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How Wood Chip Size Affects Pretreatment Effectiveness of Woody Biomass for
Biological Processing

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

Master of Science

in

Chemical and Environmental Engineering

by

Jerry Tam

August 2013

Thesis Committee:

Dr. Charles E. Wyman, Chairperson

Dr. Phillip Christopher

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2013

The Thesis of Jerry Tam is approved:

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University of California, Riverside

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ABSTRACT OF THE THESIS

How Wood Chip Size Affects Pretreatment Effectiveness of Woody Biomass for
Biological Processing

by

Jerry Tam

Master of Science, Graduate Program in Chemical and Environmental Engineering
University of California, Riverside, August 2013
Dr. Charles E. Wyman, Chairperson

Woody biomass is particularly resistant to breakdown and must be size reduced to make pretreatment with steam, acid, or other chemicals effective. However, because mechanical size reduction of woody biomass can consume significant amounts of energy, it is important to understand how to minimize size reduction, while still realizing high sugar yields from the combined operations of pretreatment and enzymatic hydrolysis. Thus, this study focused on determining how sugar yields change with particle size. Downscaled composition analysis and enzymatic hydrolysis were applied to different sized wood chips and powder of *Populus tremuloides* to examine whether wood chips could perform similarly to powder. It was found that above a certain pretreatment severity range ($R_0=3.8$), large wood chips could give higher enzymatic sugar yields, so that size reduction is not always required, thus saving energy. Furthermore, models were applied to better understand whether heat transfer affected pretreatment effectiveness and establish maximum dimensions for high sugar release.

Table of Contents

Acknowledgements	iv
Abstract	v
Table of Contents	vi
List of Figures	viii
List of Tables	x
Chapter 1 - Introduction	1
1.1. Background	1
1.2. Production of ethanol from cellulosic biomass	2
1.3. Challenges and opportunities for biofuels	4
1.4. Thesis objectives	6
1.5. Thesis outline	7
1.6. References	8
Chapter 2 - Literature Review	10
2.1. Promising Feedstocks to Produce Bioethanol	10
2.2. Structure of Lignocellulosic Biomass	12
2.3. The Effects of Lignocellulosic Biomass Pretreatment	14
2.4. Factors Affecting Enzymatic Digestibility	17
2.5. Effects of Particle Size on Pretreatment Effectiveness	19
2.6. Conclusions	21
2.7. References	23
Chapter 3 - The Effect of Chip Size on the Effectiveness of Hydrothermal Pretreatment of Woody Biomass	28
3.1. Abstract	28
3.2. Introduction	29
3.3. Material and Methods	31
3.3.1. Feed Materials	31
3.3.2. Pretreatment	32
3.3.3. Material Preparation	33
3.3.4. Composition Analysis	33
3.3.5. Enzymatic hydrolysis	35
3.4. Results and Discussion	35
3.4.1. Severity Factor	35
3.4.2. Composition	36
3.4.3. Sugar left in pretreated solids	39
3.4.4. Enzymatic Hydrolysis	41
3.4.5. Effect of chip size on sugar removal in enzymatic hydrolysis	45
3.4.6. Effect of chip orientation on sugar removal in hydrothermal pretreatment	47
3.5. Conclusions	49
3.6. References	50

Chapter 4 -	Modeling the Effect of Heat Transfer on Temperature Distribution vs. Time for Wood Particles of Different Sizes	52
4.1.	Abstract	52
4.2.	Introduction	52
4.3.	Modeling Heat Conduction in Chips	53
4.4.	Conclusions	61
4.5.	References	62
Chapter 5 -	Estimation of the Impact of Size Reduction on Costs for Hydrothermal Pretreatment of Aspen Wood Chips	63
5.1.	Abstract	63
5.2.	Introduction	63
5.3.	Factors affecting energy requirement for wood chips size reduction	64
5.3.1.	Milling machine	64
5.3.2.	Post-pretreatment wood size reduction	65
5.3.3.	Effect of chemical pretreatment	65
5.3.4.	Final particles size	66
5.4.	Energy consumption for size reduction	67
5.5.	Operating cost for steam explosion pretreatment	70
5.6.	Conclusions	71
5.7.	References	72
Chapter 6 -	Summary of Key Findings and Future Work	74
6.1.	Summary of thesis objectives	74
6.2.	Key finding and future works	75

List of Figures

Figure 1.1	Composition of cellulosic biomass types (McMillan, 1994).....	3
Figure 1.2	Schematic representation of an SSF process (Oloffon et al., 2008).....	4
Figure 1.3	The renewable energy production and requirement (Hoekman, 2009).....	5
Figure 2.1	Global ethanol production by country or region, from 2007 to 2011 (U.S. Department of Energy).....	11
Figure 3.1	Schematic diagram shows mechanical size reduction of wood applied to (a)pre- and (b) post-chemical pretreatment to reduce energy consumption (Zhu et al., 2010).....	30
Figure 3.2	Sequence of steps for biomass sugar yield analysis for different wood Sizes.....	31
Figure 3.3	(A) woven metal mesh basket. (B) top basket was used to place milled material, and bottom nets were used to place woody chips. (C) Steam boiler (D) steam gun reactor used for pretreatment.....	33
Figure 3.4	(A) A 1.5 mL high recovery glass HPLC vial (B) Discovery Tools reactor block that can hold up to 48 glass vials.....	34
Figure 3.5	Compositions of solids (wt.%, dry basis) following pretreatment at different pretreatment conditions: (A) glucan content at 180°C, (B) xylan content at 180°C, (C) lignin content at 180°C, (D) glucan content at 200°C, (E) xylan content at 200°C, and (F) lignin content at 200°C.....	38
Figure 3.6	Sugar left in solids after steam explosion pretreatment: (A) unwashed materials; (B) and (C) washed materials. Note: On the x-axis in (A) and (B) S denotes small chips and L- large chips.....	40
Figure 3.7	Glucose plus xylose yields from enzymatic hydrolysis for 72 h of large chips, small chips, and powder for pretreatment temperatures of (A) 180°C and (B) 200°C. Note- enzymatic hydrolysis was performed at 10g/L glucan loading in 50 mM citrate buffer for 72 h at cellulase plus xylanase loading of 30 plus 10 mg/g glucan+ xylan in unpretreated solids.....	42

Figure 3.8	Glucose yields following enzymatic hydrolysis of solids from hydrothermal pretreatment of Aspen at 180°C for (A) 4 min, (B) 8 min, (C)14 min, and (D) 60 min. Note- enzymatic hydrolysis was performed at 10 g/L glucan loading in 50 mM citrate buffer for 72 h at cellulase plus xylanase loading of 30 plus 10 mg/g glucan+ xylan in unpretreated solids.....	44
Figure 3.9	Sugar removal from small and large chips as a percent of the total amount of sugars in the original untreated chips plotted vs (A) multiple pretreatment condition (B) pretreatment severity factor for different temperatures.....	46
Figure 3.10	The chip on the left (Chip F) was cut so that the grain direction was in the direction of the chip width (x direction). The chip on the right (Chip A) was cut so that the grain direction was in the direction of the chip thickness (z direction).....	47
Figure 3.11	Mass loss during hydrothermal pretreatment at 180°C of chips with grain running against the grain and following the grain.	48.
Figure 3.12	Sugar left in solids during hydrothermal pretreatment at 180°C of chips with grain running against the grain and following the grain.....	48
Figure 4.1	Predicted temperature (Eq 4.1) at the center of chips of small chips and large chips versus pretreatment time at 180°C.....	55
Figure 4.2	Predicted temperature (Eq 4.1) at the center of chips of small chips with different orientations of grain versus pretreatment time at 180°C.....	56
Figure 4.3	Predicted time for the center of different dimensions and orientations chips reach within 3°C of the target temperature (180°C).....	57
Figure 4.4	Predicted temperature at the center of chips of small chips and large chips versus pretreatment time at 180°C employed in Equation 4.2	59
Figure 5.1	Energy consumption to produce different final particle sizes using hammer and knife mills. (Cadoche and Lopez, 1989)	67

List of Tables

Table 1.1	Potential benefits and challenge of biofuels (Hoekman, 2009).....	4
Table 2.1	Composition of various lignocellulosic raw materials (Jeewon, 1997)	13
Table 2.2	Effects of various pretreatment methods on the three fractions of cellulosic material (Mosier et al., 2005).....	15
Table 2.3	Summary of relationship between structural features and digestibility (Zhu et al., 2008).....	19
Table 3.1	Energy requirements for mechanical comminution of lignocellulosic materials to different size ranges (Cadoche and Lopez, 1989).....	29
Table 3.2	Severity factor for different reaction time and temperature.....	36
Table 4.1	Parameters employed for heating of wood chips employed in Equation 4.1.....	54
Table 4.2	Parameters for small chips employed in Equation 4.2.....	58
Table 4.3	Parameters for large chips employed in Equation 4.2.....	59
Table 5.1	Effects of various pretreatments on lodgepole pine wood chip size reduction energy consumption (Zhu et al., 2010).....	66

Chapter 1

Introduction

1.1 Background

The growing energy crisis is a global problem. Petroleum is the largest source of energy, serving 36 percent of U.S. energy demand, and 70 percent is used in the transportation sector (U.S. Environmental Protection Agency). However, global petroleum consumption increases nearly every year, accelerating pressure on finite oil reserves. Therefore, there is mounting urgency to develop renewable energy to reduce our dependence on petroleum. Furthermore, consumption of fossil fuels releases greenhouse gases such as carbon dioxide, methane, and nitrous oxide that impact the environment and especially cause global warming (Goldemberg, 2007). Carbon dioxide is the major greenhouse gas, accounting for about 84% of all U.S. greenhouse gas emissions from human activities (U.S. Environmental Protection Agency). Carbon dioxide emissions have increased about 12 percent in last 20 years, with total carbon dioxide emissions of about 5,800 million metric tons in 2010. The increase in carbon dioxide emissions is mainly due to an expanding population and economy which require more energy. Thus, it is important to find routes to produce more energy from renewable sources such as bio-fuels to reduce greenhouse gas emissions and reduce our dependence on petroleum

In 2010, approximately 48% of U.S. renewable energy was from biomass, but half of that was from direct combustion for electricity production (U.S. Environmental

Protection Agency). New techniques to use biomass are being researched, and bioethanol produced from cellulosic biomass can become a sustainable energy sources. Use of biofuels not only successfully reduces greenhouse gas emissions but also increases agricultural income for rural areas (Mustafa and Havva, 2009). Bioethanol can be blended directly into gasoline and used by vehicles without modifying their engine. More than 95% of the gasoline in U.S. was blended with ethanol, predominantly at the E10 (ten percent ethanol) level. Recent approval of higher level blends with gasoline, E15 (fifteen percent ethanol), should see use of more biofuels in domestic markets in the months and years ahead (RFA, 2012 Ethanol Industry Outlook).

Over the last 25 years, corn and grain sorghum are the primary feedstocks to produce bio-ethanol in the United States (about 13.9 billion gallons in 2012) (RFA, 2012 Ethanol Industry Outlook). Since bio-ethanol production increased year by year, the feedstock requirement to produce bio-ethanol has increased as well (RFA, 2012 Ethanol industry Outlook). However, the production of bio-ethanol from corn or other grains tends to be expensive and drive up the price of food and animal feed. Lignocellulosic biomass, including forestry and agricultural residues, woody and grassy crops, and solid waste, are attractive materials for bio-ethanol production due to their relative abundance and low costs (Wyman, 1999)

1.2 Production of ethanol from cellulosic biomass

It is more difficult to produce ethanol from cellulosic biomass than from sugar or starch because of its resistant nature to breakdown (Huber et al., 2006). Cellulosic

biomass is composed mostly of cellulose, hemicellulose, and lignin, and different types of cellulosic biomass have different structures and compositions (Figure 1.1).

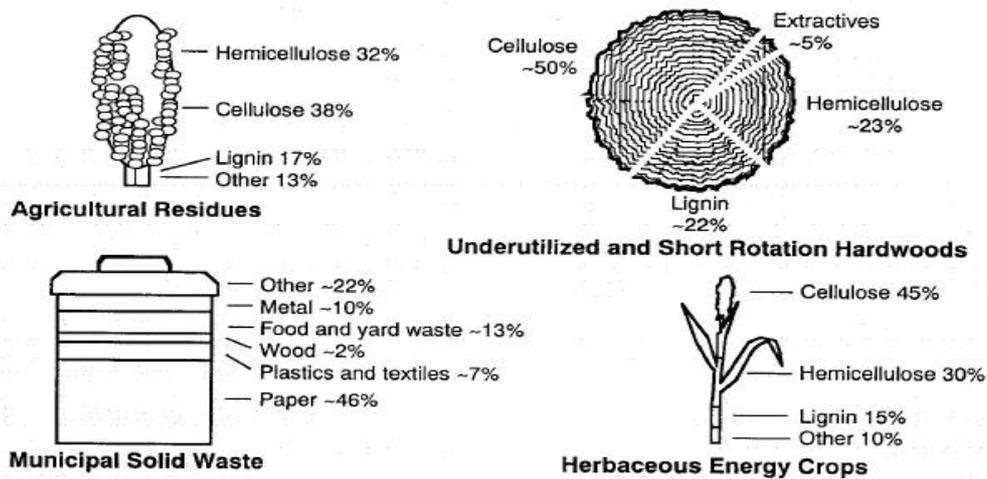


Figure 1.1. Composition of various cellulosic biomass types (McMillan, 1994).

Processing of cellulosic biomass to bio-ethanol consists of four major steps: pretreatment, hydrolysis, fermentation, and product separation/distillation (Balat and Balat, 2009; Mustafa and Havva, 2009). In order to increase hydrolysis yields, it is necessary to have a pretreatment step to open up the biomass and break down cell structures to a large extent, increasing accessibility of enzymes to cellulose (Hamelinck et al., 2005). Figure 1.2 illustrates a hydrolysis process using enzymes or acids to break down the chains of five and six carbon sugars into simple sugars for fermentation. However, because products of hydrolysis such as cellobiose and glucose are strong inhibitors of enzymes, simultaneous saccharification and fermentation (SSF) is often preferred. In SSF, enzymatic hydrolysis and fermentation are carried out simultaneously in one vessel, so glucose is fermented to ethanol immediately as it is released into

solution by enzymes (Wright et al., 1987; Wyman et al., 1992; Spindler et al., 1991).

Next product separation/distillation separates ethanol from the other components.

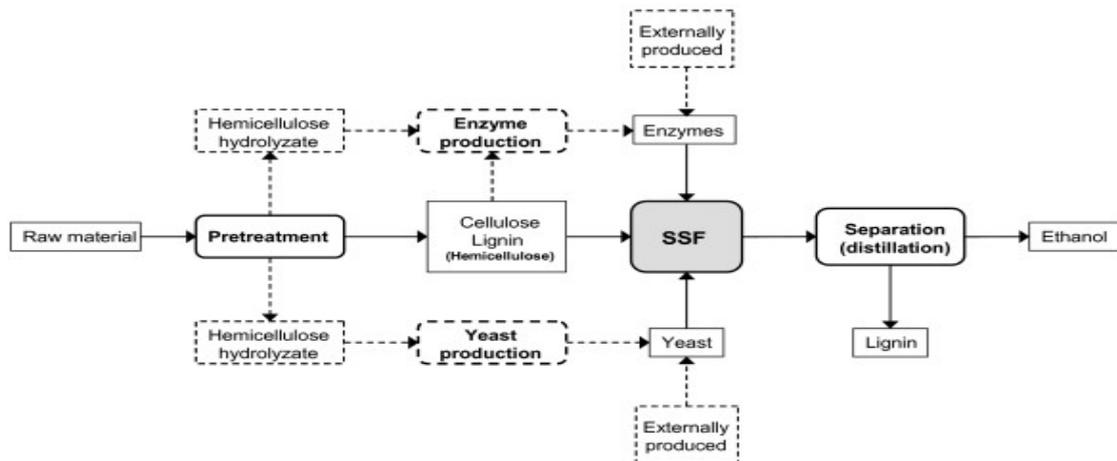


Figure 1.2. Schematic representation of an SSF process (Oloffon et al., 2008)

1.3 Challenges and opportunities for biofuels

Table 1.1 shows some of the potential benefits and challenges of making and using biofuels.

Table 1.1 Potential benefits and challenges of biofuels (Hoekman, 2009).

Improved energy security	Economic productivity	Environmental impacts
<ul style="list-style-type: none"> • Domestic supply • Distributed resources • Supply reliability • Petroleum reduction 	<ul style="list-style-type: none"> • Price stability • Increased rural development • Reduced trade deficit • Improved global competitiveness 	<ul style="list-style-type: none"> • Land and water use • Criteria air pollutants • Greenhouse gases • Wildlife habitat • Biodiversity • Carbon sequestration

The Energy Policy Act of 2005 (EPA 2005) presents a plan to use 14 billion gallons of biofuels and contains many provisions related to energy efficiency and

conservation and promotion of both traditional and renewable energy. EPCA 2005 includes production incentives to promote annual ethanol production from lignocellulosic biomass to 1 billion gallons in 2015 (Hoekman, 2009). The currently dominant feedstock to produce biofuels in the U.S. is corn, and Figure 1.3 shows how the annual ethanol production increased from 1.5 billion gallons per year in 1999 to 6.5 billion gallons per year in 2006, and around 14 billion in 2012 (RFA, 2012 Ethanol Industry Outlook), but further increase in corn ethanol will be restrained by the availability of agricultural land, water resources, and the debate between food versus fuel (Hoekman, 2009). In addition, there are concerns about the energy balance and greenhouse gas benefits of using corn (Hoekman, 2009). Therefore, producing bioethanol from lignocellulosic biomass becomes more important to provide clean energy.

