the reaction. The rate constant was assumed to have an Arrhenius dependence on temperature and include the acid concentration:

$$k' = AC_a^{\ m} \exp[-E_a / RT] \tag{4.3}$$

where A is the frequency factor, C_a is the acid concentration, T is the absolute temperature, m is an arbitrary constant that varies with the type of biomass being pretreated, assumed to be 1 in this study, and E_a is the activity energy which is independent of temperature and acid concentration. However, the acid concentration in this study was not constant and is also affected by temperature, especially for the second dissociation of sulfuric acid (Dickinson et al. 1990, Marshall and Jones 1966). Although a pH meter can measure hydrogen ion activity, it is very difficult to measure pH during the reaction due to the high temperature and pressure. However, the hydrogen ion activity can be estimated by the following equation (Lloyd and Wyman 2004):

$$a_{H^+} = \left[-\left(\frac{K_2^o}{\gamma_{SO_4^=}} - M + N \right) + \left(\left(\frac{K_2^o}{\gamma_{SO_4^=}} - M + N \right)^2 + 8\frac{K_2^o}{\gamma_{SO_4^=}} M \right)^{1/2} \right] \frac{\gamma_{H^+}}{2} \tag{4.4}$$

where a_{H^+} is the hydrogen ion activity and M is the acid concentration in mol/L. N is the neutralizing capacity of the substrate, mol/L, that was used to compensate for the neutralizing effect by minerals in biomass. Because the xylooligomer enriched solution was separated from biomass after pretreatment in this study, the amount of minerals available for neutralization is limited and was therefore not included in the kinetics for this study. K_2^o is the thermodynamic equilibrium constant for dissociation of the second proton that was calculated by the following equation (Marshall and Jones 1966):

$$\log K_2^o = 56.889 - 19.8858 \ \ \log T - 2307.9/T - 0.006473 \ \ T, \tag{4.5}$$

in which T is the absolute temperature in Kelvin. The activity coefficient γ_i was calculated by the modified Debye-Hückel limiting law (Davies 1930):

$$-\log \gamma_i = Az^2\left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.2I\right), \tag{4.6}$$

in which

I = Ionic Strength $= \frac{1}{2}\sum n_i z_i^2$

γ_i = Ionic Activity Coefficient

A = Debye – Huckel Constant $= 1.825x10^6(\varepsilon T)^{-1.5}$

ε = Dielectric Constant of Water $= 132.88 - .208T$

z = Ionic Charge

n = Ion Molarity

T = Temperature, K

Therefore, equation 4.2 can be rewritten as:

$$\frac{k'}{a_{H^+}} = A\exp[-E_a/RT] = k \tag{4.7}$$

in which k is rate constant independent of acid concentration.

4.4.2Determination of reaction order

Chapter 3 results show that the degradation rate of xylose for dilute sulfuric acid hydrolysis of the xylooligomer solution produced by hydrothermal pretreatment of corn stover at 200℃ for 14.3 minutes was very slow over the temperature range of 101℃ to 130℃. Therefore, the xylose degradation pathway was not included in the kinetic model. To test whether the reaction order depended on the concentration of xylooligomers, a dilute xylooligomer solution (50% DI water and 50% xylooligomer solution from hydrothermal pretreatment) was also subjected to acid hydrolysis.

Figures 4.1 and 4.2 show xylose yields versus time for hydrolysis of the original and diluted solutions over a range of sulfuric acid concentrations. For both 110℃ and 120℃, the concentration of xylose formed from xylooligomer hydrolysis increased linearly until the yields reached around 90%. The slope of the yield versus time lines in the beginning linear increasing section at 110℃ and 120℃ given in Table 4.1 for different acid concentrations reveals that the hydrolysis rate of oligomers-to-xylose increased with higher acid but displayed on a small difference between results for dilute and original xylooligomer concentrations. For example, the slopes at 101℃for 1% w/w acid concentration were 0.82 and 0.88, about a 7% difference.

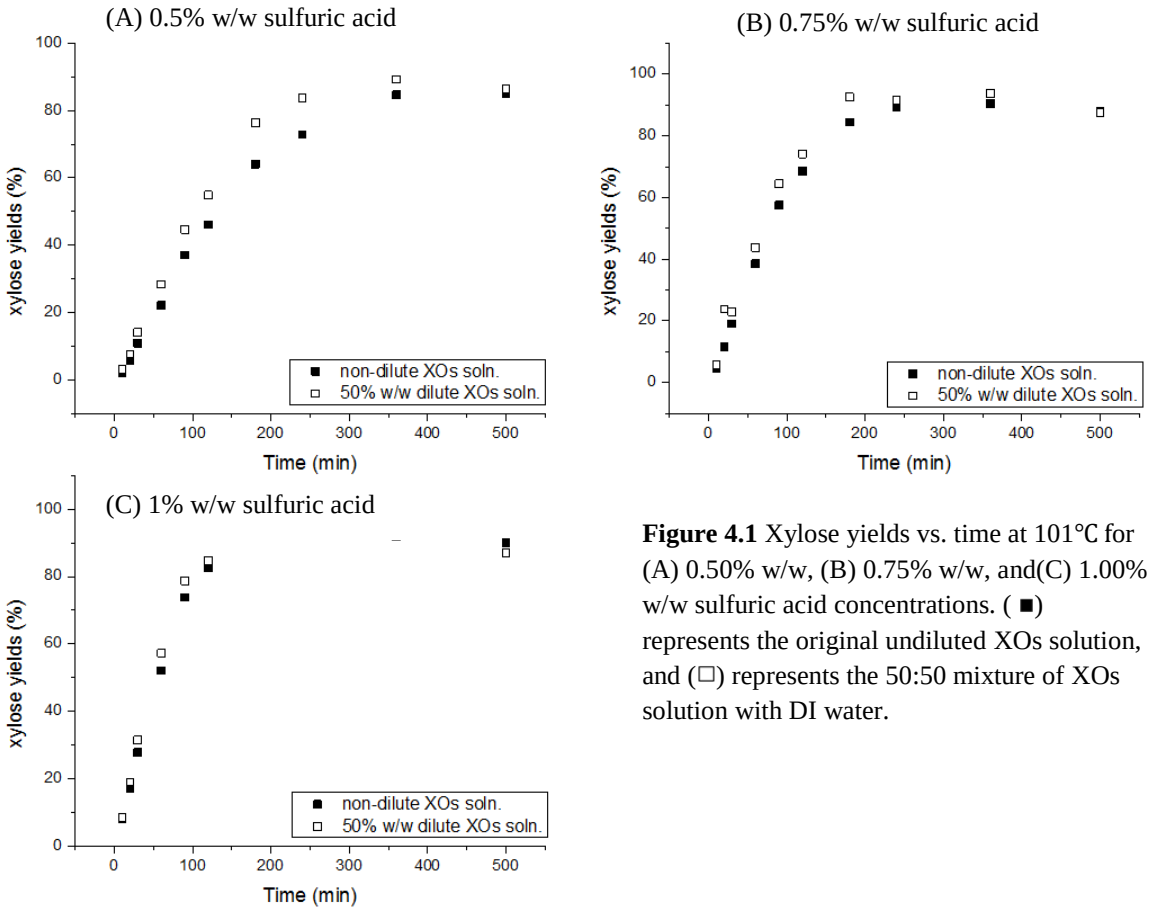


Figure 4.1 Xylose yields vs. time at 101℃ for (A) 0.50% w/w, (B) 0.75% w/w, and(C) 1.00% w/w sulfuric acid concentrations. (■) represents the original undiluted XOs solution, and (□) represents the 50:50 mixture of XOs solution with DI water.

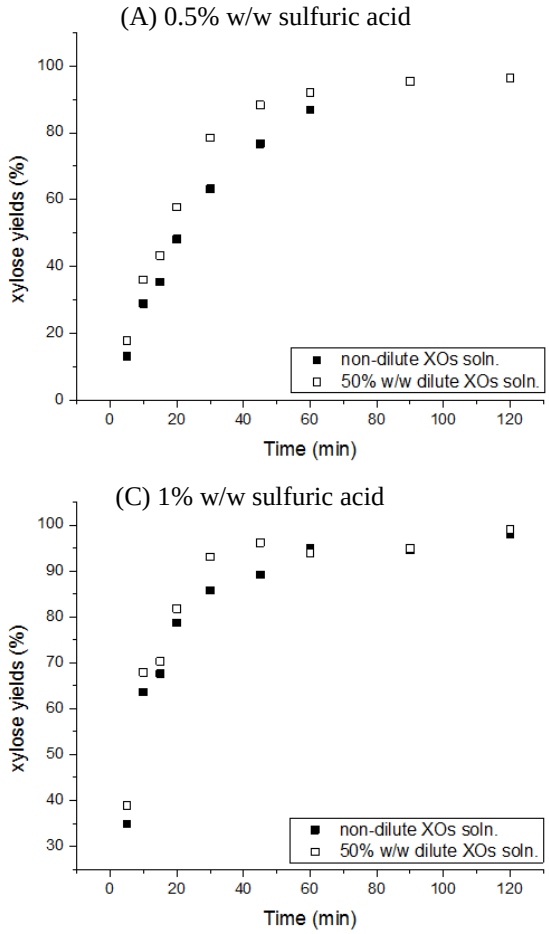


Figure 4.2 Xylose yields vs. time at 120℃ for (A) 0.50% w/w, (B) 0.75% w/w, and(C) 1.00% w/w sulfuric acid concentrations. (■) represents the original undiluted XOs solution, and (□) represents the 50:50 mixture of XOs solution with DI water.

