SIZE REDUCTION OF CELLULOSIC BIOMASS FOR BIOFUEL MANUFACTURING

by

MENG ZHANG

B.S., Dalian University of Technology, 2009

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Industrial & Manufacturing Systems Engineering
College of Engineering

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2014

Abstract

Currently, transportation is almost entirely dependent on petroleum-based fuels (e.g. gasoline, diesel fuel, and jet fuel). Increasing demands for sustainable sources of liquid transportation fuels make it imperative to develop alternatives to petroleum-based fuels. Biofuels derived from cellulosic biomass (forest and agricultural residues and dedicated energy crops) have been recognized as promising alternatives to petroleum-based liquid fuels. Cellulosic biofuels not only reduce the nation's dependence on foreign petroleum but also improve the environment through reduction of greenhouse gas emissions.

In order to convert cellulosic biomass into biofuels, cellulosic biomass must go through a size reduction step first, because large size cellulosic biomass (whole stems of herbaceous biomass or chunks of woody biomass) cannot be converted to biofuels efficiently with the current conversion technologies. Native cellulosic biomass has limited accessibility to enzyme due to its structural complexity. Size reduction can reduce particle size and disrupt cellulose crystallinity, rendering the substrate more amenable to enzymatic hydrolysis.

The purpose of this research is to provide knowledge of how size reduction alters biomass structural features, and understand the relationships between these biomass structural features and enzymatic hydrolysis sugar yield. This research is also aimed to investigate the impacts of process parameters in biomass size reduction on the conversion of cellulosic biomass to biofuels to help realize cost-effective manufacturing of cellulosic biofuels.

This dissertation consists of eleven chapters. Firstly, an introduction of this research is given in Chapter 1. Secondly, Chapters 2 presents a literature review on cellulosic biomass size reduction. Thirdly, a preliminary experimental study is included in Chapter 3. Chapters 4 to 6 present a three-phase study on confounding effects of two important biomass structural features:

particle size and biomass crystallinity. Chapters 7 and 8 investigate effects of sieve size used in size reduction of woody and herbaceous biomass, respectively. Chapters 9 and 10 focus on the relationship between particle size and sugar yield. Chapter 11 studies effects of cutting orientation in size reduction of woody biomass. Finally, conclusions and contributions are given in Chapter 12.

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Chapter 1 - Introduction

1.1 Energy crisis and need for cellulosic biofuels

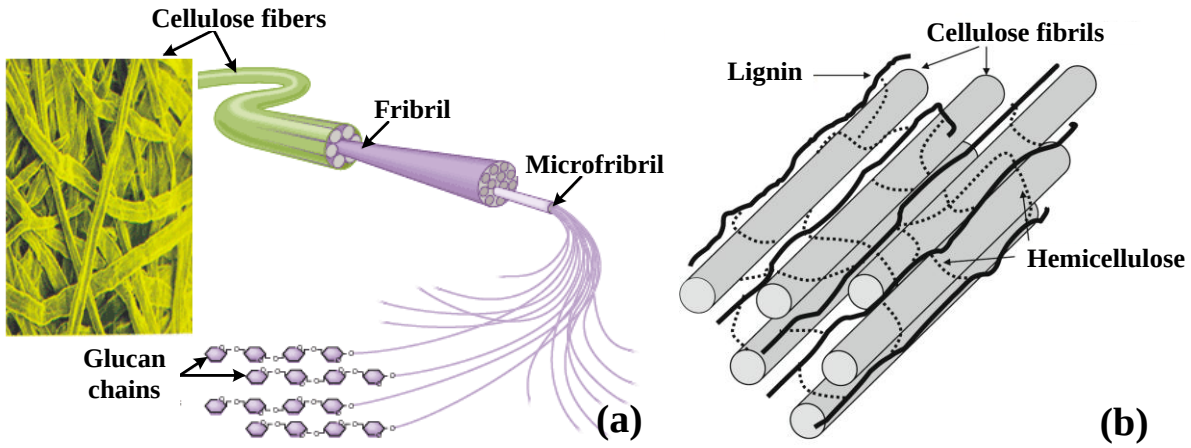
In the United States and worldwide, economies have been depending on fossil fuels (including petroleum), which are finite, nonrenewable energy sources. Fossil fuels currently provide more than 85% of all the energy consumed in the U.S., and virtually all of the liquid transportation fuels [1]. Conventional liquid transportation fuels (e.g. gasoline, diesel fuel, and jet fuel) are distilled from petroleum. Petroleum-based transportation fuels take a large proportion of the nation's total energy consumption and the increasing demand for liquid transportation fuels in the U.S. has been far beyond the domestic production capacity [2]. Meanwhile, consuming petroleum-based transportation fuels contributes to the accumulation of greenhouse gases (CO_2, SO_2, and NO_x) in the atmosphere [3]. Increasing demands for sustainable sources of liquid transportation fuels make it imperative to find alternatives to conventional transportation fuels.

Biofuels produced from cellulosic biomass (forest and agricultural residues and dedicated energy crops) are alternatives to conventional transportation fuels. Land resources in the U.S. are sufficient for sustainable production of over 1 billion dry tons of biomass annually [4,5]. This amount of biomass is sufficient to produce 90 billion gallons of liquid fuels that can replace about 30% of the nation's current annual consumption of conventional petroleum-based transportation fuels [5]. Producing and using cellulosic biofuels can reduce the nation's dependence on foreign oil, create new jobs, improve rural economies, and reduce greenhouse gas emissions [6]. Furthermore, advances in agriculture and biotechnology have made it possible to produce cellulosic biofuels at costs that are significantly lower than petroleum-based transportation fuels [7].

1.2 Overview of composition and structure of cellulosic biomass

Composition of cellulosic biomass includes approximately 40-50% cellulose, 20-30% hemicellulose, and 15-20% lignin [8,9]. As shown in Figure 1.1(a), cellulose, the principal carbohydrate component, is organized into fibrils. A fibril is further formed by microfibrils. A microfibril is an aggregate of glucan chains consisting of many glucose units. These sheets of glucan chains stacking on top of each other give cellulose its highly ordered crystalline characteristic [10].

Figure 1.1 Illustration of structure of cellulosic biomass (after [15])



Surrounding cellulose fibrils is hemicellulose that forms a matrix by bonding with cellulose and other hemicellulose molecules as shown in Figure 1.1(b). Hemicellulose consists of various sugar units. The dominant carbohydrate components of hemicellulose are xylan and glucomannan [11]. Cellulose and hemicellulose are polysaccharides that can be hydrolyzed to sugars fermentable to ethanol biofuels.

Lignin is considered a filler in spaces between cellulose and hemicellulose. Lignin performs an important role in strengthening cell walls of cellulosic biomass by cross-linking polysaccharides (primarily hemicellulose) and providing support to structural elements in a plant

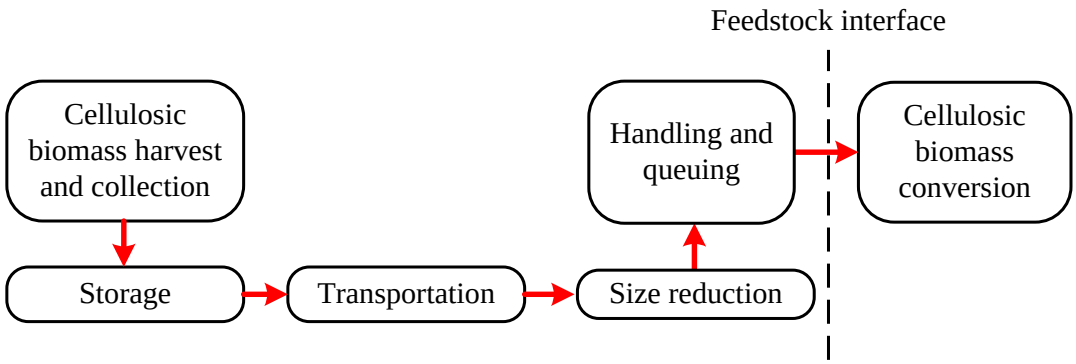
body [12]. This extensive cross-linking of lignin and other polysaccharides limits the accessibility of enzyme to cellulosic biomass. Lignin contains no sugar components and it cannot be digested by enzyme [13]. Lignin can be used to produce compounds for pharmaceutical purposes or can be burned to produce electricity and heat [11,14].

1.3 Role of cellulosic biomass size reduction

As shown in Figure 1.2, before converting to biofuels, cellulosic biomass feedstock has to be processed through a size reduction process to reduce particle size by mechanical methods (e.g., milling, cutting, and chipping) [15]. The position of cellulosic biomass size reduction in the feedstock supply system can be different from that shown in Figure 1.2. For example, size reduction can also be conducted before storage or transportation [16,18].

Cellulosic biomass size reduction is a crucial process with significant impacts on both supply and conversion systems. First, size reduction can aid to increase the bulk density and improve flowability of biomass feedstocks [17]. The properties of biomass chips or particles produced influence decisions on biomass storage and transportation. Second, size reduction is guided by the biofuel conversion requirements [15]. Large size cellulosic biomass (whole stems of herbaceous biomass or chunks of woody biomass) cannot be converted to biofuels efficiently with current conversion technologies [17]. Size reduction is helpful to increase the digestibility of cellulosic biomass in biofuel conversion [18-20]. Third, size reduction is an energy intensive process with potentially significant cost implication in both feedstock supply and biomass conversion systems [15].

Figure 1.2 Illustration of cellulosic biomass feedstock supply system interfacing with biomass conversion system (after [22])



1.4 Objectives of this research

The purpose of this research is to provide knowledge of how size reduction alters biomass structural features, and understand relationships between these biomass structural features and enzymatic hydrolysis sugar yield. This research is also aimed to investigate the impacts of process parameters in biomass size reduction on conversion of cellulosic biomass to biofuels to help realize cost-effective manufacturing of cellulosic biofuels.

Specific research tasks are as follows:

1. Investigate confounding effects of biomass particle size and crystallinity on biomass enzymatic hydrolysis sugar yield. Separate the confounding effects, and study the effects of biomass particle size and crystallinity on sugar yield independently.
2. Evaluate the effects of sieve size used in size reduction equipment on energy consumption in size reduction, cellulose recovery rate in pretreatment, and sugar yield in enzymatic hydrolysis.

3. Study the relationship between cellulosic biomass particle size and sugar yield specified by different sugar yield definitions.
4. Examine the effects of milling orientation in woody biomass size reduction on biomass structural features and sugar yield.

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Chapter 2 - Literature Review - Cellulosic Biomass Size Reduction

Paper title:

Effects of mechanical comminution on enzymatic conversion of cellulosic biomass in biofuel manufacturing: a review

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Abstract

It is imperative to develop alternative fuels to replace current petroleum-based liquid transportation fuels. Biofuels produced from cellulosic biomass (forest products and residues, agricultural residues, and dedicated energy crops) is one such alternative. Manufacturing biofuels from cellulosic biomass requires reduction of the material size using mechanical comminution methods. This paper reviews these mechanical comminution methods. It presents their effects on biomass particle size, cellulose crystallinity, and sugar yield. It also discusses the characteristics of each method and future research directions.

Keywords: Biofuel, Cellulosic biomass, Energy manufacturing, Mechanical comminution, Milling, Sugar yield

2.1 Introduction

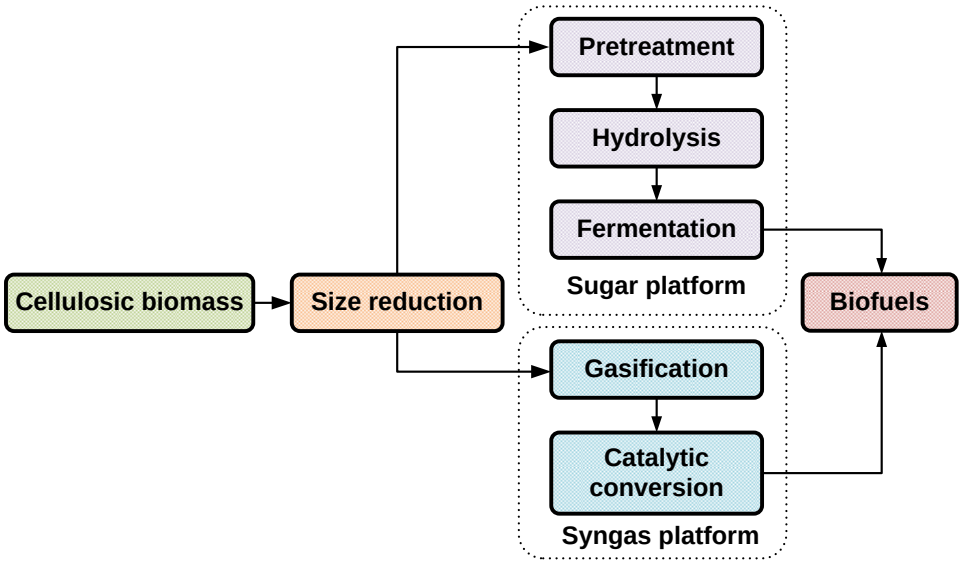
Energy and environment issues are listed highly among the top 10 major concerns facing the global community for the next 50 years [1]. Transportation fuels take a large proportion of energy consumption and the increasing demand for transportation liquid fuels in the U.S. has been far beyond U.S. domestic production capacity [2]. In the near future, the heavy use of petroleum (fossil fuels) for transportation fuels will not change [3].

Renewable fuels, such as biofuels, are becoming more and more important considering the greenhouse effect of fossil fuels, gradual depleting of oil reserves and the dependency on imported oil [4-6]. The U.S., Brazil and China are the top three countries that produce the largest amount of ethanol (one type of biofuels). Most ethanol is now produced from corns. A major drawback of corn-based ethanol is that it causes a competition between ethanol and food/feed products for the limited agricultural farm land and other resources [7,8].

Cellulosic biofuels made from cellulosic biomass will not use food crops. Cellulosic biomass consists of forest products and residuals, agricultural residues and by-products, and energy crops [9]. According to the U.S. Department of Energy, more than 1 billion dry tons of biomass (80% are cellulosic biomass) could be sustainably harvested from the U.S. fields and forests, enough to replace 30% of the nation's annual petroleum consumption for transportation fuels [10]. Therefore, cellulosic biomass has great potential as a feedstock for alternative liquid fuel manufacturing.

Currently, cellulosic biomass can be converted to biofuels using either sugar platform or syngas platform [5,9], as illustrated in Figure 2.1. This review paper concerns only the sugar platform. Cellulosic biomass mainly consists of three components: cellulose, hemicellulose, and lignin. Cellulose can produce fermentable sugar through enzymatic hydrolysis and the sugar can be converted to biofuel (ethanol) by fermentation. However, cellulose usually is sealed by a highly ordered structure formed by hemicellulose and lignin.

Figure 2.1 Two platforms for biofuel production from cellulosic feedstocks (after [9]).



In the sugar platform, the cellulosic biomass (after comminution) is pretreated first. The overall purpose of pretreatment is to break down the shield formed by hemicelluloses and lignin. Pretreatment can help to make the cellulosic feedstock more accessible to enzymatic hydrolysis; thus, can speed up the conversion rate of cellulose to sugar and increase the yield of fermentable sugars (such as glucose, xylose, arabinose, galactose, and mannose). Afterwards, fermentation will convert sugars into biofuel (ethanol) [9,11].

In the syngas platform, the cellulosic feedstocks are taken through a gastification process. In this process, heat and chemicals are used to break biomass into synthesis gas or syngas (CO and H_2). Syngas can then be converted into biofuels [12].

Mechanical comminution methods are needed in both platforms. It is important to know the effects of cellulosic biomass comminution on subsequent steps of biofuel manufacturing; especially, on the enzymatic conversion of cellulosic biomass to sugars. However, no review papers in the literature are focused on this topic. This paper will review the effects of mechanical comminution methods on the enzymatic conversion of cellulosic biomass. It will cover the following aspects: reduction of biomass particle size, disruption of cellulose crystallinity, and improvement of sugar yield. It also discusses the characteristics of each method and future research directions.

2.2 Evaluation parameters for effects of mechanical comminution

Mechanical comminution methods significantly reduce particle size, disrupt cellulose crystallinity, resulting in better microorganism accessibility, rendering the substrate more amenable to subsequent enzymatic hydrolysis to increase sugar yield [11,13]. Mechanical comminution methods include various milling methods: ball milling [14-18], compression

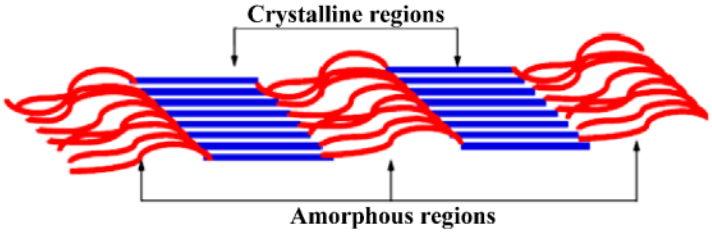
milling [19,20], hammer milling [21,22], and other types of milling (such as fluid energy milling and colloid milling) [11,20].

Material particle size can be determined by microscopy examination. Sizes ranging from 0.5 – 5000 microns can be measured by optical microscope. For fine particles (<0.1 μm), scanning or transmission electron microscopy is advised [23]. Sieving is also a common method to determine the particle size for dry biomass materials [23,24].

Cellulose in cellulosic biomass is usually organized into microfibrils, which are the fundamental structural unit of the cell wall, each measuring about 3 to 6 nm in diameter [25]. As shown in Figure 2.2, cellulose consists of crystalline regions and amorphous regions. The highly ordered crystalline regions of cellulose are less susceptible to enzymatic hydrolysis than the amorphous regions [26-29]. As a result, the crystalline regions of cellulose constitute a major obstacle for the conversion of cellulosic biomass to biofuels [17].

Crystallinity is determined as the percentage of crystalline material in the biomass and expressed as the crystallinity index (CI). Higher CI indicates that the percentage of the crystalline material is higher, which may result in a poor susceptibility to enzymatic hydrolysis. CI is usually determined by an X-ray diffractometer [30].

Figure 2.2 Illustration of cellulose microfibril (after [4]).

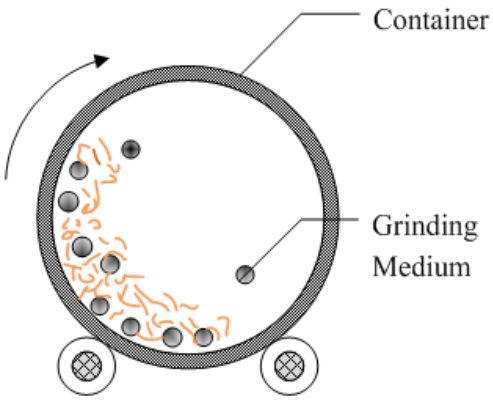


Fermentable sugars after hydrolysis are determined by High Performance Liquid Chromatography (HPLC). Determination of sugar can be done by following the standard procedure described by National Renewable Energy Laboratory (NREL) [31].

2.3 Effects of ball milling

As illustrated in Figure 2.3, a ball mill consists of a cylindrical container rotating around a horizontal axis, partially filled with the material to be ground and the grinding medium (such as ceramic balls, pebbles, and stainless steel balls). When the ball mill is running in the critical speed range, the balls go around on the surface of the container until they get to the top and then fall in a cascade. This internal cascading effect reduces the material to fine powder [32, 33].

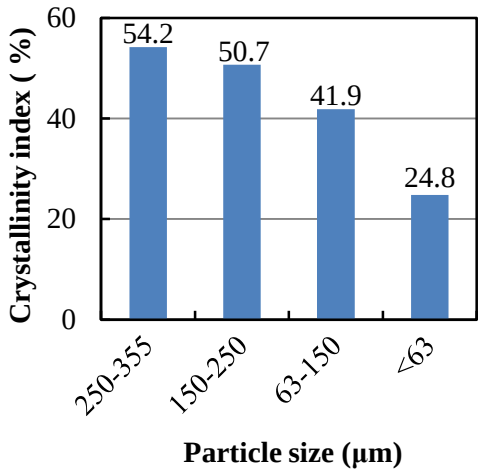
Figure 2.3 Illustration of ball mill (after [32]).



Yoshida et al. [18] studied effects of ball milling on cellulose crystallinity and enzymatic hydrolysis of miscanthus sinensis (a kind of biomass material harvested in Japan) to monosaccharides (fermentable sugar). In this study, air dried biomass material (miscanthus sinensis) was ground by ball-milling for 24 hours. The powder obtained was passed through sieves of different mesh sizes, and separated into four groups: 250–355, 150–250, 63–150, and < 63 μm.

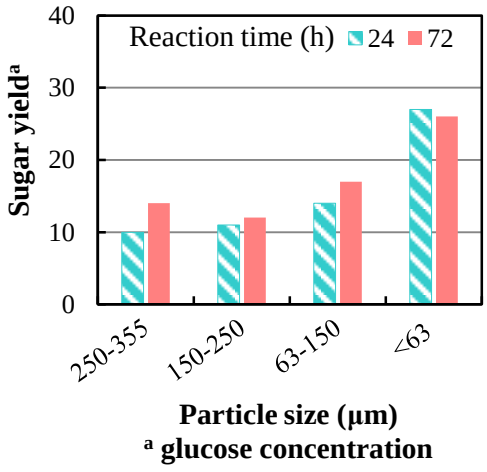
X-ray diffraction analysis indicated that the crystallinity of the biomass declined as the particle size decreased (Figure 2.4).

Figure 2.4 Relation between particle size and crystallinity after ball milling (after [18]).



Each group was hydrolyzed with commercially available cellulase (enzyme used for cellulose hydrolysis). As shown in Figure 2.5, after 24 h and 72 h of reaction, sugar yield increased significantly when biomass particle size was reduced below 63 μm. Sugar yield increased also with the reduction in biomass crystallinity.

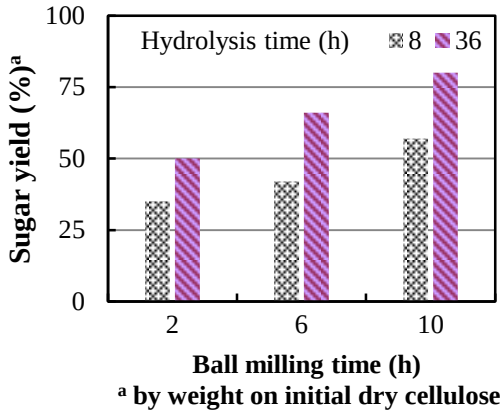
Figure 2.5 Sugar yield for different particle sizes (after [18]).



Sidiras and Koukios [17] studied effects of ball-milling using barley straw. First, the barley straw sample was ground using a hammer mill with a 0.85 mm screen. Then the sample was further ball milled at room temperature in a ball mill with corundum balls.

They found that crystallinity index (CI) of the barley straw cellulose decreased linearly with increasing ball milling time.

Figure 2.6 Sugar yield after different ball milling time (after [18]).



They also conducted hydrolysis on barley straw. As shown in Figure 2.6, after 8 h and 36 h of hydrolysis, straw cellulose prepared with longer ball milling time resulted in a higher sugar yield.

Table 2.1 Effect of ball milling on crystallinity index (after [15]).

Substrate	Initial CI	CI after ball milling	
		Wet ball milling	Dry ball milling
Avicel	82.8	78.9	48.1
Bagasse	60.2	55.6	51.9
Cardboard	70.8	68.6	52.8
Mill Waste	65.8	60.3	64.2
Newspaper	57.4	60.4	53.1
Rice Straw	55.9	52.9	54.2
Sludge	67.3	69.4	38.7

Rivers and Emert [15] studied effects of wet and dry ball milling. Materials used in the study included: one purified cellulose and six waste lignocellulose substrates (bagasse, rice straw, pulp mill primary clarifier sludge, newspaper, corrugated card-board, and saw mill waste). A ball mill was used in either wet or dry mode to comminute materials to ultrafine (<10 μm in diameter) particles. They reported that CI after ball milling ranged from approximately equal to the initial CI (CI before ball milling treatment) to greatly reduced (Table 2.1).