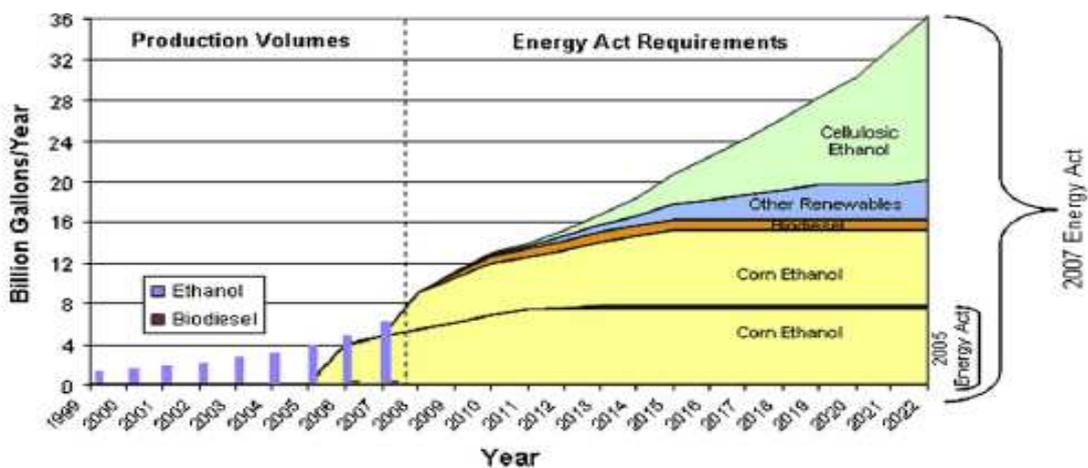


Figure 1.3. The renewable energy production and requirement (Hoekman, 2009).

Although lignocellulosic biomass is the most abundant and inexpensive global source (Wyman, 1999), the cost of producing bioethanol from this material is still high. The pretreatment step has been shown to be the most expensive single operation followed

by cellulase production, enzymatic hydrolysis, and sugar fermentation (Aden et al., 2002). For biofuels to become commercially successful, it is necessary to reduce the cost of overall process. In order to reach this goal, it is important to improve pretreatment technologies, lower enzyme costs, and develop better fermentation processes. Furthermore, a better understanding of the relationship between the recalcitrance of biomass and pretreatment and enzymatic hydrolysis are critical as well.

1.4 Thesis objectives

Cellulosic biomass can be utilized to produce ethanol, and ethanol is being considered as an important sustainable transportation fuel for the future. However, biomass must be pretreated prior to biological conversion to ethanol to overcome its natural resistance to sugar release. Woody biomass is particularly resistant to breakdown and must be size reduced to make pretreatment with steam, acid, or other chemicals that hydrolyze biomass to sugars effective. However, because mechanical size reduction of woody biomass can consume a significant amount of energy, it is important to understand how to minimize size reduction, while still realizing high sugar yields from the combined operations of pretreatment and enzymatic hydrolysis. Thus, this study focused on determining how sugar yields from pretreatment and subsequent enzymatic hydrolysis change with particle size. Hydrothermal pretreatment, downscaled composition analysis, and enzymatic hydrolysis were applied to different sized wood chips and fine powder of *Populus tremuloides* to determine the impact of wood size on sugar release. Furthermore, heat transport models were developed to better understand the results and figure out how

heat transfer affects pretreatment effectiveness and establish maximum dimensions to high sugar release.

1.5 Thesis outline

To support the outline above, Chapter 2 presents a literature review to summarize the promising feedstocks to produce bio-ethanol, key features of lignocellulosic biomass, and current leading pretreatment technologies. This chapter also introduces some key factors that affect the enzymatic digestibility and summarize some important literature that relate to the effects of particle size on pretreatment effectiveness. Chapter 3 shows the effects of different particle size on sugar left in solid after steam explosion pretreatment, enzymatic yield, and total sugar left. This chapter also tests different dimension of chips to see their effectiveness on mass loss after pretreatment. Chapter 4 used heat transfer models to better understand the results and figure out how heat transfer affects pretreatment effectiveness. Chapter 5 introduces some important factors that will affect the energy consumption in both size reduction and pretreatment process; it also estimates the energy consumption for size reduction and steam pretreatment process with different particle size. Chapter 6 summarizes key conclusions of this study and also provides some recommendations for future work.

1.6 References

Aden, A., Ruth, M., Ibsen, K., Jechura, J., Neeves, K., Sheehan, J., Wallace, B., 2002. Lignocellulosic Biomass to Ethanol Process Design and Economics Utilizing Co-Current Dilute Acid Prehydrolysis and Enzymatic Hydrolysis for Corn Stover. National Renewable Energy Laboratory, NREL/TP-510-32438.

Balat M., Balat H., 2009. Recent trends in global production and utilization of bioethanol fuel. *Appl. Energy* 86:2273–2282.

George W.H., Sara I., Avelino C., 2006. Synthesis of Transportation Fuels from Biomass: Chemistry, Catalysts, and Engineering. *Chem. Rev.* 106: 4044-4098

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

Hoekman S., 2009. Biofuels in the U.S. – Challenges and Opportunities. *Renewable energy* 34: 14-22

Hamelinck C.N., Geertje van Hooijdonk, Andre' PC Faaij. 2005. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. *Biomass and Bioenergy* 28: 384–410

Huber G.W., Iborra S., Corma A., 2006. Synthesis of Transportation Fuels from Biomass: Chemistry, Catalysts, and Engineering. *Chem. Rev.* 106:4044:4098

Kádár. Zs. Szengyel, Zs. Réczey K., 2004. Simultaneous saccharification and fermentation (SSF) of industrial wastes for the production of ethanol, *Industrial Crops and Products* 20:103–110

McMillan J.D., 1994. Pretreatment of lignocellulosic biomass. *American Chemical Society*, Ch15: 292-324

Mustafa B., Havva B., 2009. Recent trends in global production and utilization of bio-ethanol fuel. *Applied Energy* 86:2273-2282

Oloffon K., Magnus B., Gunnar L., 2008. A short review on SSF – an interesting process option for ethanol production from lignocellulosic feedstocks. *Biotechnology for Biofuels* 1:7 doi:10.1186/1754-6834-1-7

Spindler D.D., Wyman C.E., Grohmann K., 1991. Simultaneous saccharification and fermentation of pretreated woody crop to ethanol. *Appl. Biochem. Biotechnol* 28/29:773-836.

Wright J.D., Wyman C.E., Grohmann K., 1987. Simultaneous saccharification and fermentation of lignocelluloses: process evaluation. *Appl. Biochem. Biotechnol* 18:75-90

Wyman C.E., Spindler D.D., Grohmann K., 1992. Simultaneous saccharification and fermentation of several lignocellulosic feedstocks to fuel ethanol. *Biomass Bioenergy* 3(5): 301-7

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

Chapter 2

Literature Review

2.1. Promising Feedstocks to Produce Bioethanol

Biomass is seen as an important resource to produce renewable energy, and the bio-fuels produced from biomass can be used to reduce use of petroleum fuels. A number of environmental and economic benefits are claimed for bio-fuels, including reducing consumption of fossil fuels and lowering greenhouse gas emissions. There are many different applications to convert biomass into bio-fuels, but fermentation biomass into ethanol is the most widely used and researched approach. Figure 2.1 shows that from 2007 to 2011, U.S. ethanol production increased every year to reach nearly 14 billion gallons in 2011, and the U.S. is the largest ethanol producer in the world followed by Brazil (U.S. Department of Energy).

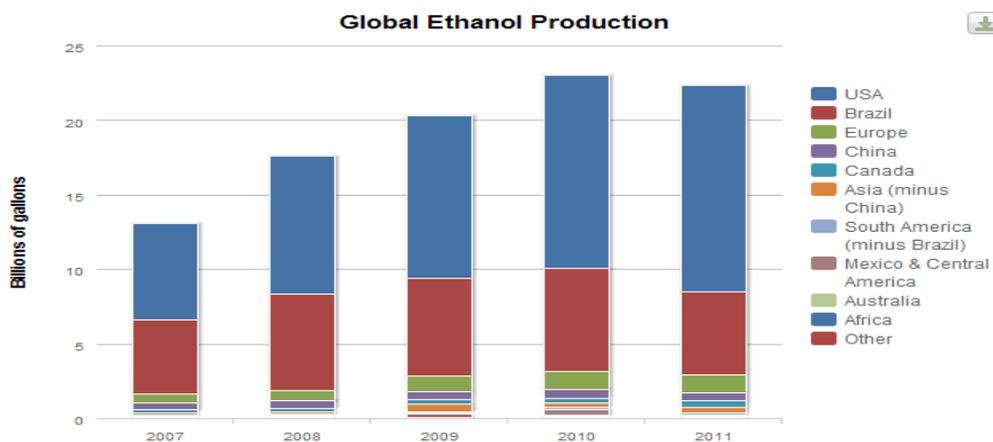


Figure 2.1. Global ethanol production by country or region, from 2007 to 2011 (U.S. Department of Energy).

Bio-ethanol can be produced from many different kinds of agricultural raw materials such as simple sugars (e.g., from sugar cane, sugar beets, sweet sorghum, and fruits), starch (e.g., corn, wheat, rice, potatoes, cassava, sweet potatoes, and barley), and lignocellulosic biomass (e.g., wood, straw, and grasses) (Smith, 2008; Lynd et al., 1999). Brazil is the largest producer of cane sugar in the world. During the period 2006 to 2007, 6.45 million hectares of its land were cultivated for sugar cane crops, and over three million of them was used to produce bio-ethanol (Trostle, 2008). However, the price of cane sugar has significant effects on the cost of ethanol production in that the cost of sugar represents more than 30% of the total production costs. Starch-rich crops such as corn and wheat are the main feedstock for ethanol production in the United States and Canada. In order to produce ethanol from starch, it is necessary to breakdown the α -1,4 and α -1,6 glycoside linkages of starch to form glucose which can be converted into ethanol by yeast (Klass, 1998, Mustafa and Havva, 2009). In general, the ethanol yield per mass of biomass from starch-rich crops is higher than from sugar cane. However, starch-rich crops have lower annual yields per cultivated hectare and require using more land to make the same amount of fuel (Sa'nchez and Cardona, 2008). On the other hand, lignocellulosic biomass can be the most promising feedstock for ethanol production because it offers lower price and is the most abundant globally. However, it is much more difficult to produce ethanol from cellulosic biomass than from sugar or starch because of its resistant nature to breakdown, but improvements in pretreatment, hydrolysis, and fermentation technologies have made it easier to overcome its recalcitrance with lower energy requirement (Wyman, 1999; Lynd et al., 2008).

2.2. Structure of Lignocellulosic Biomass

Lignocellulosic biomass is the most abundant and inexpensive global source of biomass but has a complex structure that is mainly composed of cellulose (35-50%), hemicellulose (15-35%), lignin (5-30%), and usually smaller amount of protein, extractives, and ash (Lynd et al., 2002). In general, the composition of lignocellulosic biomass depends on its species, growing region, weather, soil and other factors. Table 2.1 summarizes representative compositions of different lignocellulosic raw materials.

Table 2.1. Composition of various lignocellulosic raw materials (Lee, 1997)

	Corn stover	Wheat straw	Rice straw	Rice hults	Bagasse fiber	Cotton gin trash	Newsp rint	Populus tristis	Doug las fir
Carbohydrate (% of sugar equivalent)									
Glucose	39	36.6	41	36.1	38.1	20	64.4	40	50
Mannose	0.3	0.8	1.8	3	NA	2.1	16.6	8	12
Galactose	0.8	2.4	0.4	0.1	1.1	0.1	NA	NA	1.3
Xylose	14.8	19.2	14.8	14	23.3	4.6	4.6	13	3.4
Arabinose	3.2	2.4	4.5	2.6	2.5	2.3	0.5	2	1.1
Non-carbohydrate (%)									
Lignin	15.1	14.5	9.9	19.4	18.4	17.6	21	20	28.3
Ash	4.3	9.6	12.4	20.1	2.8	14.8	0.4	1	0.2
Protein	4	3	NA	NA	3	3	NA	NA	NA

Cellulose usually makes up the largest fraction of plant cell walls and is composed of a long chain of typically 100-14,000 glucose molecules attached to one another by β -(1,4) glycosidic linkages (Van Wyk, 2001). Intra-molecular hydrogen bonds between adjacent cellulose chains form crystalline microfibrils, and these microfibrils are the most important construction component of plant cell walls in terms of giving the plant strength. However, these microfibrils are not uniform: some amorphous regions are located between crystalline regions and are more easily hydrolyzed compared to highly crystalline regions (Congrove, 1998; Newman and Davidson, 2004; Edward, 2008).

Hemicellulose is the second most abundant constituent of lignocellulosic biomass and is often composed of short chains of five-carbon sugars (xylose, arabinose), six carbon sugars (glucose, mannose, galactose), acetic acid, and uronic acids. Unlike cellulose, hemicelluloses are not chemically homogeneous or crystalline. The main component of hemicelluloses from hardwoods is usually xylan, but the dominant component from softwoods is galactoglucomannans (Fengel and Wegener, 1984; Saha, 2003). Hemicellulose usually forms amorphous structures that make it more easily hydrolyzed into monomeric sugars by thermochemical pretreatment. However, hemicelluloses also play an important role in connecting lignin and cellulose fibers to form a cross-linked fibrils network that also protects cellulose from enzymatic attack (Laureano-Perez et al., 2005; Hendriks and Zeeman, 2009)

Lignin is a cross-linked aromatic polymer with relatively high molecular weight. Lignin is composed of three different phenylpropene units: *p*-hydroxyphenyl, guaiacyl, and syringyl. Lignin not only gives physical strength to the cell walls but also provides structural support and impermeability. Since lignin is not digestible by animal enzymes and also non-water soluble, it provides resistance to insects and pathogens to plant cell wall, but the same characteristics make lignin hard to biologically deconstruct (Laureano-Perez et al., 2005; Hendriks and Zeeman, 2009).

2.3. The Effects of Lignocellulosic Biomass Pretreatment

It is far more difficult to produce ethanol from cellulosic biomass than from sugar or starch because of its resistant nature to breakdown. The four primary operations to convert cellulosic biomass to ethanol are pretreatment, hydrolysis, fermentation, and product separation/distillation (Balat and Balat, 2009; Mustafa et al. 2009). The purpose of pretreatment is to open up the biomass structure to increase enzyme accessibility. It is important that the pretreatment process maximizes the total sugar yields from pretreatment and enzymatic hydrolysis together and minimize sugar losses and formation of inhibitory products (Modenbach et al., 2012). Because pretreatment can be one of the most expensive steps, it must be energetically and chemically efficient, so the overall biomass conversion process can be profitable (Mosier et al., 2005). Numerous pretreatments processes have been devised, and each of them has advantages and disadvantages. Table 2.2 summarizes the effects of different pretreatment methods.

Table 2.2. Effects of various pretreatment methods on the three fractions of lignocellulosic material (Mosier et al., 2005)

	Increases accessible surface area	Decrystallizes cellulose	Removes hemicellulose	Removes lignin	Alters lignin structure
Uncatalyzed 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 Recycled Percolation

Pretreatment methods can be categorized into biological, physical and chemical or their combinations. Physical pretreatment refers to the reduction of feedstock size or other changes in biomass structure by mechanical means to increase the surface areas and realize better enzyme accessibility (Zhu et al., 2010). Milling (e.g., ball milling, two-roll milling, hammer milling, colloid milling, and vibro energy milling) and irradiation (e.g., by gamma rays, electron beams, or microwaves) are the most common types of physical pretreatments (Taherzadeh et al., 2008, Yang et al., 2008, Da Costa Sousa et al., 2009). However, physical pretreatments have major disadvantages in that these pretreatment processes require lots of energy and do not remove lignin or other biomass components from the feedstock (Chandra et al., 2007).

Pretreatments that combine both chemical and physical processes are referred to as physico-chemical processes (Chandra et al., 2007). Steam explosion pretreatment is a hydrothermal technology that rapidly heats biomass by high pressure steam without adding chemicals to treat biomass from few seconds to several min and suddenly reduces the pressure at the end of process to make biomass undergo an explosive decompression that also disrupts its physical structure (Taherzadeh et al., 2008). During steam explosion pretreatment, most of the hemicelluloses are removed, thus improving enzyme digestibility. However, reaction for longer times would degrade sugar and also form inhibitors to decrease the yield of enzymatic hydrolysis and fermentation. In order to reduce the formation of the degradation products, steam pretreatment can be performed with addition of sulfur dioxide (SO₂). Impregnation of SO₂ before pretreatment can enhance carbohydrate hydrolysis rate by increasing the accessibility of cell walls (Chandra et al., 2007), and shorter reaction times reduce formation of degradation

products. Steam explosion pretreatment has low energy requirement compare to physical size reduction pretreatment and no use of toxic chemicals, thus reducing the environmental impact. It is the most commonly used pretreatment method for lignocellulosic biomass (Sun and Cheng, 2002).

Ammonia fiber expansion (AFEX) is another physico-chemical pretreatment. In this process, biomass is exposed to liquid ammonia at relatively high temperature (90°C-100°C) and pressure for a period of time, with the pressure suddenly reduced at the end of process (Sun and Cheng, 2002; Mohammad et al.2008). The overall concept for AFEX is similar to steam explosion, but most of the biomass components following AFEX pretreatment remain in the solid material and not the liquid fraction. The advantages of AFEX include that it can successfully leave the hemicellulose and cellulose fractions intact. AFEX also forms less by-products inhibitory to downstream biological processes (Lau et al., 2009). However, the ammonia must be recycled after pretreatment to reduce the process cost and protect the environment (Sun and Cheng, 2002; Mohammad et al., 2008).

Acid pretreatment has been developed for many years. Concentrated acids such as H₂SO₄ and HCl are toxic, corrosive, and hazardous and expensive to recover as required to be cost-effective (Sun and Cheng, 2002) Therefore, dilute acid pretreatment is the most commonly applied option among the chemical pretreatment methods. During dilute acid pretreatment, hemicellulose is hydrolyzed to fermentable sugars that dissolve in the liquid phase, and less severe conditions have been developed to remove over 90% of hemicelluloses. Although dilute acid pretreatment only removes limited lignin and glucose from the solid material, it still significantly increases enzyme accessibility

because most of hemicelluloses are removed and lignin structure is disrupted (Wyman, 1996; Mosier et al., 2005). Dilute acid pretreatment also has some disadvantages such as higher capital cost for reactors that are resistant to corrosion and the need for neutralization before enzymatic hydrolysis or fermentation (Sun and Cheng, 2002).