Table 4.1 Slopes of xylose yields versus time lines at 101℃ and 120℃ for 0.5%, 0.75% and 1% wt. sulfuric acid concentration

	slopes			
	101°C		120°C	
acid concentration	non-dilute XOs soln.	50% w/w dilute XOs soln.	non-dilute XOs soln.	50% w/w dilute XOs soln.
0.5% w/w	0.43	0.52	5.97	6.30
0.75% w/w	0.66	0.58	6.60	6.71
1% w/w	0.82	0.88	8.27	8.72

These data support representing the hydrolysis of xylooligomers as first order in terms of oligomer concentration to give a rate equation (eq. 4.1) of the form:

$$\frac{d[XOs]}{dt} = -k'[XOs] \tag{4.8}$$

4.4.3 Estimation of rate constant k and activity energy E_a

In equation 4.2, k' was assumed to be a function of acid concentration and temperature from which the value of k can be estimated. To determine k', the natural logarithm of the ratio of the instantaneous xylooligomer concentration to the original concentration is plotted versus time in Figure 4.3. These results further support the idea that oligomer hydrolysis is a first order reaction whose rate constant k' is strongly depends on acid concentration and temperature. From such plots, the k' values at different temperature and acid concentration reported in Table 4.2 were determined. From this, we can see that k' values continually increased with acid concentration, as illustrated by results at 101℃ in the order 0.0016, 0.0059, 0.0106, and 0.0156 for0.25%, 0.5%, 0.75% and 1% w/w acid concentrations, respectively. Moreover, k' values also increased with temperature, with the values at 1.0% acid concentrations in Table 4.2 of 0.0156, 0.0321, 0.0693, and 0.2623 at 101℃, 110℃, 120℃ and 130℃, respectively, illustrating this trend.

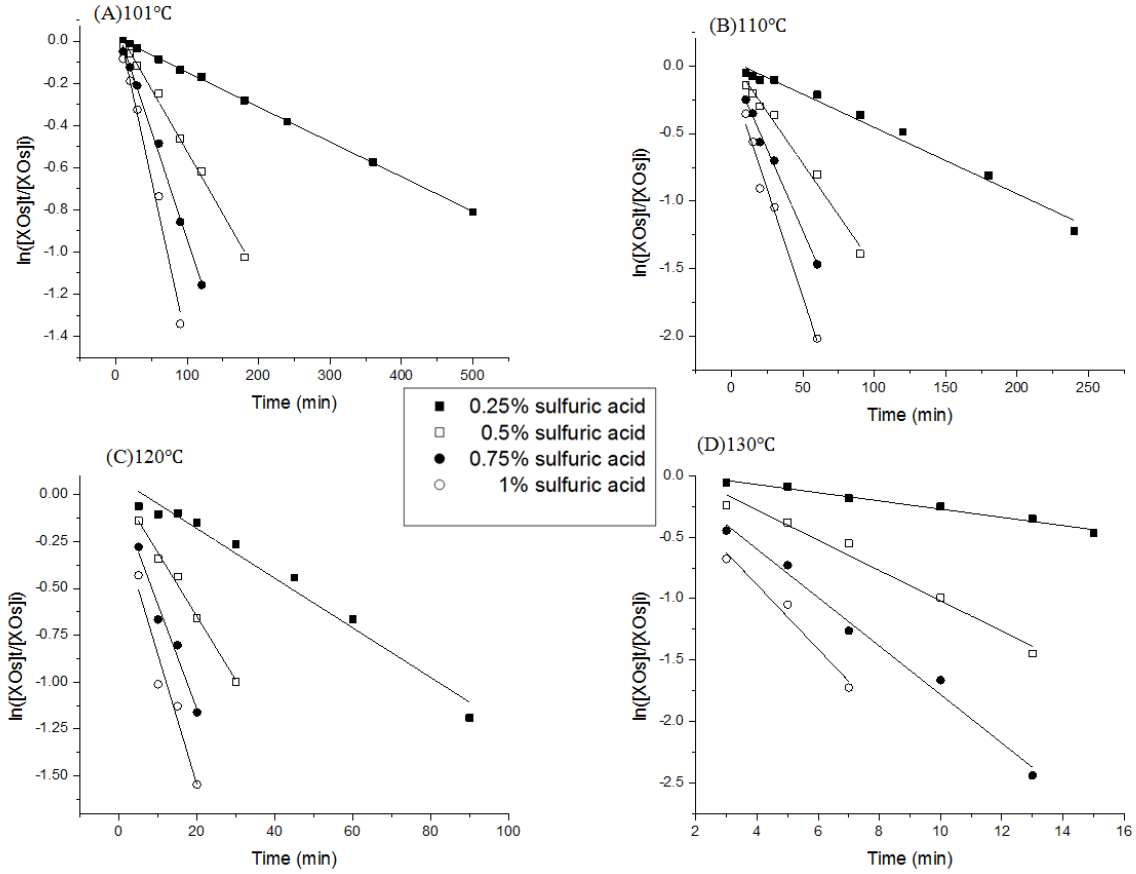


Figure 4.3 $\ln([XOs]_t/[XOs]_i)$ vs. time (A). 101℃ (B). 110℃ (C). 120℃ (D). 130℃, $[XOs]_t$: xylooligomer concentration at time t; $[XOs]_i$: initial xylooligomers concentration.

Table 4.2 Rate constant k' values at 101°C, 110°C, 120°C and 130°C for 0.25%, 0.5%, 0.75% and 1% sulfuric acid as determined from ln plots of oligomer concentrations vs. time.

k' value min^{-1}				
	Temperature [°C]			
Acid conc.	101°C	110°C	120°C	130°C
0.25%	0.0016	0.0049	0.0132	0.0334
0.50%	0.0059	0.0153	0.0340	0.1233
0.75%	0.0102	0.0242	0.0557	0.1975
1.00%	0.0156	0.0321	0.0693	0.2623

Once k' values were determined, k rate constants based on an Arrhenius dependence on temperature were calculated by dividing k' by the hydrogen ion activity calculated from equation 4.6 to produce the resulting k values shown in Table 4.3.

Table 4.3 Rate constants k calculated at 101, 110, 120, and 130°C for 0.25, 0.50, 0.75, and 1.00% w/w sulfuric acid.

	k value			
	Temperature [°C]			
Acid conc.	101°C	110°C	120°C	130°C
0.25%	0.0740	0.2327	0.6438	1.6691
0.50%	0.1513	0.4013	0.9123	3.3803
0.75%	0.1843	0.4463	1.0489	3.7951
1.00%	0.2190	0.4590	1.0107	3.9002

These resulting rate constants clearly showed temperature dependence for 0.50, 0.75, and 1.00% w/w sulfuric acid concentrations, and the k values were calculated as 0.140, 0.369, 1.032, and 2.741 min^{-1} at 101, 110, 120, and 130°C, respectively. However, the rate constant k for 0.25% sulfuric acid was inconsistent with the others. This deviation likely results from the calculation of the hydrogen ion activity at low acid concentrations. In particular, the hydrogen ion activity as estimated by Lloyd's approach (Lloyd and Wyman 2004) is highly dependent on the initial acid concentration (M in equation 4.3). Furthermore, the k value for a 0.25% acid concentration was much smaller than others due to an overestimation of the a_{H^+} value for 0.25% acid concentration. However, despite this inconsistency with rate constants calculated for other acid concentrations, the rate constants calculated for the 0.25% acid concentration still varied linearly with the reciprocal of the absolute temperature, consistent with an Arrhenius

dependence as shown in Figure 4.4.These results are consistent with an activity energy calculated from equation 4.6 as 15.5[kJ/mol].