It has been implicated that crystallinity is a major deterrent to enzymatic hydrolysis of cellulose; therefore, the substrate with the lowest CI might in the highest conversion yield of sugar. This, however, was not the case in the study by Rivers and Emert [15]. Their results indicated the complexity of the native lignocelluloses matrix (Table 2.2).

Table 2.2 Effects of ball milling on sugar yield (after [15]).

Substrate	Wet ball milling		Dry ball milling	
	CI	Sugar	CI	Sugar
Avicel	78.9	70.5	48.1	65.5
Bagasse	55.6	25.0	51.9	49.2
Cardboard	68.6	56.8	52.8	70.5
Mill Waste	60.3	35.0	64.2	46.1
Newspaper	60.4	66.7	53.1	57.4
Rice Straw	52.9	59.8	54.2	56.2
Sludge	69.4	48.3	38.7	49.2

Like CI, substrate particle size has long been considered a major factor in enzymatic hydrolysis of lignocelluloses [34,35]. River and Emert's data (shown in Table 2.3) indicated that native substrates are more resistant in a wet ball milling environment. Dry ball milling resulted in a better size reduction. Their study did not provide result of how particles sizes affected enzymatic hydrolysis of cellulose.

Some researchers proposed that effects of reduced CI after ball milling on hydrolysis rate might be a consequence of increased surface area [36] or decreased particle size [37]. However, these structural features of cellulosic biomass are closely associated. The change in one structural feature may also lead to changes in other features.

Table 2.3 Effects of ball milling on material particle size (after [15]).

Average size (μm)		percentage of particles passed 53 μm sieve	
wet	Dry	Wet	Dry
410	41	20	93.9
443	224	15	67.1
465	137	7.5	73.8
248	36	16	97.6
356	97	7.5	90.1
406	133	5.9	70.6
469	445	25	43.7

In Chang et al.'s study [38] on effects of particle size after ball milling on switchgrass digestibility, they found that there was little benefit of reducing particle size below mesh 20 (841 μm). However, from particle size mesh 4 to mesh 20, sugar yield increased as the particles became finer. This is consistent with other studies on different biomass materials [38].

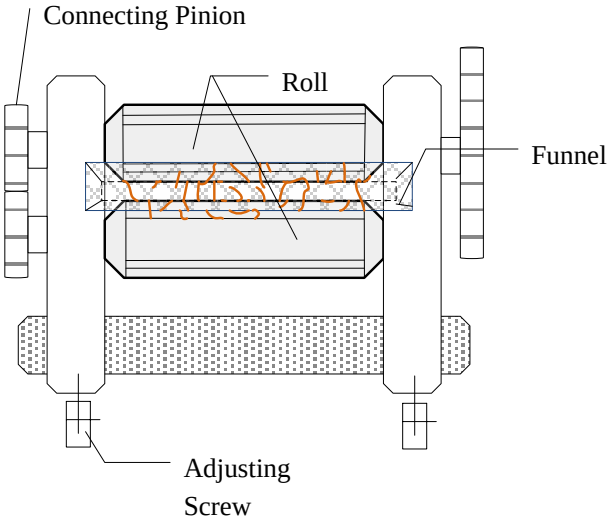
Chang et al. also studied effects of CI on sugar conversion of poplar wood. In order to minimize the effects of particle sizes, poplar wood was milled using a ball mill and sieved through a mesh 40 screen. Then, ball milling was employed to decrystallize the biomass for different lengths of time to make the biomass into different CI levels.

Poplar wood was enzymatically hydrolyzed for 3 days. Sugar yield increased as the CI decreased. The result indicated that enzyme effectiveness depends significantly on CI, and ball milling is an effective method to reduce CI of cellulosic biomass materials.

2.4 Effects of compression mill

A compression mill (Figure 2.7) (also called roll mill) consists of two metal rolls placed horizontally. The rolls are set close together, and gap setting between the rolls are adjustable by screws [19].

Figure 2.7 Illustration of compression mill (after [19,20]).



Tassinari and Macy [20] reported that compression mill was effective for increasing the susceptibility of cellulose to enzymatic hydrolysis.

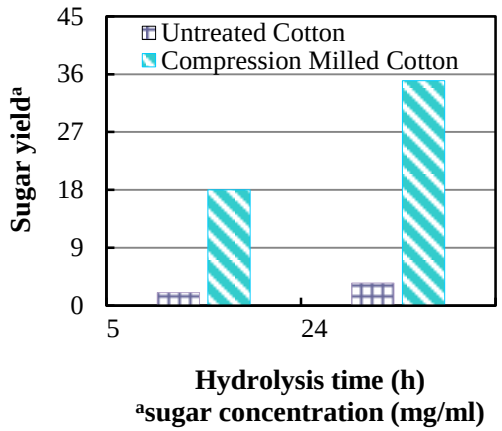
In their study, several materials were investigated: cotton, newspaper, softwoods and hardwoods containing different amounts and types of lignin and hemicelluloses.

During compression milling of cotton, substantial compressive and shearing forces exerted by the rolls were responsible for a decrease in CI.

After 5 h and 24 h enzymatic hydrolysis of cotton, cotton that was processed on the compression mill for 3 min yielded much more sugar than untreated cotton (Figure 2.8).

The authors also studies milling of soft wood (eastern white pine) and hard wood (sugar maple). The compression milled maple yield 17 times more sugar than untreated control after a 24 h hydrolysis while the pine showed a sevenfold improvement over the untreated control. The same increase was observed when comparing the sugar yield of milled and untreated samples in other materials studied in this paper.

Figure 2.8 Effects of compression milling on sugar yield of cotton (after [20]).



2.5 Effects of hammer mill

A hammer mill (Figure 2.9) is essentially a steel drum containing a horizontal rotating shaft on which hammers are mounted. The hammers are fixed to the rotor. The comminution process is performed through an impact-induced material fragmentation [33,39]. Material leaving contact with the hammers reaches the sieve and will fall through the sieve if small enough; if too big, material is recirculated [40] and mixed with the fed material and the milling process resumes.

Mandels et al. [22] milled newspaper with hammer mill through different screen sizes. They reported that sugar yield did not increase as the screen size decreased. Hammer milling gave good size reduction without increasing the availability of cellulose in newspaper. Prolonged hammer milling may actually reduce the availability of the cellulose (Table 2.4).

Figure 2.9 Illustration of hammer mill (after [33]).

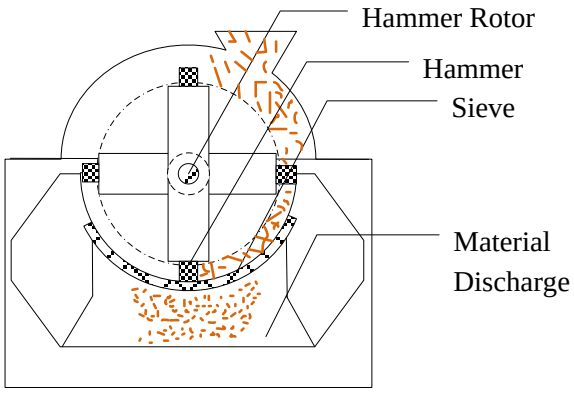


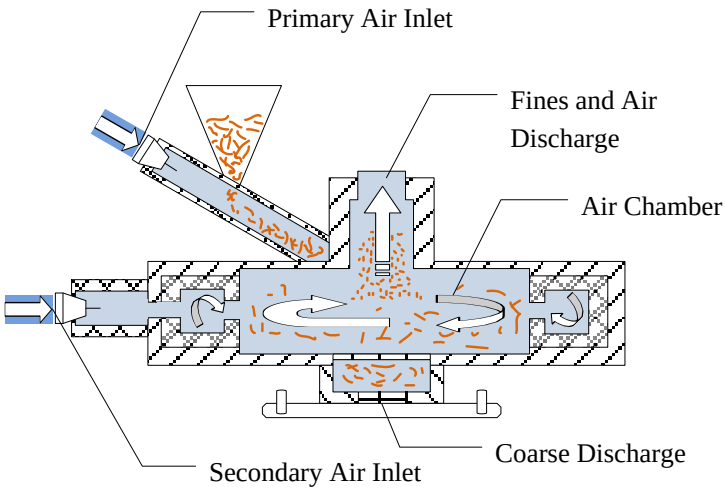
Table 2.4 Effects of screen size in hammer milling and hydrolysis time on sugar yield (after [22]).

Screen size (in)	Sugar yield (%)			
	1 h	4 h	24 h	48 h
0.25	5.6	14.0	24.1	25.6
0.12	7.8	15.0	19.6	26.8
0.02	5.9	12.6	24.5	24.8
0.006	3.4	8.6	14.9	17.1

2.6 Effects of fluid energy mill

In the operation of a fluid energy mill (Figure 2.10), gas of high energy content is introduced into an air chamber. Feed materials in the chamber are caused to impinge upon themselves at high velocities while entrained in the gas stream, causing a reduction in particle size [40].

Figure 2.10 Illustration of fluid energy mill (after [40]).



Mandels et al. [22] presented the fluid energy milling method. The feed to the mill was done pneumatically using air at approximately 42 psi, at a rate of 48 SCFM (standard cubic feet per minute).

This mill gave considerable size reduction. It also resulted in an increase in sugar yield after 1 hr of hydrolysis. This advantage decreased as hydrolysis proceeded (Table 2.5).

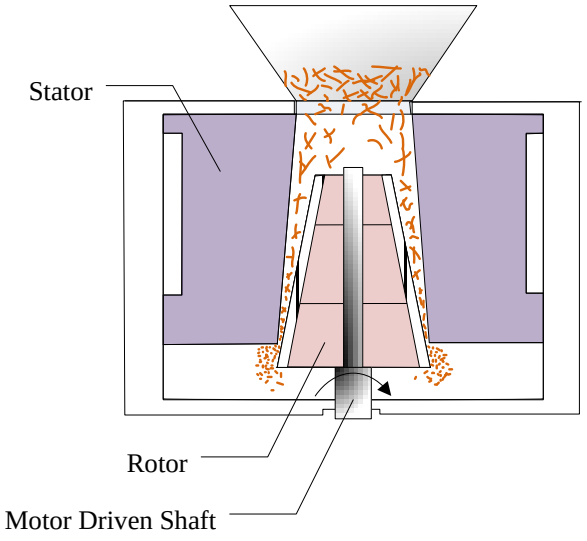
Table 2.5 Effects of feed rate in fluid energy milling and hydrolysis time on sugar yield (after [22])

Material feed rate (kg/hr)	Sugar yield (%)			
	1h	4h	24h	48h
0.272	10.3	16.4	26.3	31.7
0.726	10.8	14.6	25.6	29.2
2.540	8.1	11.3	24.1	29.2
2.812	7.4	11.0	20.9	23.4
1.089	8.1	11.3	20.9	25.6
Untreated	5.8	11.0	20.9	21.6

2.7 Effects of colloid mill

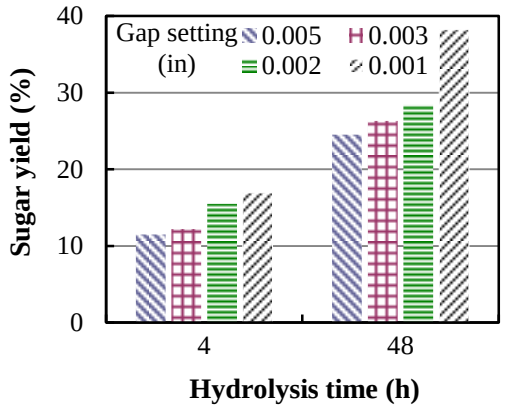
A colloid mill (Figure 2.11) works on the principle of hydraulic shear. It can apply a tremendous amount of energy on a small portion of material in the form of a thin film. This will reduce particles to ultra fine size [41,42]. A colloid mill consists of a stator, rotor and a motor-driven shaft system. The gap between the stator and the rotor can be adjusted.

Figure 2.11 Illustration of colloid mill (after [41]).



Mandels et al. [22] studied effects of colloid milling on sugar yield of newspaper in enzymatic hydrolysis. The colloid milled materials showed an increase in sugar yield for the entire 48 h hydrolysis period. The results also indicated that sugar yield increased as the gap setting decreased (Figure 2.12).

Figure 2.12 Effects of colloid milling on sugar yield of newspaper in enzymatic hydrolysis (after [22]).



2.8 Concluding remarks

Mechanical comminution methods have long been used for biomass feedstocks in biofuel manufacturing. Besides size reduction, mechanical comminution can modify physical structure of biomass materials; thus, can make feedstocks more amenable to enzymatic hydrolysis and increase sugar yield.

Extensive research has been done to investigate effects of mechanical comminution on cellulosic biomass enzymatic conversion. Most of the studies were conducted at particle size of about hundreds of microns or finer. Few studies were conducted at or above millimeter particle size. In most of the studies, in order to achieve biomass particles to micron size level, several days of ball milling is needed. Moreover, the productivity is relatively low. In order to make progress to large-scale production, future studies are needed to explore the effects of mechanical comminution on cellulosic biomass enzymatic conversion at or above millimeter particle size level.

Fundamental understanding of how mechanical comminution changes biomass structural features is needed. Currently, mechanical comminution equipment has been considered as a

"black-box" system in reported investigations. Interactions between biomass materials and comminuting media (such as hammers and balls) are not clear.

Further understanding of how biomass structural features affect enzymatic conversion is necessary. In fact, biomass structural features are closely associated. This makes structural features such as crystallinity, specific surface area and particle size etc. confounded. Efforts have been made to separate some confounding factors, but these factors have not been fully separated. How to untangle these factors and study them separately remains unsolved.

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Chapter 3 - A Preliminary Investigation on Biomass Size Reduction using a Metal-cutting Milling Machine

Paper title:

Size reduction of cellulosic biomass in biofuel manufacturing: effects of milling orientation on sugar yield

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Abstract

Cellulosic biofuels can reduce greenhouse gas emissions and the nation's dependence on foreign oil. In order to convert cellulosic biomass into biofuels, size reduction of biomass is a necessary step. Most related studies in the literature claimed that smaller particles produced higher sugar yields. However, some researchers reported that this claim was not always true. The literature does not have satisfactory explanations for the inconsistence. This paper presents an experimental study on size reduction of poplar wood using a metal cutting process (milling). The results provided one explanation for this inconsistence. It was found for the first time that milling orientation had a strong effect on poplar wood sugar yield. Although smaller poplar particles had a higher sugar yield when they were milled from the same orientation, this trend did not exist for particles milled from different orientations.

Keywords: Cellulosic biofuel, Hydrolysis, Poplar wood, Pretreatment, Size reduction

3.1 Introduction

Currently, transportation is almost entirely dependent on petroleum-based fuels [1]. Increasing demands for sustainable sources of liquid transportation fuels make it imperative to develop alternatives to petroleum-based fuels [1].

Biofuels produced from cellulosic biomass (forest resources, agricultural residues and by-products, and energy crops) offer an alternative to petroleum-based liquid transportation fuels. They can reduce green house gas emissions and the nation's dependence on foreign petroleum while continue to meet the nation's transportation energy needs [2,3]. More than 1 billion dry tons of biomass could be sustainably harvested from U.S. fields and forests, which is enough to displace 30 percent of the nation's current annual petroleum consumption for transportation fuels

[4]. Unlike other feedstocks (e.g. corn, sugar cane, and soybean) for biofuels, cellulosic biomass does not compete for limited agricultural land [5,6].

Size reduction is a necessary step for biofuel manufacturing using cellulosic biomass. For a size reduction process, it usually takes more energy to produce smaller particles [7]. Therefore, it will save energy in size reduction step if larger particles are produced.

Particle size also affects sugar yield, and sugar yield is proportional to biofuel (ethanol) yield [8,9]. In the literature, it was claimed that smaller particles would produce higher sugar yields [10-12]. However, some researchers reported that this claim was not always true [13-16]. The literature does not have satisfactory explanations for the inconsistence.

This paper presents an experimental study on size reduction of poplar wood using a metal cutting process (milling). The results provided one explanation for this inconsistence.

The remainder of this paper is organized as follows: Section 2 presents background information on biofuel manufacturing using poplar wood. The experimental conditions are described in Section 3. Evaluation parameters and their measurement procedures are discussed in Section 4. In Section 5, experimental results are presented. Conclusions and future research are presented in Section 6.

3.2 Background information on biofuel manufacturing using poplar wood

3.2.1 Characteristics of poplar wood

Poplar refers to trees in the genus populus (a genus of 25–35 species), such as cottonwoods, aspens, hybrid poplars, and white poplars [17]. Poplar trees grow fast and can produce 4 to 10 dry tons of wood per acre annually with a very wide distribution in North America [18-20].

Poplar wood consists of cellulose shielded in a hemicellulose/lignin matrix, and a small fraction of other components. Cellulose is a linear polysaccharide polymer with many glucose (a six-carbon sugar) units that can be converted into ethanol [21]. Hemicellulose is a highly-branched complex polymer composed mainly of xylose and other five-carbon sugars [22]. The conversion of five-carbon sugars to ethanol is difficult [23]. Lignin is a polymer filling the spaces between cellulose and hemicellulose, and cannot be converted into biofuels by current technologies [24].

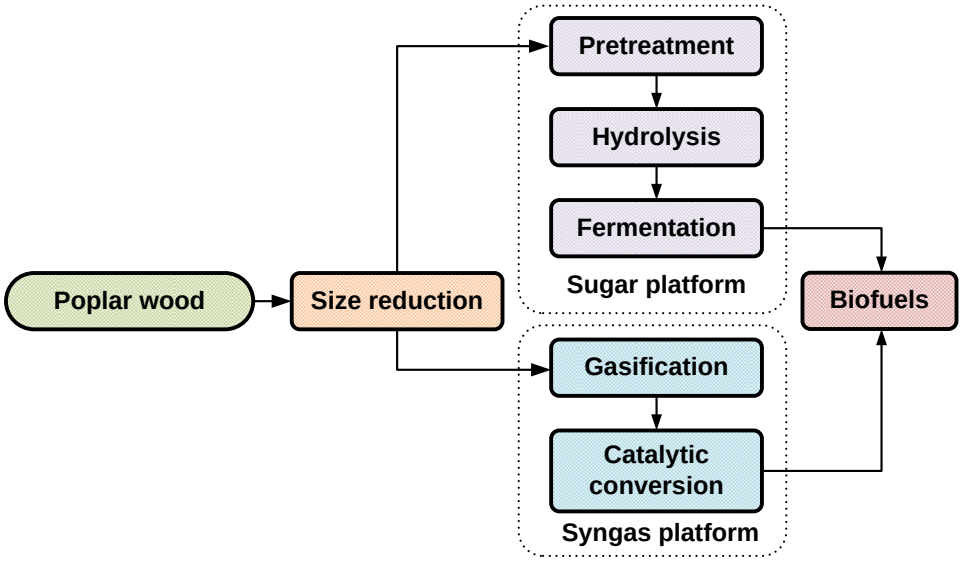
Poplar wood contains about 50% of cellulose and about 20% of lignin [19]. Poplar wood has a porous structure with shorter fibers and smaller cells compared with many other hardwoods [25], resulting in higher biofuel yields [19,26].

3.2.2 Major steps in conversion of poplar wood to biofuels

Poplar wood can be converted to biofuels through either sugar platform or syngas platform [10,27], as illustrated in Figure 3.1. Size reduction of poplar wood is required on both platforms. It is usually conducted on hammer mills, ball mills, compression mills [28], etc.

On the sugar platform, pretreatment helps to make biomass more accessible to enzymatic hydrolysis and, thus, can speed up the conversion rate. Hydrolysis reduces cellulose into fermentable sugars. Afterwards, fermentation converts sugars into biofuels (ethanol) [27]. On the syngas platform, gasification process breaks biomass into syngas (CO and H_2). Syngas can then be converted into biofuels [29].

Figure 3.1 Two platforms to produce biofuels from poplar wood (after [27]).



3.3 Experimental conditions

3.3.1 Materials and machine

Biomass materials used in this study were commercial poplar boards (2 × 6 ×25 inch) purchased from The Home Depot Inc. (Manhattan, KS). Size reduction of poplar wood (as shown in Figure 3.2) was conducted on a metal cutting machine − a plain milling machine (Model No. 2, Brown & Sharpe MFG. Co., Providence, RI) with a high-speed steel slab milling cutter (Figure 3.3). The diameter of the cutter was 4 inch, and the length of the cutter was 6 inch. The cutter had a helical angle of 45° and a rake angle of 10°.

3.3.2 Milling orientation

Three milling orientations (O1, O2 and O3) were employed in this study, as illustrated in Figure 3.4. These three milling orientations were determined by the three directions of wood. The longitudinal direction was parallel to the long axis of the stem, the radial direction was

perpendicular to both the growth rings and the long axis of the stem, and the tangential direction was tangent to the growth rings.

Figure 3.2 Poplar wood size reduction process.

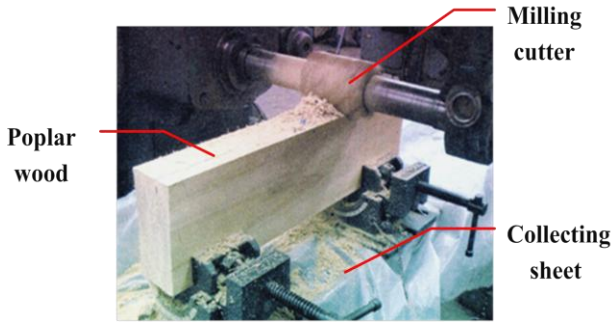


Figure 3.3 Milling cutter.

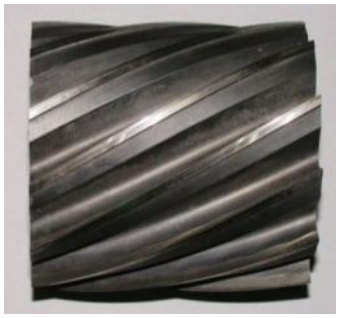
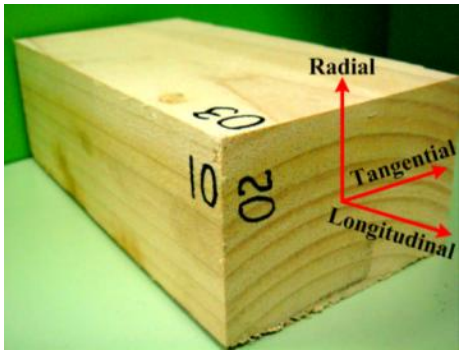


Figure 3.4 Illustration of three milling orientations.



Orientation 1 was the surface parallel to the longitudinal and radial directions, Orientation 2 was the surface parallel to the radial and tangential directions, and Orientation 3 was the surface parallel to the longitudinal and tangential directions.

3.3.3 Milling conditions

From each orientation, two groups of poplar particles were obtained under two different levels of depth of cut: 0.025 inch and 0.25 inch. Feedrate was kept constant at 4.5 inch·min^{-1}. Tool rotation speed was kept constant at 225 rev·min^{-1}. In total, six groups of poplar particles under six different milling conditions were prepared, as listed in Table 3.1.

Table 3.1 Poplar milling conditions.

Condition No.	Milling orientation	Depth of cut (inch)
1	1	0.025
2	1	0.25
3	2	0.025
4	2	0.25
5	3	0.025
6	3	0.25

3.3.4 Sample collection

For each test condition, poplar particles that fell onto the plastic sheet underneath the workpiece were collected. Particles were first sieved through a No. 18 mesh size sieve to get rid of very fine particles. However, poplar particles milled from Condition #2 (Orientation 2 and 0.025 inch depth of cut) were not sieved because these particles were very fine. Then 25 g of poplar particles under each condition were kept in individual sealed Ziploc® bag.

3.4 Evaluation parameters and their measurement

3.4.1 Poplar particle size observation

A small amount of poplar particles were randomly picked from each Ziploc® bags. Digital pictures of these six groups of particles were taken and observed by naked eyes.