2.4. Factors Affecting Enzymatic Digestibility

Enzymatic hydrolysis, a process converts lignocellulosic biomass into fermentable sugars, requires milder operating conditions (pH 4.8 and temperature 45°C-50°C) than chemical processes and do not have a severe corrosion problem. Thus, its utility cost is lower than for acid or alkaline hydrolysis (Yang et al., 2011, Sun and Cheng, 2002). Enzymatic hydrolysis also has other advantages such as higher yield, higher selectivity, and lower energy cost (Yang et al., 2011). However, the primary challenge is the high cost of enzymes.

In order to improve the efficiency of enzymatic hydrolysis to reduce enzyme costs, it is important to understand enzyme interactions with lignocellulosic biomass. There are few major factors that have been identified that affect the digestibility or reactivity of cellulosic biomass by enzymes: porosity, cellulose fiber crystallinity, lignin content, and hemicellulose content (McMillan, 1994; Yang et al., 2011). The effects of such structural features on biomass digestibility are summarized in Table 2.3. Porosity is the accessible surface area for the enzyme, but it is hard to quantify the enzymatic hydrolysis rate as a function of accessible surface area because surface area is not easy to independently manipulate in pretreatment. Many techniques have been used to estimate surface area, including nitrogen adsorption, mercury porosimetry, and dye adsorption (McMillan,

1994; Lynd et al., 2002). Various studies have shown that accessible surface area plays an important role in biomass enzymatic hydrolysis (Gharpuray et al., 1983; Sinitsyn et al., 1991).

It is broadly believed that the crystalline cellulose have negative effects on the efficiency of enzymatic hydrolysis. Physical pretreatment is the common way to destroy its crystalline structure and increase surface area as well (Chandra et al., 2007). However, some studies have shown that further reduction of particle size below 40-mesh does not increase hydrolysis more (Chang et al., 1997; Zhu et al., 2008).

Lignin and hemicelluloses are two major inhibitors to enzyme attack on cellulose. Lignin binds cellulosic fibers together to give physical strength to the cell walls and provides structural support but also reduces the accessibility of cellulose to enzymes (Yang et al., 2011; Kumar and Wyman, 2009). Many studies have shown that the biomass digestibility improved with lignin removal. Delignification disrupts the lignin structure and subsequently leads to an increase in internal surface area, thus increasing the enzymatic hydrolysis rate (Fan et al., 1987; Gharpuray et al., 1982; Zhu et al., 2008). Removing hemicelluloses also significantly improves the digestibility of lignocellulosic biomass by increasing the surface area accessible to enzymes (McMillan, 1994; Jeoh et al., 2007; Jeoh et al., 2005). Studies of dilute acid pretreatment have shown that hemicelluloses removal improves digestibility, even though dilute acid pretreatment only removed a limited amount of the lignin (Grohmann et al., 1985, 1986).

Table 2.3. Summary of relationship between structural features and digestibility (Zhu et al., 2008)

Structural features		Relationship between structural feature and digestibility	Reference
Physical	Surface area	Positive	Grethlein (1985), Sinitsyn et al. (1991)
	Crystallinity	Negative	Caulfield and Moore (1974), Fan et al. (1981)
		No correlation	Grethlein, (1985), Puri (1984)
	Degree of polymerization	Negative	Puri (1984)
		No correlation	Sinitsyn et al. (1991)
	Pore volume	Positive	Grethlein (1985), Weimer and Weston (1985)
Particle size	No correlation	Draude et al. (2001), Sinitsyn et al. (1991)	
Chemical	Lignin	Negative	Draude et al. (2001), Mooney et al. (1998)
	Hemicellulose	Negative	Grohmann et al. (1989), Kim et al. (2003)
	Acetyl group	Negative	Grohmann et al. (1989), Kong et al. (1992)

2.5. Effects of Particle Size on Pretreatment Effectiveness

Pretreatment is a necessary step for enzymatic hydrolysis to have reasonable sugar yields (Hendriks and Zeeman, 2009; Mosier et al., 2005). In order to produce ethanol economically, the energy demand should be minimized (Laser et al., 2002). In addition, using larger wood chips in pretreatment can save a lot of energy in the size reduction step prior the pretreatment (Cadoche and Lopez, 1989, Zhu et al., 2010), thus it is essential to know the effects of particle size on pretreatment effectiveness. Steam explosion is a relatively mature and the most common technology that has been used for lignocellulosic biomass pretreatment, and there are many studies that have provided the information about the relationship between particle size and overall sugar yield for steam

explosion pretreatment (Cullis et al., 2004; Ballesteros et al., 2000; Ballesteros et al., 2002; Maria et al., 2003; Brownell et al., 1986; Liu et al., 2013; Bernardo et al., 2011)

However, there are only a few studies indicating that larger particle size is a better choice during steam explosion pretreatment. For example, Cullis et al., (2004) in a study used Douglas fir (softwood) with the following dimensions: < 0.422 mm, 1.5×1.5 cm, and 5×5 cm, and pretreated them with steam explosion with SO₂ as a catalyst. Ballesteros et al., (2000) who treated pine (softwood) by steam explosion pretreatment with just water, but milled feedstock to smaller particle size (2-5 mm, 5-8 mm, and 8-12 mm). These two studies reported similar results that larger size chips achieved higher cellulose and hemicelluloses recovery as well as better enzymatic digestibility.

Ballesteros et al., (2002) used herbaceous (*Brassica carinata residues*) as a feedstock, milled it to different particle sizes (2-5 mm, 5-8 mm, and 8-12 mm), and applied steam explosion pretreatment. Their results indicated that the best pretreatment conditions for large particle size were 210°C for 4-8 min giving >80% of overall yield, however, for smaller particle sizes the yield was less than 70%. However, 5-8 mm particle size had the highest overall yield at less severe pretreatment conditions (190°C for 4-8 min). In another study, working with corn stover, Liu et al. (2013) showed that larger biomass particle (2.5 cm L×1.0 cm W×1.0 cm H) gave higher sugar yields during enzymatic hydrolysis. Furthermore, Maria et al. (2003) indicated that steam exploded poplar (hardwood) showed no significant effect of particle size on both enzymatic digestibility in hydrolysis and simultaneous saccharification and fermentation (SSF). However, Brownell et al., (1986) represented that higher pretreatment temperature with longer reaction time may cause uneven cooking of the larger chips, i.e., overcooking the

exterior of the chips and undercooking in the center, therefore, forming more degradation product and lower sugar yield. Although according to these studies, larger particles had similar or higher overall sugar yields than smaller materials when pretreated at high severity, however, most of the studies used different biomass as feedstock and applied different pretreatment conditions. Thus it can not be firmly concluded that larger particles will always be the better choice at all pretreatment conditions. Furthermore, little work has been done to look at the larger particle size (>50 mm). Therefore, it is necessary to have a more systematic study of wider range of particle size under different pretreatment conditions, using various feedstocks (Bernardo et al., 2011)

2.6. Conclusions

Lignocellulosic biomass is the most abundant and inexpensive source of renewable carbon globally and has important benefits as a feedstock to produce bioethanol. However, lignocellulosic biomass must be pretreated prior to biological conversion to ethanol to overcome its natural resistance to sugar release. The cost of ethanol produced from lignocellulosic biomass is still more expensive than petroleum based fuels due to the high costs of pretreatment and enzymes. Thus, it is vital to reduce processing costs but still realize nearly theoretical sugar yields. Several factors have been identified to affect the biomass digestibility and reactivity by enzymes that include porosity, cellulose fiber crystallinity, lignin content, and hemicelluloses content (McMillan, 1994; Yang et al., 2011). The different pretreatment methods that have been developed to reduce biomass recalcitrance have their own advantages and disadvantages, and it is critical to choose pretreatment based on the feedstock used and the design goal. For example, steam explosion has been recognized as one of the most effective

pretreatment methods for hardwoods, but it is ineffective for softwoods because the latter contain relatively larger amounts of condensed type lignin (Chikako et al., 2012). Evident from literature represented that particle size directly influences overall sugar yield. Larger size of biomass normally has higher or similar yields compared to smaller particle size. However, all literatures researched different feedstock in different pretreatment conditions, so more systematic study of wider range of particle size under different pretreatment conditions, using various feedstocks, will be required to better understand the effects of particle size on pretreatment effectiveness. In order to achieve low cost ethanol from lignocellulosic biomass, it is necessary to devise more economical pretreatments, figure out the best operating conditions, and choose low-cost feedstocks.

2.7. References

- Bin, Y., Ziyu, D., Shi-You, D., Wyman, C.E., 2011. Enzymatic hydrolysis of cellulosic biomass, *Biofuels* 4(2):421-450
- Balat M., Balat H., 2009. Recent trends in global production and utilization of bioethanol fuel. *Appl Energy* 86:2273–2282.
- Ballesteros, I., Oliva, J.M., Navarro, A.A., Gonzalez, A., Carrasco, J., Ballesteros, M., 2000. Effect of chip size on steam explosion pretreatment of softwood. *Applied Biochemistry and Biotechnology*, 84– 86, 97–110.
- Bernardo, C.V. Jr., Bruce, S.D., K.C. Ting, Vijay Singh. 2011. Influence of feedstock Particle Size on Lignocellulose Conversion- A Review. *Biochemical Biotechnology* 164: 1405-1421
- Brownell, H.H., Yu, E.K.C., Saddler, J.N., 1986. Steam-explosion pretreatment of wood: effect of chip size, acid, moisture content and pressure drop. *Biotechnol. Bioeng.* 28:792-801
- Chandra, R., Bura, R., Mabee, W., Berlin, A., Pan, X., Saddler, J., 2007. Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics. *Adv. Biochem. Eng. Biotechnol.*, 108, 67-93.
- Chang, V.S., Burr, B., Holtzapple, M.T., 1997. Lime pretreatment of switchgrass. *Appl. Biochem. Biotechnol.* 63–65, 3–19.
- Chikako A., Chizuru S., Yoshihiro U., Jun S., Yoshitoshi N., 2012. Effect of steam explosion pretreatment with ultra-high temperature and pressure on effective utilization of softwood biomass. *Biochemical engineering journal* 60:25-29
- Cosgrove, D. J., 1998. Cell Walls: Structures, Biogenesis, and Expansion. In: *Plant Physiology*. , In L. Taiz and E. Zeiger, eds. Sunderland: Sinauer Associates, Inc.
- Cullis, I.F., Saddler, J.N., Mansfield, S.D., 2004. Effect of initial moisture content and chip size on the bioconversion efficiency of softwood lignocellulosics. *Biotechnology and Bioengineering*, 85: 413-421
- Da Costa Sousa, L., Chundawat, S.P.S., Balan, V., Dale, B.E., 2009. `Cradle-to-grave' assessment of existing lignocellulose pretreatment technologies. *Curr. Opin. Biotechnol.*, 20(3), 339-347.
- Dufey A., 2006. Biofuels production, trade and sustainable development: emerging issues. *Environmental Economics Programme, Sustainable*

Markets Discussion Paper No. 2, International Institute for Environment and Development (IIED), London.

Duff, S.J.B., Murray, W.D., 1996. Bioconversion of forest products industry waste cellulose to fuel ethanol: a review. *Bioresource technology* 55:1-33

Edward M.R., 2008. Genomics of cellulosic biofuels, *Nature* 14: 841-845.

Fan, L.T., Lee, Y.H., Beardmore, D.H., 1981. The influence of major structural features of cellulose on rate of enzymatic hydrolysis. *Biotechnol. Bioeng.* 23, 419–424.

Fengel, D., Wegener, G., 1984. *Wood: Chemistry, Ultrastructure, Reactions*. De Gruyter, Berlin.

Gharpuray, M.M., Fan, L.T., Lee, Y., 1982. Caustic pretreatment study for enzymatic hydrolysis of wheat straw. In: Soltes, E.J. (Ed.), *Wood and Agriculture Residues – Research on Use for Feed, Fuels, and Chemicals*. Academic Press, New York, pp. 369–389.

Gharpuray, M.M., Lee, Y.H., Fan, L.T., 1983. Structural modification of lignocellulosics by pretreatments to enhance enzymatic hydrolysis. *Biotechnol. Bioeng.* 25, 157–172.

Grohmann, K., Torget, R., Himmel, M., 1985. Optimization of dilute acid pretreatment of biomass. *Biotechnol. Bioeng. Symp.* 15, 59–80.

Grohmann, K., Torget, R., Himmel, M., 1986. Dilute acid pretreatment of biomass at high solid concentrations. *Biotechnol. Bioeng. Symp.* 17, 135–151.

Hendriks A.T.W.M., Zeeman G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass, *Bioresource Technology* 100:10–18

International Risk Governance Council (IRGC), *Governing the risks and opportunities of bioenergy*, IRGC's bioenergy Project, Geneva, Switzerland, 2007. [cited; available from: [/www.irgc.org/irgc/IMG/pdf/IRGC_ConceptNote_Bioenergy_1408S](http://www.irgc.org/irgc/IMG/pdf/IRGC_ConceptNote_Bioenergy_1408S)]

Jeewon Lee. 1997. Biological conversion of lignocellulosic biomass to ethanol, *Journal of Biotechnology* 56: 1–24.

Jeoh, T., Ishizawa, C.I., Davis, M.F., Himmel, M.E., Adney, W.S., Johnson, D.K., 2007. Cellulase digestibility of pretreated biomass is limited by cellulose accessibility. *Biotechnol. Bioeng.*, 98(1), 112-122.

- Jeoh, T., Johnson, D.K., Adney, W.S., Himmel, M.E., 2005. Measuring cellulase accessibility of dilute-acid pretreated corn stover. Preprints of Symposia - American Chemical Society, Division of Fuel Chemistry, 50(2), 673-674.
- Klass, D.L., 1998. Biomass for Renewable Energy, Fuels and Chemicals. San Diego: Academic Press.
- Kumar, R., Wyman, C.E., 2009. Cellulase adsorption and relationship to features of corn stover solids produced by leading pretreatments. Biotechnol. Bioeng., 103(2), 252-267.
- Laureano-Perez, L., Teymouri, F., Alizadeh, H., Dale, B.E., 2005. Understanding factors that limit enzymatic hydrolysis of biomass. Appl. Biochem. Biotechnol., 1081-1099.
- Lau, M.W., Dale, B.E., 2009. Cellulosic ethanol production from AFEX-treated corn stover using *Saccharomyces cerevisiae* 424A(LNH-ST). Proc. Natl. Acad. Sci., 106(5), 1368-1373.
- Lynd, L.R., Wyman, C.E., Gerngross, T.U. 1999. Biocommodity Engineering. Biotechnol. Prog., 15(5), 777-793.
- Lynd, L.R., Weimer, P.J., van Zyl, W.H., Pretorius, I.S., 2002. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiology and Molecular Biology Reviews, 66, 506.
- Lynd, L.R., Laser, M.S., Bransby, D., Dale, B.E., Davison, B., Hamilton, R., Himmel, M., Keller, M., McMillan, J.D., Sheehan, J., Wyman, C.E. 2008. How biotech can transform biofuels. Nat. Biotechnol., 26(2), 169-72.
- Modenbach, A.A., Nokes, S.E., 2012. The use of high-solids loadings in biomass pretreatment- A review, Biotechnology and Bioengineering 109:1430-1442.
- Maria, J.N., Paloma M., Ignacio B., Jose M.O., Araceli C., Mercedes B., 2003. Hydrothermal Pretreatment Conditions to enhance Ethanol Production from Poplar Biomass. Biochemistry and Biotechnology 105-108: 87-100
- McMillan, J.D., 1994. Pretreatment of lignocellulosic biomass, Bioprocessing branch, Alternative fuels division, National renewable energy laboratory.
- Mohammad J.T., Keikhosro K., 2008. Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. International Journal of Molecular Sciences 9:1621-1651
- Mosier, N., Wyman, C.E., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol., 96(6), 673-686.

- Mustafa B., Havva B., 2009. Recent trends in global production and utilization of bio-ethanol fuel. *Applied Energy* 86:2273-2282
- Newman R., Davidson T., 2004. Crystalline forms and cross-sectional dimensions of cellulose microfibrils in the Florideophyceae (Rhodophyta). *Botanica Marina* 47: 490-495.
- Sánchez ÓJ & Cardona CA (2008) Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresour Technol* 99(13):5270-5295.
- Saha, B.C., 2003. Hemicellulose bioconversion. *J. Ind. Microbiol. Biotechnol.* 30, 279–291.
- Sinitsyn, A.P., Gusakov, A.V., Vlasen, E.Y., 1991. Effect of structural and physico-chemical features of cellulosic substrates on the efficiency of enzymatic hydrolysis. *Appl. Biochem. Biotechnol.* 30, 43–59.
- Smith A.M., 2008. Prospects for increasing starch and sucrose yields for bioethanol production. *Plant J* 54:546–58.
- Soccol C. R., Vandenberghe L.P.D.S., Medeiros A.B.P., 2010. “Bioethanol from lignocelluloses: status and perspectives in Brazil,” *Bioresource Technology* 101: 4820–4825.
- Sun Y., Cheng J.Y., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology* 83:1-11
- Taherzadeh M.J., Karimi K., 2008. Pretreatment of lignocellulosic waste to improve ethanol and biogas production: a review. *Int. J. Mol. Sci.* 9: 1621:1651.
- Trostle R.G., 2008. Agricultural supply and demand: factors contributing to the recent increase in food commodity prices. USDA Economic Research Service, Report WRS-0801, Washington, DC
- Van Wyk J.P., 2001. Biotechnology and the utilization of biowaste as a resource for bioproduct development. *Trends Biotechnol.* 19: 172–177
- Wyman, C.E., 1996. *Handbook on bioethanol: production and utilization*; Taylor & Francis: Washington DC, USA,
- Wyman C.E. 1999. Biomass ethanol: Technical progress, opportunities, and commercial challenges. *Annual Review of Energy and the Environment* 24: 189-226.
- Yang B, Dai Z, Ding S-Y, Wyman C.E., 2011. Enzymatic hydrolysis of cellulosic biomass. *Biofuels* 2(4):421-450.