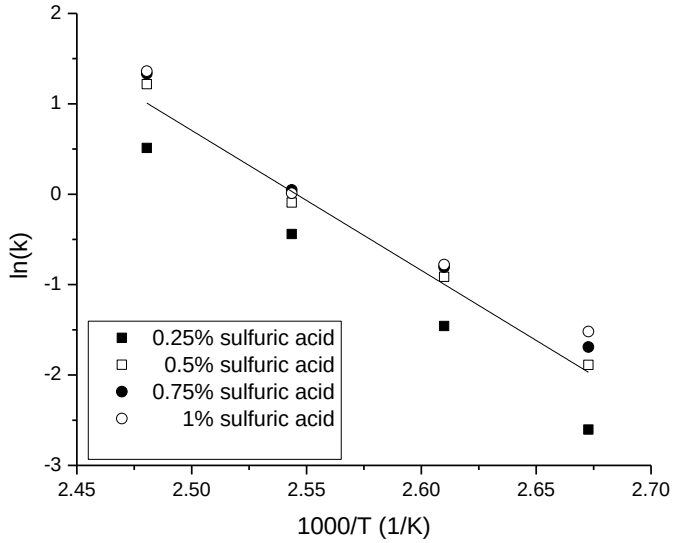


Figure 4.4. Arrhenius plot of rate constants for different acid concentrations.

Xylose degradation has been researched for many years, and some recent studies showed that the activity energies of around 120 kJ/mol to 160 kJ/mole for xylose degradation to furfural and degradation to other products are much higher than for xylooligomers hydrolysis (Borrega et al. 2011, Kim et al. 2011, Morinelly et al. 2009, Weingarten et al. 2010). Thus, xylose degradation at these relatively low temperatures (110℃ to 130℃) and low acid concentrations (0.25% to 1% w/w) would be expected to be much slower than oligomer hydrolysis, resulting in potentially high xylose yields.

4.4.4 Prediction of xylose yields from oligomer hydrolysis

The combined severity parameter provides a rapid tool with which to relate yields over a wide range of reaction conditions (Chum et al. 1990):

$$CSF = \log R_0 - pH \tag{4.9}$$

$$R_0 = t \cdot \exp[(T_H - T_R)/14.75] \tag{4.10}$$

where is the reaction time in minutes, T_H is the reaction temperature in ℃ , and T_R is a reference temperature, usually 100℃. However, as shown by Figure 3.8 in Chapter 3, the data reveals that the optimal value of CSF as defined above changes with temperature. Thus, rather than relying on the simple temperature correlation inherent in the definition of CSF, Figure 4.5 includes the activation energy in the correlation of the xylose yield data to the combination of reaction time, hydrogen ion activity, and temperature. In this case, the data is more consistent over the temperature range of 110℃ to 130℃, allowing a more accurate correlation of xylose yields at different conditions.

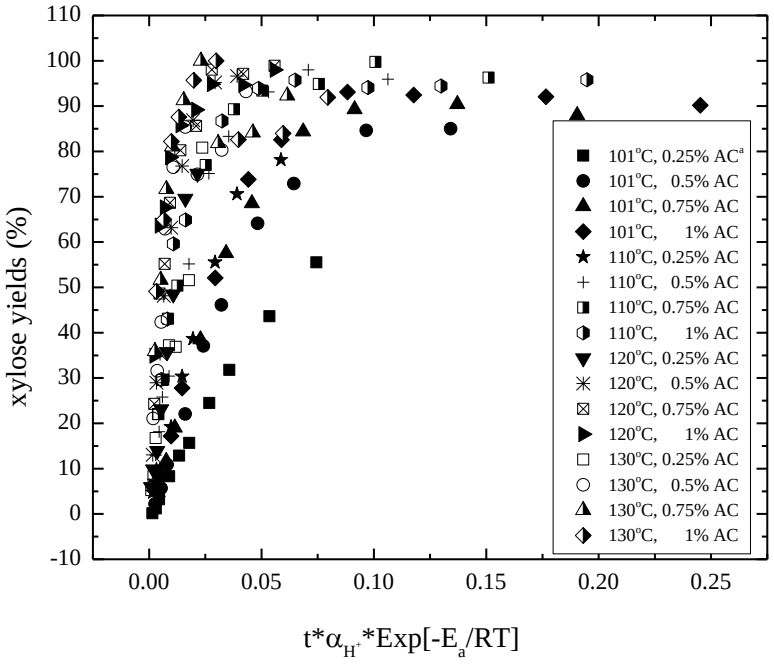


Figure 4.5 Correlation of xylose yields from oligomer hydrolysis over a range of times, temperatures, and acid concentrations based on integrating the activity energy into the combined severity parameter expression.

4.5 Conclusions

Data for hydrolysis of xylooligomers to xylose by dilute sulfuric acid over a range of sulfuric acid concentrations followed a first order rate law versus time at 101, 110, 120, and 130℃.On that basis, rate constants for the reaction were estimated and found to follow an Arrhenius dependence on temperature with an activity energy of 15.5kJ/mol. This activity energy is much lower than others have reported for xylose degradation, with the result that xylose degrades much more slowly at these relatively low temperatures compared to breakdown of oligomers. This results reinforces why high xylose yields are possible at lower temperatures. Although yields of xylose from xylooligomer solutions were not accurately correlated by the conventional combined severity factor, the

relationship among yield results was improved by incorporating the activity energy into the correlation.

4.6 References

Borrega M, Nieminen K, Sixta H. 2011. Degradation kinetics of the main carbohydrates in birch wood during hot water extraction in a batch reactor at elevated temperatures. Bioresource Technology 102: 10724-10732.

Chum H, Johnson D, Black S, Overend R. 1990. Pretreatment catalyst Effects and the combined severity parameter. Applied Biochemistry and Biotechnology 24-5: 1-14.

Davies CW. 1930. The Conductivity of Solutions and the Modern Dissociation Theory. New York, N. Y.: J. Wiley & Sons, Inc.

Dickinson A, Wesolowski D, Palmer D, Mesmer R. 1990. Dissociation-constant of bisulfate ion in aqueous sodium-chloride solutions to 250-degrees C. Journal of Physical Chemistry 94: 7978-7985.

Garrote G, Dominguez H, Parajo JC . 2001a. Autohydrolysis of corncob:study of non-isothermal operation for xylooligosaccharide production. Journal of Food Engineering: 211-218.

Garrote G, Dominguez H, Parajo J. 2001b. Kinetic modelling of corncob autohydrolysis. Process Biochemistry 36: 571-578.

Jacobsen S, Wyman C. 2000. Cellulose and hemicellulose hydrolysis models for application to current and novel pretreatment processes. Applied Biochemistry and Biotechnology 84-6: 81-96.

Kim S, Lee M, Park E, Lee S, Lee H, Park K, Park M. 2011. Kinetic study of the dehydration of D-xylose in high temperature water. Reaction Kinetics Mechanisms and Catalysis 103: 267-277.

Kobayashi T, Sakai Y. 1956. Hydrolysis rate of pentosan of hardwood in dilute sulfuric acid. Bulletin of the Agricultural Chemical Society of Japan 20: 1-7.

Kumar R, Wyman C. 2008. The impact of dilute sulfuric acid on the selectivity of xylooligomer depolymerization to monomers. Carbohydrate Research 343: 290-300.

Li X. 2002. Degree of Polymerization and Kinetics of Oligomer Released in Xylan Hydrolysis, *Thayer School of Engineering*: 96. Dartmouth College: Hanover, NH

Liu X, Lu M, Ai N, Yu F, Ji J. 2012. Kinetic model analysis of dilute sulfuric acid-catalyzed hemicellulose hydrolysis in sweet sorghum bagasse for xylose production. Industrial Crops and Products 38: 81-86.

Lloyd T.A., Wyman C.E. 2004. Predicted effects of mineral neutralization and bisulfate formation on hydrogen ion concentration for dilute sulfuric acid pretreatment. Applied Biochemistry and Biotechnology 113: 1013-1022.

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 96: 1967-1977.

Marshall W, Jones E. 1966. 2nd dissociation constant of sulfuric acid from 25 to 350 degrees evaluated from solubilities of calcium sulfate in sulfuric acid solutions. Journal of Physical Chemistry 70: 4028-4035.

Morinelly J, Jensen J, Browne M, Co T, Shonnard D. 2009. Kinetic characterization of xylose monomer and oligomer concentrations during dilute acid pretreatment of lignocellulosic biomass from forests and switchgrass. Industrial & Engineering Chemistry Research 48: 9877-9884.

Puls J. 1997. Chemistry and biochemistry of hemicelluloses: Relationship between hemicellulose structure and enzymes required for hydrolysis. Macromolecular Symposia 120: 183-196.

Saeman J. 1945. Kinetics of wood saccharification - hydrolysis of cellulose and decomposition of sugars in dilute acid at high temperature. Industrial and Engineering Chemistry 37: 43-52.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D. 2006. Determination of sugars, byproducts, and degradation products in liquid fraction process samples, Laboratory analytical Procedure (LAP): National Renewable Energy Laboratory.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D. 2008. Determination of structural carbohydrates and lignin in biomass, Laboratory Analytical Procedure (LAP): National Renewable Energy Laboratory.