3.4.2 Sugar yield and its measurement

3.4.2.1 Definition of sugar yield

In this study, sugar yield was expressed in the percentage of cellulose digested into glucose. Sugar yield was calculated by dividing the amount of cellulose digested by enzymes by the initial cellulose content in dry biomass loaded. The following formula was used for sugar yield calculation:

$$\text{Sugar yield} = \left(\frac{\text{Mass of cellulose digested}}{\text{Mass of cellulose loaded}} \right) \times 100\% \quad (1)$$

3.4.2.2 Sugar yield measurement

Figure 3.5 Four steps in sugar yield measurement

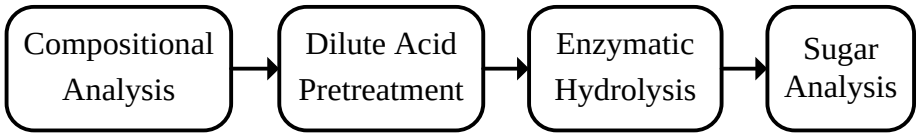


Figure 3.5 shows the measurement procedure of sugar yield. Compositions of poplar particles were firstly analyzed to determine the amount of cellulose and lignin. Second, pretreatment was employed to expose cellulose in biomass to make it more accessible to enzymes. After pretreatment, enzymatic hydrolysis for 96 hours broke down cellulose to

fermentable sugar (glucose). In this study, the amount of glucose was determined using HPLC (Shimadzu, Kyoto, Japan). HPLC is a chromatographic technique that can identify and quantify individual components of a liquid mixture.

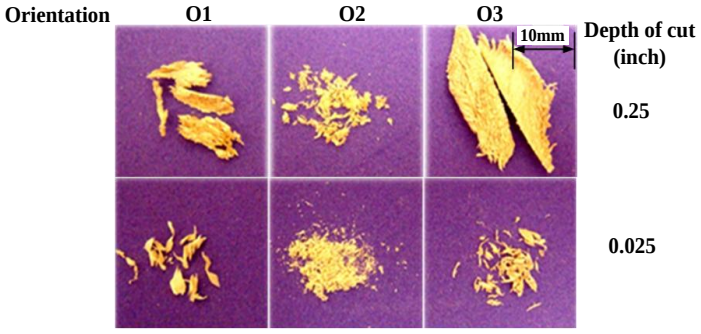
3.5 Results and discussion

3.5.1 Effects of milling condition on poplar particle size

It was observed that poplar particles obtained under the same milling condition had nearly same size. However, particles collected under different milling conditions had very different sizes.

As shown in Figure 3.6, from the same orientation, milling with 0.25 inch depth of cut resulted in larger particles than milling with 0.025 inch depth of cut. Using the same depth of cut (either 0.25 or 0.025 inch) but from different orientations, poplar particle sizes differed significantly. Among all these milling orientations, Orientation 2 resulted in smaller particle size. Poplar particles milled with 0.025 inch depth of cut from Orientation 2 were the smallest. Poplar particles milled with the larger depth of cut (0.25 inch) from Orientation 2 were smaller than those milled with the smaller depth of cut (0.025 inch) from Orientation 1.

Figure 3.6 Poplar particles milled under different conditions.



3.5.2 Effects of depth of cut on sugar yield

As shown in Figure 3.7 to 3.9, from the same orientation (for all the three milling orientations), the smaller depth of cut (0.025 inch) produced smaller particles and higher sugar yields than the larger depth of cut (0.25 inch). The results (from the same orientation) were consistent with many reported studies on effects of particle size on sugar yield [13,14].

Figure 3.7 Effects of depth of cut on sugar yield (Orientation 1).

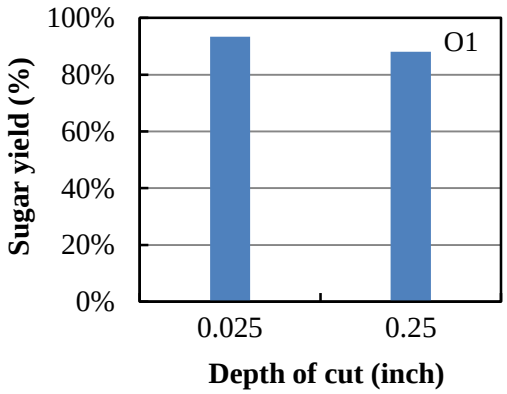


Figure 3.8 Effects of depth of cut on sugar yield (Orientation 2).

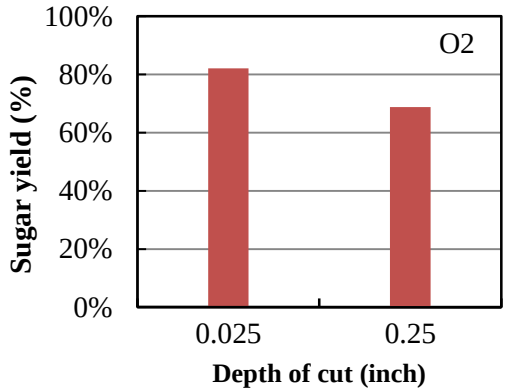
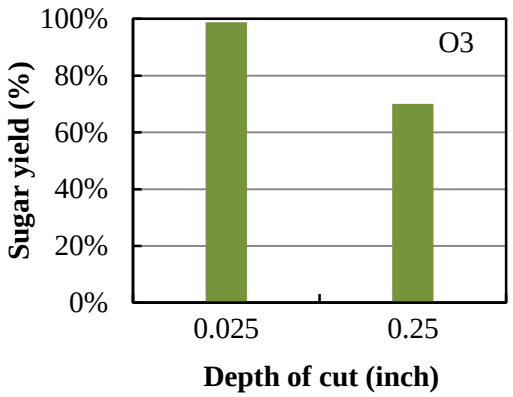


Figure 3.9 Effects of depth of cut on sugar yield (Orientation 3).



3.5.3 Effects of milling orientation on sugar yield

Comparing sugar yields of three groups of poplar particles milled with 0.025 inch depth of cut from three orientations, and three groups of poplar particles milled with 0.25 inch depth of cut from three orientations, it can be seen that the smallest particles (milled with 0.025 inch depth of cut from Orientation 2) yielded less sugar than the other two groups (from Orientations 1 and 3) milled with 0.025 depth of cut. Furthermore, among the three groups milled with 0.25 inch depth of cut, the particles from Orientation 1 had the highest sugar yield, but the particles were much larger than those from Orientation 2.

For the sugar yield measurement under each test condition, two samples were used. Sugar yield data presented in this paper were the mean values of these two samples. The variation under each condition was very small, and not presented in the graphs. Analysis of variance was performed by statistics software Minitab (Version 15, Minitab Inc., State College, PA, USA). The differences in sugar yields for different conditions were significant at the significance level of $\alpha = 0.05$.

3.6 Conclusions

Poplar particles were obtained by a metal cutting process (milling) from three different orientations. Two levels of depth of cut were employed for each orientation. It was observed that, if poplar wood was milled using the same depth of cut but from different orientations, particle sizes could be very different.

It was found for the first time that milling orientation had a strong effect on sugar yield of poplar particles. Although smaller poplar particles had a higher sugar yield when they were milled from the same orientation, this trend did not exist for particles milled from different orientations. The results provide an explanation to the inconsistent claims in the literature about the effects of particle size on sugar yield of cellulosic biomass. If all the other conditions in size reduction are kept the same, smaller particles produce higher sugar yield. However, it is possible that, if poplar particles are milled under different conditions (for example, milled from different orientation), the relation that smaller particles produce higher sugar yield does not hold true anymore.

The results in this paper also have significant practical implications for size reduction of poplar wood for biofuel manufacturing. They demonstrate some possible ways to solve this dilemma: smaller particles are desirable for higher sugar yield, but large particles are preferred for less energy consumption in size reduction step. For example, larger particles obtained by milling poplar wood from one orientation can produce the same or higher sugar yield than smaller particles obtained by milling poplar wood from other orientations.

The authors will conduct further research to answer the following questions:

1. How does milling orientation affect power consumption in size reduction step?

2. How do other milling parameters (feedrate, tool geometry, etc.) affect power consumption in size reduction step and sugar yield?
3. Why do poplar particles obtained by milling from different orientations have different sugar yields?

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Chapter 4 - Confounding Effects of Particle Size and Biomass Crystallinity: Study 1

Paper title:

Size reduction of cellulosic biomass in biofuel manufacturing: a study on confounding effects of particle size and biomass crystallinity

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Abstract

Biofuels derived from cellulosic biomass offer an alternative to petroleum-based liquid transportation fuels. In order to convert cellulosic biomass into biofuels, size reduction is a necessary step along with pretreatment, enzyme hydrolysis, and fermentation. In the literature, there are inconsistent reports about why size reduction affects sugar yield (proportional to biofuel yield). An important reason for the inconsistence is that particle formation in current size reduction methods is not well controlled, causing effects of some biomass structural parameters confounded. In this study, a metal-cutting (milling) process is used for size reduction of poplar wood, where particle formation can be well controlled to prevent the effects of multiple parameters from being confounded. The results of this study provide explanations for some inconsistent reports in the literature. These results also reveal some opportunities for future research to understand the effects of size reduction on cellulosic biofuel manufacturing.

Keywords: Biofuel, Cellulosic biomass, Crystallinity, Particle size, Poplar wood, Size reduction

4.1 Introduction

Today's transportation is almost entirely dependent on petroleum-based fuels [1]. Increasing demands for sustainable sources of liquid transportation fuels make it imperative to find alternatives to petroleum-based fuels [1].

Biofuels produced from cellulosic biomass (forest and agricultural residues and energy crops) offer an alternative to petroleum-based liquid transportation fuels. Biofuels not only reduce the nation's dependence on foreign petroleum but also improve the environment through reduction of greenhouse gas emissions [2,3]. According to the U.S. Department of Energy, land resources in the U.S. are sufficient to sustain production of enough biomass annually to replace

30% or more of the nation's current consumption of liquid transportation fuels [4]. Unlike other types of feedstocks (e.g. corn, sugar cane, and soybean) for biofuels, cellulosic biomass does not compete with food or feed production for limited agricultural land [5,6].

In order to convert cellulosic biomass into biofuels, cellulosic biomass must go through a size reduction step first [2]. Current size reduction methods include various biomass milling methods (more details will be provided in Sec. 4.2.2). In the literature, there are inconsistent reports about why size reduction affects sugar yield (proportional to biofuel yield). It is not clear which biomass structural parameters (particle size, biomass crystallinity, etc.) dominate sugar yield [7-9]. An important reason for the inconsistence is that particle formation is not well controlled in current size reduction methods. Therefore, the effects of some biomass structural parameters on sugar yield are confounded.

This paper presents the first attempt to use a metal-cutting (milling) process to produce particles with a well-controlled particle formation mechanism. It studies the confounding effects of two structural parameters (particle size and biomass crystallinity) of cellulosic biomass on sugar yield.

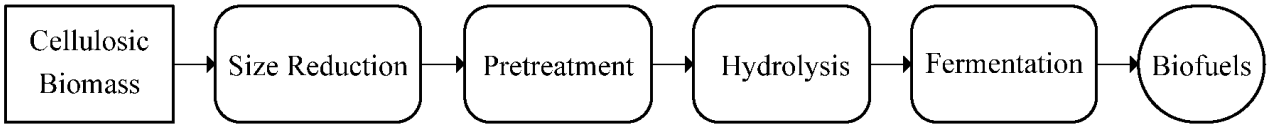
The remainder of this paper is organized as follows. Section 4.2 provides background information on biofuel manufacturing using cellulosic biomass. Section 4.3 describes experimental conditions. Section 4.4 provides definitions and measurement procedures of evaluation parameters. Section 4.5 discusses experimental results. Section 4.6 presents conclusions and future work.

4.2 Background information on biofuel manufacturing using cellulosic biomass

4.2.1 Major steps in conversion of cellulosic biomass to biofuels

Major steps in conversion of cellulosic biomass to biofuels (through sugar platform) are listed in Figure 4.1. Size reduction reduces the particle size of cellulosic biomass. Pretreatment helps to make cellulose in the biomass more accessible to enzymes in enzymatic hydrolysis. Hydrolysis depolymerizes cellulose into fermentable sugar (glucose). Afterwards, fermentation converts sugar into biofuels (ethanol) [10].

Figure 4.1 Major steps in conversion of cellulosic biomass to biofuels (after [10]).



4.2.2 Current size reduction methods

Current size reduction methods include hammer milling [11,12], ball milling [13-16], knife milling [17], fluid energy milling [18], colloid milling [18], and compressive milling [19,20]. These methods were discussed in a review paper [21].

Figure 4.2 to 4.4 illustrate three commonly used size reduction methods (hammer milling, ball milling, and knife milling), respectively. In hammer milling (Figure 4.2), hammers are mounted on a rotating steel drum. Size reduction is performed through impact-induced material fragmentation [23]. When biomass particles become smaller than the sieve size, they will fall through the sieve. Particles larger than the sieve size will be recirculated to mix with the feed biomass for continuing size reduction [24].

Figure 4.2 Illustration of hammer milling (after [22]).

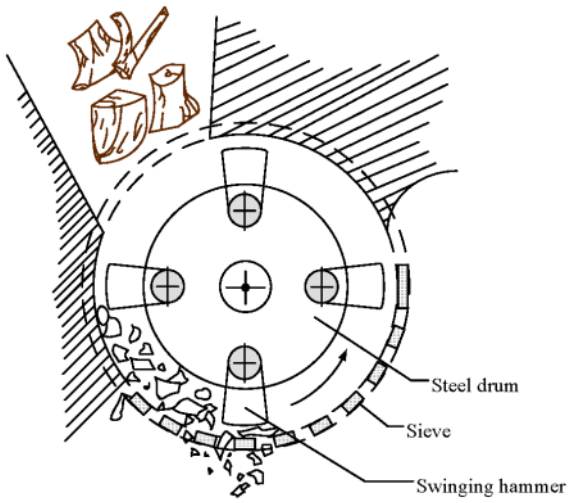
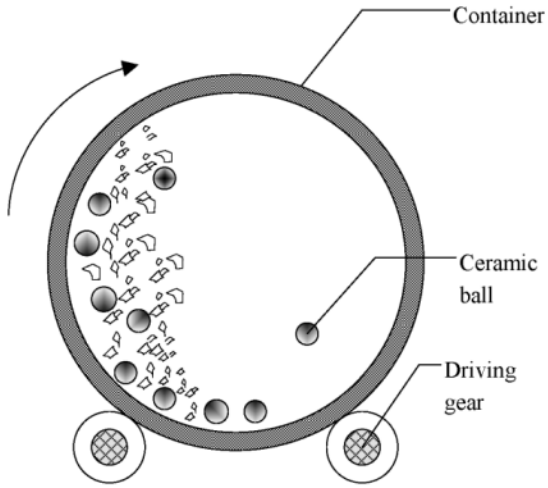


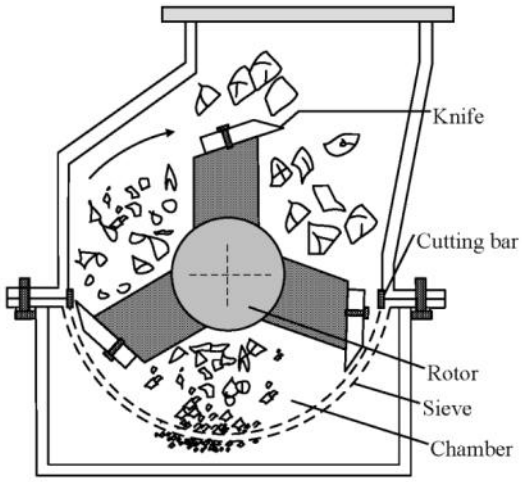
Figure 4.3 Illustration of ball milling (after [25]).



In ball milling (Figure 4.3), a cylindrical container rotates around a horizontal axis. The container is partially filled with cellulosic biomass and ceramic balls (or steel balls). When the container is rotating in a critical speed range, the ceramic balls go around on the surface of the container until they get to the top and then fall in a cascade. This internal cascading effect reduces biomass to smaller sizes [25,26].

In knife milling (Figure 4.4), cellulosic biomass comes into contact with three cutting knives equipped on a rotor in the chamber. Biomass is cut between the knives and the cutting bars. Particles that are smaller than the sieve size will pass through the openings of the sieve; those larger than the sieve size will be recirculated and continue being milled [27].

Figure 4.4 Illustration of knife milling (after [27]).



4.2.3 Confounding effects caused by current size reduction methods

Table 4.1 summarizes reported relationships between biomass structural parameters (particle size and biomass crystallinity) and sugar yield. Some researchers reported that smaller particle size produced higher sugar yields; while some other researchers did not support such relationship. Inconsistent results are also reported for the relationships between biomass crystallinity and sugar yield.

In the current size reduction methods, particle size was controlled by the sieve size [38]. In order to produce smaller particles, longer milling time was usually needed. Longer milling time would also decrease biomass crystallinity [9]. In other words, in the current size reduction

methods, biomass samples with smaller particle sizes almost always had lower biomass crystallinity. Therefore, their effects on sugar yield were confounded.

Table 4.1 Reported relationships between biomass structural parameters (biomass crystallinity and particle size) and sugar yield

Reported relationship	Reference
Smaller particle sizes produced higher sugar yield	[28-31]
No correlation between particle size and sugar yield	[32,33]
Lower biomass crystallinity produced higher sugar yield	[31,32,34-36]
No correlation between biomass crystallinity and sugar yield	[29,37]

4.3 Experimental conditions

4.3.1 Machine, Milling cutter, and workpiece material

A metal cutting (milling) machine (Model H, Kearney & Trecker Corporation, Milwaukee, WI) was employed to reduce the particle size of poplar wood.

Figure 4.5 Picture of the milling cutter.

Figure 4.5 shows the milling cutter that was installed in the tool holder of the machine. It was a Slot Master® indexable milling cutter (Model SM612158, Republic Drill APT

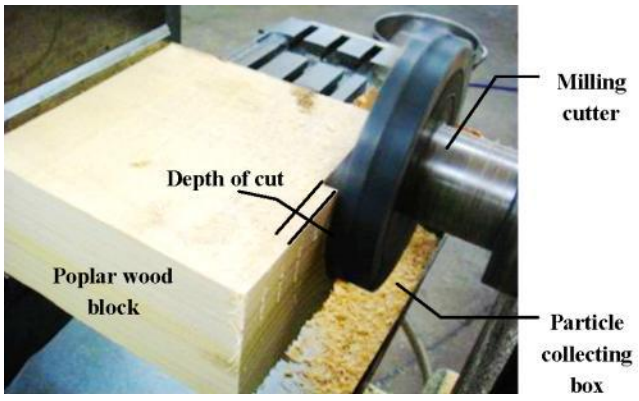
Corporation, Melrose Park, IL, USA). Twelve carbide inserts were mounted on the milling cutter. The shape of the inserts was 87 °parallelogram. The thickness of the inserts was 3/16 inch (4.76 mm), and the nose radius of the inserts was 1/32 inch (0.8 mm). The rake angle of the milling cutter was 0 °.

Cellulosic biomass used in this study was poplar wood. A 12 ×12 × 60 inch (305 × 305 × 1524 mm) wood block was purchased from a local lumber company (Griffith Lumber Company, Manhattan, KS, USA) and custom cut into small blocks of 12 ×12 × 5 inch (305 × 305 × 127 mm).

4.3.2 Experimental setup

As shown in Figure 4.6, the poplar wood block was fed towards the milling cutter that rotated at a certain speed. Particles were cut off from the poplar wood block by the milling cutter and fell down into a collecting box. Each particle was formed by only one cutting action. Under a fixed cutting condition (with fixed feedrate, tool rotation speed, and depth of cut), all the particles would be formed by the identical mechanism.

Figure 4.6 Experimental setup.



4.3.3 Cutting orientations

Three cutting orientations (O1, O2 and O3), as illustrated in Figure 4.7, were employed in this study to produce particles. They were determined by the three directions of wood. The longitudinal direction was parallel to the long axis of the stem, the tangential direction was tangent to the growth rings, and the radial direction was perpendicular to both the growth rings and the long axis of the stem.

Figure 4.7 Illustration of three directions of wood and three cutting orientations.

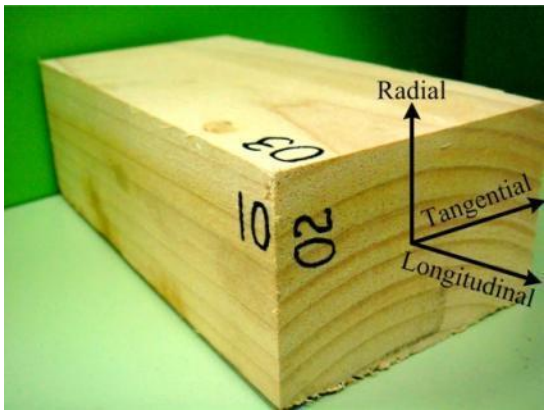


Table 4.2 Nine different cutting conditions.

Cutting orientation	Depth of cut	
	(inch)	(mm)
O1	0.10	2.5
O1	0.25	6.4
O1	0.35	8.9
O2	0.10	2.5
O2	0.25	6.4
O2	0.35	8.9
O3	0.10	2.5
O3	0.25	6.4
O3	0.35	8.9

Orientation 1 (O1) was the surface parallel to the longitudinal and radial directions. Orientation 2 (O2) was the surface parallel to the radial and tangential directions. Orientation 3 (O3) was the surface parallel to the longitudinal and tangential directions.

4.3.4 Cutting conditions

Particles were produced under nine different cutting conditions as listed in Table 4.2. For each of the three orientations, three different depths of cut were used: 0.10, 0.25, and 0.35 inch (2.5, 6.4, and 8.9 mm). Before tests under each condition, the milling cutter and the collecting box were cleaned thoroughly by blowing with compressed air.

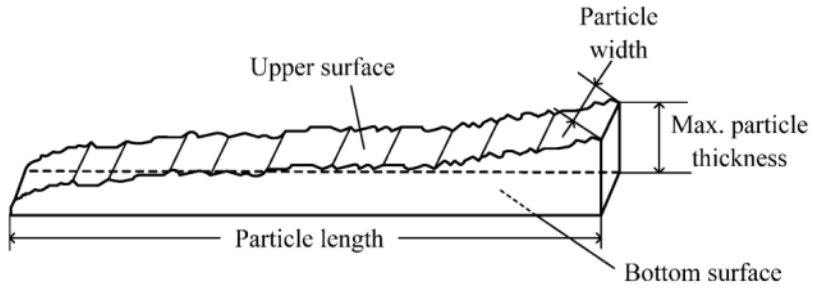
4.3.5 Sample collection

Under each condition, 50 grams of particles were collected. After collecting, they were kept in individual sealed Ziploc® bags and stored in a refrigerator at 4 ℃.

4.4 Evaluation parameters and their measurement procedures

4.4.1 Particle (chip) size

Figure 4.8 Illustration of a biomass particle (not to scale).



A particle (or chip) after size reduction is illustrated in Figure 4.8. In this paper, particle size is investigated by four definitions: width, thickness, length, and volume. Under each cutting

condition, 20 particles were randomly picked for particle size measurement. A digital caliper (Model IP-65, Mitutoyo Corporation, Kawasaki, Japan) was used to measure the thickness (the thickness being measured was the maximum thickness) and width of the particle. A metric ruler (with 10 divisions per centimeter) was used to measure its length. Figure 4.9 illustrates how these dimensions were measured.

Figure 4.9 Measurement of particle dimension (not to scale).