Yang, B., Wyman, C.E., 2008. Pretreatment: the key to unlocking low-cost cellulosic ethanol. *Biofuels, Bioprod. Biorefin.*, 2(1), 26-40.

Zhu L., Jonathan P.O., Vincent S.C., Cesar B.G., Mark T.H., 2008. Structural features affecting biomass enzymatic digestibility. *Bioresource technology* 99: 3817-3828.

Liu Z-H., Qin L., Jin M-J., Pang F., Li B-Z., Kang Y., Dale B.E., Yuan Y-J., 2013. Effects of biomass particle size on steam explosion pretreatment performance for improving the enzyme digestibility of corn stover. *Industrial Crops and Products* 44(0):176-184

Zhu J.Y., Pan X.J., 2010. Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation, *Bioresource Technology* 101:4992-5002

Chapter 3

The Effect of Chip Size on the Effectiveness of Hydrothermal Pretreatment of Woody Biomass

3.1. Abstract

Woody biomass is particularly resistant to breakdown and must be size reduced to make pretreatment with acid or other chemicals that hydrolyze biomass to sugars effectively. However, because mechanical size reduction of woody biomass can consume a significant amount of energy, it is important to understand how to minimize size reduction while still realizing high sugar yields from the combined operations of pretreatment and enzymatic hydrolysis. Thus, in this project, different sized wood chips (2×1.5×1.5", 2×1.5×0.5", as length x width x thickness, respectively, and powder) were pretreated by steam explosion at 180°C and 200°C over a range of reaction times, and the solids were subjected to enzymatic hydrolysis to examine whether yields suffer for larger chips. The results showed that above a certain pretreatment severity ($R_0=3.8$), large wood chips could give similar enzymatic sugar yields as small chips and powder, making size reduction unnecessary, thus saving around 75% of energy.

3.2. Introduction

Woody biomass can be an important feedstock for production of liquid fuels and has several advantages, such as a high density to reduce transportation costs, harvesting year round to avoid long-term storage, and low ash content (Zhu et al., 2010). However, woody biomass is recalcitrant to enzymatic sugar release and typically requires significant size reduction and severe pretreatment to achieve reasonable sugar yields (DeMartini and Wyman, 2011; Zhu et al., 2010). Due to its large size and high density, mechanical size reduction of woody biomass can consume several times more energy than required for agricultural residues (Zhu et al., 2010; Sun and Cheng, 2002; Cadoche and Lopez, 1989). This difference is illustrated by the energy requirements for mechanical comminution of different lignocellulosic materials shown in Table 3.1.

Table 3.1. Energy requirements for mechanical comminution of lignocellulosic materials to different size ranges (Cadoche and Lopez, 1989)

Lignocellulosic materials	Final size (mm)	Energy consumption (kWh/ton)	
		Knife mill	Hammer mill
Hardwood	1.60	130	130
	2.54	80	120
	3.2	50	115
	6.35	25	95
Straw	1.60	7.5	42
	2.54	6.4	29
Corn stover	1.60	NA ^a	14
	3.20	20	9.6
	6.35	15	NA ^a
	9.5	3.2	NA ^a

Because thermal or chemical pretreatment of wood chips can alter the chemical composition and physical structure by partially removing some cell-wall components such as hemicelluloses and lignin, post-chemical pretreatment size reduction can significantly lower energy consumption for size-reduction of wood chips (Zhu et al., 2010).

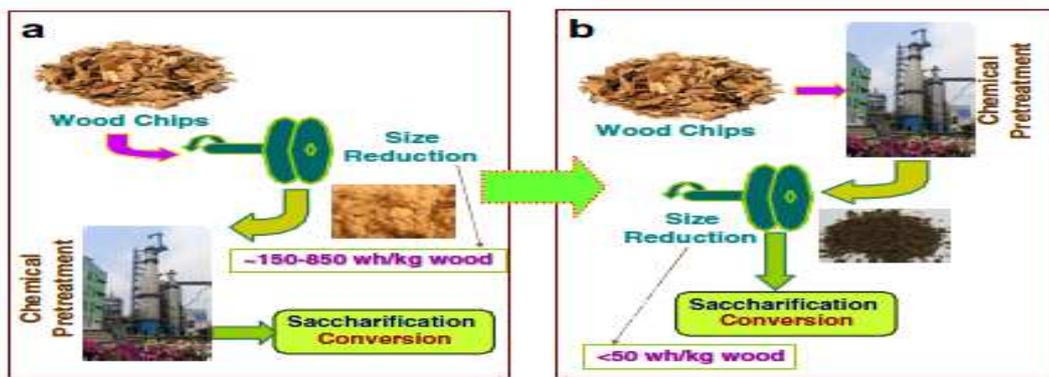


Figure 3.1. Schematic diagram shows mechanical size reduction of wood applied to (a) pre- and (b) post-chemical pretreatment to reduce energy consumption (Zhu et al., 2010).

In order to accommodate small equipment and focus on reaction kinetics, most laboratory pretreatment research has been performed with woody biomass ground to a powder (<2 mm), but grinding to this size is unlikely to be desirable in an industrial setting. Many studies have determined relationships between particle size and overall sugar yields from steam explosion pretreatment, but most studies employed particle sizes of <12 mm, as a result, the effects of steam explosion pretreatment on subsequent enzymatic hydrolysis is poorly understood for larger chips. To better understand how the sugar yields change with particle size, this study applied steam explosion pretreatment to different sizes of wood chips (2×1.5×1.5”, 2×1.5×0.5”, and powder) over a range of times and for temperatures of 180°C and 200°C.

3.3. Material and Methods

Figure 3.2 illustrates the experimental procedure employed. Wood chips and powder were prepared for steam explosion pretreatment, with about half of the pretreated material used for composition analysis and the other half for enzymatic hydrolysis.

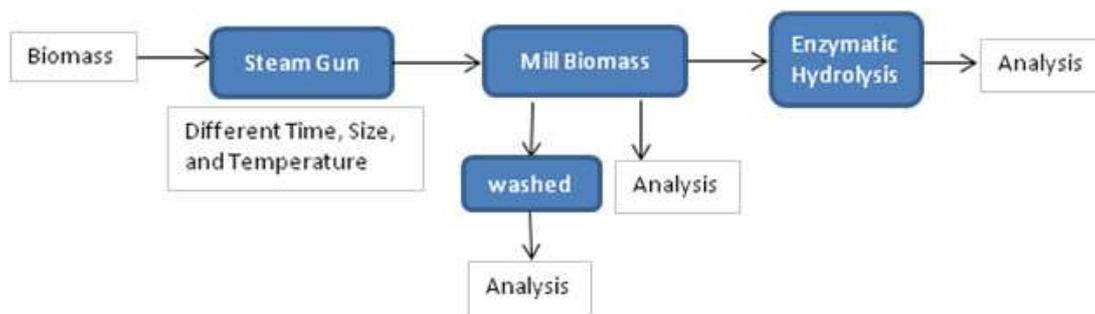


Figure 3.2. Sequence of steps for biomass sugar yield analysis for different wood sizes

3.3.1. Feed Materials

The trembling Aspen (*Populus tremuloides*) used in this study was provided by Benchmark International in High Level, Alberta, Canada. Each sample was collected as approximately 20-80 mm thick disks, with a diameter of around 38 cm. Samples were shipped to the University of California Riverside, CA, USA, and each sample was stored in plastic Ziploc bags in a -20°C freezer until use.

After thawing out a cross section, chips were made using a chisel. All chips were taken from the outer portion of the section but not including the tree bark. Chips were made with the following length, width, and thickness dimensions, respectively: 2×1.5×1.5” and 2×1.5×0.5”. All chips were placed in a 105°C oven (Heratherm OGS100, Thermo Scientific, Pittsburgh, PA, USA) until totally dry, and the weight of each chip

was recorded. A smaller portion from each cross section was milled (Thomas Wiley laboratory mill model-4, Arthur H. Thomas company, Philadelphia, PA, USA.) through a 20 mesh screen (<0.85 mm) and allowed to air dry for a few days.

3.3.2. Pretreatment

In this study, steam explosion pretreatment was employed without addition of any acids or other chemicals as catalysts. For each pretreatment, one large and one small chip was placed in a basket made from woven metal mesh (8×8 mesh particle-sifting woven 316 stainless steel 0.635 mm diameter wire cloth, McMaster Carr, CA,USA). For the powder materials, about 5 g was loaded into a cylindrical metal basket (Figure 3.3A), with its side made of woven metal mesh as well. Two woven metal mesh baskets and cylindrical basket were tied together, as shown in Figure 3.3B, and the assembly was placed into a 4L Hastelloy steam gun reactor (Figure 3.3D). Steam generated by a high pressure steam boiler (Figure 3.3C,FB-075-L, Fulton Companies, Pulaski, NY) was used to rapidly heat up the samples to 180°C for periods from 4 to 60 min, and 200°C for times of 2.5 to 18 min. At the end of the reaction, the reactor pressure was suddenly dropped by opening a valve at the bottom of the reactor, cooling the contents quickly to 100°C. Pretreatment liquid was discharged with steam and could not be collected for analysis. After cooling, the chips and powder were removed from the steam gun for further analysis.

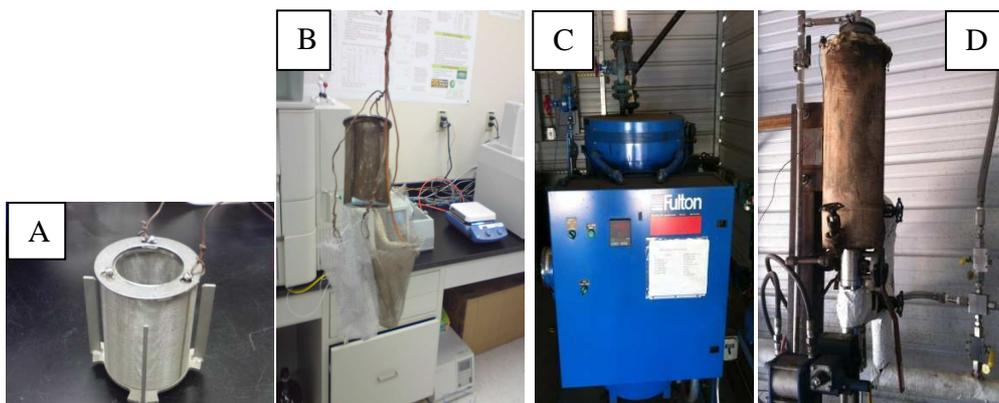


Figure 3.3. (A) Woven metal mesh basket. (B) top basket was used to place milled material, and bottom nets were used to place woody chips. (C) Steam boiler (D) steam gun reactor used for pretreatment.

3.3.3. Material Preparation

Half of the pretreated chips were placed in a 105°C oven until totally dry, and their weight recorded to measure mass loss during pretreatment. Afterward, these samples were milled through a 20-mesh screen (<0.85 mm), and 1 g of the milled material was washed with 50 mL DI water to remove any soluble sugars or other components that may have remained inside the chips. Washed and unwashed samples were stored in different plastic Ziploc bags in a freezer (0°C) until further analysis. Another half of the pretreated chips was directly milled before drying, and the milled materials were stored in plastic Ziploc bags in a freezer (0°C) until enzymatic hydrolysis.

3.3.4. Composition Analysis

The composition of each sample was measured by the downscaled compositional analysis method (DeMartini et al., 2011). Other than using centrifugation rather than filtration for solid liquid separation, this procedure is similar to the standard NREL method (Sluiter et al., 2008, Sluiter et al., 2005) but requires 100 times less biomass. A solid and liquid dispensing robotics platform (Core Module Standard Configuration 2 equipped with Sartorius WZA65-CW balance and six 25-mL biomass-dispensing hoppers, Symyx Technology Sunnyvale, CA) distributed 3 mg of each sample into 1.5 mL high recovery glass HPLC vials (Figure 3.4 A, Agilent, Santa Clara, CA) and placed them in the orange reactor block shown in Figure 3.4B that can hold a maximum of 48 1.5-2 mL glass vials. Sugar concentrations were determined by high performance liquid chromatography (HPLC, Model Alliance 2695, Waters Corporation, Milford, MA) using a model 2414 RI detector and an Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA). Klason lignin content was measured by the gravimetric method, which provided a good estimate for materials due to the low ash contents of *Populus tremuloides* (DeMartini et al., 2011).

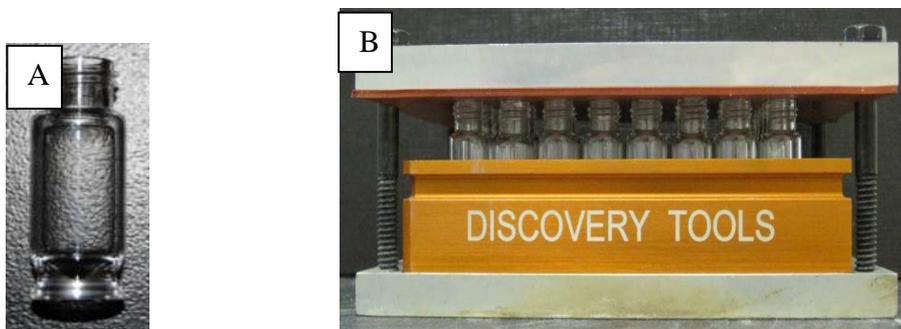


Figure 3.4. (A) A 1.5 mL high recovery glass HPLC vial (B) Discovery Tools reactor block that can hold up to 48 glass vials

3.3.5. Enzymatic hydrolysis

Samples without drying were used for enzymatic hydrolysis. 0.1 g biomass on a dry basis was loaded into 25 mL narrow-mouth Erlenmeyer flasks, followed by pipetting 0.1 mL of sodium azide (10 g/L), 5 mL of 0.1M citric buffer (pH 4.60), and DI water (amount of it depend on samples' moisture content) into each flask. The enzyme loading applied was 30 mg Cellic[®] CTec2 cellulase (Novozymes, Wilmington, DE, USA, Protein content-175mg/ml) plus 10 mg Cellic[®] HTec2 hemicellulase (Novozymes, Wilmington, DE, USA, Protein content -168 mg/ml) per gram of glucan plus xylan in the raw material prior to pretreatment. All flasks were sealed with rubber stoppers and placed in an incubation shaker (Multitron Infors-HT, ATR Biotech, Laurel, MD, USA) for reaction at 50°C and 200 rpm. Samples were collected for sugar and other analysis at 24, 48, and 72 h.

3.4. Results and Discussion

3.4.1. Severity Factor

Previous work by Foody (1980) pointed out that the right combination of treatment time and temperature are critical for successful steam explosion pretreatment. Based on this observation, Abatzoglou et al., 1992 developed the following simple mathematical relationship they named the severity factor to capture the effects of both temperature and reaction time on yields:

$$\text{Severity factor, } \text{Log } (R_0) = \text{Log } \{t \times \exp[(T_H - T_R)/14.75]\} \quad (3.1)$$

In this relationship, t is reaction time in min, T_H is the reaction temperature in °C, and T_R is the reference temperature, usually 100°C. Thus, the severity factor captures tradeoffs between treatment temperature and residence time in one value to provide an approximate composite indication of pretreatment conditions. Low severity conditions result in limited improvement in enzymatic hydrolysis yields, but high severity conditions results in formation of degradation products that reduce yields and also inhibit subsequent enzymatic hydrolysis and fermentation steps. This equation was employed to select the range of reaction times appropriate for pretreatment temperatures of 180°C and 200°C. The following table provides the severity factor for different reaction time and temperature applied in the study.

Table 3.2. Severity factor for different reaction time and temperature.

Temperature (180°C)							
Reaction time (min)	60	45	28	17.5	14	8	4
Severity factor	4.13	4	3.8	3.6	3.5	3.25	2.95
Temperature (200°C)							
Reaction Time (min)	18	12	7	4.5	3.5	2	
Severity factor	4.2	4	3.8	3.6	3.5	3.24	

3.4.2. Composition

The composition of the raw untreated aspen wood was measured to be 45.6% glucan, 19.0% xylan, 19.2% acid insoluble residue (AcIR), and 16.22% others. AcIR includes acid insoluble ash and lignin, but because the ash content of *Populus tremuloides* was quite small (<4%), the AcIR content provides a very good estimate of the Klason lignin content.

Chips and milled materials were pretreated at 180°C for 4 min, 8 min, 14 min, 17.5 min, 28 min, 45 min, and 60 min, and at 200°C for 2 min, 3.5 min, 4.5 min, 7 min, 12 min, and 18 min corresponding to severity factors shown in Table 3.2. Figure 3.5A-F displays the glucan, xylan, and AcIR contents of the solids left after pretreatment at these conditions. Additionally, the composition of the raw material is shown at the far left end of the x-axis. In general, glucan content increased, while xylan content decreased with increasing reaction time. The data also show that the glucan content was always higher in the powder than in the chips and in the small chips than in the large chips. On the other hand, the xylan content in the powder was lower than in the chips and lower in the small chips than the large chips. For example, the average glucan content for powder, small chips, and large chips after pretreatment for 45 min at 180°C was 70.6%, 55.5%, and 50.9%, respectively, but the average xylan content for powder, small chips, and large chips at the same pretreatment conditions was 3.7%, 9.4%, and 11%, respectively. These results show that smaller particle size and longer pretreatment times increased hemicelluloses solubilization during steam explosion pretreatment and correspondingly increased the relative glucan content. The average lignin contents were 20-25% for pretreatment at 180°C and 25-30% for pretreatment temperature at 200°C. Furthermore, the lignin contents remained quite constant at either temperature regardless of reaction time or materials size.