Studer M, DeMartini J, Brethauer S, McKenzie H, Wyman C.E. 2010. Engineering of a high-throughput screening system to identify cellulosic biomass, pretreatments, and enzyme formulations that enhance sugar release. Biotechnology and Bioengineering 105: 231-238.

Vazquez M, Alonso J, Dominguez H, Parajo J. 2000. Xylooligosaccharides: manufacture and applications. Trends in Food Science & Technology 11: 387-393.

Weingarten R, Cho J, Conner W, Huber G. 2010. Kinetics of furfural production by dehydration of xylose in a biphasic reactor with microwave heating. Green Chemistry 12: 1423-1429.

Yang B, Wyman C.E. 2008. Characterization of the degree of polyperization of xylooligomers produced by flowthrough hydrolysis of pure xylan and corn stover with water. Bioresource Technology: 5756-5762.

Chapter 5

Cost estimation for hydrothermal pretreatment of corn stover followed by dilute acid post hydrolysis of oligomers

5.1 Introduction

In this study, the biochemical conversion process developed by the National Renewable Energy Laboratory (NREL) was adapted to estimate the cost of a process for dilute acid hydrolysis of xylooligomers produced by hydrothermal pretreatment of corn stover to fermentable monomers. The pretreatment section of the NREL process was modified to describe a two stage xylan-to-xylose conversion process, and both capital and operating costs were estimated.

5.2 NREL biochemical ethanol conversion process of corn stover

The NREL biochemical conversion process was reported in 2011 and is based on designs reported in 1999 and 2002 by NREL and its subcontractors (Wooley et al., 1999; Aden et al., 2002). The process was designed to convert corn stover to ethanol by a series of conversion operations: dilute acid pretreatment, enzymatic hydrolysis and co-fermentation. This process also included ancillary areas such as biomass handing, ethanol recovery, wastewater treatment, solid waste combustion, and utilities. A process

flow diagram for the NREL approach is shown in Figure 5.1 (Humbird et al., 2011). Moreover, the report contains detailed material balances, energy balances, capital and operating cost estimates.

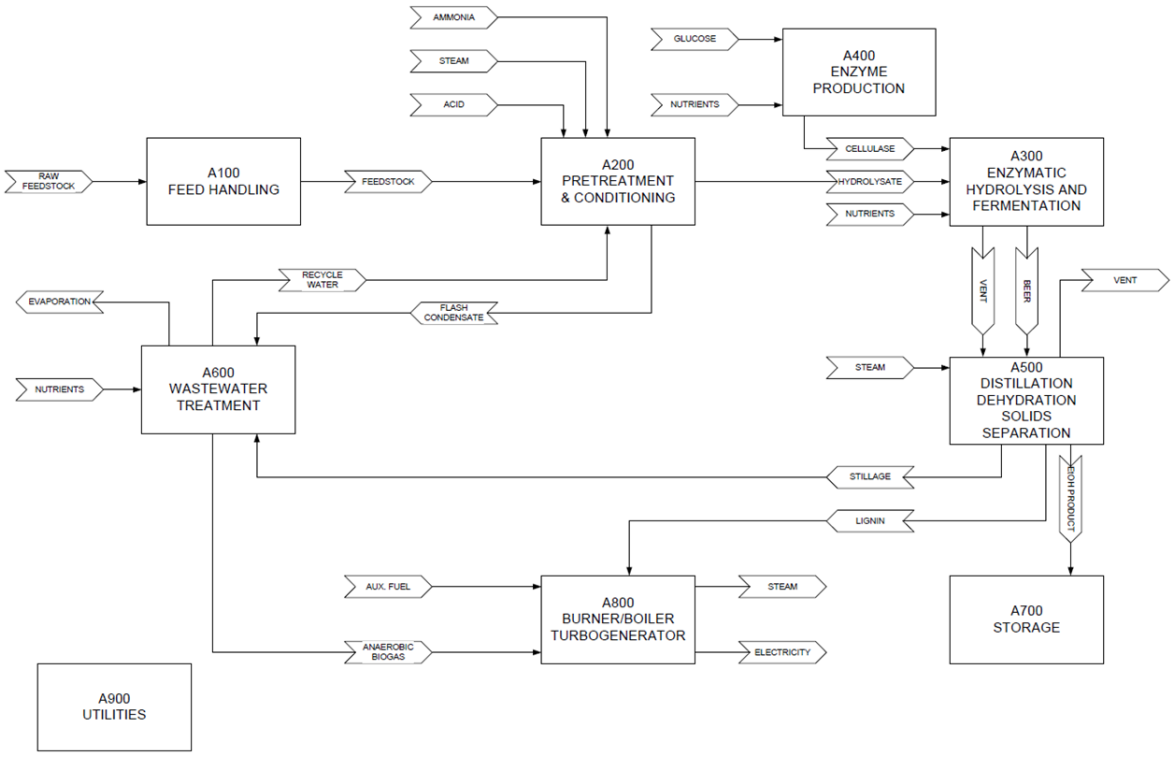


Figure 5.1 Overall NREL process of biochemical ethanol conversion process (Humbird et al., 2011)

5.2.1 Feedstock and feed handling

Corn stover, which is the most abundant agriculture residue in the U.S., was used as the feedstock in the NREL process based on the following composition: 32% glucan, 19% xylan, 3% arabinose, 1.5% galactan, 0.3% mannan, 13% lignin, 4% ash. Total structural carbohydrates that also include arabinose, mannose, and galactose added up to around 59% of dry biomass. The washed and milled (<0.25 in.) feedstock was transported to the facility from a storage location with 20% moisture content. The feed

rate of biomass to the process was 104,200 kg/h including moisture, and the cost of biomass was assumed to be $58.50/dry ton including the costs of growing, collection, processing, transportation, and storage.

5.2.2 Dilute acid pretreatment

The second step of the biochemical ethanol conversion process is pretreatment. In this step, most of the pentose (xylose and arabinose) and a small portion of hexose (glucose, galactose, and mannose) and some lignin were released hemicellulose into the liquid phase. The resulting cellulose structure disruption gives high enzyme accessibility. Dilute sulfuric acid was used as catalyst in the pretreatment process, and high pressure steam provided the heat to maintain the reaction temperature. In the NREL design process, pretreatment was carried out in two stages to convert as much as possible of the xylan to monomeric xylose and minimize formation of degradation products. The pretreatment flow diagram is shown in Figure5.2 (Humbird et al., 2011)

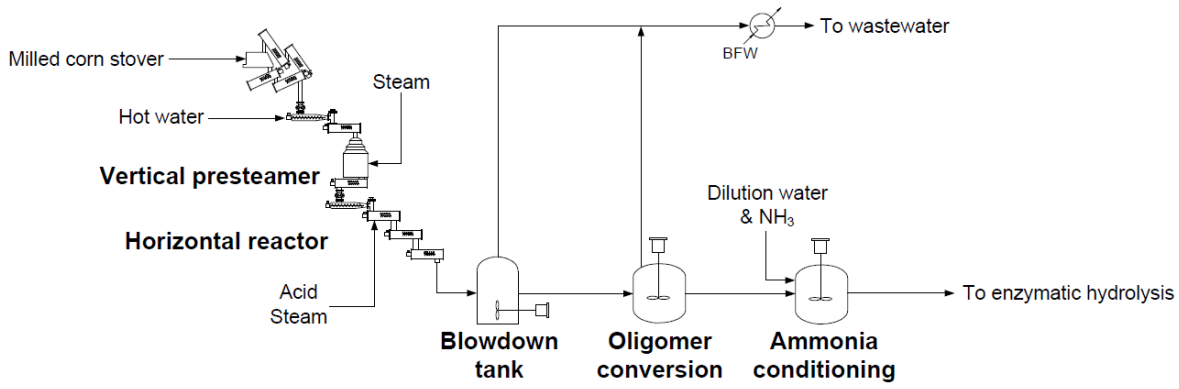


Figure 5.2 Flow diagram of two stages dilute acid pretreatment (Humbird et al., 2011)

The milled biomass and water were mixed together at a 30wt% solids loading with preheating process to 100℃, and then the slurry was delivered to the two stage pretreatment. The first stage heated the slurry with steam to 158℃ for 5 minutes with 0.81% wt. sulfuric acid, hydrolyzing hemicellulose to sugar oligomers and monomers. The second stage converted the oligomers from the first stage to monomers at lower temperature with a longer reaction time (130℃, 20~30 minutes). After hemicellulose hydrolysis, the pH level of the slurry was adjusted to 5 by ammonia solution and cooled to 75℃. The cooling and pH adjustment steps provide better conditions for the following enzymatic saccharification process. Conversions of carbohydrates and sugar degradation during pretreatment were assumed to be: 92% xylan-to-xylose conversion (including 90% xylose and 2.4% xylooligomers), 10% glucan-to-glucose conversion, 5% xylose degradation and 0.3% glucose degradation. The estimate of the capital costs for pretreatment and conditioning was $30 MM, and the cash costs of sulfuric acid and ammonia were determined to be $1.5 MM and $4 MM per year, respectively.