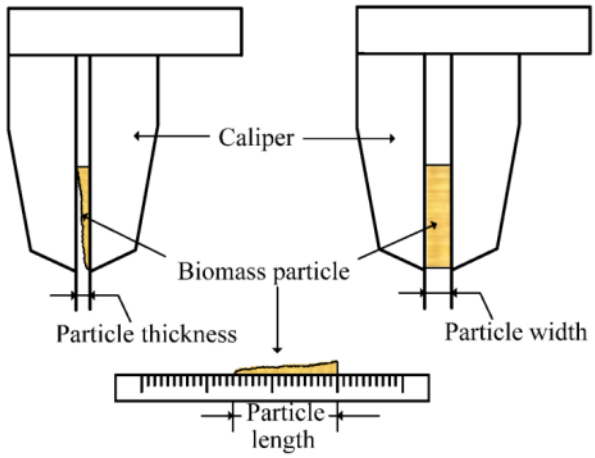
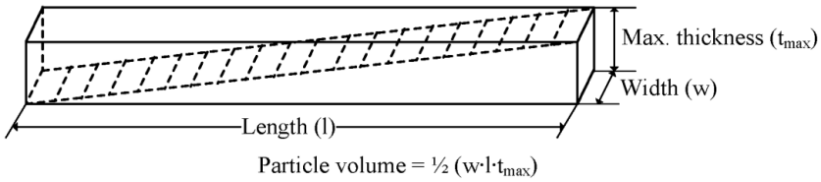


Figure 4.10 shows how the particle volume was calculated. Particle volume was half of the volume of the cube determined by length (l), width (w) and maximum thickness (t_{max}).

Figure 4.10 Simplified calculation of particle volume.

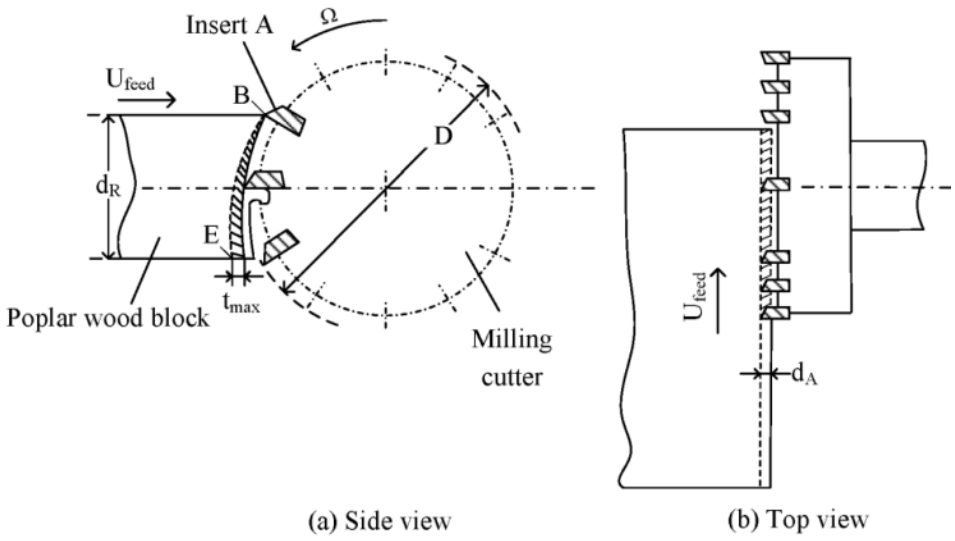


4.4.2 Deformation severity (measured by chip thickness ratio)

4.4.2.1 Maximum uncut particle (chip) thickness

Figure 4.11 illustrates the particle formation in the cutting process. The workpiece (poplar wood block) was feeding towards the milling cutter at a feedrate of U_{feed}, and the feeding direction was horizontal and normal to the rotation axis of the milling cutter. The position of the milling cutter was fixed. Material of the workpiece was removed by a depth of cut of d_A over a width of d_R (thickness of the workpiece). The milling cutter had N inserts and a diameter of D. The rotation speed of the milling cutter was Ω. Insert A entered the workpiece at point B and left the workpiece at point E. In this study, d_R = 5 inch (127 mm), U_{feed} = 4.5 inch·min^{-1} (114.3 mmmin^{-1}), N = 12, Ω = 250 rev·min^{-1}, D = 6 inch (152 mm).

Figure 4.11 Illustration of particle (chip) formation.



The uncut particle (chip) thickness is the particle (chip) thickness prior to deformation. In this study, it increased from 0 at the start (at Point A in Figure 4.11) to the maximum value (at Point E in Figure 4.11). The maximum uncut particle (chip) thickness (t_{max}) was equal to the feed

of the workpiece towards the milling cutter during one revolution divided by the number of the inserts mounted on the milling cutter. Thus, t_{max} can be calculated by Equation (1)

$$t_{\max} = \frac{U_{feed}}{N\Omega} \tag{1}$$

Since U_{feed} = 4.5 inch·min^{-1} (114.3 mm·min^{-1}), N = 12, Ω = 250 rev·min^{-1}, t_{max} = 1.5×10^{-3} inch (0.038 mm).

4.4.2.2 Particle (chip) thickness ratio

It was observed that measured maximum particle (chip) thickness t_a was larger than the calculated uncut particle (chip) thickness t_{max}. Particle (chip) thickness ratio is:

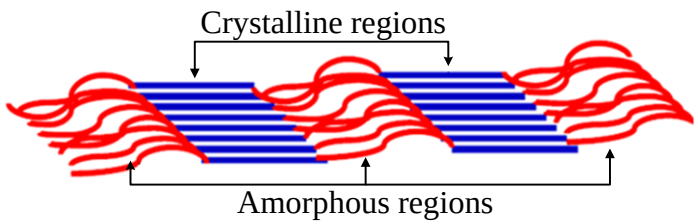
$$r = \frac{t_{\max}}{t_a} \tag{2}$$

A smaller particle (chip) thickness ratio means that the particle has gone through more severe deformation during the process [39].

4.4.3 Biomass crystallinity

As shown in Figure 4.12, cellulose consists of crystalline regions and amorphous regions. Crystallinity is determined as the percentage of crystalline material in the biomass and expressed as crystallinity index (CI) [41].

Figure 4.12 Crystalline and amorphous regions in cellulose (After [40]).

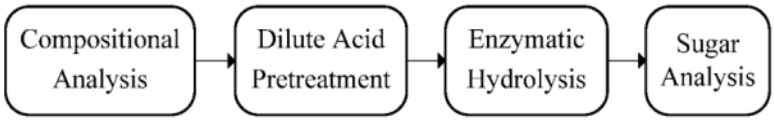


In this study, crystallinity of biomass particles was measured by an X-ray diffractometer (Model MiniFlex II, Rigaku Americas Corporation, The Woodlands, TX, USA). Crystallinity index was calculated using analysis software Rigaku PDXL Version 1.6.0.0.

4.4.4 Sugar yield

In this study, sugar yield was expressed as the concentration of glucose in a sample. Its unit was mg/mL. Figure 4.13 shows the four steps in measurement of sugar yield. Compositions of poplar particles were analyzed first to determine the amount of cellulose in the sample. Second, dilute acid pretreatment was employed to break up biomass structure and thus allow better access of enzymes to cellulose. After pretreatment, enzymatic hydrolysis (72 hours) depolymerized cellulose to fermentable sugar (glucose). In this study, the amount of glucose was determined using HPLC (Shimadzu Corporation, Kyoto, Japan). HPLC is a chromatographic technique that can identify and quantify individual components of a liquid mixture [42].

Figure 4.13 Four steps in sugar yield measurement.



Each sugar yield data point (under each condition) presented in this paper was the mean of the two samples. The variation under each condition was very small, and therefore, was not presented in the graphs.

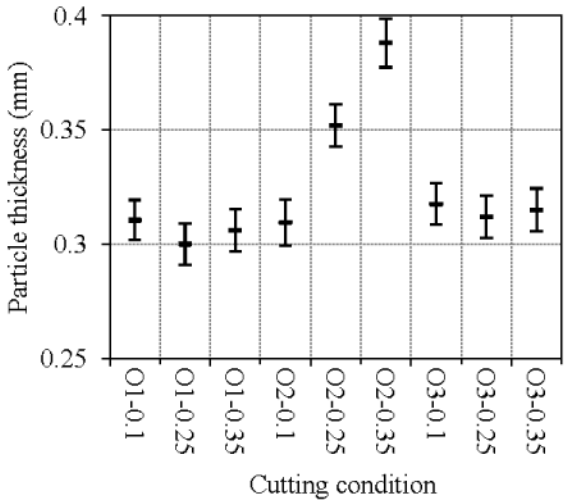
4.5 Results and discussion

4.5.1 Particle size

4.5.1.1 Particle thickness

Figure 4.14 shows particle thickness data. In this figure, as well as in Figures 4.15-4.18, error bars for each data point were drawn using the 95% confidence interval of the mean. For particles produced in Orientations 1 and 3, particle thickness almost remained constant under three different values of depth of cut. For particles produced in Orientation 2, particle thickness increased as depth of cut became larger.

Figure 4.14 Results on particle thickness, cutting orientation: O1, O2, and O3; depth of cut: 0.1, 0.25, and 0.35 inch (2.5, 6.4, and 8.9 mm).

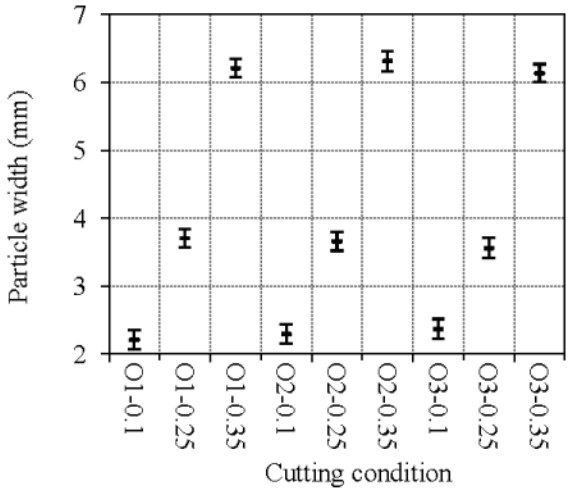


4.5.1.2 Particle width

Figure 4.15 shows particle width data. In all three cutting orientations, particle width increased as depth of cut became larger. As can be seen in Figure 4.6, particle width was

determined by depth of cut using this experimental setup. However, the measured particle width was smaller than the depth of cut.

Figure 4.15 Results on particle width, cutting orientation: O1, O2, and O3; depth of cut: 0.1, 0.25, and 0.35 inch (2.5, 6.4, and 8.9 mm).



4.5.1.3 Particle length

Figure 4.16 Results on particle length, cutting orientation: O1, O2, and O3; depth of cut: 0.1, 0.25, and 0.35 inch (2.5, 6.4, and 8.9 mm).

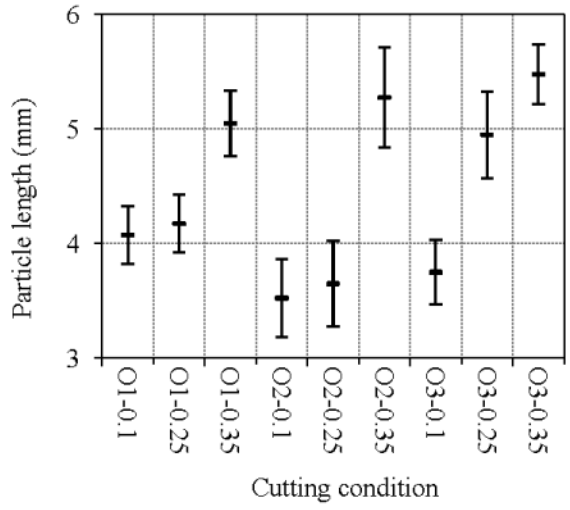


Figure 4.16 shows particle length data. Larger values of depth of cut tended to produce longer particles (chips). There were large variations in particle length.

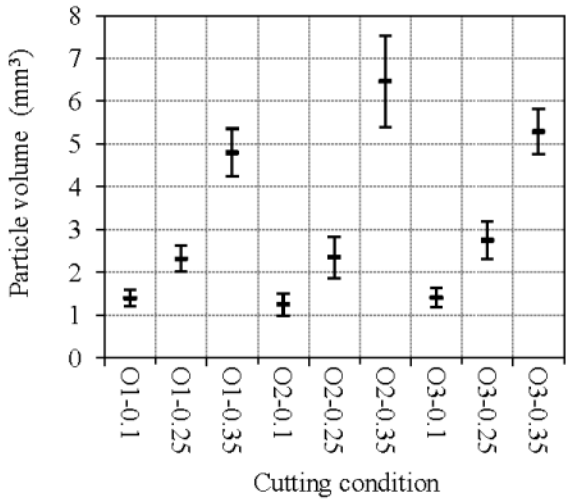
4.5.1.4 Particle volume

Figure 4.17 shows particle volume results. In all three cutting orientations, particle volume increased as depth of cut increased.

In the literature, biomass particle size was usually controlled by sieve size [38]. It was difficult to define what particle size was referred to as (diameter, length, or width) in current size reduction methods because particle formation was not well controlled.

In this study, particles were produced using a metal-cutting (milling) process. Since the particle formation was well controlled, particles produced under the same cutting condition had approximately the same shape and dimensions. Therefore, it became possible to describe particle size by four definitions: thickness, width, length, and volume.

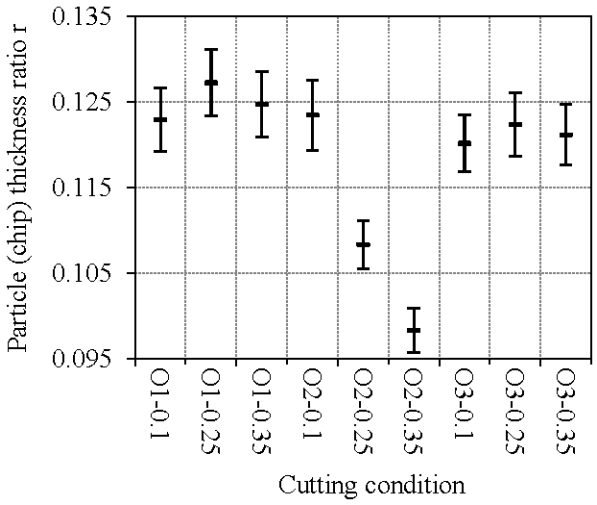
Figure 4.17 Results on particle volume, cutting orientation: O1, O2, and O3; depth of cut: 0.1, 0.25, and 0.35 inch (2.5, 6.4, and 8.9 mm).



4.5.2 Deformation severity measured by particle (chip) thickness ratio

Figure 4.18 shows results on particle (chip) thickness ratio. Compared with the calculated maximum uncut particle thickness (0.038 mm), the measured particle thickness was 8-10 times larger. It can be seen that, in Orientations 1 and 3, particle (chip) thickness ratio remained roughly constant as depth of cut increased. However, in Orientation 2, particle (chip) thickness ratio decreased significantly as depth of cut increased. Particles produced in Orientation 2 and with depth of cut of 0.35 and 0.25 inch had the two lowest thickness ratios (0.098 and 0.108, respectively). Thickness ratio under other cutting conditions was about 0.125.

Figure 4.18 Results on particle (chip) thickness ratio, cutting orientation: O1, O2, and O3; depth of cut: 0.1, 0.25, and 0.35 inch (2.5, 6.4, and 8.9 mm).

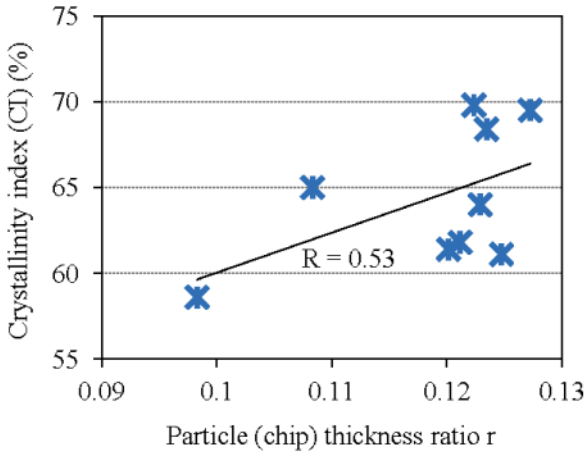


4.5.3 Relationship between biomass crystallinity and particle (chip) thickness ratio

It can be seen in Figure 4.19 that there are some correlations between biomass crystallinity and particle (chip) thickness ratio. As particle (chip) thickness ratio increased, biomass crystallinity increased. This trend probably can lead to the following conjectures: (1) deformation severity of poplar particles (chips) can be measured by particle (chip) thickness

ratio, and (2) more severe deformation can result in lower biomass crystallinity. However, further research is needed in order to confirm these conjectures.

Figure 4.19 Relationship between biomass crystallinity index and particle (chip) thickness ratio.



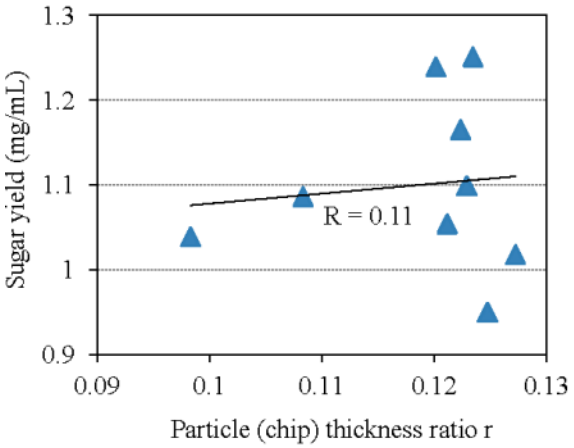
4.5.4 Relationship between sugar yield and particle (chip) thickness ratio

It can be seen from Figure 4.20 that the correlation between sugar yield and particle (chip) thickness ratio was very weak (R = 0.11). R is the Pearson product moment correlation coefficient measuring the strength of the linear relationship between two variables. The range of R values is from −1 to 1. The absolute value of R represents the strength of the relationship. The sign represents the direction of the relationship. Thus R = 0 means the two variables are not linear related, while R = 1 signifies a perfect positive relationship, and R = −1 is obtained for a perfect negative relationship [43]. The weak correlation probably indicates that deformation severity of poplar particles was not the dominating factor for sugar yield.

4.5.5 Relationship between sugar yield and biomass crystallinity

Figure 4.21 shows the relationship between sugar yield and biomass crystallinity. There was no strong correlation between sugar yield and biomass crystallinity (R = 0.32). This result does not agree with the reported trend that biomass with low crystallinity would have a high sugar yield [31,36]. Ball milling was the size reduction method used in the reported studies. This method decreased particle size and biomass crystallinity simultaneously [34]. Therefore, the trend that reducing biomass crystallinity helped to increase sugar yield might actually be a consequence of decreasing particle size [29].

Figure 4.20 Relationship between sugar yield and particle (chip) thickness ratio.



4.5.6 Relationship between biomass crystallinity and particle size

Figure 4.22 to 4.25 show relations between crystallinity index and particle (chip) thickness, length, width, and volume, respectively. It can be seen that, although not very strong, there seemed to be some correlations between biomass crystallinity and particle size (thickness, length, width, or volume): as particle size increased, biomass crystallinity decreased. These correlations are different from most reported results in the literature. Most reported results agreed that, after

size reduction (ball milling, hammer milling, or knife milling), smaller particles would have a lower crystallinity [11-16,19,20].

This result shows that, after size reduction by the metal-cutting (milling) method, particle size and biomass crystallinity did not have a strong correlation. Therefore, their effects on sugar yield will not be confounded. This makes it possible to study their effects on sugar yield separately. It is noted that this would be impossible with current size reduction methods.

Figure 4.21 Relationship between sugar yield and biomass crystallinity index.

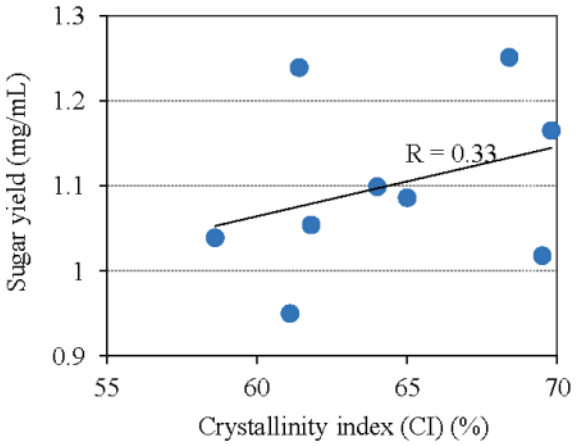


Figure 4.22 Relationship between biomass crystallinity index and particle thickness.

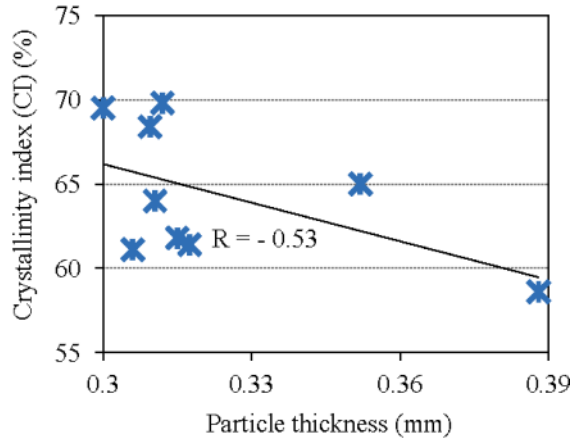


Figure 4.23 Relationship between biomass crystallinity index and particle length.

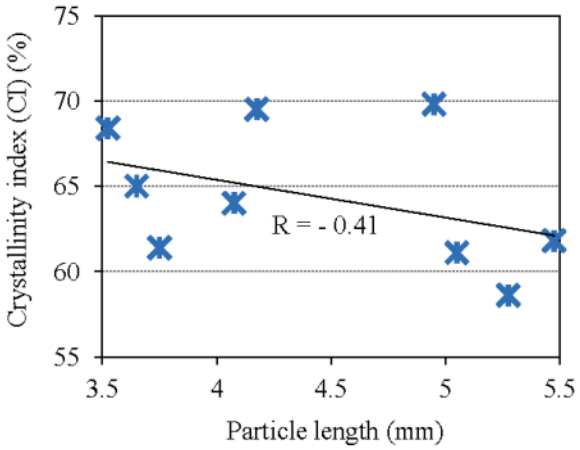
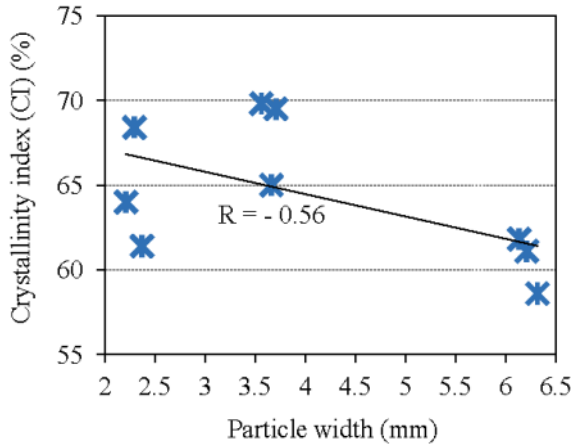


Figure 4.24 Relationship between biomass crystallinity index and particle width.



4.5.7 Relationship between sugar yield and particle size

Figure 4.26 to 4.29 show relationship between sugar yield and particle size (thickness, length, width, and volume). The general trend was that sugar yield increased as particle size decreased. However, correlation between sugar yield and particle thickness was very weak (R = − 0.11). This trend is consistent with many reported studies that sugar yield would increase as particle size became smaller [28-30].

Figure 4.25 Relationship between biomass crystallinity index and particle volume.

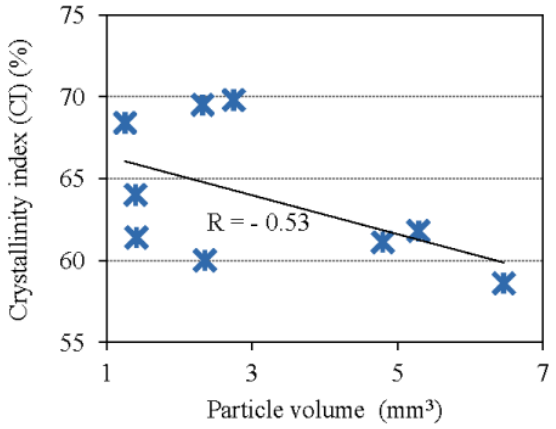


Figure 4.26 Relationship between sugar yield and particle thickness.