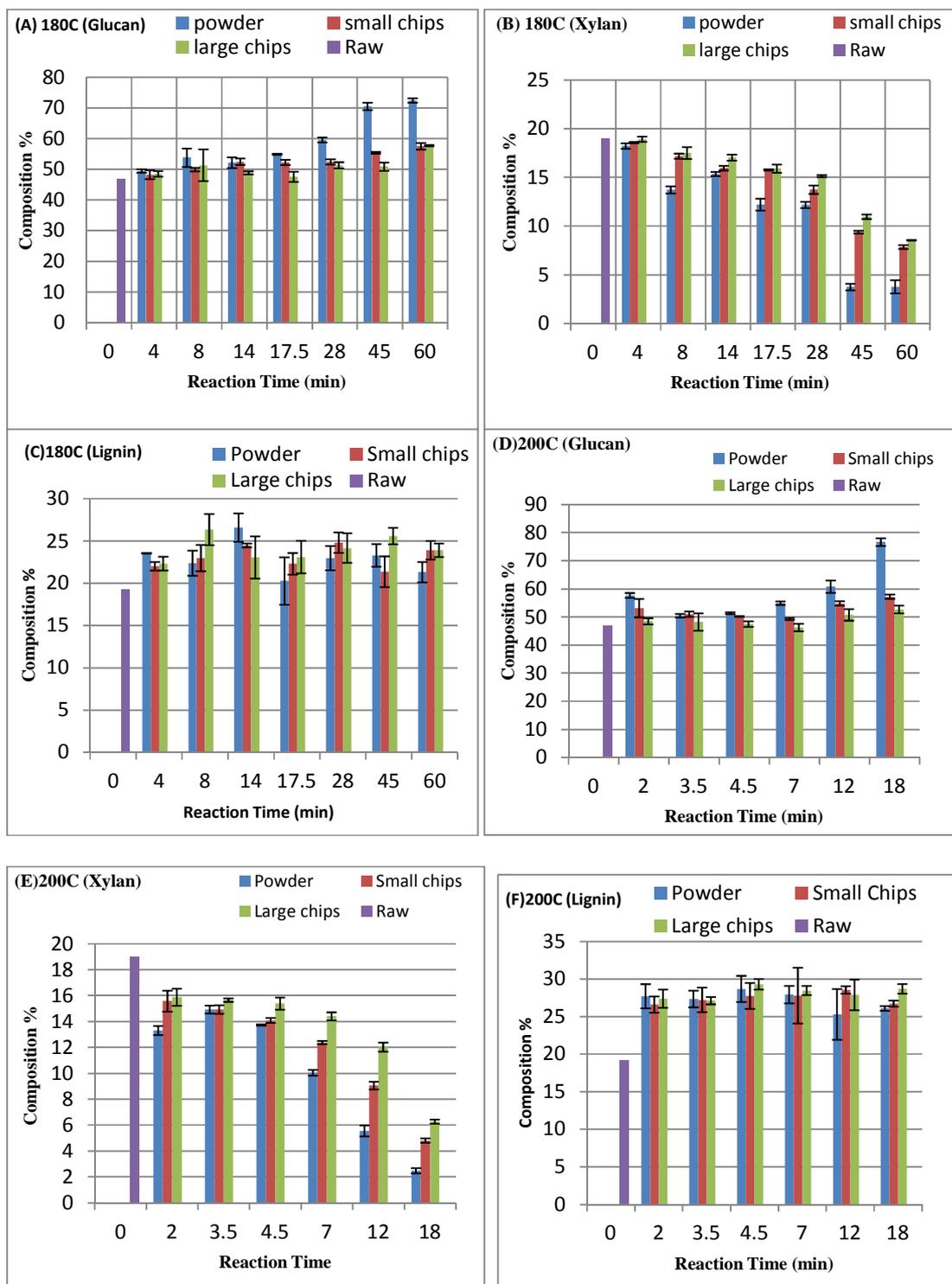


Figure 3.5. Compositions of solids (wt.%, dry basis) following pretreatment at different pretreatment conditions: (A) glucan content at 180°C, (B) xylan content at 180°C, (C) lignin content at 180°C, (D) glucan content at 200°C, (E) xylan content at 200°C, and (F) lignin content at 200°C

3.4.3. Sugar left in pretreated solids

The weight of dry materials after pretreatment was measured to determine chips mass loss and allow calculation of the mass of sugars left in the solid fraction. The solids recovery expressed as mass of solids remaining after pretreatment per 100 g of dry raw material decreased with increasing reaction time. As an example for the 200°C pretreatment, the solid recovery for large chips at 3.5 min was 88.5% but dropped to 79.7% at 12 min.

In order to measure the amount of hydrolyzate sugar retained in solid, sugar left in washed and unwashed solid were measured by the downscaled compositional analysis method. In general, non-washed materials had a higher percentage of sugar left in pretreated solids than washed materials, but the glucan and lignin left in solids were only slightly different for washed vs. non-washed materials. However, the percentage of xylan left in the washed solids was about 15-20% lower than for unwashed materials. This result shows that although hemicellulose was clearly the major component extracted by pretreatment, some of it remained inside the wood chips and required washing to remove it. The data in Figure 3.6 also show that small chips were more fully pretreated, resulting in greater extraction of hemicelluloses than for large chips.

$$\text{Glucan recovery (\% in solid)} = 100 \times \frac{\text{Glucan}_{\text{Pretreated solid},g}}{\text{Glucan}_{\text{Feedstock},g}} \quad (3.2)$$

$$\text{Xylan recovery (\% in solid)} = 100 \times \frac{\text{Xylan}_{\text{Pretreated solid},g}}{\text{Xylan}_{\text{Feedstock},g}} \quad (3.3)$$

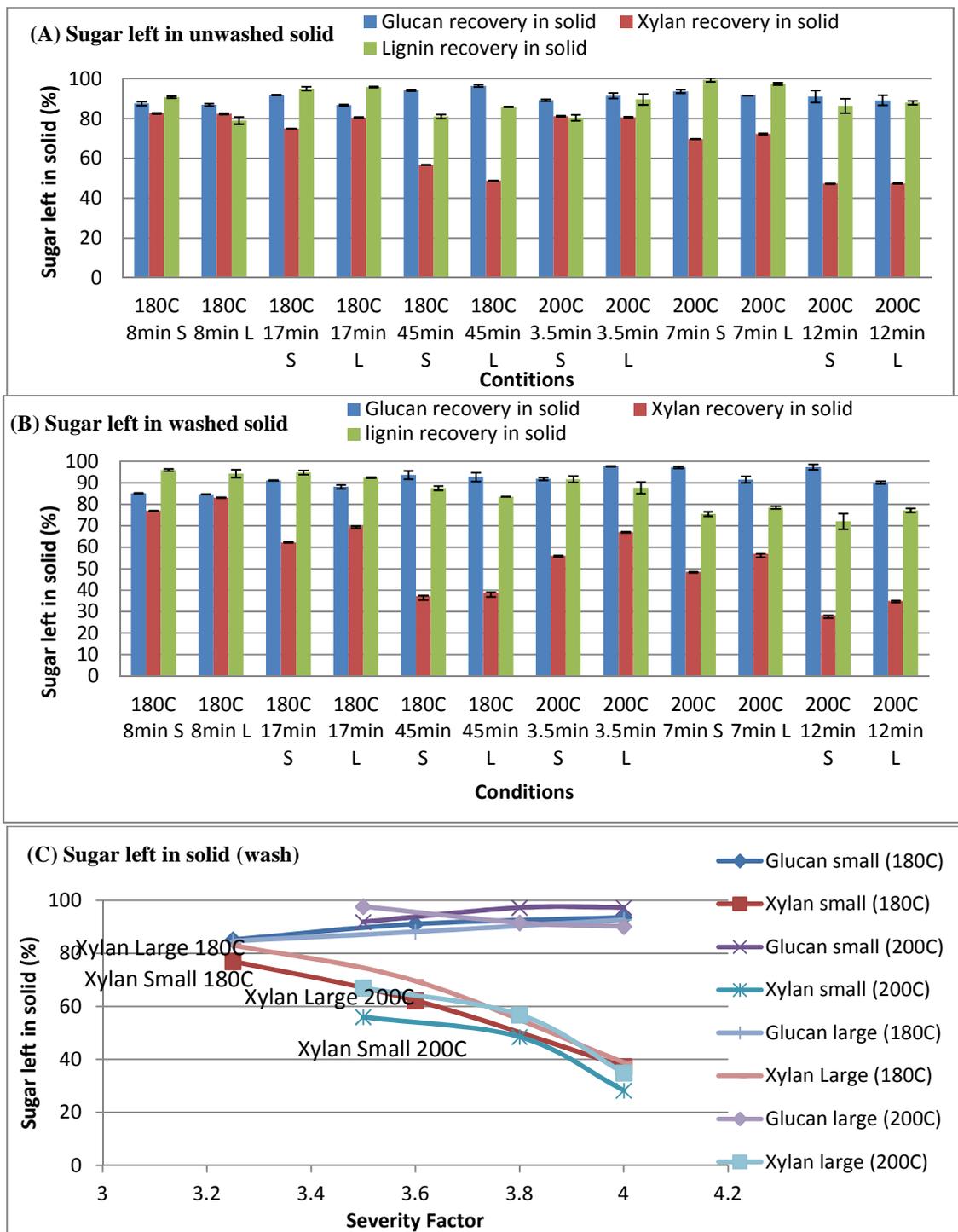


Figure 3.6. Sugar left in solids after steam explosion pretreatment: (A) unwashed materials; (B) and (C) washed materials. Note: On the x-axis in (A) and (B), S denotes small chips and L- large chips.

3.4.4. Enzymatic Hydrolysis

Solids from each pretreatment condition were enzymatically digested to determine how sugar release by enzymes was impacted by pretreatment time and chip size. Figure 3.7 plots the total glucose plus xylose (total sugar) yields following 72 h of enzymatic hydrolysis for the two pretreatment temperatures. In this case, the yields are defined as the amount of sugar released in enzymatic hydrolysis as a percent of the total amount of sugar available in the pretreated biomass solids.

$$\text{Glucose hydrolysate yield (\%)} = 100 \times \frac{\text{Glucose}_{\text{Hydrolysate},g}}{\text{Glucose}_{\text{Pretreated solid},g}} \quad (3.4)$$

$$\text{Xylose hydrolysate yield (\%)} = 100 \times \frac{\text{Xylose}_{\text{Hydrolysate},g}}{\text{Xylose}_{\text{Pretreated solid},g}} \quad (3.5)$$

$$\begin{aligned} \text{Glucose+Xylose hydrolysate yield (\%)} = \\ 100 \times \frac{\text{Glucose}_{\text{Hydrolysate},g} + \text{Xylose}_{\text{Hydrolysate},g}}{\text{Glucose}_{\text{Pretreated solid},g} + \text{Xylose}_{\text{Pretreated solid},g}} \end{aligned} \quad (3.6)$$

The data show that the total sugar yield increased with increasing reaction time, so that the shortest reaction time resulted in lower overall sugar yields. However, the sugar yields did not increase at the same rate as pretreatment time passed: the sugar yield increased by about 20-30% between the 4 min and 8 min reaction times at 180°C, but the rate dropped when the yields were close to 80% yield for powder and small chips. For large chips at 180°C, the sugar yield increased until it reached 100% in 28 min reaction time.

It is important to note that powder exhibited higher yields at the shorter pretreatment times, e.g., overall sugar yields for powder, small chips, and large chips

were 55.4%, 45.6%, and 37.5%, respectively, for solids that resulted from pretreatment for 4 min at 180°C. However, at longer pretreatment times, chips gave similar or higher sugar yields in enzymatic hydrolysis as powder. For example, small and large chips pretreated for 28 min at 180°C gave overall sugar yields of 101.2% and 101.5%, respectively, whereas the yield for powder was only 94.7%. However, pretreatment for excessive times would lose sugars to degradation during pretreatment, and the resulting degradation products such as pseudo-lignin (Kumar et al., 2013) would inhibit enzymatic hydrolysis, thereby further hurting yields. This trend is illustrated in Figure 3.7 below in which total sugar yields from pretreatment of powder at 200°C increased as the pretreatment time was increased from 2 to 12 min but then dropped slightly from 88.6% to 80.4% when the reaction time was extended to 18 min.

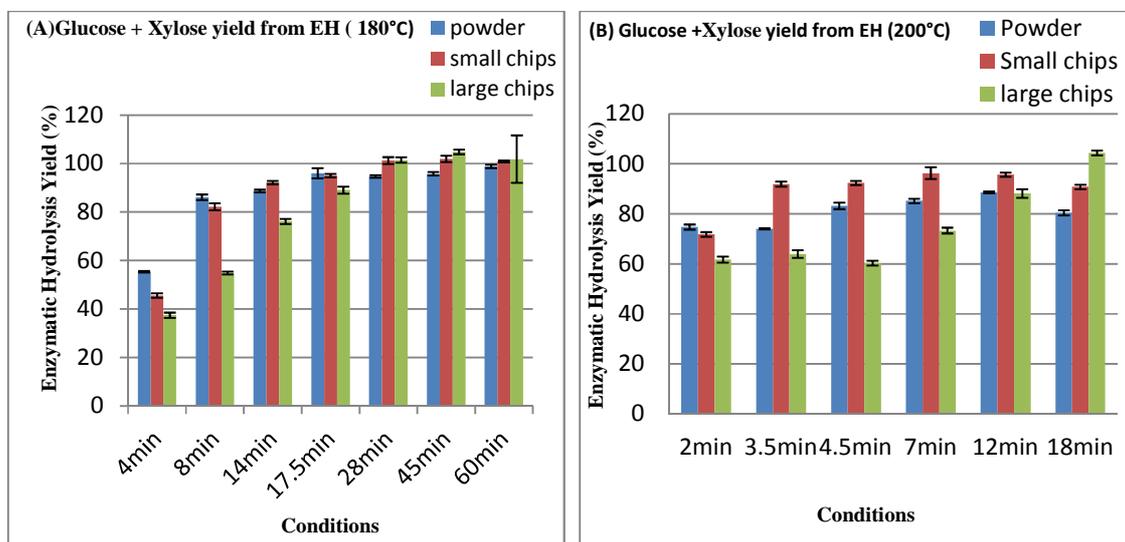


Figure 3.7. Glucose plus xylose yields from enzymatic hydrolysis for 72 h of large chips, small chips, and powder for pretreatment temperatures of (A) 180°C and (B) 200°C. Note- enzymatic hydrolysis was performed at 10 g/L glucan loading in 50 mM citrate buffer for 72 h at cellulase plus xylanase loading of 30 plus 10 mg/g glucan + xylan in unpretreated solids.

Figure 3.8 shows the increase in glucose yields with enzymatic hydrolysis time for all pretreated chips and powder samples. For a 4 min pretreatment time at 180°C, the different sized materials did not show significant difference in yields from enzymatic hydrolysis until after 48 h. However, for 14 min pretreatment time at 180°C, differences in glucose yields with particle size became apparent after only 24 h of enzymatic hydrolysis and continued to further differentiate until 72 h. When the pretreatment time was increased to 60 min, the biggest difference in enzymatic hydrolysis yields for the different size materials occurred at 24 h of enzymatic hydrolysis, and then the difference became smaller and smaller, with powder and the two sizes of chips achieving almost 100% glucose yields after 72 h of enzymatic hydrolysis.

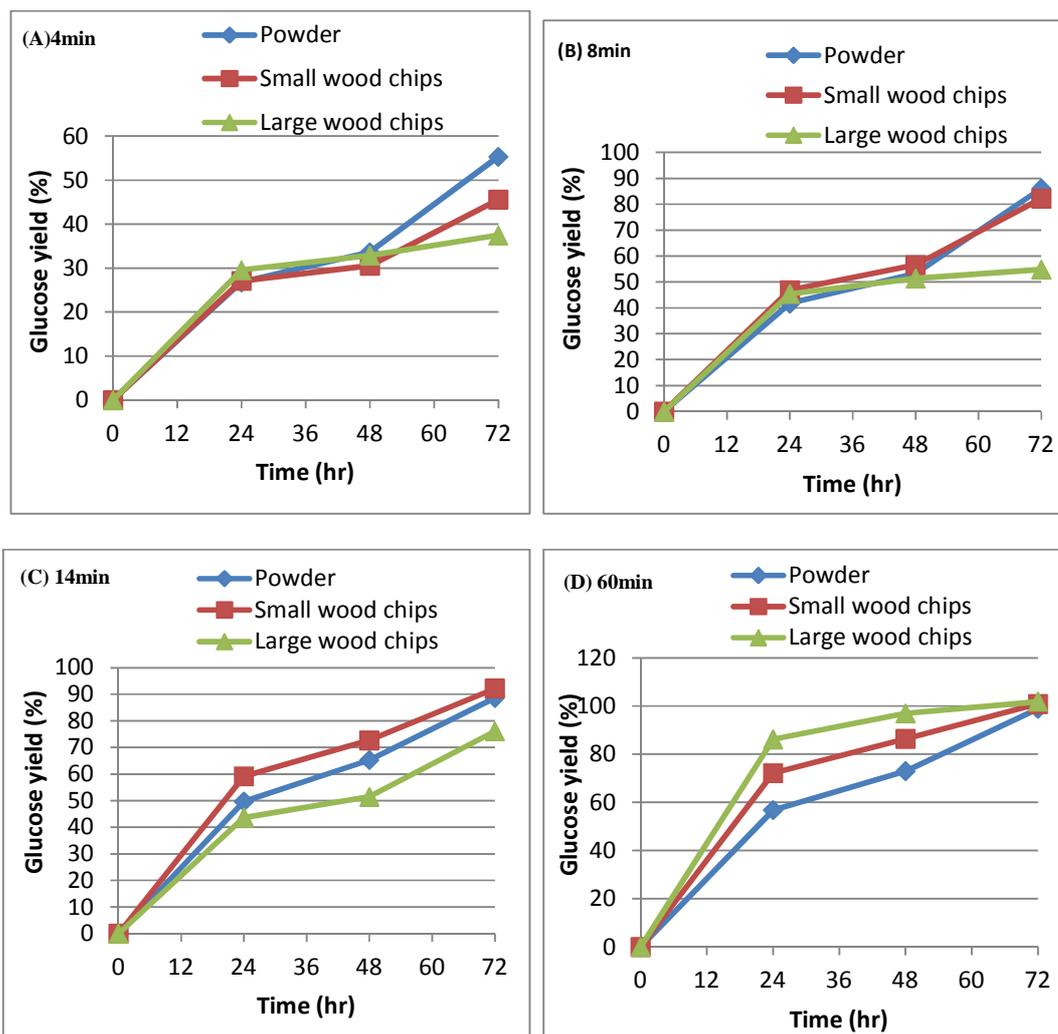


Figure 3.8 Glucose yields following enzymatic hydrolysis of solids from hydrothermal pretreatment of Aspen at 180°C for (A) 4 min, (B) 8 min, (C) 14 min, and (D) 60 min. Note- enzymatic hydrolysis was performed at 10 g/L glucan loading in 50 mM citrate buffer for 72 h at cellulase plus xylanase loading of 30 plus 10 mg/g glucan+ xylan in unpretreated solids.