5.2.3 Enzymatic hydrolysis and fermentation

The pretreatment slurry was then sent to enzymatic hydrolysis and fermentation process to convert the cellulose fiber into glucose by enzyme and ferment the xylose and glucose to ethanol by *Zymomonas mobilis*. In NREL's design, a separate hydrolysis and fermentation process (SHF) was applied due to different optimum operating temperatures for enzymatic hydrolysis and fermentation. In the first stage, the slurry was mixed with cellulase (20mg protein/g cellulose) at 48℃ for 84 hours with around 90% glucose yields.

At this hydrolysis temperature, the enzyme activity is higher so the less enzyme amount is required. The slurry enriched with glucose and xylose was cooled down to the fermentation temperature of 32℃ and underwent co-fermentation of glucose and xylose to ethanol (second stage). The co-fermentation process was assumed to take 36 hours to convert 90% of the glucose and 80% of the xylose to ethanol. The capital costs of enzymatic hydrolysis and fermentation were estimated to be around $31MM, and the operating costs were $12MM per year.

5.3 Process modification

The NREL ethanol conversion process used dilute sulfuric acid to convert hemicellulose to monomeric xylose in a two stage pretreatment (higher and lower severity). In Chapter 3, we introduced a different two stage hemicellulose hydrolysis approach to achieve 90+% xylose yields in the hydrolyzate by applying hydrothermal pretreatment followed by post hydrolysis with dilute sulfuric acid. Several sets of conditions could achieve this result: 101℃ with 1% and 0.75% wt. acid concentrations; 110℃ with 1%, 0.75% and 0.5% wt. acid concentrations; 120℃ with 1%, 0.75% and 0.5%; and 130℃ with 1%, 0.75% and 0.5% wt. acid concentrations. The pretreatment severity in this study was milder than for the NREL process to reduce the capital and operating costs of the ethanol conversion process. To estimate cost differences between the two approaches, the more detailed process flow diagram of section A200 in Figure 5.1 for the NREL process design shown in Figures 5.3 and 5.4 was modified (Humbird et al., 2011). However, first, the pretreatment procedure for this study has to be known.

First, the biomass was mixed with just water and reacted at 200℃ for 14.3 minutes (hydrothermal pretreatment). Then the slurry was subjected to the different combinations of dilute sulfuric acid concentrations and times that were found to give the highest xylose sugar yields. After dilute acid hydrolysis, the slurry was neutralized with an ammonia solution (more details are in Chapter 3). Figure 5.5 presents a simple flow diagram for pretreatment and shows differences in the pathway between the NREL process and the process of this study. Sulfuric acid was not added during stage 1 but was applied in stage 2.

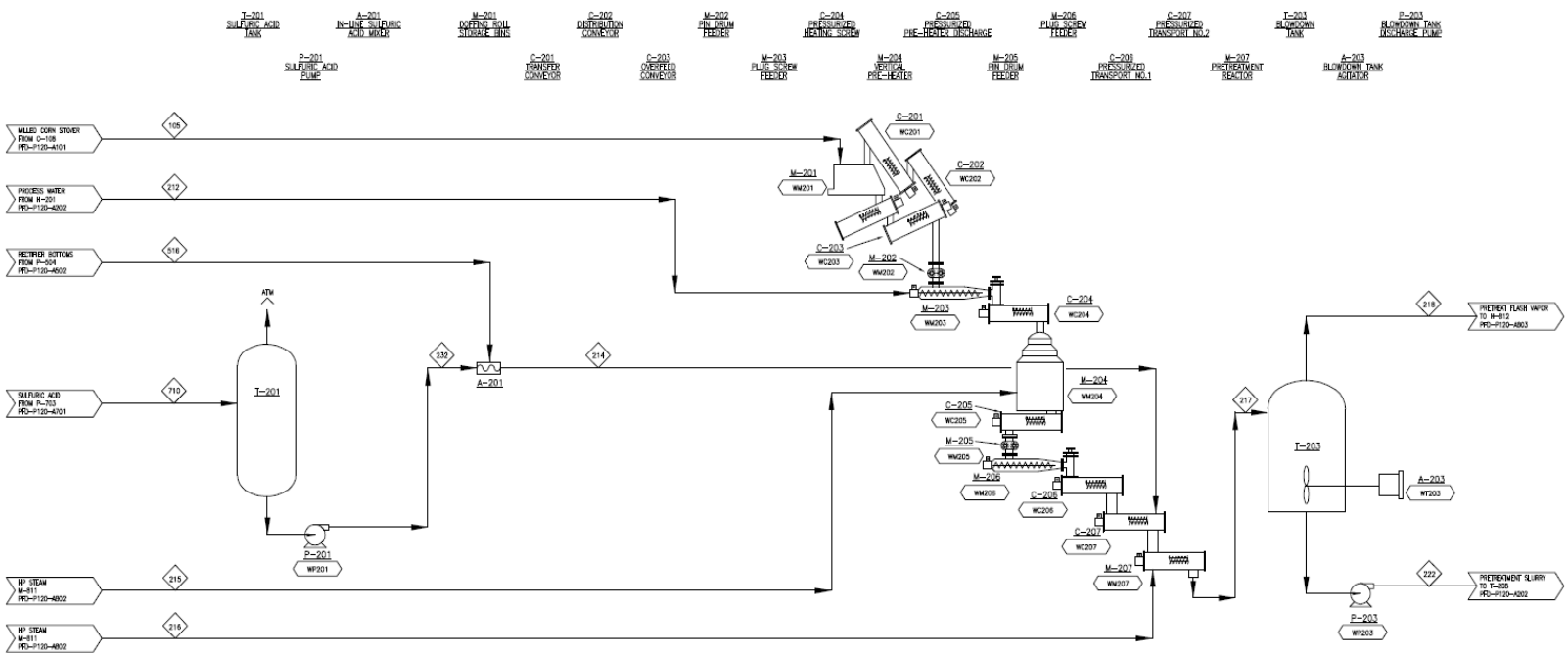


Figure 5.3 Flow diagram of stage 1 pretreatment section of NREL biochemical ethanol conversion process (Humbird et al., 2011)