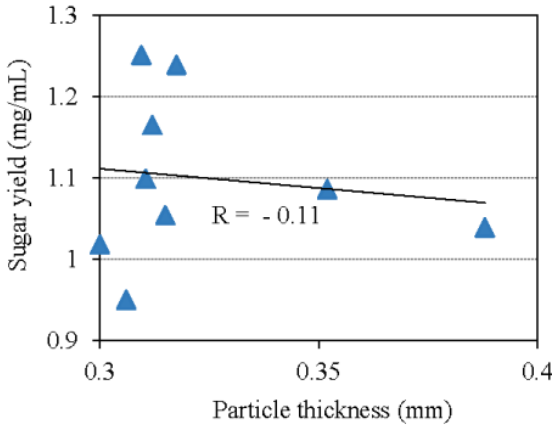


Figure 4.27 Relationship between sugar yield and particle length.

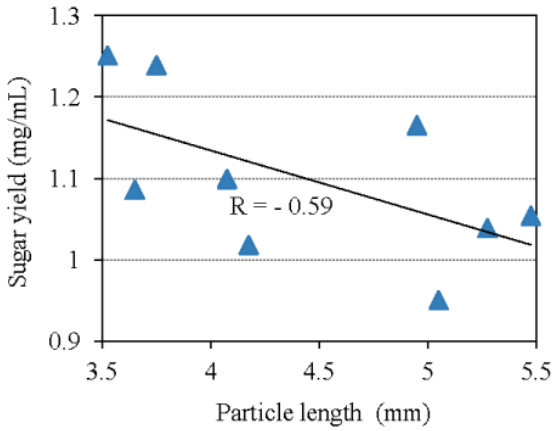
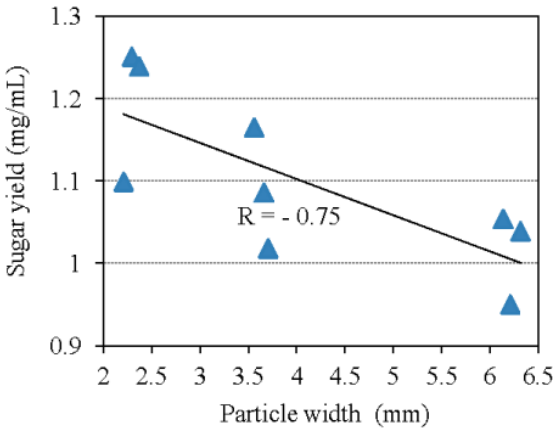
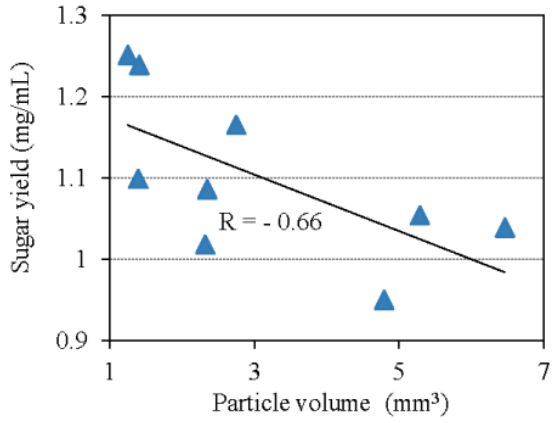


Figure 4.28 Relationship between sugar yield and particle width.



In the reported studies, biomass particle size was measured using sieves. There was no knowledge to answer such a question: which dimension is the most critical to increasing sugar yield? In this study, particle size was investigated by three dimensions (thickness, length, and width) and volume. The results seem to show that particle width had more significant effects on sugar yield than particle length. Particle thickness had the minimum effects on sugar yield. The results also show that particle volume correlated well with sugar yield (a smaller particle volume tended to produce a higher sugar yield).

Figure 4.29 Relationship between sugar yield and particle volume.



4.6 Conclusions and future work

A study on confounding effects of particle size and biomass crystallinity was conducted. A metal-cutting (milling) process was used for size reduction of poplar wood. Because particle formation was well controlled, effects of particle size and biomass crystallinity were not confounded. Particle size was investigated by four definitions: particle width, thickness, length, and volume. The relationships among particle size, biomass crystallinity, and sugar yield were studied. The following conclusions can be drawn:

1. Sugar yield increased as particle size decreased.
2. Poplar particles with lower biomass crystallinity did not produce higher sugar yield. This result is different from many reported results in the literature. In those studies, lower biomass crystallinity would have a higher sugar yield. It is noted that, in the reported studies, lower biomass crystallinity was always confounded with smaller particle size.

In this study, the variation in particle (chip) length was very large. This might contribute to the low value of R in some graphs. In the future, particle length will be better controlled to avoid its large variation. For example, milling cutters with larger rake angles can be used to produce more continuous and uniform-sized particles. Particle length can be controlled by changing workpiece thickness or adjusting the number of carbide inserts on the milling cutter.

In this study, particles with about the same biomass crystallinity but different sizes were produced. However, the sizes of particles with different biomass crystallinity were not well controlled. In the future, the authors will produce particles of the same size but different crystallinity. Producing such material will make it possible to further investigate the effects of biomass crystallinity on sugar yield independently from particle size. In future experiments, milling cutters with different rake angles will be employed. It is assumed that using milling

cutters with different rake angles will introduce different thickness ratios and produce particles with different crystallinity.

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Chapter 5 - Confounding Effects of Particle Size and Biomass Crystallinity: Study 2

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Abstract

Increasing demands and concerns for reliable supply of liquid transportation fuels make it important to find alternative sources to petroleum-based fuels. Cellulosic biofuels provide one such alternative in the short to medium term. Size reduction is the first step for converting biomass into biofuels. In the literature, there are inconsistent reports about the effects of particle size and biomass crystallinity on sugar yield (proportional to biofuel yield). An important reason for this inconsistence is that particle formation in current size reduction methods is not well controlled, causing the effects of these two variables confounded. One paper investigating the confounding effects of particle size and biomass crystallinity using a metal-cutting (milling) process was previously published in this journal. This paper presents a follow up study. In this study, a lathe was used to produce poplar wood particles with the same crystallinity but different sizes, making it possible to study the effects of particle size on biofuel yield independently without being confounded by the effects of biomass crystallinity. Results showed that, for the three levels of particle size used in this study, sugar yield increased as particle size became smaller. This study also revealed future research opportunities to understand the effects of size reduction and biomass crystallinity in cellulosic biofuel manufacturing.

Keywords: Biofuel, Cellulosic biomass, Crystallinity, Hydrolysis, Particle size, Size reduction

5.1 Introduction

Transportation fuels in the United States account for over 70% of the nation's total petroleum consumption, and 57% of the petroleum consumed by the nation's transportation sector is imported [1]. In addition, use of petroleum-based fuels contributes to accumulation of greenhouse gas (GHG) in the atmosphere. The concerns of energy security and GHG emissions

make it important to develop domestic sustainable alternatives to petroleum-based transportation fuels [2].

One such alternative is biofuels produced from cellulosic biomass (such as forest and agricultural residues, and energy crops including short-rotation woody crops and switchgrass). Cellulosic biofuels can reduce the nation's dependence on foreign petroleum, and cut GHG emissions while continuing to meet the nation's need for liquid transportation fuels [3,4]. Over 1 billion dry tons of biomass with more than 80% of cellulosic biomass can be sustainably produced yearly in the United States [5]. This amount of biomass is sufficient to produce 90 billion gallons of liquid fuels that can replace about 30% of the nation's current annual consumption of petroleum-based transportation fuels [5]. In contrast to biofuels based on grains (such as corn), cellulosic biofuels do not compete with food or feed for the limited agriculture land [6].

Major processes of cellulosic biofuel manufacturing include size reduction, pretreatment, hydrolysis, and fermentation. Size reduction reduces the particle size of cellulosic biomass. Pretreatment helps to make cellulose in the biomass more accessible to enzymes during hydrolysis. Hydrolysis depolymerizes cellulose into its component sugars (glucose). Afterwards, fermentation converts glucose into biofuel (ethanol) [7].

Size reduction is necessary because, without it, cellulosic biomass cannot be converted to biofuels efficiently using current conversion technologies [8-10]. Size reduction can affect cellulosic biomass primarily in two ways: reducing particle size and decreasing biomass crystallinity. The literature contains inconsistent reports regarding the effects of particle size and biomass crystallinity on sugar yield (proportional to biofuel yield) [11].

An important reason for the inconsistence reported in the literature is that particle formation is not well controlled in current size reduction methods. These methods (such as knife milling and hammer milling) were discussed in a review paper. In order to produce smaller particles, longer milling time is usually needed. In the meanwhile, longer milling time will also decrease biomass crystallinity [12]. In other words, in current size reduction methods, biomass with smaller particle size almost always has lower biomass crystallinity. Therefore, their effects on sugar yield are confounded.

In order to clearly understand how biomass milling affects sugar yield, it is desirable to separate the confounding effects of particle size and biomass crystallinity. One paper investigating the confounding effects was previously published in this journal [11]. In that study, a metal-cutting (milling) process was employed to perform size reduction of poplar wood. The study demonstrated the feasibility of separating the confounding effects and explained the inconsistent reports in the literature. However, the particles produced under the same condition did not have the same size (specifically, the length).

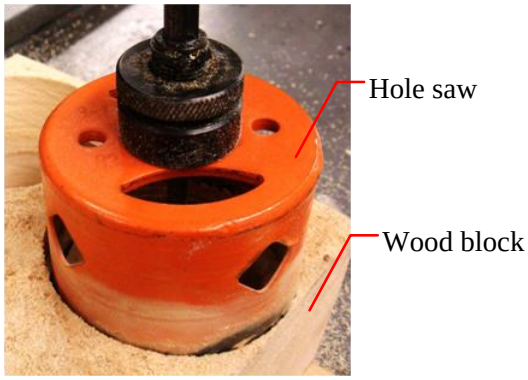
This paper presents a follow-up work. A lathe was used for size reduction of poplar wood to separate the confounding effects of particle size and biomass crystallinity on sugar yield. In this study, particles of three size levels were produced. All particles of the same size level had (statistically) the same size. In addition, all the particles were produced under the same particle formation mechanism and, therefore, had the same biomass crystallinity. In other words, particles with the same crystallinity but different particle sizes were produced, making it possible to study the effects of particle size on sugar yield independently without being confounded with the effects of biomass crystallinity.

5.2 Experimental condition

5.2.1 Sample preparation

Cellulosic biomass used in this study was poplar wood. The poplar wood was purchased from a local lumber company (Griffith Lumber Co., Manhattan, KS, USA) and cut into small blocks (173 mm × 173 mm × 77 mm). A hole saw (No. 7027, Ridge Tool Co., Elyria, OH, USA) with the diameter of 83 mm was used to cut cylinders out of poplar wood blocks, as shown in Figure 5.1. Then the wood cylinder was fixed on a lathe (Monarch Machine Tool Co., Sidney, OH, USA) using a 3-jaw chuck. The diameter of the wood cylinder was reduced to 61.40 mm by a turning tool (NKLNR-121B tool holder, KC850 tungsten carbide insert, Kennametal Inc., Latrobe, PA, USA). A center hole with the diameter of 38.26 mm was drilled (using a drill bit mounted on the tailstock of the lathe) to prepare the workpiece (a hollow cylinder with the wall thickness of 11.57 mm) for size reduction experiments.

Figure 5.1 Preparation of wood cylinders using a hole saw.



The experimental setup for size reduction using a lathe is shown in Figure 5.2. The cutting tool used in this study was custom made with AISI T8 high speed steel. The tool geometry is shown in Figure 5.3.

Figure 5.2 Experimental setup.

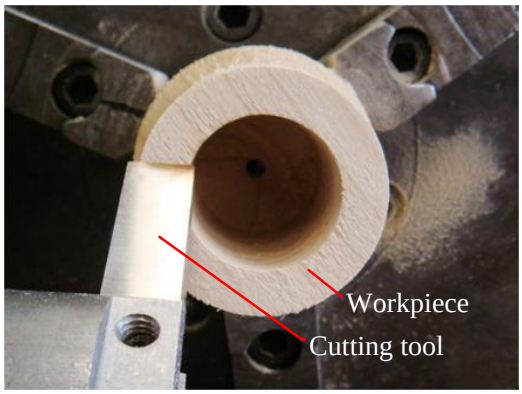
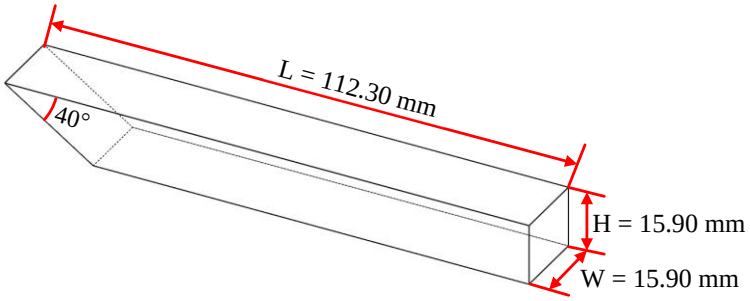


Figure 5.3 Geometry of the cutting tool.



5.2.2 Experimental condition

Figure 5.4 illustrates the cutting process. Preliminary tests were conducted to find the feasible values of process variables to produce continuous chips. In order to produce particles (instead of continuous chips) with controlled length, different numbers of slots were cut into the wood cylinder. As shown in Figure 5.5, four slots (with 1 mm wide for each slot) were cut using a hacksaw, dividing the wood cylinder into four equal parts. The continuous chip would break (into particles) at the locations of these slots. When the lathe spindle rotated one revolution, four particles with the same length were produced.

Three different numbers of slots (4, 8, and 16) were used to produce particles with three different length levels. Values of process variables were listed in Table 5.1. Please note that the depth of cut was the same as the wall thickness of the hollow cylinder workpiece.

Table 5.1 Process variables and their values.

Process variable	Value
Spindle rotation speed (Ω)	532 rev.·min^{-1}
Feedrate (f)	0.01 inch rev.$^{-1}$ (0.25 mm rev.$^{-1}$)
Depth of cut (hollow cylinder wall thickness) (d)	11.57 mm
Cutting tool rake angle (α)	30°

Ten grams of particles were collected for every number of slots (4, 8, or 16 slots). After the particles were collected, they were kept in individual sealed Ziploc® bags and stored in a refrigerator at 4 ℃.

Figure 5.4 Illustration of the cutting process.

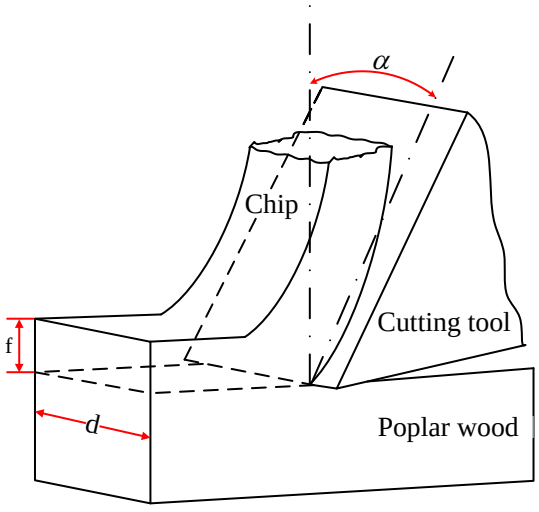
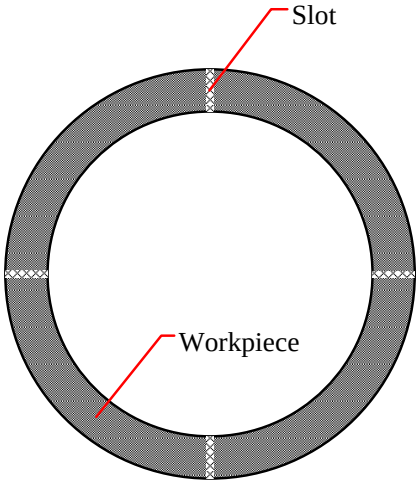


Figure 5.5 Controlling of particle length using different numbers of slots (four slots in this illustration).



5.3 Evaluation parameters and their measurement procedures

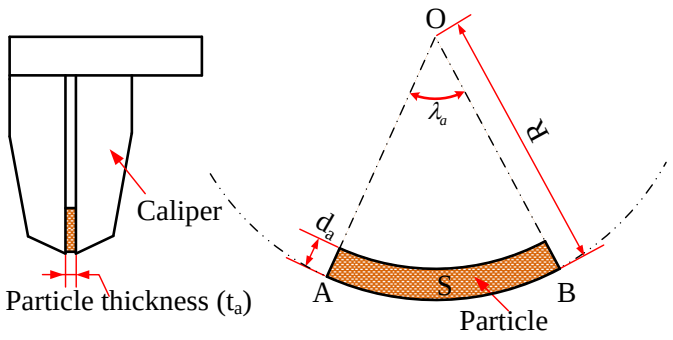
5.3.1 Particle size

In this paper, particle size is investigated by two parameters: particle thickness (t_a) and surface area (S), as shown in Figure 5.6. S is the surface area of the particle top (or bottom) face. Ten particles were randomly picked for measurement from particles produced under each condition. Particle thickness was measured by a digital caliper (Model IP-65, Mitutoyo Corp., Kawasaki, Japan). To measure the dimension needed for surface area (S) calculation, a particle was placed against the arc on a circle (center at O, radius R = 30.70 mm). The central angle λ_a was measured by a protractor (minimum scale = 0.5 °), and its unit was radian. Particle width (d_a) was measured by the caliper.

The surface area of a particle was determined using Equation (1):

$$S = \frac{\lambda_a}{2}[R^2 - (R - d_a)^2] \quad (1)$$

Figure 5.6 Illustration for particle size measurement.



5.3.2 Deformation severity

The deformation severity of particles in this study was measured by chip (particle) thickness ratio. It was observed that the thickness of produced particle was larger than the uncut chip thickness. Let t = uncut chip thickness, and t_a = actual particle thickness measured. In this study, t equals to feedrate (f = 0.25 mm rev.$^{-1}$). Then, the chip (particle) thickness ratio is:

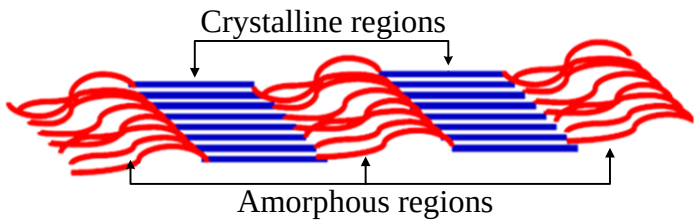
$$r = \frac{t}{t_a} \tag{2}$$

A smaller chip (particle) thickness ratio means that the chip (particle) has gone through more severe deformation during the process [13].

5.3.3 Biomass crystallinity

As shown in Figure 5.7, cellulose in cellulosic biomass consists of crystalline regions and amorphous regions. Crystallinity is determined as the percentage of crystalline material in cellulose and expressed as crystallinity index (CI) [14].

Figure 5.7 Crystalline and amorphous regions in cellulose (After Hu, 2008).



To measure biomass crystallinity, three particles under each condition were randomly picked for measurement using X-ray diffractometer (Model MiniFlex II, Rigaku Americas Corp., The Woodlands, TX, USA). Crystallinity index was calculated using analysis software Rigaku PDXL (Version 1.6.0.0).

5.3.4 Sugar yield

Sugar yield after hydrolysis was the amount of glucose produced from hydrolyzing cellulose by enzymes. It was expressed as the concentration of glucose (mg/ml) in the measured sample.

For pretreatment, 10 g of biomass and 200 ml of 2% sulfuric acid were loaded in the 600 ml vessel of a Parr pressure reactor (Model 4760A, Parr Instrument Co., Moline, IL, USA). Pretreatment time was 30 minutes, and pretreatment temperature was 140 °C.

After pretreatment, biomass was washed three times with 300 ml of hot deionized water (85 ℃) using a centrifugal (Model PR-7000M, International Equipment Co., Needham, MA, USA). The rotation speed of the centrifugal was 4500 rpm. The purpose of biomass washing was to remove the acid residues and inhibitors (substances that would bind to enzymes and decrease their activity to depolymerize cellulose to glucose [15]) formed during pretreatment.

Accellerase 1500^{TM} (Danisco US Inc., Rochester, NY, USA) enzyme complex was used for hydrolysis of wood particles into sugars in the sodium acetate buffer solution (50 mM, pH 4.8) with 0.02% (w/v) sodium azide to prevent the microbial growth during hydrolysis. Enzymatic

hydrolysis was carried out in 125 mL flasks with 50 mL of slurry in the water bath shaker (Model C76, New Brunswick Scientific, Edison, NJ, USA) with agitation speed of 110 rpm at 50 °C for 72 hours. The dry mass content of the hydrolysis slurries was 5% (w/v) and the enzyme loading was 1 mL/g of dry biomass. After enzymatic hydrolysis, samples were ready for sugar analysis.

Sugar analysis was done using a HPLC (Shimadzu Corp., Kyoto, Japan) equipped with an RPM-monosaccharide column (300 × 7.8 mm; Phenomenex, Torrence, CA, USA) and a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan). The mobile phase was 0.6 mL/min of double-distilled water, and oven temperature was 80 ℃. HPLC can identify and quantify individual components of a liquid mixture [16].

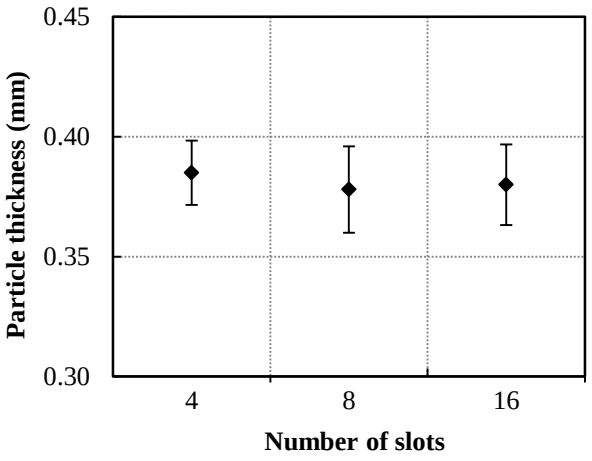
5.4 Results and discussion

5.4.1 Particle size

5.4.1.1 Particle thickness

Figure 5.8 shows results on particle thickness. Analysis of variance was performed by statistics software Minitab (Version 15, Minitab Inc., State College, PA, USA). In this figure as well as in Figure 5.11 to 5.13, error bars for each data point were drawn using the 95% confidence interval of the mean. The results show that, for particles produced with three different numbers of slots, particle thickness was approximately the same.

Figure 5.8 Results on particle thickness.



5.4.1.2 Surface area

Figure 5.9 shows three particles produced with three numbers of slots. Figure 5.10 shows results on surface area. The surface area of particles produced with 4 slots was twice as large as that of particles produced with 8 slots and four times as large as that of particles produced with 16 slots. Under each of the three conditions, the variance in surface area was very small.

Figure 5.9 Pictures of produced particles using three different numbers of slots.