3.4.5. Effect of chip size on sugar removal in enzymatic hydrolysis

Another measure applied to judge the effect of particle size on performance was the amount of sugar removed from the original hardwood during enzymatic hydrolysis as a percent of the total amount of sugar available in the raw materials. This metric did not include the sugars recovered in the liquid fraction during pretreatment because of the inability to capture these sugars in the open steam gun system. For enzymatic hydrolysis for 72 h with an enzyme loading of 30 mg cellulase plus 10 mg xylanase per gram of glucan plus xylan in the raw material prior to pretreatment, there was a clear trend of increasing total sugar removal from shortest to longest pretreatment time. Of particular note to the objective of this study, enzymatic hydrolysis was able to remove more sugars from small chips than from large chips, but the advantage in sugar removal from small chips dropped with increasing pretreatment time. For example, as shown in Figure 3.9, 68% of the sugar was removed from small chips compared to only 46% for large chips for an 8 min pretreatment time at 180°C, a 22% advantage for small chip, but the difference dropped to essentially zero (~ -2%) for a 45 min pretreatment at this temperature. Furthermore, although the greatest amount of sugar removed was observed for a 45 min pretreatment of the large chips at 180°C, the longest reaction time did not maximize sugar removal for pretreatment at 200°C because sugar degradation and possibly formation of inhibitors hurt enzymatic hydrolysis yields.

$$\text{Glucose removed (\%)} = 100 \times \frac{\text{Glucose}_{\text{Hydrolysate,g}}}{\text{Glucose}_{\text{Feedstock,g}}} \quad (3.7)$$

$$\text{Xylose removed (\%)} = 100 \times \frac{\text{Xylose}_{\text{Hydrolysate,g}}}{\text{Xylose}_{\text{Feedstock,g}}} \quad (3.8)$$

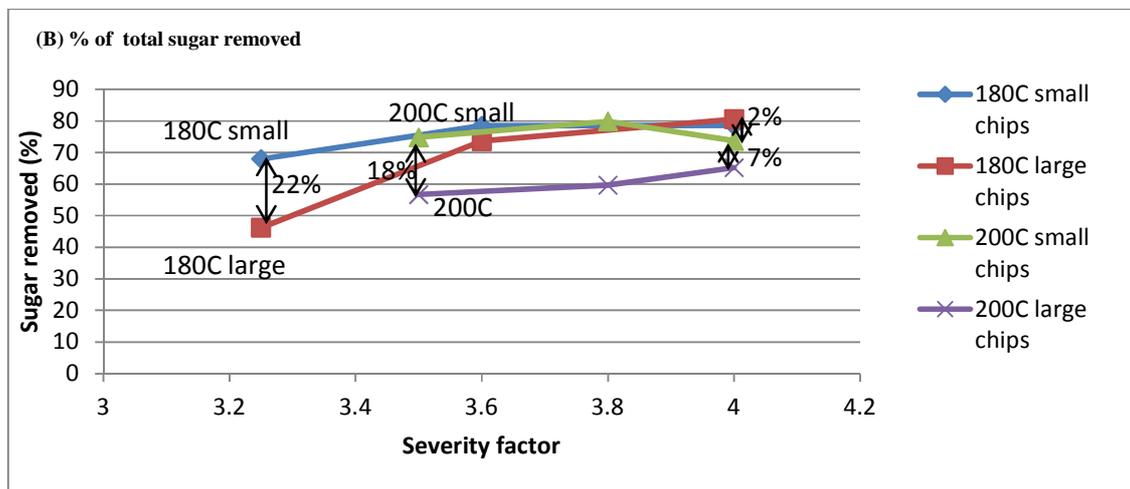
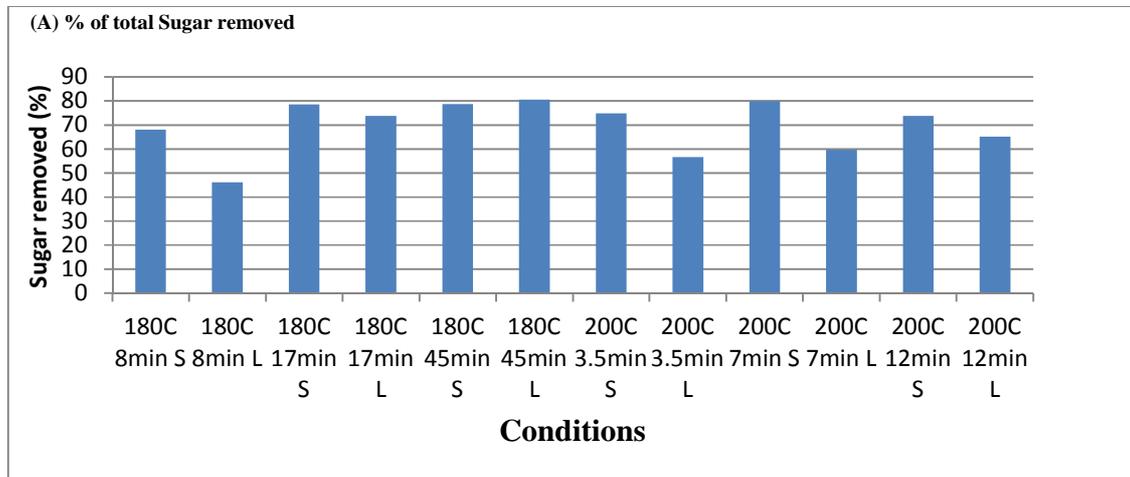


Figure 3.9. Sugar removal from small and large chips as a percent of the total amount of sugars in the original untreated chips plotted vs. (A) multiple pretreatment condition (B) pretreatment severity factor for different temperatures

3.4.6. Effect of chip orientation on sugar removal in hydrothermal pretreatment

Two sets of chips were prepared with the same dimensions (2×1.5×0.5” as length x width x thickness, respectively) but cut in different orientations with respect to the wood grain direction, as shown in Figure 3.10, to determine the effect of pore orientation on yields for hydrothermal pretreatment. Based on previous findings, these chips were pretreated at 180°C for 8, 17, and 45 min. Chips that were chiseled so that the cross section followed the grain direction were designated as Chips F, while those chiseled against the grain direction were designated as Chips A. Figure 3.11 shows that the mass loss were virtually the same for either chip at all pretreatment times. In addition, similar percentages of glucan and xylan were left in Chips F and Chips A as shown in Figure 3.12, reinforcing that grain orientation had little effect on sugar yields.

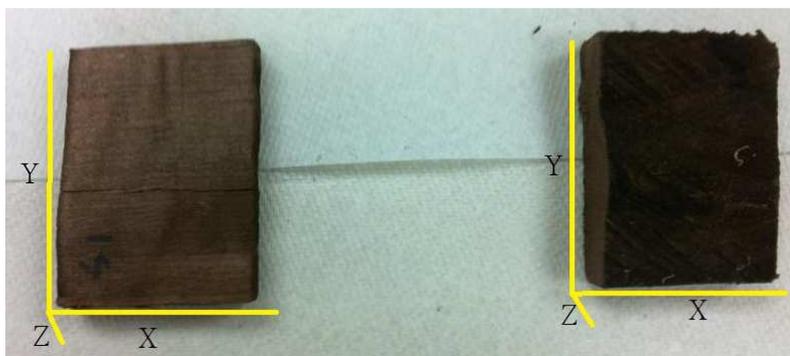


Figure 3.10. The chip on the left (Chip F) was cut so that the grain direction was in the direction of the chip width (x direction). The chip on the right (Chip A) was cut so that the grain direction was in the direction of the chip thickness (z direction).

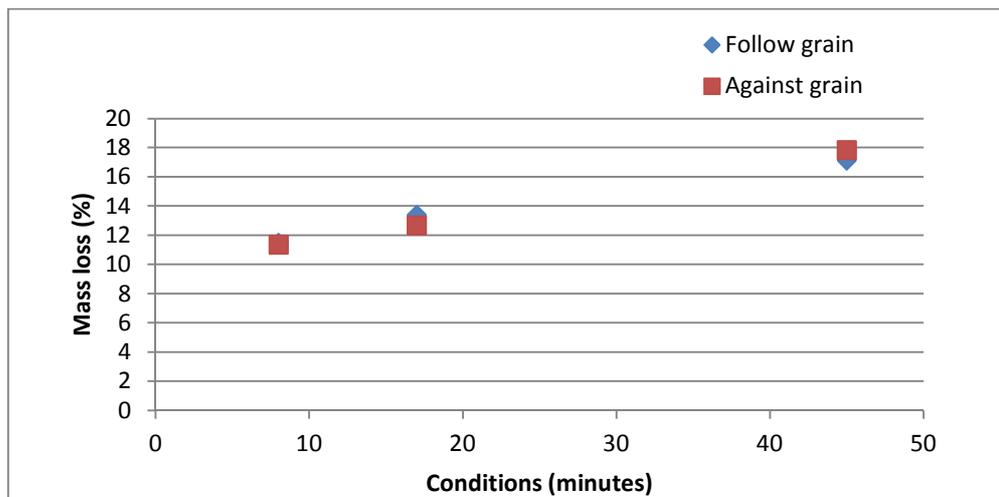


Figure 3.11. Mass loss during hydrothermal pretreatment at 180°C of chips with cutting against the grain and following the grain.

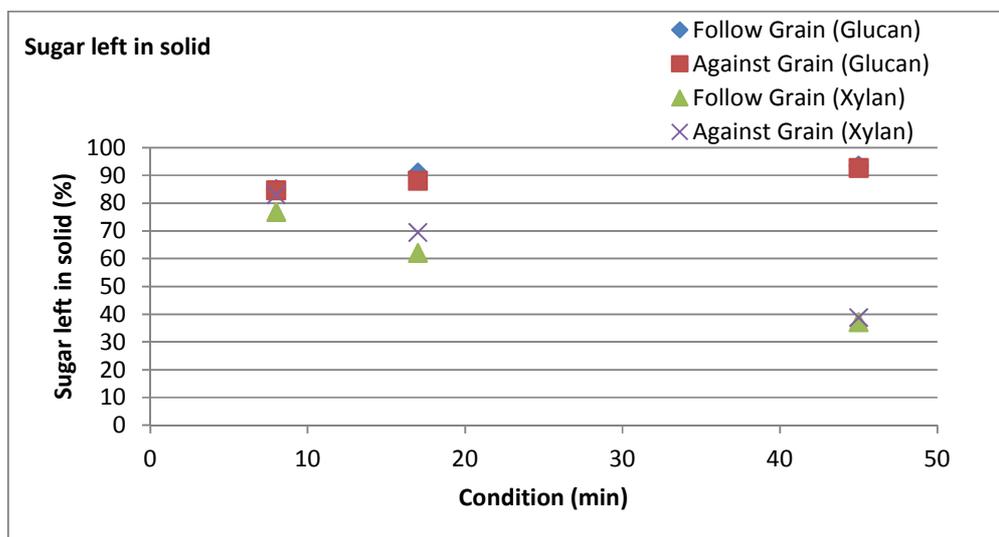


Figure 3.12. Sugar left in solids during hydrothermal pretreatment at 180°C of chips with cutting against the grain and following the grain.

3.5. Conclusions

In general, longer reaction time removed more xylose, but it did not have a significant effect on glucose and lignin contents in pretreated materials. The data also indicated that the enzymatic sugar yields increased with increasing reaction time, so that the shortest reaction time resulted in lower overall sugar yields. However, small chips that were pretreated at 180°C for 8 min or longer only barely increased its sugar yield, and it took 28 min for large chips to achieve uniform enzymatic sugar yields.

These experiments showed that more sugar (Glucose + Xylose) was removed in pretreatment coupled with enzymatic hydrolysis from powdered Aspen wood than from larger Aspen chips at shorter reaction times. However, above a certain pretreatment severity ($R_0=3.8$) similar enzymatic sugar yields were achieved from wood chips as from powder, possibly because larger chips require longer pretreatment times to reach to break down its physical structure for enzymatic attack. Thus, although more sugars were removed from smaller particles, yields advantages of small particles will drop with increasing reaction time. In conclusion, for longer reaction times, chips can give as good yields as powder. Thus, it is desirable to determine if the capital cost to provide longer pretreatment times for larger chips is less than the cost of energy required for size reduction to powder. This tradeoff will be evaluated and discussed in Chapter 5.

3.6. References

Abatzoglou, N., Chornet, E., Belkacemi, I., Overend, R., 1992. Phenomenological kinetics of complex systems: The development of a generalized severity parameter and its application to lignocellulosics fractionation. *Chem. Eng. Sci.* 47(5), 1109-1122.

Cadoche, L., Lopez, G.D., 1989. Assessment of size reduction as a preliminary step in the production of ethanol from lignocellulosic wastes. *Biol. Wastes* 30, 153–157

Chornet, E., Overend, R.P., 1988. Phenomenological Kinetics and Reaction Engineering Aspects of Steam/Aqueous Treatments. *Proceedings of the International Workshop on Steam Explosion Techniques: fundamentals and Industrial Applications* 21-58.

DeMartini, J.D., Studer, M.H., Wyman, C.E., 2011. Small-scale and automatable high-throughput compositional analysis of biomass. *Biotechnol. Bioeng.*, 108(2), 306-312

DeMartini J.D., Wyman C.E., 2011. Change in consumption and sugar release across the annual rings of populus wood and implication on recalcitrance. *Bioresource Technology* 102: 1352-1358.

Foody, P., 1980. Optimization of Steam Explosion Pretreatment. Final Report to DOE, Contract AC02-79ET23050.

Kumar, R., Hu, F., Sannigrahi, P., Jung, S., Ragauskas, A.J., Wyman, C.E., 2013. Carbohydrate derived-pseudo-lignin can retard cellulose biological conversion. *Biotechnol. Bioeng.*, 110(3), 737-753.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2005. Determination of Ash in Biomass. National Renewable Energy laboratory, Golden, Colorado.

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. National Renewable Energy Laboratory, Golden, Colorado.

Sun, Y., Cheng, J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology* 83:1-11

Zhu, J.Y., Pan, X.J., Zalesny, R.S., 2010. Pretreatment of woody biomass for biofuel production: Energy efficiency, technology and recalcitrance. *Appl. Microbiol. Bioethanol.* 87:847-857

Zhu, J.Y., Pan, X.J., 2010. Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation, *Bioresource Technology* 101:4992-5002

Chapter 4

Modeling the Effect of Heat Transfer on Temperature Distribution vs. Time for Wood Particles of Different Sizes

4.1. Abstract

A heat transfer model was applied to estimate whether differences in temperature could be a factor limiting the effectiveness of steam explosion pretreatment of chips. Two models were applied to estimate the heating time for the center of two sets of chips with the same dimensions ($2 \times 1.5 \times 0.5$ " in length \times width \times thickness, respectively) but cut in different orientations with respect to the wood grain direction to reach within 3°C of the target temperature, and large chips ($2 \times 1.5 \times 1.5$ " in length \times width \times thickness, respectively) were applied to estimate the heating time as well. The resulting temperature profile was used to estimate the effect of heat transfer on pretreatment and the resulting hemicellulose removal and enzymatic hydrolysis yield trends reported in the previous chapter. The result indicates that smaller wood particle had better heat transfer, and result in removing more hemicellulose than larger wood particle.

4.2. Introduction

Heat transfer and migration of moisture in wood have been well studied in the literature (Kocaefe et al., 2006; Plumb et al., 1985; Simpson, 2006; Pavlo et al., 2003). High temperature treatment of wood can remove moisture from wood and increase its

rigidity and stability, thereby increasing its protection from biological degradation (Kocaefe et al., 2006). Moreover, high temperature treatment can be needed to kill insects or pathogens before transfer of wood chips between regions in trade (Simpson, 2001). However, heat treatment cannot only remove moisture and kill insects and pathogens but also modifies and breaks down the physical structure of wood to reduce its hemicelluloses content (Pavlo et al., 2003). Heat transfer models can be valuable tools to estimate the amount of time required to heat wood to a target temperature that will kill insects or pathogens. In our study, a heat transfer model was used to predict the amount of time required to achieve uniform pretreatment in chips. MacLean published results for heat conduction in wood, and the heat conduction equation was used here to estimate heating times in rectangular wood cross-sections with steam as the heating source (Simpson, 2006)

4.3. Modeling Heat Conduction in Chips

To estimate whether heat transfer could be a factor limiting the effectiveness of the steam explosion pretreatment of chips, the solution developed by MacLean (1932) for two-dimensional heat conduction through a rectangular cross section was employed. Because the series solution converges quickly, only the first seven terms were needed to estimate the temperature profile across the chip thickness, as follows (Simpson, 2011):

$$\begin{aligned}
T = T_s + (T_0 - T_s) & (16/\pi^2) \\
& \times \{ \sin(\pi x/a) \sin(\pi y/b) \exp[-\pi^2 t(\alpha_x/a^2 + \alpha_y/b^2)] \\
& + (1/3) \sin(3\pi x/a) \sin(\pi y/b) \exp[-\pi^2 t(9\alpha_x/a^2 + \alpha_y/b^2)] \\
& + (1/3) \sin(\pi x/a) \sin(3\pi y/b) \exp[-\pi^2 t(\alpha_x/a^2 + 9\alpha_y/b^2)] \\
& + (1/5) \sin(5\pi x/a) \sin(\pi y/b) \exp[-\pi^2 t(25\alpha_x/a^2 + \alpha_y/b^2)] \\
& + (1/5) \sin(\pi x/a) \sin(5\pi y/b) \exp[-\pi^2 t(\alpha_x/a^2 + 25\alpha_y/b^2)] \\
& + (1/7) \sin(7\pi x/a) \sin(\pi y/b) \exp[-\pi^2 t(49\alpha_x/a^2 + \alpha_y/b^2)] \\
& + (1/7) \sin(\pi x/a) \sin(7\pi y/b) \exp[-\pi^2 t(\alpha_x/a^2 + 49\alpha_y/b^2)] \\
& + \dots \}
\end{aligned} \tag{4.1}$$

in which T_s is the surface temperature (which for steam heating is assumed to be attained immediately), T_0 is the initial temperature, a and b are the cross-sectional dimensions in meters (a is the shorter side against the grain and b is the direction with grain), α_x and α_y are the thermal diffusivities in the x and y directions (x is the direction against the grain and y is the direction follow the grain) that were obtained by Abasaheed et al. (1991), and t is the time in seconds. In order to calculate the temperature at the center of the wood chips, x and y were assumed to be $a/2$ and $b/2$. Furthermore, the thermal diffusivity was assumed to be the same in the radial and tangential directions since both run against the grain (Simpson, 2001). All the parameters applied to the small chips are listed in the following table:

Table 4.1. Parameters employed for heating of wood chips employed in Equation 4.1

T_s	180°C
T_0	22°C
a	0.0127m (0.5 inch)
b	0.0381m (1.5 inch)
x	0.00635m
y	0.01905m
α_x	$1.27 \times 10^{-7} m^2/s$
α_y	$2.63 \times 10^{-7} m^2/s$

This model was then used with the parameters in Table 4.1 to predict how many min of pretreatment time it would require for the temperature at the center of the chips to reach within 3°C of the target temperature. The result is shown in Figure 4.1.