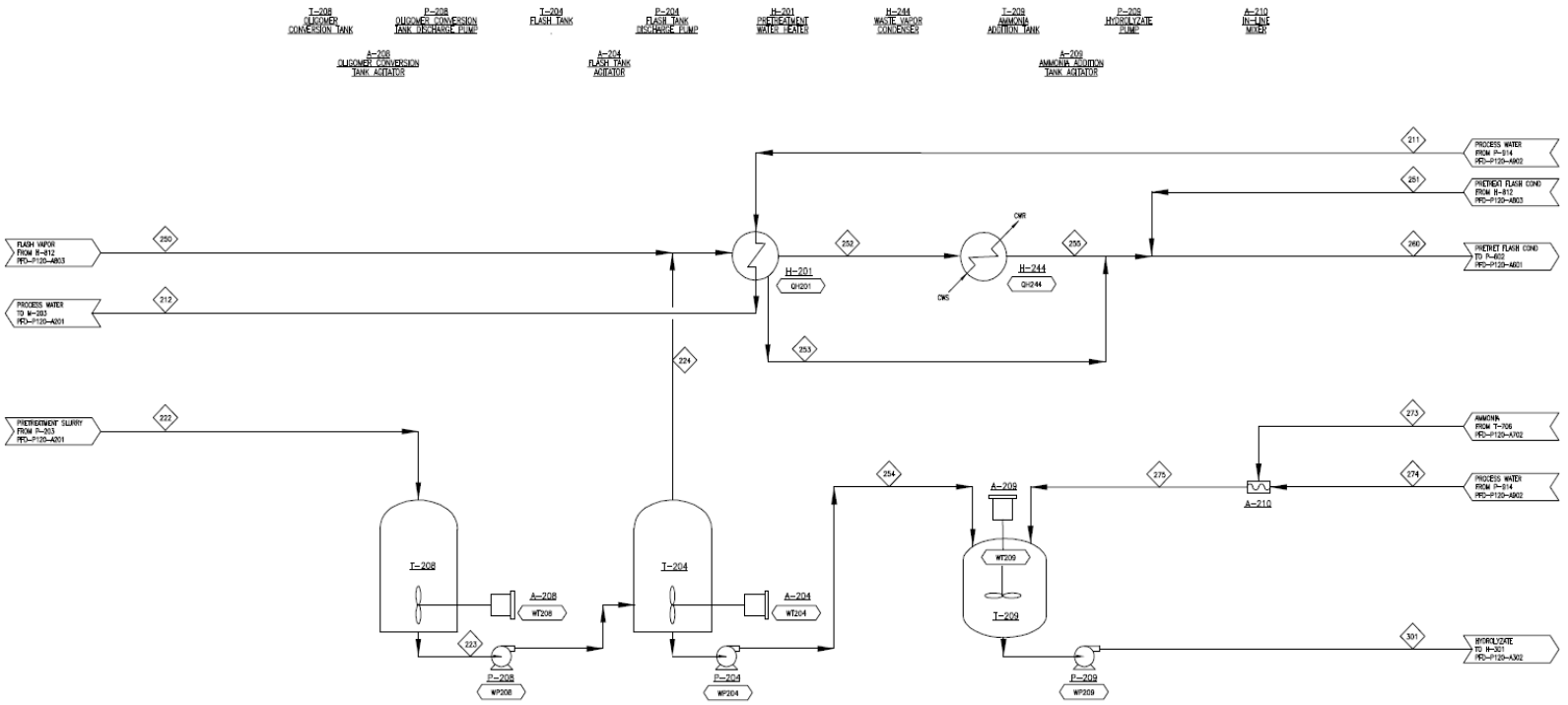


Figure 5.4 Flow diagram of stage 2 xylooligomers converting section of NREL biochemical ethanol conversion process (Humbird et al., 2011)

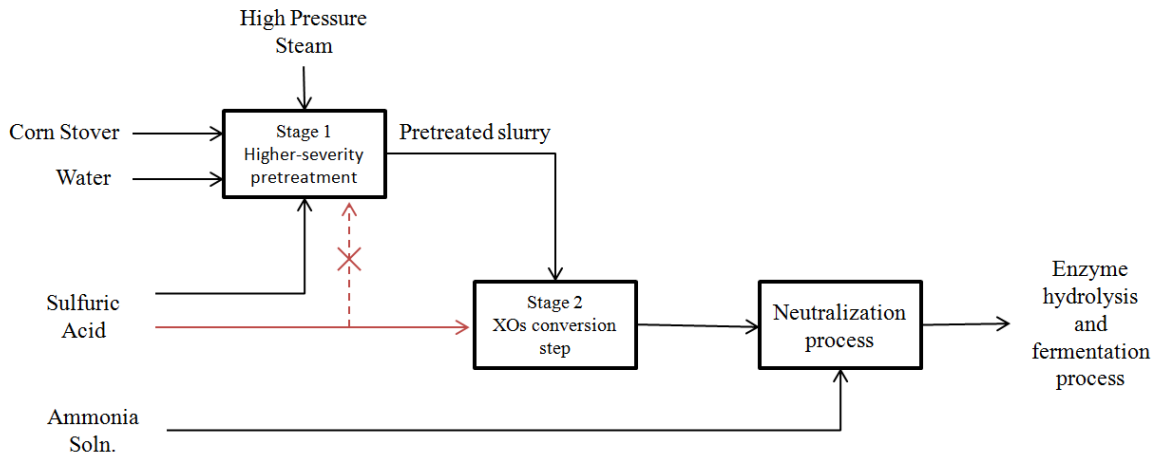


Figure 5.5 Flow diagram of two stage pretreatment process to point out differences between the original NREL process (black) and approach for this study (red). XOs : xyloologomers

5.4 Conditions optimization and cost estimation

5.4.1 Optimization of pretreatment conditions

NREL targeted 90% xylan-to-xylose yields with 5% degradation in their two stage pretreatment process. In 2007, 75% of xylan-to-xylose was achieved based on results from a batch reactor at the bench scale and horizontal reactor in continuous pilot-scale operation (Weiss et al., 2007). After modification of horizontal reactor, 79.6% xylan-to-xylose conversion was achieved with 6.4% degradation, 5% unreacted xylan, and 9% xyloologomers that did not convert to monomeric xylose (Nagle et al. 2009). At UCR, 77.3% of xylan was hydrolyzed to xylose and xylooligomers during first stage hydrothermal pretreatment (Zhang, TY unpublished data, 2012). Then, 90+% of the oligomers were converted to monomeric xylose by a second stage dilute acid

xylooligomer hydrolysis process (results shown in chapter 3). Although the conversion is little lower than for the NREL approach, less degradation products were produced in this two stages pretreatment than in the NREL two stage dilute acid pretreatment because no acid was employed in stage1 and a lower temperature was applied in stage 2. Unreacted xylan could also be potentially hydrolyzed to monomeric xylose in the following enzymatic hydrolysis operation.

We target at least a 90% oligomers-to-xylose conversion yield in the stage 2 post hydrolysis operation, with reaction times to reach the target yields estimated based on the xylose yield data in Chapter 3. The results with 0.25% acid concentration were not considered because the xylose yields were below 90% for every temperature at the end of the reaction time. Table 5.1 shows the reaction time needed to achieve the highest xylose yield for each temperature/acid concentration combination.

Table 5.1 the optimal reaction time (min) of 90% oligomers-to-xylose conversion for 0.5%,0.75% and 1% wt acid concentration at 101℃ to 130℃

	Temperature,℃			
Acid conc.,%	101	110	120	130
0.5	450	160	70	25
0.75	290	95	40	13
1	200	70	35	10

5.4.2 Capital costs estimation

The reactor system such as pretreatment reactor, transporter (C206, C207 in figure 5.3) ,and screw feeder of the NREL stage 1 dilute acid pretreatment was

constructed of carbon steel clad with Incoloy 825 to provide acid resistance. The rest of the equipment including stage 2 xylooligomers conversion process was mostly made of 316 stainless steel. Since the residence times are both changed in the second step, the size of the tank needed to be estimated (T 208 in Figure 5.4). The new size of the tank can be estimated by the following equation (Humbird et al., 2011):

$$\text{New Size} = \frac{t_{new}}{t_{base}} \text{ x (Base size)} \tag{5.1}$$

where t_{base} is residence time for the NREL process of 30 minutes for the second step. The base size of the tank in the second step was 30,000 gallon. According to the NREL process report, an exponential scaling expression was used to estimate the new cost of the reactor (Humbird et al., 2011).

$$\text{New Cost} = \text{(Base Cost)} \left(\frac{\text{New Size}}{\text{Base Size}}\right)^{n} \tag{5.2}$$

where n is a characteristic scaling exponent that is 0.7 for the oligomer conversion tank. Figure 5.6 shows the cost estimates for each operating condition.

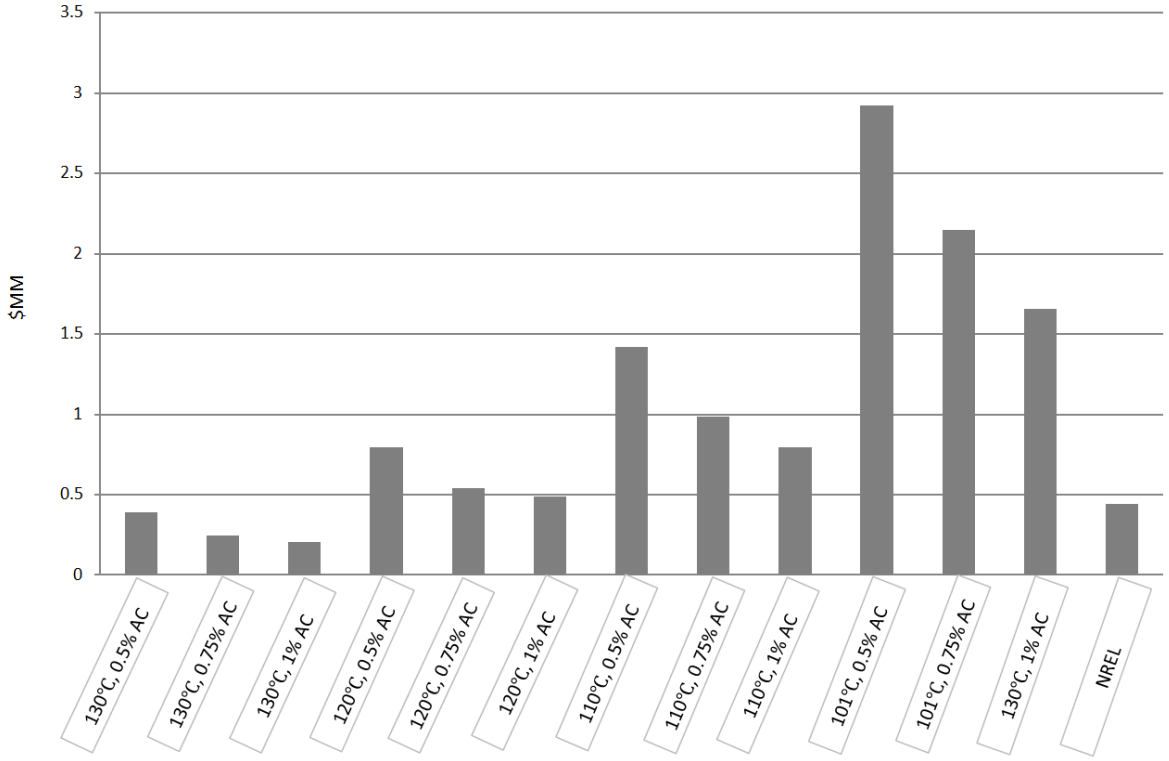


Figure 5.6 Capital cost of second step pretreatment at different temperatures and acid concentrations. AC: acid concentration in weight percentage