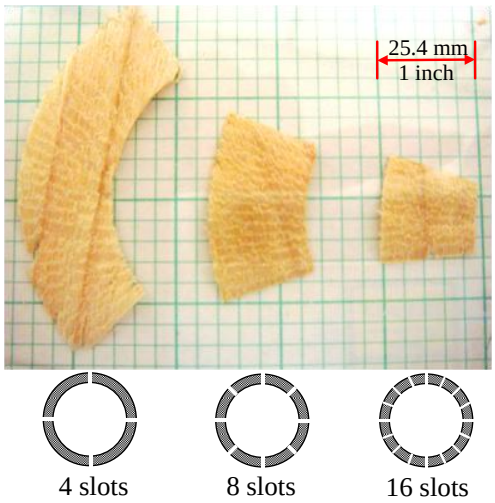
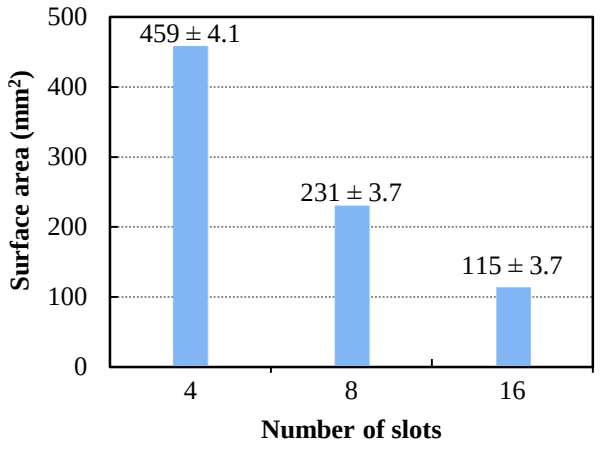


Figure 5.10 Results on particle surface area.

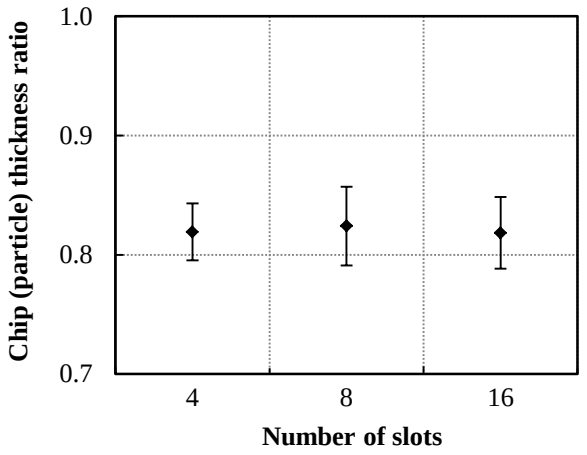


5.4.2 Deformation severity

Figure 5.11 shows results on deformation severity measured by chip (particle) thickness ratio. It can be seen that chip (particle) thickness ratio produced by using three different numbers of slots were approximately the same. The overall average in chip (particle) thickness ratio was 0.82. This value indicated that the average (measured) particle thickness $\overline{t_a}$ was about 1.2 times the uncut chip thickness t. This indicates that these particles underwent approximately the same deformation severity during machining. Because deformation of biomass during size reduction can affect biomass crystallinity, these particles having the same deformation severity would possibly have the same crystallinity.

Biomass crystallinity results are shown in Figure 5.12. Statistical tests (two-sample T-tests) were performed to test if biomass crystallinity was significantly different for different numbers of slots (Table 5.2). The null hypothesis was that the means of biomass crystallinity for two different numbers of slots were not significantly different. The results of statistical tests failed to reject the null hypothesis. This indicates that the particles produced in this study had different surface area while having approximately the same crystallinity.

Figure 5.11 Results on chip (particle) thickness ratio.



5.4.3 Biomass crystallinity

Figure 5.12 Results on biomass crystallinity.

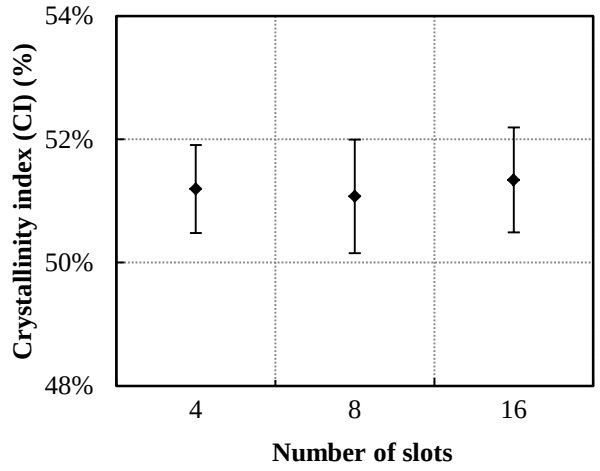


Table 5.2 Hypothesis testing using two-sample T-tests.

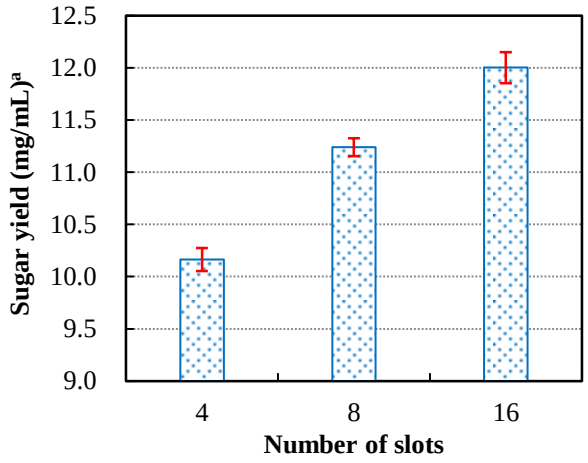
Null hypothesis	P value	If the difference between the two means was significant
$\mu_{4\ slots} = \mu_{8\ slots}$	0.85	No
$\mu_{4\ slots} = \mu_{16\ slots}$	0.81	No
$\mu_{8\ slots} = \mu_{16\ slots}$	0.70	No

In current size reduction methods, the main cause for the confounding effects of particle size and biomass crystallintiy is that biomass with smaller particle size almost always has lower biomass crystallinity. In the previous study reported in this journal [11], such confounding was avoided by employing a metal cutting (milling) process for size reduction. However, the particles produced did not have the same crystallinity, and the particle size (length) was not well controlled.

5.4.4 Sugar yield

Figure 5.13 shows sugar yield results of particles with three levels of surface area (produced by three different numbers of slots). Sugar yield increased as particles became smaller (with a smaller surface area produced with a larger number of slots). Sugar yield of particles produced with 16 slots was 18% higher than that of particles produced with 4 slots, and 7% higher than that of particles produced with 8 slots.

Figure 5.13 Results on sugar yield.



5.5 Conclusions and future work

In this study, a lathe was used to produce poplar wood particles with well-controlled particle formation mechanism to separate the confounding effects of particle size and biomass crystallinity. Particles with three levels of size (surface area) were produced. Biomass crystallinity and sugar yield were measured. The main conclusions are as follows:

1) Particle size (surface area) was well controlled by different numbers of slots. Surface area of particles produced with 4 slots was twice that of particles produced with 8 slots and four times that of particles produced with 16 slots.
2) There was no significant difference in biomass crystallinity for the particles with three levels of particle size (surface area).
3) The ability to separate the confounding effects of particle size and biomass crystallinity in this study made it possible to investigate the effects of particle size on sugar yield independently. Results showed that, for the three levels of particle size used in this study, sugar yield increased as particle size (surface area) became smaller.

In the future, in order to understand the effects of biomass crystallinity on sugar yield, biomass particles of the same size but with different levels of crystallinity have to be produced. To achieve different levels of biomass crystallinity, the authors propose to use cutting tools of different rake angles. According to metal cutting theory, when cutting with a larger rake angle, the material undergoes less deformation [17]. It is reasonable to make the hypothesis that this theory can also be applied to wood cutting. The difference in material deformation severity induced by different tool rake angles can produce particles with different levels of crystallinity. Sugar yield study using particles of the same size but with different levels of crystallinity will be

conducted to investigate the effects of biomass crystallinity on sugar yield independently without being confounded with the effects of particle size.

Acknowledgements

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Chapter 6 - Confounding Effects of Particle Size and Biomass Crystallinity: Study 3

Paper title:

Size reduction of poplar wood using a lathe for biofuel manufacturing: effects of biomass crystallinity on sugar yield

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Abstract

Poplar wood can be used as a feedstock for manufacturing cellulosic biofuel (ethanol) as an alternative to petroleum-based liquid transportation fuel. Producing biofuel from poplar wood involves reducing poplar wood into small particles (known as size reduction), hydrolyzing cellulose inside poplar particles to fermentable sugars, and converting these sugars to ethanol biofuel. Size reduction is usually done by wood chipping and biomass milling. In the literature, there are inconsistent reports about effects of particle size and biomass crystallinity on sugar yield (proportional to ethanol yield). An important reason for this inconsistence is that effects of these two biomass structural features (particle size and biomass crystallinity) on sugar yield are confounded with current size reduction methods. In this study, a lathe was used to produce poplar wood particles with (statistically) the same particle size (thickness) but different levels of biomass crystallinity, making it possible to investigate effects of biomass crystallinity on sugar yield without being confounded with effects of particle size. Results from this study show that, for the three levels of biomass crystallinity tested, sugar yield increased as biomass crystallinity decreased.

Keywords: Biofuel, Cellulosic biomass, Crystallinity, Poplar wood, Size reduction, Sugar yield

6.1 Introduction

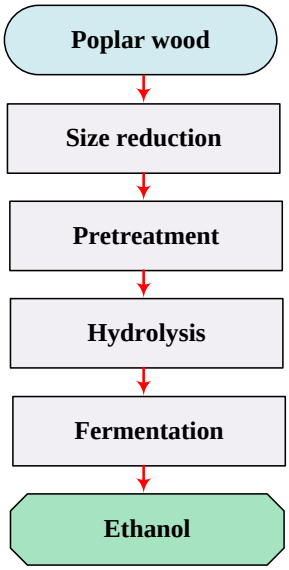
Liquid transportation fuels currently used in the U.S. are mainly petroleum based [1–3]. In 2011, the U.S. transportation sector consumed about 18.95 million barrels of petroleum per day, 45% of them were imported [4]. The dependence on foreign petroleum threatens the nation's energy security. Another issue of consuming petroleum-based transportation fuels is greenhouse

gas (GHG) emissions. One-third of the total carbon dioxide emissions in the U.S. are from the use of petroleum-based transportation fuels [5].

Biofuels are critical to addressing these issues. Biofuels have the potential to reduce GHG emissions by as much as 86% [6]. Because biofuels are made from plant-based feedstocks, the carbon dioxide released during combustion is "recycled" by plants as they grow [7]. In addition, cellulosic biofuels are produced from cellulosic biomass, including agricultural and forestry residues and dedicated energy crops. Unlike other types of feedstocks (e.g. corn, sugar cane, and soybean) for biofuels, cellulosic biomass does not compete with food production for the limited agricultural land [8,9].

Major processes of biofuel manufacturing from poplar wood are listed in Figure 6.1. First, the size of poplar wood needs to be reduced [10–13]. Pretreatment helps to make cellulose in the biomass more accessible to enzymes during hydrolysis. Hydrolysis depolymerizes cellulose into its component sugars (glucose). Afterwards, fermentation converts glucose into biofuel (ethanol) [14–16].

Figure 6.1 Major processes of biofuel manufacturing from poplar wood (after [14]).



Size reduction of poplar wood is necessary because large-size woody biomass cannot be converted to biofuels efficiently with current conversion technologies [17]. Size reduction of poplar wood usually involves two steps. The first step is wood chipping. Machines available for wood chipping include disk, drum, and V-drum chippers [17]. The second step is biomass milling to further reduce the wood chips into small wood particles. This step is usually conducted on hammer mills [11,18], knife mills [17,19,20], compression mills [11], or ball mills [21].

Two important structural features of cellulosic biomass are biomass crystallinity and particle size [22]. Reported relationships between sugar yield and these two features are summarized in Table 6.1. It can be seen that reported relationships are inconsistent. Some researchers reported that lower biomass crystallinity produced higher sugar yield, while some other researchers did not support such a relationship. Inconsistent results are also reported for relationships between particle size and sugar yield as analyzed by Zhang et al. in their review paper [32].

Table 6.1 Reported relationship between structural feature and sugar yield.

Structural feature	Relationship between structural feature and sugar yield	Reference
Biomass crystallinity	As biomass crystallinity decreased, sugar yield increased	[21, 23-26]
	No correlation	[27-29]
Particle size	As particle size decreased, sugar yield increased	[21, 27-28, 30]
	No correlation	[23, 31]

An important reason for this inconsistence is that, with current size reduction methods, effects of these two features on sugar yield are confounded. Current size reduction methods tend to change particle size and biomass crystallinity simultaneously. With current size reduction methods, in order to produce smaller particles, longer milling time is usually needed. In general,

longer milling time also decreases biomass crystallinity by generating more impact and deformation to disrupt the crystalline structure of cellulose in the biomass [22].

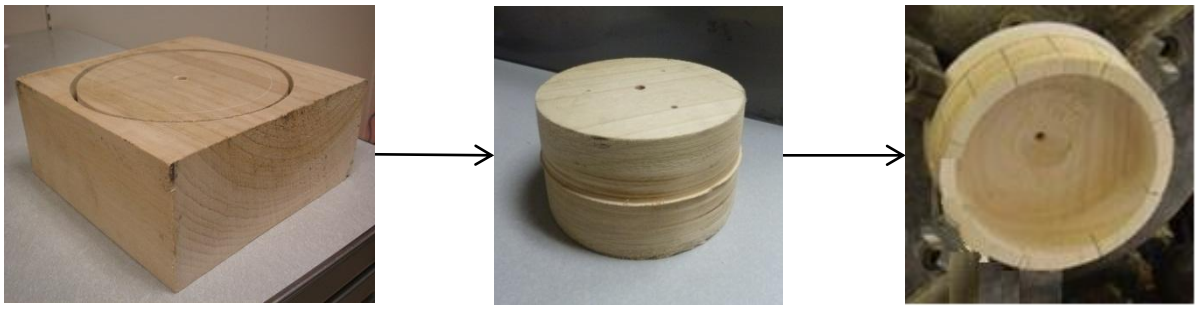
In this study, a metal-cutting machine (lathe) was used to produce poplar wood particles with (statistically) the same particle size (thickness) but different levels of biomass crystallinity. This effort made it possible to study effects of biomass crystallinity on sugar yield without being confounded with effects of particle size.

6.2 Experimental set-up and measurement procedures

6.2.1 Poplar wood material

The poplar wood used in this study was purchased from a local lumber company (Griffith Lumber Co., Manhattan, KS, USA). The size of the poplar lumber boards was 156 mm × 156 mm × 1,000 mm. As shown in Figure 6.2, poplar wood logs were cut from the lumber board using a hole saw (Milwaukee Electric Tool Co., Brookfield, WI, USA) with an inner diameter of 146 mm, on a drilling machine. Then the poplar wood log was fixed on a lathe (Monarch Machine Tool Co., Sidney, OH, USA) using a three-jaw chuck. A center hole with the diameter of 38.26 mm was drilled (using a twist drill mounted on the tailstock of the lathe) into the wood log to obtain the hollow cylinder workpiece. The inner surfaces of the hollow cylinders were machined by a boring tool to reduce the wall thickness (the distance between outer and inner radii of the hollow cylinder). The hollow cylinders were used for size reduction experiments.

Figure 6.2 Poplar wood workpiece preparation.



a. A poplar wood log was cut by a hole saw from a lumber board. Since the maximum cutting depth of the hole saw was smaller than the thickness of the lumber board, the wood log was produced after two cuts, each cut from each side of the lumber board.

b. A center hole was first drilled using a twist drill mounted on the tailstock of the lathe. The inner surface of the hollow cylinder was machined by a boring tool.

6.2.2 Experimental set-up and conditions

The experimental setup is shown in Figure 6.3. Size reduction experiments were conducted on a lathe (Monarch Machine Tool Co. Sidney, OH, USA). The cutting tool was custom made with AISI T8 high speed steel. The tool geometry is shown in Figure 6.4. The rake angle of the tool could be adjusted by rotating the tool holder (NKLNR-121B, Kennametal Inc., Latrobe, PA, USA) along its axial direction. Eight slots (with 1 mm wide for each slot) were cut into the workpiece using a hacksaw, dividing the hollow cylinder workpiece into eight equal parts, as illustrated in Figure 6.5. The continuous chip would break (into particles) at the locations of these slots. When the lathe spindle rotated one revolution, eight particles with the same length were produced. The experimental conditions are listed in Table 6.2. No coolant was used. A large white paper board was placed on the lathe guide to collect poplar wood particles. After the particles were collected, they were kept in zip bags and stored in a refrigerator at 4℃ before further use.

Figure 6.3 Experimental setup.

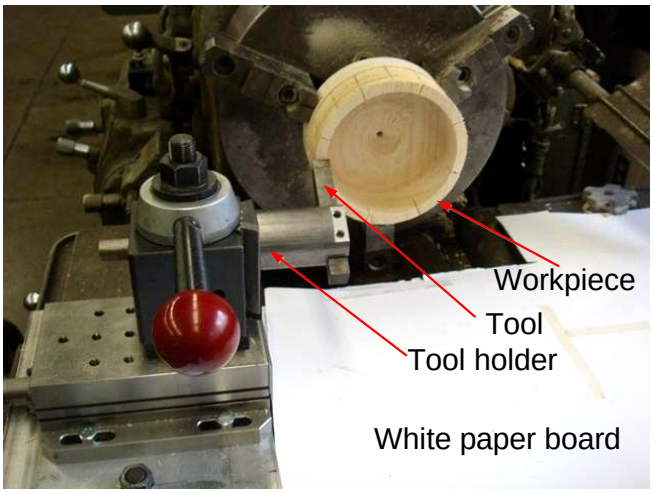


Figure 6.4 Dimensions of the cutting tool.

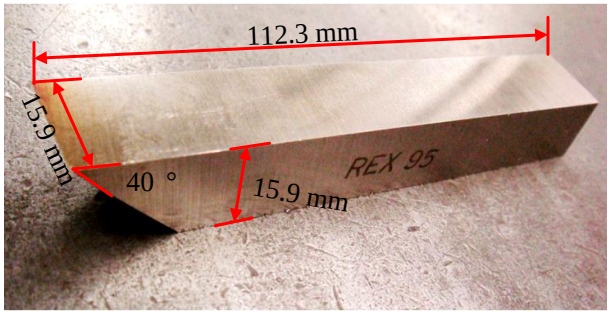
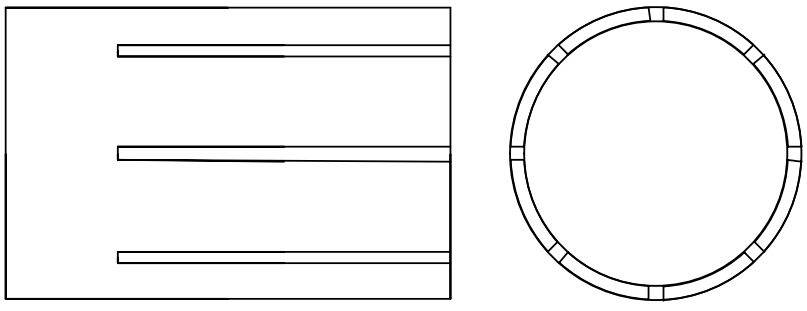


Figure 6.5 Illustration of eight slots on the workpiece.



Front view Side view

Table 6.2 Experimental conditions.

Process variable	Value
Tool rake angle	20 °, 25 °, 30 °
Cutting speed = 4.0 m/s	
Feedrate = 0.508 mm/r	
Wall thickness = 8.67 mm	

Figure 6.6 Illustration of poplar particle thickness measurement (not to true scale).

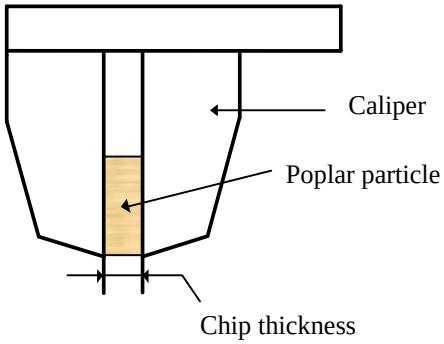


Figure 6.7 Pictures of a poplar particle.

(a) After cutting (b) After flattening

6.2.3 Measurement of particle size

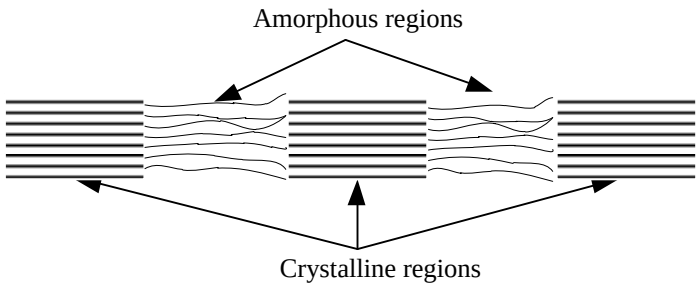
In this study, particle size is represented by particle thickness (a_0). It was measured using a caliper (Model IP-65, Mitutoyo Corp., Kawasaki, Japan), as shown in Figure 6.6. A typical particle was shown in Figure 6.7. Particles were curved when cut off from the wood cylinder workpiece, as shown in Figure 6.7 (a). If they were manually flattened, they would look like the

one shown in Figure 6.7 (b). Thirty particles under each condition were randomly picked for measurement of particle thickness.

6.2.4 Measurement of biomass crystallinity

Cellulose in cellulose biomass consists of amorphous regions and crystalline regions, as illustrated in Figure 6.8. Biomass crystallinity is used to describe the percentage of crystalline regions of cellulose and expressed as crystallinity index (CI) [33]. A higher CI means that cellulose in cellulose biomass has a higher percentage of crystalline regions. It has been suggested that amorphous regions of cellulose degrades more easily than crystalline regions [29, 34, 35]. Therefore, a higher CI would result in lower enzyme accessibility, and, hence, lower sugar yield. CI was measured by an X-ray diffractometer (MiniFlex II, Rigaku Americas Corp., The Woodlands, TX, USA) and calculated using analysis software PDXL (Version 1.6.0.0, Rigaku Americas Corp., The Woodlands, TX, USA). For each test condition, three particles were randomly picked for CI measurement. For each measurement, one poplar particle was placed on the sample holder of the X-ray diffractometer.

Figure 6.8 Amorphous and crystalline regions in cellulose (after[25]).



6.2.5 Measurement of sugar (glucose) yield

Prior to sugar yield measurement, collected poplar particles were treated using dilute acid pretreatment. The pretreatment was carried out in the 600-ml reaction vessel of a Parr pressure reactor (4760A, Parr Instrument Co., Moline, IL, USA). Poplar particles were mixed with diluted sulfuric acid to obtain biomass slurry with 5% solid content (10 g of poplar particles in 200 mL of 2% diluted sulfuric acid). Pretreatment time was 30 min, and pretreatment temperature was 140 ℃.

After pretreatment, biomass was washed three times with 300 mL of hot deionized water (85 ℃) using a centrifuge (PR-7000M, International Equipment Co., Needham, MA, USA). The rotation speed of the centrifuge was 4,500 rpm. The purpose of biomass washing was to remove acid residues and inhibitors (substances that would bind to enzymes and decrease their activity to depolymerize cellulose to glucose) formed during pretreatment.

Then, the pretreated biomass was processed by enzymatic hydrolysis, following the National Renewable Energy Laboratory (NREL) analytical procedure [36]. Enzyme complex Accellerase 1500^{TM} (Danisco US Inc., Rochester, NY, USA) was used in the sodium acetate buffer solution (50 mM, pH 4.8) with 0.02% (w/v) sodium azide to prevent microbial growth during hydrolysis. Enzymatic hydrolysis was carried out in 125-mL flasks with 50 mL of slurry in a water bath shaker (C76, New Brunswick Scientific, Edison, NJ, USA) with the agitation speed of 110 rpm at 50 °C for 72 hours. The dry mass content of the hydrolysis slurries was 5% (w/v) and the enzyme loading was 1 mL/g of dry biomass. After 72 hours of enzymatic hydrolysis, the hydrolysis slurries were sampled by withdrawing 0.1 mL of slurry from each flask. Sample slurries were then mixed with 0.9 mL of double-distilled water in 1.5-mL vials. The vials were placed into boiling water for 15 min to deactivate the enzyme. Then, the sample slurries were

centrifuged in a micro centrifuge (RS-102, REVSCI Co., Lindstrom, MN, USA) at 10,000 rpm for 15 min. The supernatants were filtered into 2-mL autosampler vials through 0.2-μm syringe filters (EMD Millipore Corp., Billerica, MA, USA). The filtered samples in the autosampler vials were ready for sugar analysis.