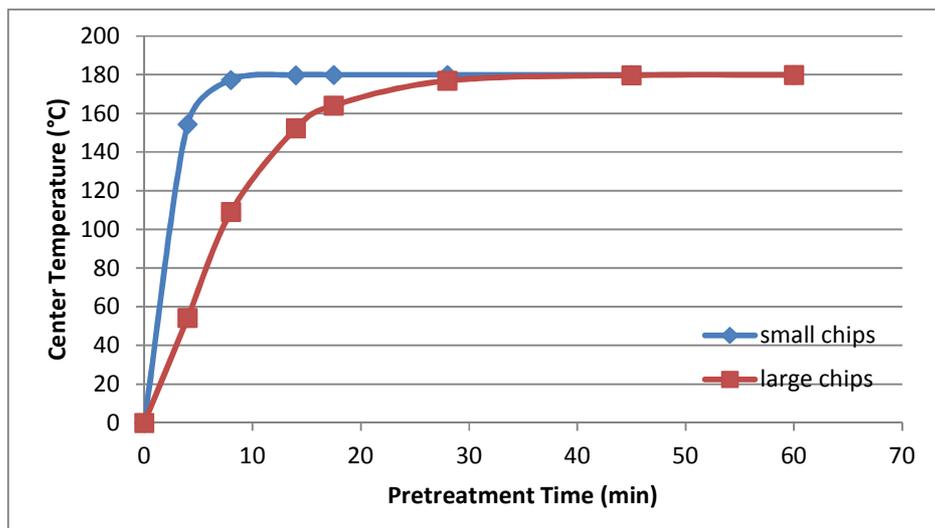


Figure 4.1 Predicted temperature at the center of small and large chips versus pretreatment time at 180°C, employed in Equation 4.1

The figure indicates that the temperature at the center of the small chip will reach 177°C in 8 min but requires 28 min to reach this same temperature for large chips. The results also indicate that the temperature at the center of wood chips increased rapidly during the first few min but then slowly approach the target temperature of 180°C.

This model was also used to estimate the center temperature for different chip orientations with respect to the wood grain direction, with results plotted versus pretreatment time in Figure 4.2. This figure shows that the small Chip A with the grain direction in the direction of the chip thickness reached the target temperature or close to it faster than Chip F that was the same size but had the grain direction in the direction of the chip width. However, the model shows that the center of small chips can reach the target temperature in less than 8 min regardless of grain orientation. Because the minimum pretreatment time in the study was equal to or greater than 8 min, the model predictions can explain why mass loss and the amount of sugar left in pretreated biomass does not show significant differences for both chip orientations. For the chips F, it only took around 8 min to reach 80% of the enzymatic hydrolysis yield (as shown in Figure 3.7). Thus, it can be expected that 8 min pretreatment time is long enough to achieve high yield for both chip orientation.

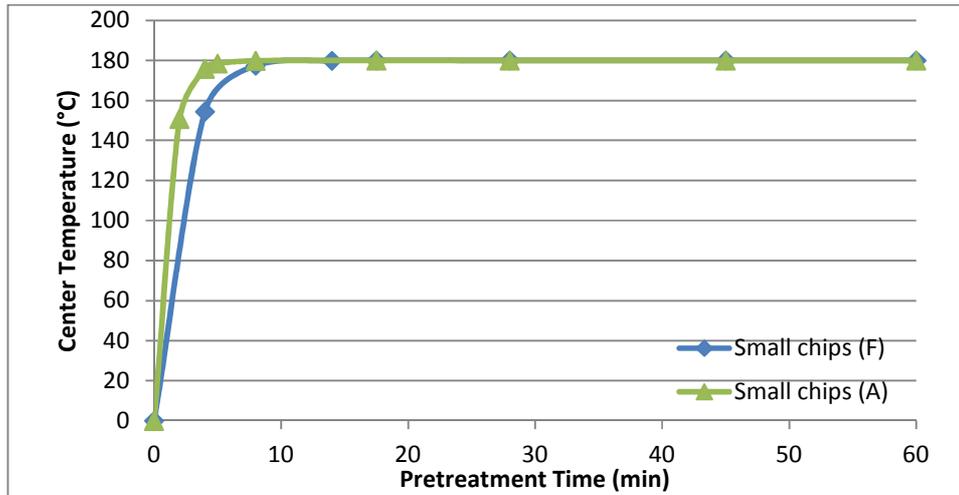


Figure 4.2 Predicted temperature at the center of small chips with different orientations of grain versus pretreatment time at 180°C, employed in Equation 4.1

The models also predict that different grain orientations in the chips will not have a major effect on heat transfer rates for the small chips used in this study. According to the Figure 4.3, for chips size smaller than the dimension of 0.5×1.5” as width×thickness, chip A reached the target temperature or close to it did not significant faster than the chip F. After increasing the chips size to 1×2”, the figure indicates that it only took around 15 min for the center of the chip A reach to within 3°C of the target temperature of 180°C, but it took 25 min for chip F to reach the same temperature. In this situation, chips A will have higher enzymatic hydrolysis yield than chips F for the pretreatment time shorter than 25 min. Thus, the orientations of grains will play an important role for the dimension of chips larger than 1×2” as width×thickness

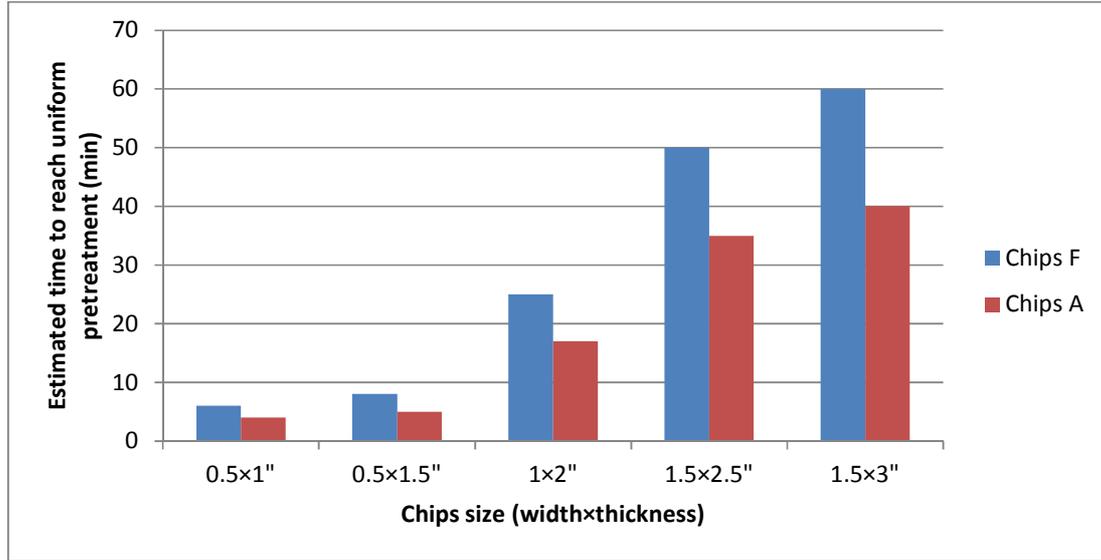


Figure 4.3 Predicted time for the center of different dimensions and orientations chips to reach within 3°C of the target temperature of 180°C

As a check on these predictions, a second empirical model was also applied to predict the heating time for the center of chips to reach the target temperature. In this case, a model by Simpson (2006) was applied based on a large number of variables including chip size, temperature, moisture, and wood specific gravity, with the heating time calculated by the following multiple regression model:

$$t = a(T_{ht})^b(T_{ctr})^c(T_{init})^d(TH)^eW^fM^gG^h \quad (4.2)$$

Where t is the estimated time for the center of wood chip reach target temperature (min), T_{ht} is the heating temperature, T_{ctr} is the target temperature, T_{init} is the initial temperature, TH is the chip thickness, W is the wood chip width, M is the moisture content (%), G is the wood chip specific gravity, and a to h are pre-determined regression coefficients. All the parameters applicable to the chips employed in this study are listed in the Tables 4.2 and 4.3 for small and large chips, respectively.

Table 4.2. Parameters for small chips employed in Equation 4.2

T _{ht}	180°C
T _{ctr}	177°C
T _{init}	22°C
TH	0.5 inch
W	1.5 inch
M	6%
G	0.44
a/b/c/d/e/f/g/h	66.44/-3.032/3.080/-0.2662/1.720/ -0.2560/-0.0945/0.2156

Table 4.3. Parameters for large chips employed in Equation 4.2

T _{ht}	180°C
T _{ctr}	177°C
T _{init}	22°C
TH	1.5 inch
W	1.5 inch
M	6%
G	0.536
a/b/c/d/e/f/g/h	66.44/-3.032/3.080/-0.2662/1.720/ -0.2560/-0.0945/0.2156

With these parameters, Equation 4.2 predicts that it would take 6.8 min and 45 min for the centers of the small wood chips and large chips, respectively, to reach within 3°C of the target temperature (180°C) as shown in Figure 4.4.

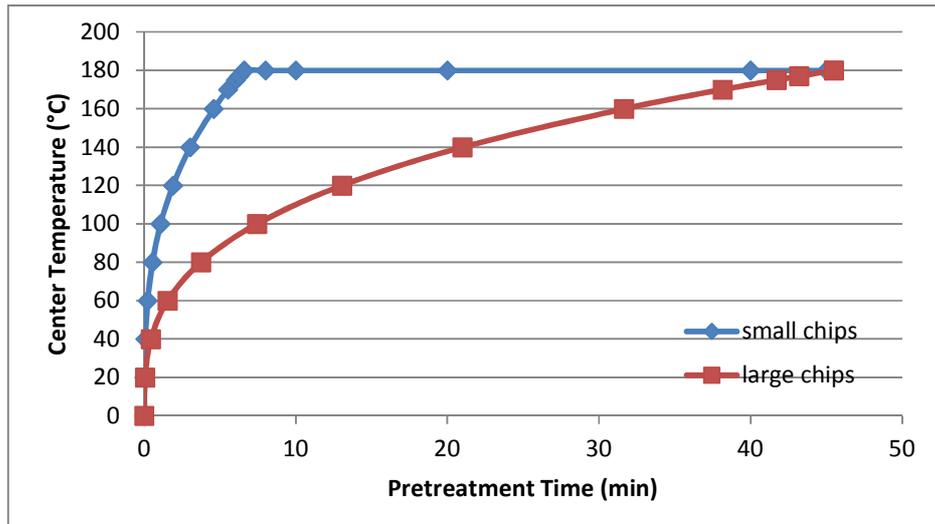


Figure 4.4 Predicted temperature at the center of small chips and large chips versus pretreatment time at 180°C, employed in Equation 4.2

These two models indicate that it should take between about 6.8 and 8 min for the center of small wood chips to reach the target temperature or close to it but that it would require about 28 to 45 min for large chips to reach within 3°C of the target temperature. These predictions can explain why the improvements in enzymatic hydrolysis yield tends to slow after 8 min of pretreatment for small chips and 28 min of pretreatment for large chips. Hemicellulose is removed more rapidly at higher temperature, we would expect small chips to release more hemicellulose in less time, and it also can explain why small chips have higher mass loss than large chips shown in Fig 3.6.

4.4. Conclusions

The solutions to the two-dimensional heat conduction equation and a multiple regression equation were applied to estimate the center temperature of wood chips of two different sizes. Both results indicate that the temperature at the center of wood chips increased rapidly during the first few min of pretreatment and then slowly approach the target temperature of 180°C. The center of small chips reached within 3°C of the target temperature in only about 7 to 8 min while the large chips needed 28 to 45 min to reach the same temperature. Because hemicellulose is removed more rapidly at higher temperatures, we would expect small chips to release more hemicellulose in less time. Furthermore, if we expect hemicellulose removal to be a marker of enzymatic hydrolysis as reported in the literature (Jeoh et al., 2007; Jeoh et al., 2005), these modeling results suggest that enzymatic hydrolysis yields from pretreated chips would significantly increase with time, but the differences of enzymatic hydrolysis yield would be reduced for longer pretreatment times. The models also predict that different grain orientations in the chips will not have a major effect on heat transfer rates for the small chips used in this study. Thus, we would not expect to see significant differences in hemicellulose removal and associated enzymatic hydrolysis for the small chips studied. Research with larger wood chips or shorter pretreatment times is recommended for further research.

4.5. References

- Abasaheed, A. E., Lee, Y. Y., Watson, J. R., 1991. Effect of transient heat transfer and particle size on acid hydrolysis of hardwood cellulose. *Bioresource Technology* 35: 15-21.
- Jeoh, T., Ishizawa, C.I., Davis, M.F., Himmel, M.E., Adney, W.S., Johnson, D.K., 2007. Cellulase digestibility of pretreated biomass is limited by cellulose accessibility. *Biotechnol. Bioeng.*, 98(1), 112-122.
- Jeoh, T., Johnson, D.K., Adney, W.S., Himmel, M.E., 2005. Measuring cellulase accessibility of dilute-acid pretreated corn stover. *Preprints of Symposia - American Chemical Society, Division of Fuel Chemistry*, 50(2), 673-674.
- Kocafe, D., Younsi R., Chaudry B., Kocafe Y., 2006. Modeling of heat and mass transfer during high temperature treatment of aspen. *Wood Sci Technol* 40:371-391
- Maclean, J.D., 1932. *Proceeding of American wood preservers' Assoc.* 28:303-329
- Pavlo, B., Niemi, P. 2003. Effect of temperature on color and strength of spruce wood. *Holzforschung* 12 539-546
- Plumb, O.A., Spolek, G.A., Olmstead, B.A., 1985. Heat and mass transfer in wood during drying. *J. Heat Mass Transfer* 28(9):1669-1678
- Simpson, W.T., 2001. Heating Times for Round and Rectangular Cross Sections of Wood in Steam. *USDA Technical Report FPL-GTR-130.*
- Simpson, W.T., 2006. Estimating heating times of wood boards, square timbers, and logs in saturated steam by multiple regression. *Forest prod* 56 (7/8): 26-28

Chapter 5

Estimation of the Impact of Size Reduction on Costs for Hydrothermal Pretreatment of Aspen Wood Chips

5.1. Abstract

The energy consumption in size reduction and total cost of steam were estimated in this chapter. The result showed that the energy consumption for size reduction followed by further size reduction for powder, small chips, and large chips will be 400 Wh/kg, 109.2 Wh/kg, and 94.4 Wh/kg, respectively. NREL process was used as a base line to calculate the total cost of steam, and result indicated that total cost of steam is \$0.11M per year regardless of wood particle size.

5.2. Introduction

Woody biomass is particularly resistant to breakdown and must be size reduced to increase the surface areas and realize effective pretreatment and better enzyme accessibility. However, size reduction requires high energy inputs, and it is vital that extra costs for size reduction be justified in terms of added sugar release. Thus, it is very important to understand the relationship between size reduction, pretreatment, and enzymatic hydrolysis in reducing recalcitrance. Although it is not difficult to use low energy consumption for size reduction, it is challenging to also achieve high sugar yield

at low energy consumption (Zhu et al., 2010). In this chapter, some of the important factors that affect energy consumption during size reduction and pretreatment will be delineated, and tradeoffs between costs for size reduction and pretreatment conditions examined to minimize energy consumption while still realizing high sugar yields.

5.3. Factors affecting energy requirement for wood chips size reduction

When wood logs arrive at a mill, large rotating drums are typically employed to remove bark from the wood (Klugman et al., 2007). After debarking, the wood is chopped into smaller pieces of around 10-50 mm in two dimensions and about 5-15 mm in the third dimension in a chipper. Then, the chips are further milled to powder, fibers, fiber bundles, or chips (Klugman et al., 2007; Zhu et al., 2010).