The cost of the oligomer conversion tank can be reduced by about 12%, 46%, and 55% for 0.5%, 0.75% and 1wt % acid concentrations, respectively, at 130℃. In addition, the change in materials of construction also reduced the capital cost which can be estimated by the following equation:

$$\text{New costs of reactor system} = \frac{(\text{Price of new material})}{(\text{Price of original material})} \times (\text{Costs of reactor system of NREL process}) \quad (5.3)$$

The materials for the first step of the reactor system can be replaced from carbon steel clad with Incoloy 825 with lower cost steel because no acid is used in that step for our design. According to equation 5.1 and 5.2, the new cost of the reactor system for the first stage is around 1.8 times higher than NREL's first step reactor system. Therefore, if the cost of the new material can compensate for the size effect, the capital cost of whole pretreatment section is dramatically decreased since almost 90% of costs are contributed by the first step. In the second stage, 316 stainless steel was used for the oligomers conversion tank T208 (Figure 5.4) in the NREL process. However, because the temperature for the xylooligomer hydrolysis process in this study covered a range of 101℃ to 130℃, the materials for the reaction tank had to be modified as appropriate for each situation. Because of the similar reaction condition to the NREL process, the same materials were assumed to be needed at 130℃, 120℃ and 110℃. However, at 101℃, the conversion process was at essentially atmospheric pressure, so that plastic or low strength metal could be applied at this condition for further capital cost reductions.

5.4.3 Operating costs estimation

The operating costs of the pretreatment section only include chemicals and high pressure steam, with the primary differences between each operating condition being the amount of chemicals (sulfuric acid and ammonia), temperature for the amount of steam, and time and pressure for the amount of electricity (less power to pump against lower pressure). The NREL process generates electricity and provides power for entire facility by burning the biomass residue. The boiler produces 239,000 kg/h steam, and 12% of it

is sent to the pretreatment section to provide all the steam required for two stages at 268℃ and 175 psi. To estimate the total amount of steam used in two stage pretreatment for our design, we chose 130℃ with a 0.5% acid concentration (Table 5.1) as a base line to estimate the steam cost of other operating conditions of stage 2 since it has similar operating conditions to the NREL process. Thus, for this situation, the difference between the NREL and our design is for the first stage (158℃, 5min vs. 200℃, 14.3 min). The annual cost of steam is then calculated by the following equation:

$$\text{Total cost of Steam} = \left(\frac{Q_1}{Q_2}\right) \text{x} \left(\frac{t_1}{t_2}\right) \text{x (flow rate of steam) x (price of steam)} \quad (5.4)$$

where

$Q_i = C_{p,268℃}\,(268℃ - T_i)$ [kJ/kg]

$C_{p,268℃} = 2.26$ [kJ/kg K] (NBS/NRC steam tables)

t_i = resident time of reaction [min]

T_i = reaction temperature [K]

The flow rate of steam for the NREL process is 467.07 kg/min, and the operating cost of generating steam is $179.59 per hour (does not include the cost of water) (Humbird et al., 2011). Thus, the price of steam can be calculated to be 0.075¢/ kg to give the resulting costs of steam in Figure 5.7.

Another important operating cost is for chemicals used in the process: sulfuric acid and ammonia. The acid concentration used for the NREL pretreatment process was 0.81% wt. sulfuric acid to give an annual cost of chemicals of $5.46 MM. Thus, the chemicals cost for 0.5%, 0.75% and 1% wt acid concentrations can be calculated as:

$$\text{New cost of chemicals} = \left(\frac{C_a}{0.81}\right) \text{x (chemicals costs of NREL process)} \qquad (5.5)$$

where C_a is the acid concentration in weight percentage.

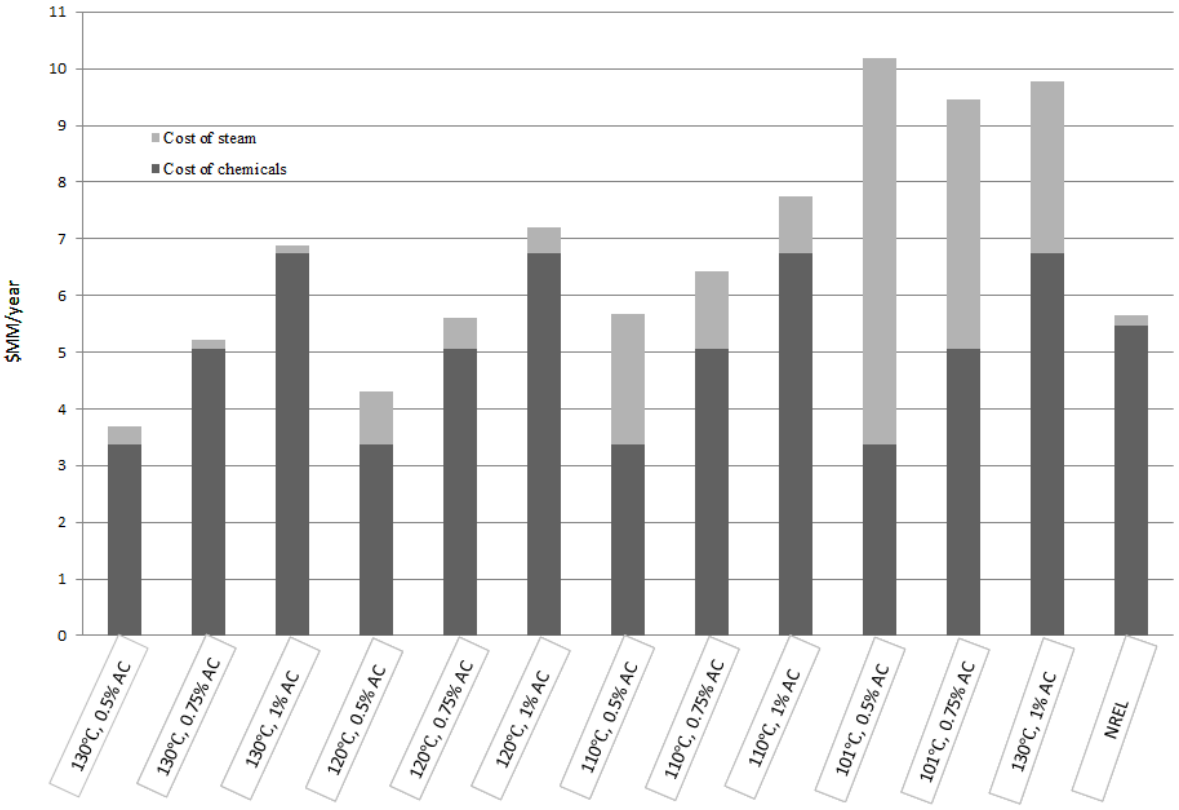


Figure 5.7 Operating cost of pretreatment at different temperatures and acid concentrations. AC: acid concentration in weight percentage

Figure 5.7 shows that the costs for our approach are less than those for the NREL for four sets of post hydrolysis conditions: 130℃ with 0.5% sulfuric acid; 130℃, 0.75%; 120℃, 0.5%; and 120℃, 0.75%. Thus, the operating cost is about 35% less for 130℃ at a 0.5% acid concentration. In addition, the operating cost can be reduced if the material of oligomer conversion tank is well insulated since the usage of steam is much smaller and the differences in operating costs between every reaction temperatures are not significant.

5.4.4 Overall cost estimation

The overall estimated cost of the second stage is shown in Figure 5.8, and the best operating conditions for the oligomer conversion process are at 130℃ with 0.5% wt. acid concentration. The overall cost was reduced 33% compared to the NREL design. The overall cost was also lower for 130℃, 0.75% wt. acid concentration and 120℃, 0.5% wt. acid concentration (reduced 10% and 16% respectively).