Sugar analysis was done using a high performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan) equipped with an RPM-monosaccharide column (300 × 7.8 mm; Phenomenex, Torrence, CA, USA) and a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan). The mobile phase was 0.6 mL/min of double-distilled water, and oven temperature was 80 ℃. HPLC can identify and quantify individual components of a liquid mixture.

Sugar yield represents the amount of glucose produced from cellulosic biomass in enzymatic hydrolysis. A higher sugar yield means that more glucose is obtained. In this paper, sugar yield was determined by the following equation:

$$\text{Sugar yield} = \frac{G_{EH} \times V}{M_{EH}} \times 100\,\% \tag{1}$$

where G_{EH} is the glucose concentration (g/L) of slurry in the flask after hydrolysis, M_{EH} is the dry weight (g) of cellulosic biomass loaded in the flask before enzymatic hydrolysis, V is the total volume (L) of slurry in the flask in enzymatic hydrolysis.

6.3 Results and discussion

Results on particle size (thickness) are shown in Figure 6.9. For particles produced with different tool rake angles, there are no significant differences in particle sizes (thickness). Error bars for each data point in Figure 6.9 (and Figures 6.11-6.13) were drawn using the 95% confidence interval of the mean. Means of data for each response variable under different experimental conditions were compared by one-way analysis of variance (ANOVA) using

software Minitab (Version 15, Minitab, Inc., State College, PA, USA). The following assumptions are used: (a) response variables are normally distributed; (b) samples are independent; and (c) variances of populations are equal.

Figure 6.9 Effects of tool rake angle on particle thickness.

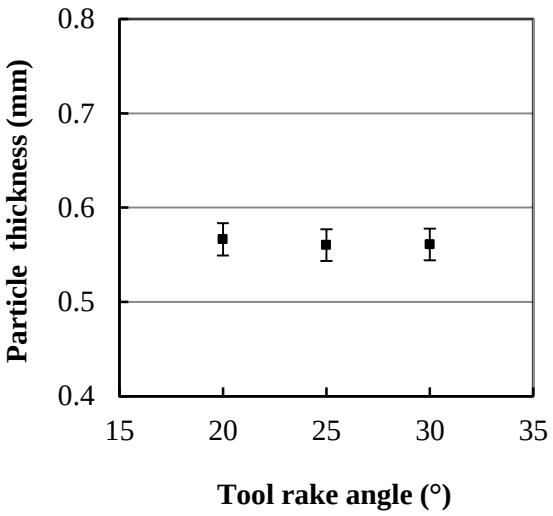


Figure 6.10 illustrates particle (chip) formation model in orthogonal cutting. This model was used for both metal cutting [37-39] and wood machining [40-41]. As shown in Figure 6.10, the cutting edge of a wedge-shaped tool is perpendicular to the cutting direction. The cutting edge of a wedge-shaped tool is perpendicular to the cutting direction. As the tool is forced into the workpiece material, the particle (chip) is formed by shear deformation along a shear plane oriented at an angle φ (shear angle) with the workpiece surface. Along the shear plane, plastic deformation of the workpiece material occurs. Shear angle is an indirect measure of the deformation severity of the produced particles. Shear angle φ is determined by Equation (2) [37]:

$$\tan\phi = \frac{\frac{a_c}{a_0}\cos\gamma_0}{1-\frac{a_c}{a_0}\sin\gamma_0} \tag{2}$$

where a_c is the uncut particle thickness, a_0 is the particle thickness, γ_0 is the rake angle of the tool. The uncut particle thickness a_c was the thickness of the layer of the workpiece material being removed per revolution of the workpiece. In this experimental setup, a_c was determined by the feedrate (mm/r). According to metal cutting theory [38,39], when cutting with a tool that has a larger rake angle, the workpiece material undergoes less severe deformation. When being cut with a tool that has a smaller rake angle, the material undergoes more severe deformation. Such a relationship was also reported for wood cutting [40]. As shown in Figure 6.11, shear angle increased when tool rake angle increased from 20 ° to 30 °. A smaller tool rake angle would produce a smaller shear angle, and cause more severe deformation in produced particles.

Figure 6.10 Illustration of particle formation in orthogonal cutting (after [37])

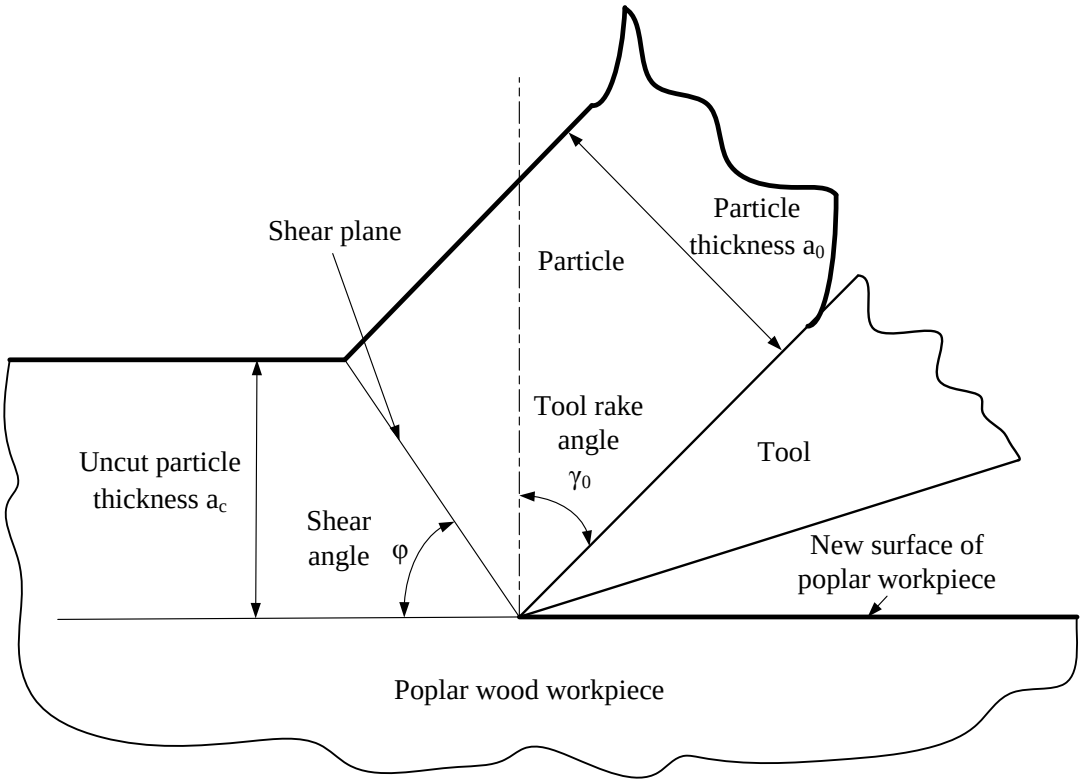
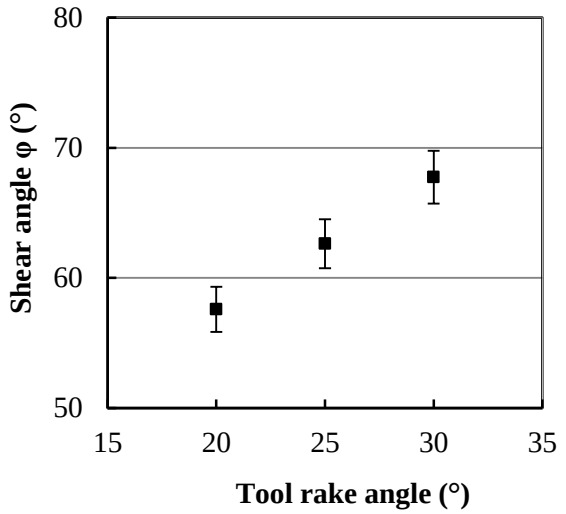


Figure 6.12 shows that biomass crystallinity index (CI) decreased as tool rake angle decreased. This observation could be explained as follows: when a smaller tool rake angle was used, larger cutting force would be applied onto the workpiece material and particles would undergo more severe deformation. This could cause crystalline regions in cellulose to deform and transform into amorphous regions [35]. Therefore, CI decreased.

Figure 6.11 Effects of tool rake angle on shear angle.



It is important to note that there was no significant difference in particle thickness when CI changed, as shown in Figures 6.9 and 6.12. Since the surface area of particles was well controlled as a constant by the slots cut into the hollow wood cylinder workpiece, biomass particles with different crystallinity but the same particle size were produced. Lower biomass crystallinity was not associated with smaller particle size. Therefore, the confounding effects of particle size and biomass crystallinity were separated.

Results on sugar yield are shown in Figure 6.13. It can be seen, from Figures 6.12 and 6.13, that sugar yield increased as biomass crystallinity decreased.

The authors have also studied effects of particle size on sugar yield independently without being confounded with biomass crystallinity [42]. In that study, poplar wood particles with different levels of particle size but the same biomass crystallinity were produced using a lathe. Experimental results show that sugar yield increased as particle size became smaller.

Figure 6.12 Effects of tool rake angle on crystallinity index.

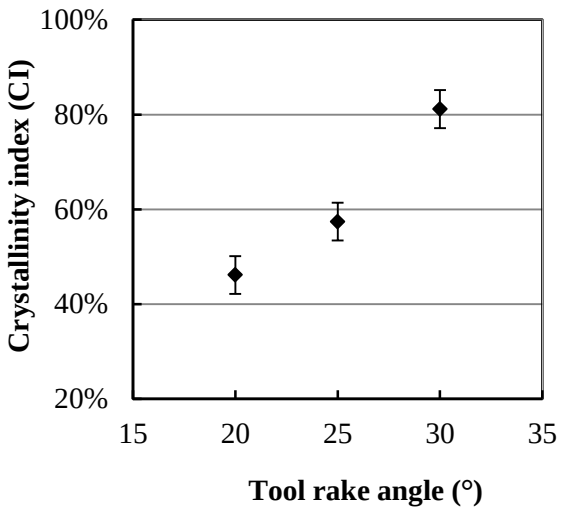
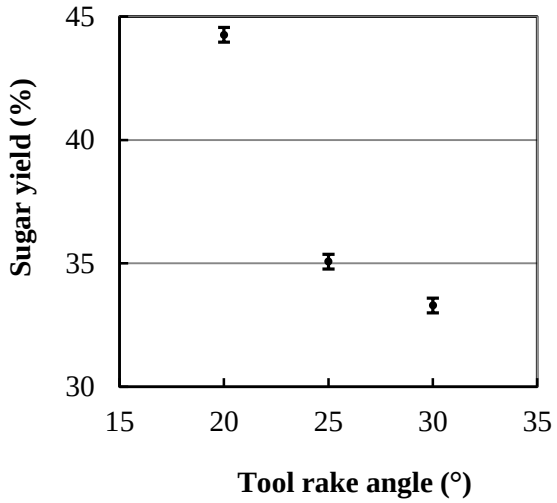


Figure 6.13 Effects of tool rake angle on sugar yield.



6.4 Conclusions

This study demonstrated an approach to separate confounding effects of particle size and biomass crystallinity. Hence, it became possible to investigate effects of biomass crystallinity on sugar yield independently. The following conclusions can be drawn.

1) Poplar wood particles produced with different tool rake angles had (statistically) the same size (thickness).
2) Poplar wood particles produced with different tool rake angles had different biomass crystallinity. Biomass crystallinity decreased as tool rake angle decreased.
3) For the three levels of biomass crystallinity tested in this study, sugar yield increased as biomass crystallinity index became smaller.

The authors plan to conduct further research to understand the mechanism responsible for the observed correlations between crystallinity index and process parameters.

Acknowledgements

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Chapter 7 - Effects of Sieve Size Used in Biomass Size Reduction: Study 1

Paper title:

Biofuel manufacturing from woody biomass: effects of sieve size used in biomass size reduction

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Abstract

Size reduction is the first step for manufacturing biofuels from woody biomass. It is usually performed using milling machines and the particle size is controlled by the size of the sieve installed on a milling machine. There are reported studies about the effects of sieve size on energy consumption in milling of woody biomass. These studies show that energy consumption increased dramatically as sieve size became smaller. However, in these studies, the sugar yield (proportional to biofuel yield) in hydrolysis of the milled woody biomass was not measured. The lack of comprehensive studies about the effects of sieve size on energy consumption in biomass milling and sugar yield in hydrolysis process makes it difficult to decide which sieve size should be selected in order to minimize the energy consumption in size reduction and maximize the sugar yield in hydrolysis. The purpose of this study is to fill this gap in the literature. In this study, knife milling of poplar wood was conducted using sieves of three sizes (1, 2, and 4 mm). Results show that, as sieve size increased, energy consumption in knife milling decreased and sugar yield in hydrolysis increased in the tested range of particle sizes.

Keywords: Cellulosic biofuel, Crystallinity, Energy consumption, Hydrolysis, Knife mill, Size reduction

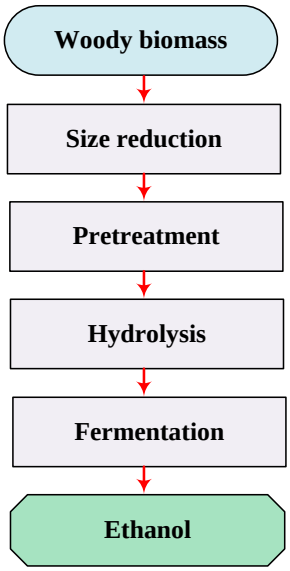
7.1 Introduction

The transportation sector of the United States accounts for over 70% of the nation's total petroleum consumption, and 57% of the petroleum is imported [1]. In addition, use of petroleum-based fuels contributes to accumulation of greenhouse gases (GHG) in the atmosphere. Due to concerns of energy security and GHG emissions, it becomes crucial to develop domestic sustainable alternatives to petroleum-based transportation fuels [2].

Biofuels produced from cellulosic biomass (herbaceous, woody, and generally inedible portions of plant matter) are a sustainable alternative to petroleum-based fuels. The United States has the resource to produce over 1 billion dry tons of biomass with more than 80% of cellulosic biomass including about 320 million dry tons of woody biomass annually [3,4]. This amount of biomass is sufficient to produce 90 billion gallons of liquid fuels that can replace about 30% of the nation's current annual consumption of petroleum-based transportation fuels [4]. In contrast to grain-based biofuels, cellulosic biofuels do not compete for the limited agricultural land with food or feed production [5].

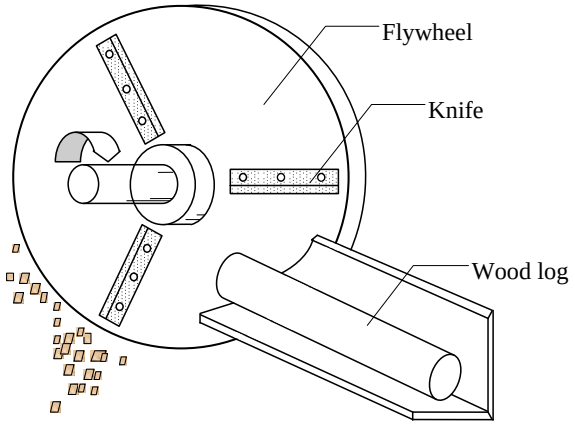
Figure 7.1 shows the major processes of converting woody biomass to ethanol (the most common form of biofuels). Size reduction reduces the particle size of woody biomass. Pretreatment helps to make cellulose in the biomass more accessible to enzymes during hydrolysis. Hydrolysis depolymerizes cellulose into its component sugars (glucose). Afterwards, fermentation converts glucose into ethanol [6].

Figure 7.1 Major processes of converting woody biomass to ethanol (after [6]).



Size reduction of woody biomass is necessary because large-size woody biomass cannot be converted to biofuels efficiently with the current conversion technologies [7-9]. Size reduction of woody biomass usually involves two steps. The first step is wood chipping [10]. Machines available for wood chipping include disk, drum, and V-drum chippers [11-13]. Figure 7.2 illustrates a disk chipper. Straight knives are mounted on a flywheel that revolves at a speed ranging from 400 to 1000 revolutions per minute (rpm). A wood log is fed to the disk chipper. Wood chips produced by wood chipping usually have sizes ranging from 5 to 50 mm [14]. Energy consumption of this step is typically about 0.05 Wh/g [15].

Figure 7.2 Illustration of a disk chipper (after [14]).



The second step is biomass milling to further reduce the wood chips into small particles. This step is usually conducted on knife mills [16] or hammer mills [17-19]. Wood particles produced by biomass milling usually have sizes ranging from 0.1 to 10 mm [19]. Energy consumption of this step ranged from 0.15 to 0.85 Wh/g [15,20,21].

Sieves are installed on knife mills and hammer mills to control the size of wood particles. During biomass milling, wood particles that were smaller than the sieve size (the size of the openings on a sieve) would pass through the sieve; those larger than the sieve size would be

recirculated and milled further. In this study, “sieves” and “sieve size” are reserved to describe the sieves installed on knife mills or hammer mills.

There are reported studies about the effects of sieve size on energy consumption in woody biomass milling using knife mills or hammer mills. A consistent observation was that energy consumption increased dramatically as sieve size became smaller [22-24]. However, these reports did not present sugar yield (proportional to ethanol yield) results using the wood particles produced by biomass milling. It was reported that woody biomass with smaller particle size had higher sugar yield [25-28]. However, particle size in these reported studies was defined differently from the sieve size in this paper. In these studies, wood particles produced by knife mills or hammer mills using a certain sieve size were separated into several size ranges by the screening method. The term “particle size” was actually the particle size range determined by the sizes of the openings on the screens. In this paper, “the size of the openings on the screen” is called “screen size”. Moreover, previously reported studies did not present energy consumption data for the biomass milling process used to produce the wood particles from which the sugar yield measurements were performed.

The lack of comprehensive studies about the effects of sieve size on energy consumption in size reduction (biomass milling) and sugar yield in hydrolysis makes it difficult to decide which sieve size should be selected in order to minimize the energy consumption in size reduction and maximize the sugar yield in hydrolysis. The purpose of this study is to fill this gap in the literature by studying the effects of sieve size on energy consumption in size reduction and sugar yield in hydrolysis simultaneously.

7.2 Experimental conditions and procedures

7.2.1 Biomass material preparation

Poplar wood chips were purchased from Petco Animal Supplies, Inc. (Manhattan, KS, USA). Since the purchased wood chips had a wide distribution in size, the wood chips were separated into three groups using two screens with screen size of 5 and 12.5 mm, respectively. Large chips are those that did not pass through the 12.5 mm screen. Small chips are those that passed through the 5 mm screen. Medium chips are those that passed through the 12.5 mm screen but not the 5 mm screen. Examples of large, medium, and small wood chips are shown in Figure 7.3. Only the medium wood chips were used in this study.

Figure 7.3 Examples of large, medium, and small wood chips.

The moisture content of the wood chips (as purchased) was 1.2%, measured by following the ASAE Standard S358.2 [29]. To adjust the moisture content of wood chips to a desired level, distilled water was added (by spraying evenly) to the wood chips. To achieve wood chips of 10% and 18% moisture content, 96 and 233 mL distilled water was added per 1000 g of original wood chips, respectively. After moisture content adjustment, the wood chips were placed in the sealed Ziploc® bags and stored in a refrigerator at 4 ℃ for at least 72 hours before knife milling.

7.2.2 Experimental set-up and procedure for knife milling

The experimental setup for knife milling of wood chips is illustrated in Figure 7.4. A Retsch knife mill (Model No. SM 2000, Retsch GmbH, Haan, Germany) was used. It was equipped with a three-phase 1.5 kW electric motor. The rotation speed of the motor was 1720 rpm.

Figure 7.5 shows the milling chamber of the knife mill. Three knives (95 mm long and 35 mm wide) were mounted on the rotor inside the milling chamber. Four cutting bars were mounted on the inside wall of the milling chamber. Wood chips were cut into particles between the knives and the cutting bars. The gap between a knife and a cutting bar was 3 mm. A sieve (145 mm long and 98 mm wide) was mounted at the bottom of the milling chamber. Sieves with three sieve sizes (4, 2, and 1 mm, respectively), as shown in Figure 7.6, were used in this study.

Figure 7.4 Experimental setup for knife milling of wood chips.

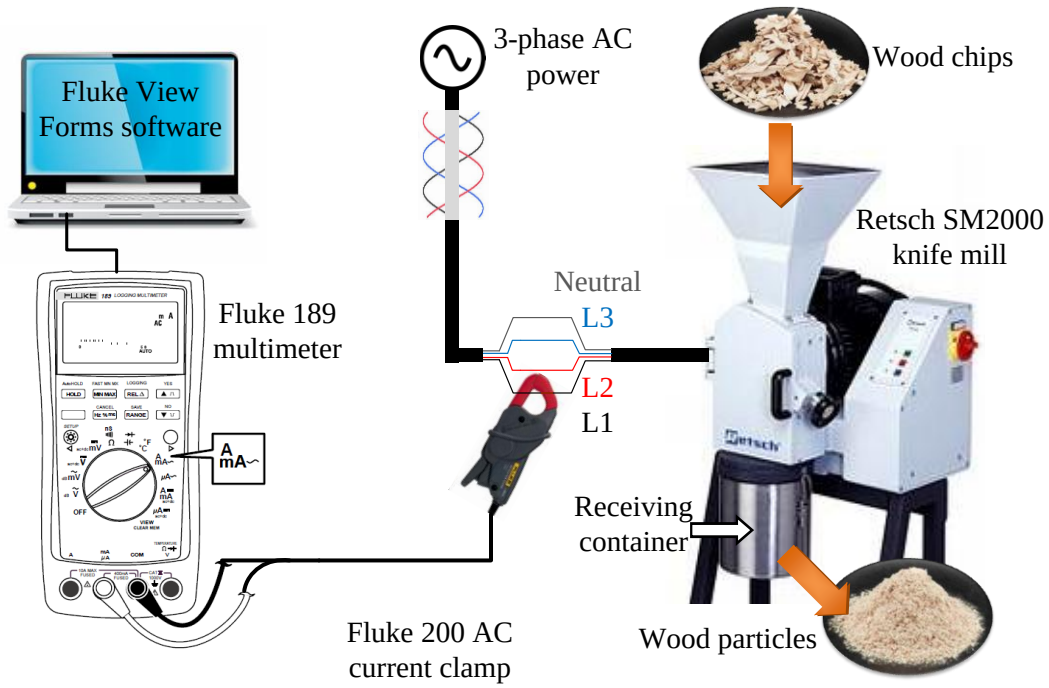


Figure 7.5 Milling chamber of the knife mill.

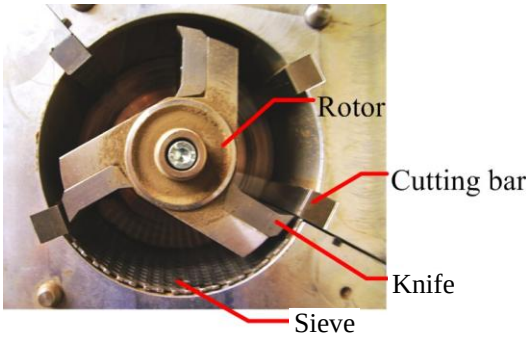
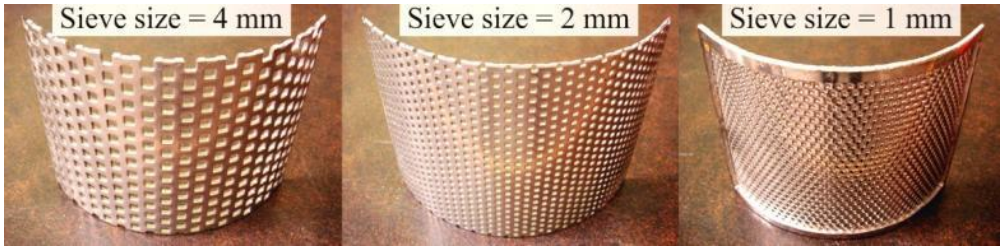


Figure 7.6 Sieves used in knife milling.