5.3.1. Milling machine

Several well developed technologies are employed for biomass size reduction, including hammer milling, knife milling, shredding, disk refining, and attrition milling (Zhu et al., 2009b). Many studies determined the relationship between energy input and size reduction for different technologies (Holtzaple et al., 1989; Cadoche and Lopez, 1989; Schell and Harwood, 1994; Naimi et al., 2006; Womac et al., 2007). The design and choice of the milling machine is important for reducing the energy input in preparing biomass. According to Schell and Harwood (1994) for aspen wood, disk mills have the highest energy consumption (200-400 kWh/ton), followed by hammer mills (90-130 kWh/ton) and knife mills (8-120 kWh/ton). However, although hammer and knife mills require lower energy inputs than disk mills, hammer mills produce particles with a larger size distribution compare to disk mill. Furthermore, hammer and knife mills are not

appropriate for wet materials (Schell and Harwood, 1994). Thus, it is difficult to generalize the choice of a particular mill for size reduction based on just energy consumption because each mill has distinct advantages and disadvantages that vary with feedstock and application. However, Zhu et al. (2010) represented that disk milling was the best choice because disk milling represents one of the best fiberization processes and results in efficient enzymatic hydrolysis. Furthermore, disk milling has been used for several decades in the pulp and paper industry (Zhu et al., 2010).

5.3.2 Post-pretreatment wood size reduction

Post-pretreatment size reduction of wood applies size reduction after thermochemical pretreatment. This approach has important advantages in that thermochemical pretreatment alters the chemical composition and physical structure of wood chips by partially removing hemicelluloses and lignin since that are important structural cell-wall components. Thus, the pretreated material becomes more friable, and as a result, less energy is needed for size reduction following chemical pretreatment (Zhu et al., 2010). For example, typical energy consumption for milling chips to powder before pretreatment is approximately 150 to 800 kWh/ton wood (Schell and Harwood, 1994; Zhu et al., 2009b; Zhu et al., 2010). However, 75% less energy can be needed for post-pretreatment wood size reduction, with the amount of energy saved depending on the choice of pretreatment method and the type of biomass.

5.3.3 Effect of chemical pretreatment

Post-pretreatment wood size reduction can significantly reduce energy consumption compared to size reduction before chemical pretreatment. Different pretreatment

technologies have different impacts on chemical composition and physical structure of the woody biomass (Mosier et al., 2005). Therefore, the energy saving in size reduction after chemical pretreatment depends on the pretreatment method. According to Kenealy and co-workers, post-steam-pretreatment can save about 30% of the energy for size reduction when wood chips were pretreated by oxalic acid (Kenealy et al., 2007). Table 5.1 indicates energy savings for size reduction of lodgepole pine wood chips pretreated by different chemical pretreatments. Based on untreated wood chips as a standard, hot water and acid pretreatment methods can save 2.7% and 41% of the energy demand for disk milling. Alternatively, it can save up to 15% of the energy for size reduction following SPORL (sulfite pretreatment to overcome recalcitrance of lignocellulose) pretreatment of wood chips (initial pH 4.2). However, this table also shows that the energy savings for size reduction can increase to about 78% when the initial pretreatment pH is 1.9.

Table 5.1. Effects of various pretreatments on lodgepole pine wood chip size reduction energy consumption (Zhu et al., 2010)

Pretreatment at 180 °C for 30 minutes ^a	Initial liquor pH	Disk-milling energy (kWh/ton wood)	Size reduction energy savings (%)
Untreated		699	
Hot water	5.0	680	2.7
Acid	1.1	412	41.0
SPORL	4.2	594	15.0
SPORL	1.9	153	78.1

5.3.4. Final particles size

The relationship between energy consumption for size reduction and final particle size is the main topic of this chapter, since this study is focused on comparing energy

consumption for chips and powder. The energy requirement in size reduction increases with decrease in the final size of chips (Cadoche and Lopez, 1989). According to Figure 5.1, the relationship between energy consumption and degree of size reduction is not always linear, and this relationship may differ for different raw materials and kinds of milling machines (Cadoche and Lopez, 1989). From other studies (Schell and Harwood, 1994; Womac et al., 2007; Zhu et al., 2010), it is obvious that larger final particle sizes save more energy in size reduction, but the percentage saving needs further definition.

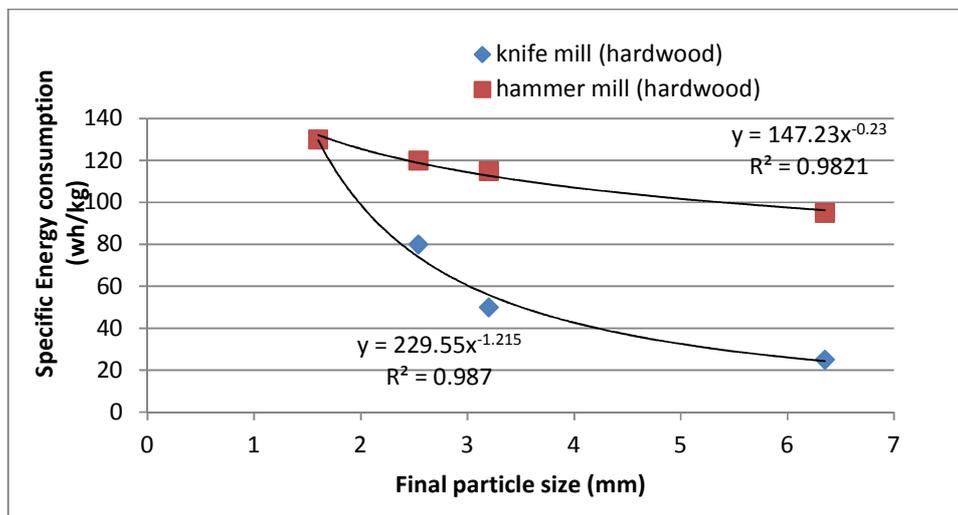


Figure 5.1. Energy consumption to produce different final particle sizes using hammer and knife mills. (Cadoche and Lopez, 1989)

5.4. Energy consumption for size reduction

According to Zhu et al., (2010), which indicates that ethanol yield from wood will be 300 L/ton with current technology, and ethanol has a thermal energy content of 24 MJ/L. Thus the total ethanol thermal energy from wood will be 7.2 MJ/kg wood. In order

to achieve reasonable net energy gain from wood ethanol production, energy consumption must be kept as low as possible.

Some of the studies have researched the relationship between final particle size and energy consumption for size reduction, with most focused on a final size below 12 mm in length. In our conversion study, the length of both chips was 50 mm, but the thickness of larger chips was 3 times wider than for smaller chips. This study will compare the energy consumption for size reduction to produce the three different sizes of chips and powder used in our study: 50 mm, 15 mm, and 0.853 mm (20 mesh).

As shown in Figure 5.1, Cadoche and Lopez, (1989) measured energy consumption for size reduction to different final particle sizes with hammer and knife mills. The resulting data can be fit well by a power function that can then be used to estimate the energy consumption as 1.98, 8.55, and 278 Wh/kg to knife mill wood to large chips, small chips, and powder of the size used in this study. The same approach estimates that 59.9, 79, and 152.7 Wh/kg will be expended to make these respective particle sizes with a hammer mill. According to Schell and Harwood, (1994), disk milling normally requires twice the energy consumption of a hammer mill to produce the same size particle, resulting in an estimated energy consumption of 120, 160, and 305 Wh/kg for large chips, small chips, and powder, respectively. However, this data was published in 1989, and technical improvements might lower the energy consumption for size reduction from these values. In a more recent study, Zhu et al. (2010) indicated that wood chips are typically made with sizes of around 10-50 mm in two dimensions and 5-15 mm in the third dimension, and that the energy consumption required is typically around 50 Wh/kg, depending on the fiberization process and degree of milling. Although their study did not

indicate the exact energy consumption to produce chips 15 mm and 50 mm in length, a reasonable estimate of energy consumption can be made from these studies (Zhu et al., 2010; Cadoche and Lopez, 1989; Schell and Harwood, 1994). First, an energy input of 50 Wh/kg was determined to be needed to produce wood chips 30 mm in length with contemporary technology. However, this is in contrast to Figure 5.1 that shows about 134 Wh/kg would be needed to produce the same size of chips with earlier technology. Thus, it appears that technical improvements may have reduced energy consumption by up to 63% from 1989 to 2010. On that basis, the energy consumption to produce large chips and small chips was estimated to be 44.4 and 59.2 Wh/kg, respectively. Sun and Cheng, (2002) represent that wood chips will likely be milled or ground to 0.2 to 2 mm in length, and Zhu et al., (2010) indicate that this would require an energy input of 150 to 800 Wh/kg wood. Based on this information, it can be estimated that the power required to produce the 0.853 mm powder employed in our study should be around 400 Wh/kg.

Pretreating the chips prior to size reduction would reduce the energy required for milling to less than 50 Wh/kg (Zhu et al., 2010). This result showed that the energy consumption for size reduction followed by further size reduction for large and small chips will be 94.4 and 109.2 Wh/kg, much less than the 400 Wh/kg needed to make powder. Based on representative electricity price data (EIA, 2013), the industrial price of electricity would be about 6.59 ¢/kWh. Thus, for a plant processing 1000 tons/day of woody feedstock (Zhu et al., 2010) and 8410 hours/year of operation, the cost for size reduction would be about \$2.27 M/year, \$2.62 M/year, and \$9.62 M/year for large chips, small chips, and powder, respectively.

5.5. Operating cost for steam explosion pretreatment

The operating costs for steam explosion pretreatment include just the cost of providing high pressure steam, and the NREL process design was used as a basis to estimate steam costs. The annual biomass feed rate for the NREL process was 773,000 dry ton per year, and a TowerPak Stirling Power Boiler was assumed to be used to generate electricity and power for entire facility. This boiler consumed 1.03×10^7 kWh and produced about 239,000 kg/h steam, with 12% of this amount extracted from the turbine at 268°C and 175 psi to be sent to the pretreatment section to provide all the pretreatment steam (Humbird et al., 2011). If NREL process bought the electricity rather than generated it by themselves as in reality, based on representative electricity prices (EIA, 2013), the price of industrial electricity is expected to be about 6.59¢/kWh. Thus, it would cost \$0.68M per year for the steam that used to heat biomass in pretreatment section for the NREL process. The NREL process employs a two stage steam pretreatment: the first stage heats the feed slurry with 158°C steam for 5 min and second stage converts oligomers from the first stage to monomers at 130°C for 20-30 min (Humbird et al., 2011). In a well insulated vessel, resident time should not be a factor affecting the cost of steam, and the factors that should be concern will be the amount of material and the final temperature. The annual cost of steam in this study was calculated by the following equation:

$$\text{Total cost of steam} = \left(\frac{Q_1}{Q_2} \right) \times (\text{total cost for NREL to generate steam}) \quad (5.1)$$

Where

$$Q_i = \text{Annual heat required for stage } i = C_{p,541K} \times m \times (541.15K - T_i) \quad [\text{kJ}]$$

$$C_{p,541K} (\text{Heat capacity at 541 K}) = 2.26 \quad [\text{kJ/kg K}]$$

m = Annual feedstock requirement [dry ton/ year]

T_i = Stage i reaction temperature, [K]

According to data in the previous chapter, 6 min would be needed for powder (<0.853 mm) to reach at least 80% enzymatic hydrolysis yield after 72 h incubation in 180°C pretreatment temperature condition, and it took 8 min and 17.5 min for small and large chips to give similar hydrolysis yields, respectively. However, resident time should not be a factor affecting the cost of steam here, therefore, the price of steam used to treat powder, small chips, and large chips will be the same. The total cost of steam is \$0.11M per year from the equation above.

5.6. Conclusions

Minimizing energy consumption is an important goal for lignocellulosic ethanol production. This chapter estimated the cost of energy to produce wood powder, small chips, and large chips that can achieve at least an 80% enzymatic hydrolysis yield. 1000 tons/day of woody feedstock can produce 100 million liters/year of ethanol. The cost of energy consumption for size reduction and steam pretreatment process were calculated to determine which size of chips will be the better option. According to our results, the yields are all about the same regardless of the size of woodchips, since resident time should not be a factor affecting the cost of steam, the operating cost for steam explosion pretreatment should be similar to treat powder, small chips, and large chips. The total cost of size reduction and steam for pretreatment of powder, small chips, and large chips were estimated to be \$9.73M, \$2.73M, and \$2.38M to reach 80% enzymatic hydrolysis

yield, and the total energy requirement to produce 1 liter of ethanol will be 1.5 kWh/L, 0.45 kWh/L, and 0.4 kWh/L, respectively. Because the size reduction cost to make powder was three times more than to make chips, it is obvious that substantial yield gains would be needed to justify producing powder before pretreatment. Although making large chips would be about \$0.35M less expensive than making small chips, the advantage of large chips was limited since the energy saving in size reduction process was not significant compare to small chips. Thus, because there is little if any yield advantage to producing powder, it would be best to use chips for hydrothermal pretreatment. Although large chips are projected to require a little bit less energy than small chips, either size would produce similar product yields making it questionable whether further size reduction is warranted.

5.7. References

Cadoche, L., Lopez, G.D., 1989. Assessment of size-reduction as a preliminary step in the production of ethanol from lignocellulosic wastes. *Biological Waste* 30 (2), 153–157

Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Sheehan, J., Aden, A., 2011. 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

Holtzapple, M.T., Humphrey, A.E., Taylor, J.D., 1989. Energy-requirements for the size-reduction of Poplar and Aspen wood. *Biotechnology and Bioengineering* 33 (2), 207–210.

Klugman, S., Karlsson, M., Moshfegh B., 2007. Scandinavian chemical wood-pulp mill. Part 1. Energy audit aiming at efficiency measures. *Applied Energy* 84:326-339

Kenealy, W., Horn, E., Houtman, C.J., 2007. Vapor phase diethyl oxalate pretreatment of wood chips: Part1. Energy saving and improved pulps. *Holzforschung* 61:224-229

Mosier, N., Wyman, C.E., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.*, 96(6), 673-686

Naimi, L.J., Sokhansanj, S., Mani, S., Hoque, M., Bi, T., Womac, A.R., Narayan, S., 2006. Cost and performance of woody biomass size reduction for energy production. In: 2006 CSBE/SCGAB Annual Conference, Edmonton, Alberta, Canada

Schell, D.J., Harwood, C., 1994. Milling of lignocellulosic biomass—results of pilot-scale testing. *Applied Biochemistry and Biotechnology* 45–46, 159–168.

Sun Ye, Jiayang Cheng. 2002. Hydrolysis of lignocellulosic materials for ethanol production. *Bioresource Technology* 83:1-11

Womac, A.R., Igathinathane, C., Bitra, P., Miu, P., Yang, T., Sokhansanj, S., Narayan, S., 2007. Biomass pre-processing size reduction with instrumented mills, Annual International Meeting of American Society of Agricultural and Biological Engineers, 2007. Minneappolis, MN, USA.

U.S. Energy Information Administration. 2013. *Electric Power Monthly*

Zhu J.Y., Pan Xuejun, Ronald S. Zalesny Jr. 2010. Pretreatment of wood biomass for biofuel production: energy efficiency, technologies, and recalcitrance. *Appl Microbiol Biotechnol* 87: 847-857

Zhu J.Y., Pan X.J. 2010. Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation, *Bioresource Technology* 101:4992-5002

Zhu J.Y, Pan XJ, Wang GS, Gleisner R .2009a. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresour Technol* 100(8):2411–2418

Zhu J.Y, Wang GS, Pan XJ, Gleisner R .2009b. Specific surface to evaluate the efficiencies of milling and pretreatment of wood for enzymatic saccharification. *Chemical Engineering Science* 64:474-485

Chapter 6

Summary of Key Findings and Future Work

6.1 Summary of thesis objectives

The main motivation of this study was to minimize the energy consumption and achieve the high sugar yield from woody biomass. Woody biomass is particularly resistant to breakdown and must be size reduced to increase the surface areas and realize better enzyme accessibility. However, size reduction pretreatment has major disadvantages in that these pretreatment processes require lots of energy. Thus, it is challenging to achieve high sugar yield at low energy consumption in size reduction.

Based on this rationale, the objectives of this study were to:

- 1) Determine the effects of particle size in steam pretreatment effectiveness (Chapter 3).
- 2) Determine the effects of grain direction of wood chips on steam explosion pretreatment (Chapter 3)
- 3) Develop heat transfer model to estimate whether differences in temperature could be a factor limiting the effectiveness of steam explosion pretreatment of different particle size (Chapter 4)
- 4) Estimate the cost in both size reduction and steam explosion pretreatment process, and determine the better size of chips to produce same amount of ethanol in lower energy consumption. (Chapter 5)

6.2 Key findings and future work

The most important finding in study was how the mass loss, enzymatic hydrolysis yield, and sugar removed for different particle size of woody biomass. These experiments showed that more sugar (Glucose + Xylose) was removed in pretreatment coupled with enzymatic hydrolysis from powdered Aspen wood than from larger Aspen chips at shorter reaction times. However, above a certain pretreatment severity ($R_0=3.8$) similar enzymatic sugar yields were achieved from wood chips as from powder because larger chips require longer pretreatment times to reach to break down its physical structure for enzymatic attack. Furthermore, the result indicated that grain orientation had little effect on sugar yields.

In this study, it did not include the sugars recovered in the liquid fraction during pretreatment because of the inability to capture these sugars in the open steam gun system. Thus, it was recommended to use close system such as parr reactor to pretreat the biomass in the future research to have better mass balance.

According to the heat transfer model in chapter 4, the center of small chips reached within 3°C of the target temperature in only about 7 to 8 min while the large chips needed 28 to 45 min to reach the same temperature. Because hemicellulose is removed more rapidly at higher temperatures, we would expect small chips to release more hemicellulose in less time. The models also predict that different grain orientations in the chips will not have a major effect on heat transfer rates for the small chips used in this study. In order to better understand the effect of grain orientation, research with larger wood chips or shorter pretreatment times is recommended for further research.

The result showed that the yields were the same regardless of the size of wood chips, and since residence time should not be a factor affecting the cost of steam during pretreatment process, the energy consumption in pretreatment process will be similar for different particle size. This study also showed that the energy consumption for size reduction for large, small chips, and powder will be 94.4, 109.2, and 400 Wh/kg, and the total energy requirement to produce 1 liter of ethanol will be 0.4 kW/L, 0.45 kW/L, and 1.5 kW/L, respectively. Larger chips will produce the same amount of ethanol as small chips and powder but with lower energy consumption making it questionable whether further size reduction is warranted.