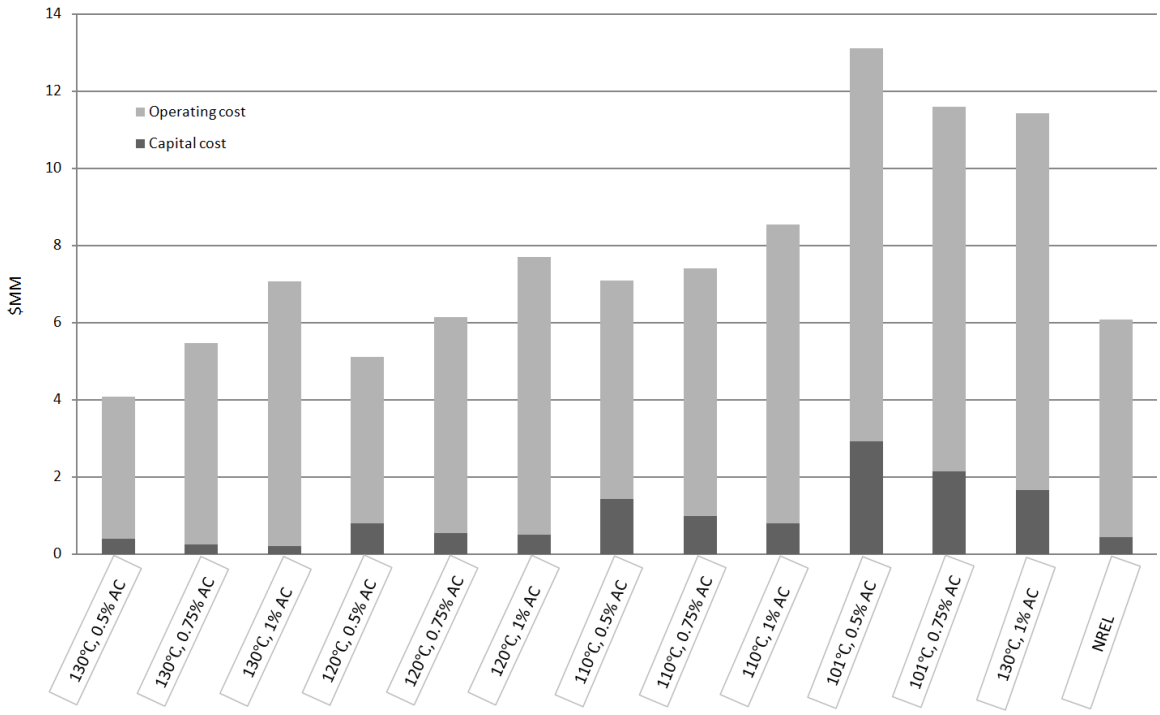


Figure 5.8 Overall cost of second step pretreatment at different temperatures and acid concentrations. AC: acid concentration in weight percentage

5.5 Conclusions

Capital and operating costs for a two stage xylan-to-xylose conversion process based on hydrothermal pretreatment followed by dilute acid hydrolysis were estimated. The capital cost of second step was reduced by 12% for 0.5% wt.; 46% for 0.75% wt. and 55% for 1%wt. sulfuric acid at 130℃. Hydrothermal pretreatment also allowed us to change all materials of construction for the first stage pretreatment reactor to lower cost steel because no acid is used in that step for our design. However, the change in materials of construction for the xylooligomers conversion tank depended on the reaction temperature. For 130℃, 120℃ and 110℃, the material was 316 stainless steel, the same as for NREL's process. Yet, materials for the tank at 101℃ can be replaced by other acid

resistance tank which has lower prices. The estimated operating cost for 130℃ with 0.5% sulfuric acid; 130℃, 0.75%; 120℃, 0.5%; and 120℃, 0.75% all were lower than for the NREL process. For 130℃, 0.5% acid concentration, the cost was reduced by about 35%. The overall cost for 130℃ with a 0.5% acid concentration was the lowest, which is about 33% less than for the NREL design.

5.6 References

Aden, A.; Ruth, M.; Ibsen, K.; Jechura, J.; Neeves, K.; Sheehan, J.; Wallace, B.; Montague, L.; Slayton, A.; Lukas, J. lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover. Report No. NREL/TP-510-32438. Golden, CO: National Renewable Energy Laboratory, June 2002.

Humbird, D.; Davis, R.; Tao, L.; Kinchin, C.; Hsu, D.; Sheehan, J.; Aden, A. process design and economics for biochemical conversion of lignocellulosic biomass to ethanol. dilute-acid pretreatment and enzymatic hydrolysis of corn stover report No. NREL/TP-5100-47764. Golden, CO: National Renewable Energy Laboratory, May 2011.

Wooley, R.; Ruth, M.; Sheehan, J.; Ibsen, K. lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis: current and future scenarios. Report No. NREL/TP-580-26157. Golden, CO: National Renewable Energy Laboratory, July 1999.

Weiss, N.; Nagle, N.; Elander, R.; Tucker, M. Achieve 75% xylose yield in laboratory scale high solids pretreatment reactor on corn stem internode. D Milestone Report, Document #9926. Golden, CO: National Renewable Energy Laboratory, 2007.

Nagle, N.; Kuhn, E.; Sievers, D.; Tucker, M.; Weiss, N.; Elander, R. Parametric study using the continuous horizontal pretreatment reactor using corn stover to determine operating conditions that achieve 80% yield of monomeric xylose. D Milestone Report, Document #10575 Golden, CO: National Renewable Energy Laboratory, September 2009.

Chapter 6

Conclusions and Recommendations

6.1 Summary of thesis objectives

The main motivation for this work was to hydrolyze most of hemicellulose into xylose without forming too much degradation products during pretreatment process. Although dilute acid can convert hemicellulose to xylose with little unconverted xylooligomers, the degradation products during this process are relatively high compared to hydrothermal pretreatment. However, most of xylose remains in oligomer form with different degree of polymerization (DP) during hydrothermal pretreatment which act as an inhibitor to enzymatic hydrolysis. The challenge is to convert these xylooligomers into xylose that are more fermentable by most organisms. Therefore, a two-step pretreatment process was applied in this study with the first stage hydrothermal hemicellulose hydrolysis (hemicellulose to xylose monomers and oligomers) and the second stage dilute acid hydrolysis of the oligomers to monomers. Based on this rationale, the objectives of this study were to:

1) Determine the effects of temperature, acid concentration, and reaction time on xylooligomer hydrolysis in the hydrolyzate from hydrothermal pretreatment (Chapter 3).

2) Develop kinetics of xylooligomers hydrolysis in the hydrolyzate from hydrothermal pretreatment (Chapter 4)

3) Estimate the cost of the two-step pretreatment compared to single stage dilute acid pretreatment (Chapter 5)

6.2 Key findings and future works

6.2.1Dilute acid xylooligomers hydrolysis at low temperature

The most important findings of this study was how xylooligomers hydrolyzed into xylose at low temperature (101℃ to 130℃). After a period of time, xylose yields reached over 90% at all temperatures with 0.5% wt., 0.75% wt., and 1% wt. acid concentrations, and over 95% with 0.75% and 1.00% w/w acid concentrations at 110℃, 120℃, and 130℃. During conversion, the degradation rate of xylose was very low. Further studies could determine xylose degradation and hydrolysis of short chain xylooligomers at low temperature and acid concentrations. Although the combined severity factor was used to predict xylose yields, the combined severity parameter did not perfectly account for the effects of temperature. This outcome suggests that the simple exponential function used in the severity parameter calculation does not fit hydrolysis data as well at these lower temperatures as it does at higher temperatures for which it was originally developed. As a result, a different rule of correlation is needed to more tightly group how temperature impacts yields.

6.2.2 The kinetics of xylooligomers hydrolysis at low temperature

The kinetics of xylooligomers hydrolysis were developed, and rate constants (k) based on first order homogeneous kinetic models were determined to be 0.140, 0.369, 1.032, and 2.741 min^{-1} at 101℃, 110℃, 120℃, and 130℃, respectively. The activity energy was 15.5kJ/min which is much lower than the activity energy of the xylose-to-furfural reaction, supporting the observed low degradation rate of xylose in this

temperature range. However, the xylose degradation rate needs to be estimated and further research of kinetics of short chain xylooligomers (DP=2~5) hydrolysis are also needed. A more robust xylooligomer hydrolysis kinetic models need to be established so we can predict xylose yields more precisely.

6.2.3 Economic evaluation of two-step pretreatment process

The economic evaluation of the two-step pretreatment process was based on the NREL biochemical ethanol conversion process. The lowest cost operating condition for oligomer conversion was at 130℃ with 0.5% wt. acid concentration, a cost about 33% less than for the NREL design. However, the capital cost may be reduced further by employing less costly materials of construction for both the first and second step. Therefore, further research should be focus on estimating the cost of lower cost materials for the reactor system.