Sieve sizes of 1 and 4 mm were selected because they were the minimum and maximum sieve size, respectively, that could be practically investigated in this study. As described in Section 7.2.1, the wood chips prior to milling had a range of 5 to 12.5 mm. If any available sieve size larger than 4 mm was used, some of the wood chips would fall through the sieve without being cut. Furthermore, based on previous experience, if any available sieve size smaller than 1 mm (the next one was 0.5 mm) was used, some of the sieve openings would be blocked by milled particles, causing significant increase in milling time and energy consumption.

At the beginning of each test, the knife mill was run for 10 seconds before loading any wood chips to avoid the current spike (this would happen if the knife mill started with wood chips already in the milling chamber). Then, 50 g of wood chips were loaded into the knife mill. This amount of wood chips was enough to keep the milling chamber approximately full (in volume). During knife milling, more wood chips were loaded into the milling chamber using a scoop as

shown in Figure 7.7. The amount of wood chips loaded by the scoop at each time was 5±1 g. These additional wood chips were loaded at a rate that would keep the milling chamber approximately full (in volume) but without causing over loading.

Figure 7.7 The scoop used for loading wood chips.

In each test, the total amount of wood chips loaded into the milling chamber was 200 g. The milling time was different under different conditions. When a smaller sieve size was used, it took a longer time to mill the same amount of wood chips.

Table 7.1 Experimental conditions.

Condition ID	Moisture content (%)	Sieve size (mm)
1	1.2	1
2	1.2	2
3	1.2	4
4	10	1
5	10	2
6	10	4
7	18	1
8	18	2
9	18	4

After each test, wood particles in the receiving container were collected, weighed, and kept in the sealed Ziploc® bags. The amount of wood particles collected by the receiving container in each test was less than 200 g, because some wood chips (or particles) did not pass through the

sieve yet when the knife mill was turned off. Before starting the next test, the milling chamber was opened and any remaining wood chips were cleaned using a brush. To allow the motor to cool down, there was a waiting period (at least five minutes) between two successive tests. Experimental conditions are listed in Table 7.1.

7.3 Evaluation parameters and measurement procedures

7.3.1 Energy consumption

In this study, energy consumption is the electricity consumed by the electric motor of the knife mill. As shown in Figure 7.4, electric current to the motor was measured using a Fluke 189 multimeter and a Fluke 200 AC current clamp (Fluke Corp., Everett, WA, USA). Current data were collected using Fluke View Forms software. The sampling rate was two readings per second. Data acquisition began after the first 50 g of wood chips were loaded into the milling chamber, and stopped when additional 150 g of wood chips were all loaded into the chamber. The knife mill was turned off right after data acquisition stopped.

The software recorded the average current (I_{AVE}) in each test. The voltage (V_{LN}) was 208 V. The energy consumed during each test (that lasted for t seconds) (E_t) was calculated using the following equation [30]:

$$E_t = \frac{\sqrt{3} \cdot I_{AVE} \cdot V_{LN} \cdot t}{3600} (Wh) \quad (1)$$

Dividing E_t by the weight (w) of the wood particles collected from the receiving container after the test gives energy consumption (E) per unit weight, as expressed in Equation 2.

$$E = \frac{E_t}{w} (Wh/g) \quad (2)$$

7.3.2 Sugar yield

Sugar yield in hydrolysis is the amount of glucose produced from hydrolyzing cellulose using enzymes. It was expressed as the concentration of glucose (mg/mL) in the measurement sample. Figure 7.8 shows the four steps in sugar yield measurement. In this study, 10 g of biomass and 200 mL of 2% sulfuric acid were loaded in the 600 mL vessel of a Parr pressure reactor (Model 4760A, Parr Instrument Co., Moline, IL, USA). Pretreatment time was 30 minutes, and pretreatment temperature was 140 ℃.

Figure 7.8 Four steps in sugar yield measurement.

Pretreatment | Biomass washing | Hydrolysis | Sugar analysis

After pretreatment, biomass was washed with hot distilled water using a centrifugal (Model PR-7000M, International Equipment Co., Needham, MA, USA). The purpose of biomass washing was to remove the acid residues and inhibitors (substances that would bind to enzymes and decrease their activity to depolymerize cellulose to glucose [31]) formed during pretreatment. The rotation speed of the centrifugal was 4500 rpm. Each biomass sample was washed three times, and each time lasted for 15 minutes.

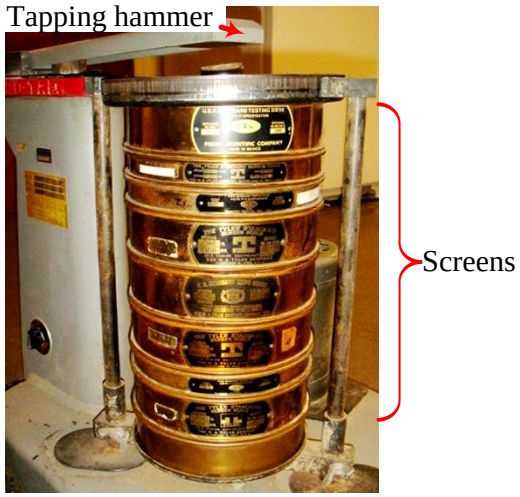
Accellerase 1500^{TM} (Danisco US Inc., Rochester, NY, USA) enzyme complex was used for hydrolysis of wood particles into sugars in solution with sodium acetate buffer (50 mM, pH 4.8) and 0.02% (w/v) sodium azide to prevent the microbial growth during hydrolysis. Enzymatic hydrolysis was carried out in 125 mL flasks with 50 mL of slurry in the water bath shaker

(Model C76, New Brunswick Scientific, Edison, NJ, USA) with agitation speed of 110 rpm at 50 °C for 72 hours. The dry mass content of the hydrolysis slurries was 5% (w/v) and the enzyme loading was 1 mL/g of dry biomass. After enzymatic hydrolysis, samples were ready for sugar analysis.

Sugar analysis was done using a HPLC (Shimadzu Corp., Kyoto, Japan) equipped with an RPM-monosaccharide column (300 × 7.8 mm; Phenomenex, Torrence, CA, USA) and a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan). The mobile phase was 0.6 mL/min of double-distilled water, and oven temperature was 80 ℃. HPLC can identify and quantify individual components of a liquid mixture [32].

7.3.3 Particle size distribution

Figure 7.9 W.S. Tyler screen shaker.



Wood particles produced by knife milling were not uniform in their size. Particle size distribution was determined using a screen shaker (Model RO-TAP® 8" RX-29, W.S. Tyler Industrial Group, Mentor, OH, USA) as illustrated in Figure 7.9. A stack of screens were arranged from the largest to the smallest in screen size. The screen sizes used were 0.1, 0.2, 0.4,

0.6, 1.2, 2.4, 5.6, and 6.3 mm. A pan (no openings) was put at the bottom of these screens. 100 g of wood particles were loaded onto the top screen.

The screen shaker provided circular motion to the stack of screens at the rate of 278 rpm. Simultaneously, the tapping hammer hit the top of the stack at the frequency of 150 times per minute. The screen shaker was on for 5 minutes. Afterwards, wood particles retained on each screen were collected and weighed. The percentage of the wood particles in each of the nine particle size ranges (< 0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6, 0.6-1.2, 1.2-2.4, 2.4-5.6, 5.6-6.3, and > 6.3 mm) was translated to particle size distribution [33].

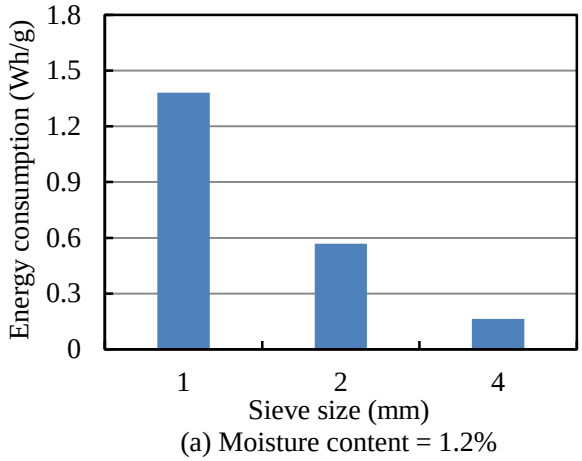
7.4 Results and discussion

7.4.1 Energy consumption in knife milling

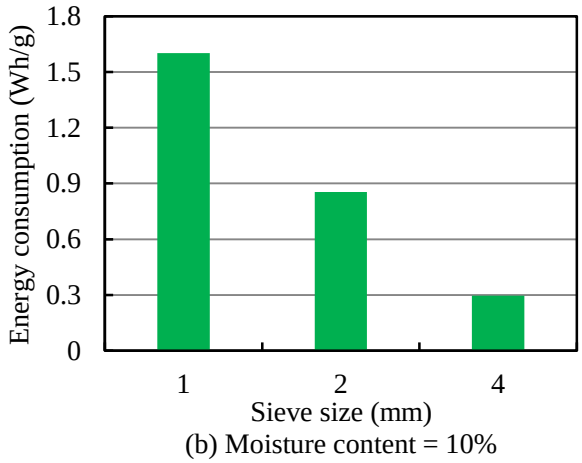
Figure 7.10 shows energy consumption in knife milling of wood chips. Energy consumption decreased dramatically with an increase of sieve size. For instance, when knife milling of wood chips with moisture content of 1.2%, energy consumption was as high as 1.38 Wh/g for 1 mm sieve size and only 0.16 Wh/g for 4 mm sieve size. The same trend was observed for the other two levels of moisture content.

In the literature, there are no reports about the effects of sieve size on energy consumption in knife milling of poplar wood chips. Phanphanich et al. [34] used a knife mill (of the same model as the one used in this study) to reduce the size of pine wood chips (including chips, branches, barks, leaves, and small particles). The moisture content of the pine wood chips was 10%. Only one sieve size (1.5 mm) was used in their study. Energy consumption in knife milling was 0.25 Wh/g. Miao et al. [23] measured energy consumption in hammer milling of willow wood chips. The hammer mill was manufactured by Sears Roebuck and Co. (Hoffman Estates, IL, USA). The

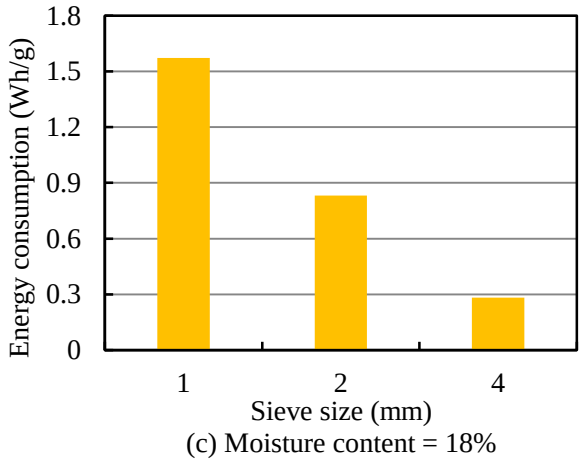
Figure 7.10 Effects of sieve size on energy consumption in knife milling.



(a) Moisture content = 1.2%



(b) Moisture content = 10%

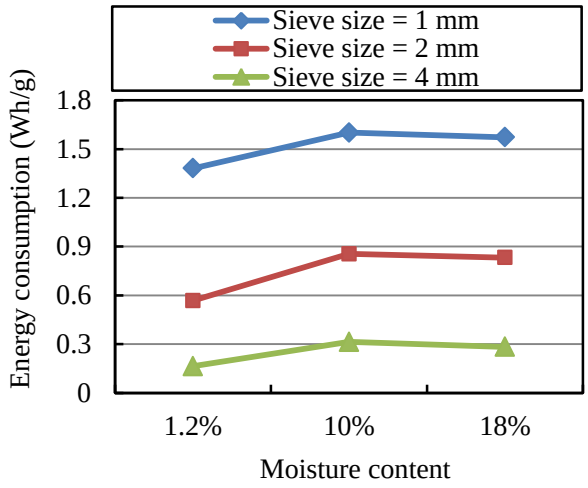


(c) Moisture content = 18%

size of the willow wood chips (three dimensions) was 13-50, 13-76, and 5-25 mm. The moisture content was 7-10%. Energy consumption in hammer milling using the 1, 2, and 4 mm sieves were 1.55, 0.66, and 0.39 Wh/g, respectively.

Moisture content of poplar wood chips also affected energy consumption in knife milling. As shown in Figure 7.11, energy consumption in knife milling increased when moisture content increased from 1.2% to 10%, and decreased slightly when moisture content increased from 10% to 18%.

Figure 7.11 Effects of moisture content on energy consumption in knife milling.



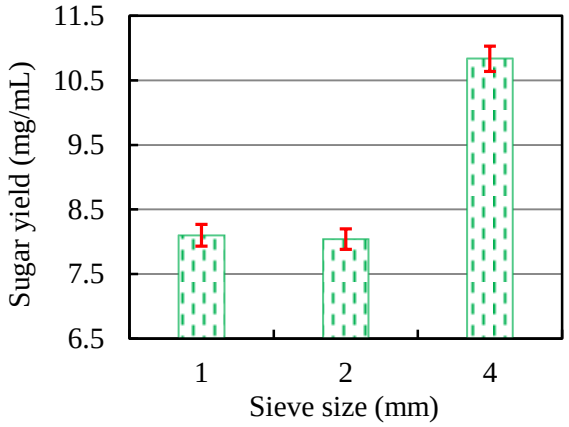
The literature does not have any reports about the effects of moisture content on energy consumption in knife milling of wood chips using the knife mill of the same model as the one used in this study. However, there are reports on these effects in knife milling of herbaceous biomass (such as miscanthus, switchgrass, and wheat straw). Miao et al. [23] investigated energy consumption in knife milling of miscanthus and switchgrass using the same model of knife mill. It was found that, when moisture content increased from 7-10% to 15%, energy consumption in knife milling increased significantly. The same trend was also found in size reduction of wheat

straw, barley straw, corn stover, and switchgrass using a hammer mill [35]. According to Mani et al. [35], an increase in moisture content of cellulosic biomass would increase the shear strength of the biomass; therefore, more energy was consumed in milling of cellulosic biomass.

7.4.2 Sugar yield

Materials used for sugar yield evaluation were the particles produced by knife milling of wood chips with the moisture content of 1.2%. For each sieve size, there were two independent samples processed for sugar yield evaluation. Figure 7.12 shows the sugar yield results. The results showed that wood particles processed using the 4 mm sieve had the highest sugar yield; while, sugar yields of wood particles processed using the 1 and 2 mm sieves were approximately the same.

Figure 7.12 Effects of sieve size on sugar yield.



There are reported investigations on the effects of sieve size on sugar yield. Zhang et al.'s results [36] are shown in Figure 7.13. Wheat straw particles milled using the 2 mm sieve had higher sugar yield than those milled using the 1 mm sieve. The knife mill used was the same model as the one in this paper. Similar results were reported by Theerarattananoon et al. [37]. In their work, wheat straw, corn stover, and big bluestem were milled using a hammer mill (Model

18-7-300, Schuttle-Buffalo Hammermill, Buffalo, NY, USA) using 3.2 and 6.5 mm sieves. For these three types of cellulosic materials, biomass particles milled using the 6.5 mm sieve yielded more sugar than those milled using the 3.2 mm sieve (Figure 7.14). Both these reported studies involved a pelleting process (agglomerating biomass particles produced by milling into pellets) before sugar yield.

Figure 7.13 Effects of sieve size on sugar yield reported by Zhang et al. [36].

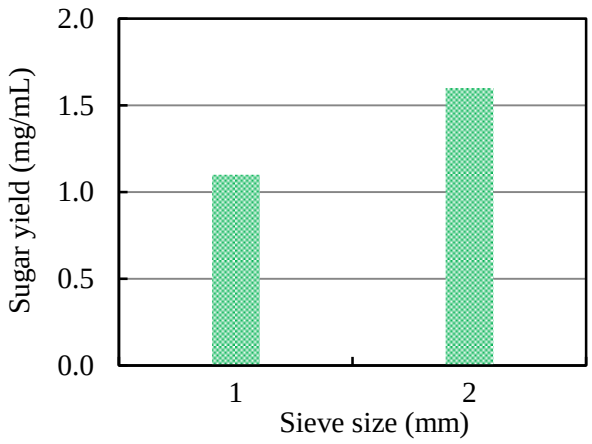


Figure 7.14 Effects of sieve size on sugar yield reported by Theerarattananoon et al. [37].

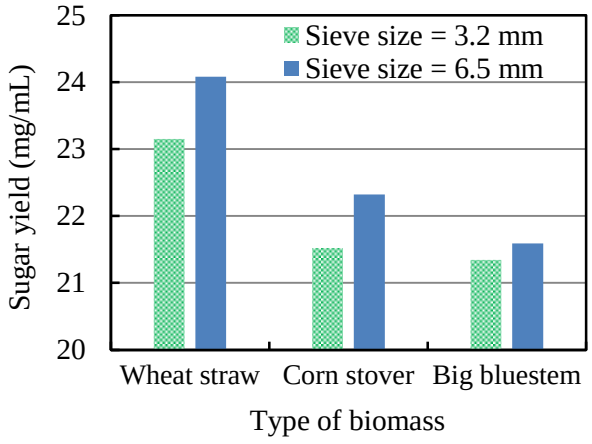


Figure 7.15 and Figure 7.16 show the effects of woody biomass particle size on sugar yield reported in the literature. In Dasari et al.'s study [27], red oak saw dust was screened into four particle size ranges. As shown in Figure 7.15, particles in the size range of 0.03-0.08 mm yielded 80% more sugar than those in the size range of 0.59-0.85 mm. In Zhu et al.'s study [26], spruce wood chips were hammer milled in three successive steps using sieve sizes of 12.7, 4.8, and 0.8 mm, respectively. After hammer milling, particles were screened into four particle size ranges. As shown in Figure 7.16, particles in the size range of smaller than 0.32 mm yielded 1.6 times more sugar than those in the size range of larger than 1.27 mm.

Figure 7.17 shows the wood particles produced by knife milling using the three different sieve sizes (4, 2, and 1 mm respectively). The particles produced using the same sieve did not have a uniform size. Their size distribution is shown in Figure 7.18. Similar distributions were reported by Himmel et al. [24]. In Himmel et al.'s study, poplar wood chips were processed by a knife mill (Mitts & Merrill Frӧmag Group, Harvard, IL, USA) using 1/16, 1/8, and 3/32 inch (1.59, 3.18, and 2.38 mm) sieves.

The results from this study, and the studies conducted by Zhang et al. [36] and Theerarattananoon et al. [37] show that biomass particles produced with larger sieve size had higher sugar yield. However, results reported by Dasari et al. [27] and Zhu et al. [26] show that wood particles in the smaller size range had higher sugar yield. At this point in time, the authors could not explain such inconsistence. However, some differences in test conditions were noticed. In the studies reported by Dasari et al. [27] and Zhu et al. [26], wood particles were from relatively narrow size ranges. In this work, wood particles were mixtures of particles that had a wide distribution in size. Further investigations will be carried out to study the effects of particle size distribution on woody biomass sugar yield.

Figure 7.15 Effects of particle size on sugar yield reported by Dasari et al. [27].

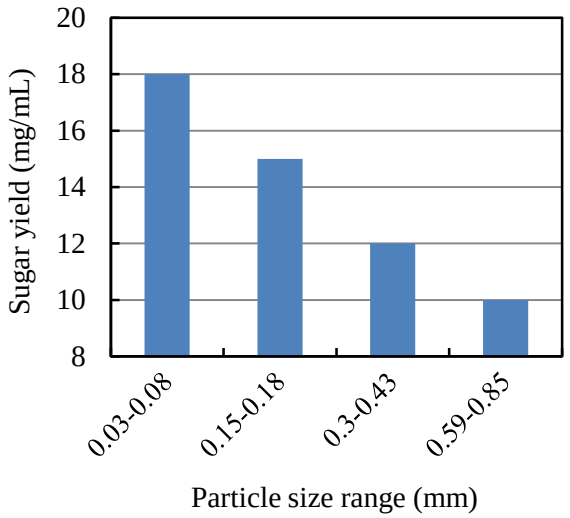


Figure 7.16 Effects of particle size on sugar yield reported by Zhu et al. [26].

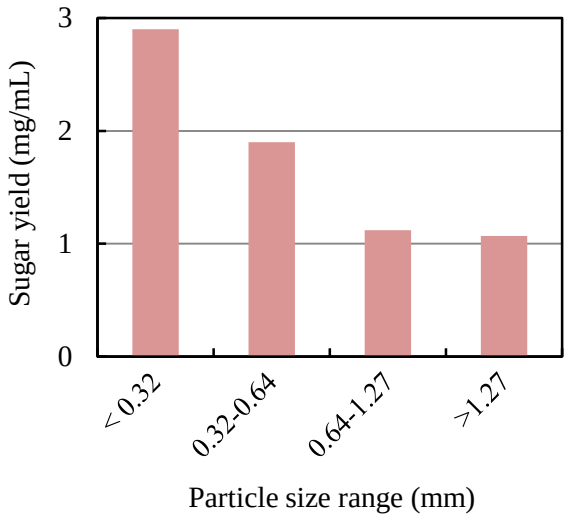
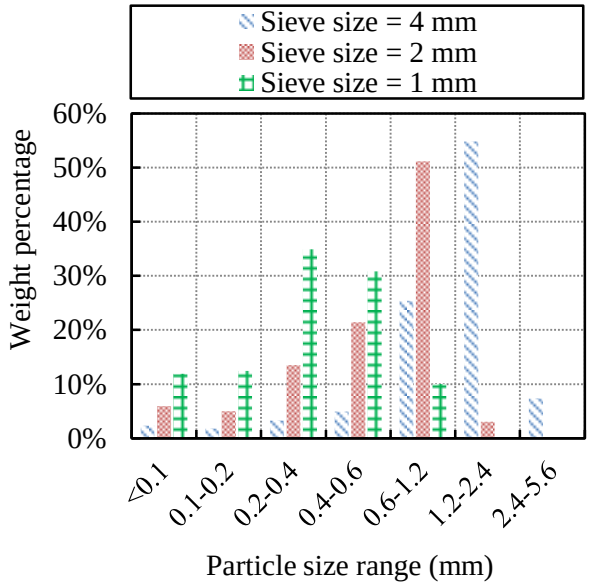


Figure 7.17 Wood particles processed using different sieve sizes (sieve size = 4, 2, and 1 mm from left to right).

Figure 7.18 Particle size distribution.



7.5 Conclusions and future work

In this study, effects of sieve size on energy consumption in knife milling of poplar wood chips and sugar yield in hydrolysis were studied. The following conclusions are drawn. Energy consumption in knife milling increased dramatically as sieve size became smaller. Poplar wood particles processed by knife milling using the 4 mm sieve had higher sugar yield than those processed by knife milling using the 1 and 2 mm sieves.

Knife milling of wood chips using the 4 mm sieve consumed less energy in size reduction than using the 1 and 2 mm sieves. The wood particles knife milled using the 4 mm sieve had higher sugar yield in hydrolysis than those milled using the 1 and 2 mm sieves. This finding is very important when deciding what sieve size is to be used in knife milling of wood chips to minimize energy consumption in size reduction and maximize sugar productivity in hydrolysis. In future study, the authors will also use 0.25, 0.5, and 8 mm sieves to further investigate the effects of sieve size on energy consumption in size reduction and sugar yield in hydrolysis. A hammer mill will be utilized to see if similar results can be obtained on different types of milling machines. More types of cellulosic materials will be tested to see if conclusions obtained in this study can be extended to different types of cellulosic biomass.

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Chapter 8 - Effects of Sieve Size Used in Biomass Size Reduction: Study 2

Paper title:

Effects of sieve size on biochemical conversion of big bluestem biomass for biofuel production

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Abstract

Biomass size reduction is the first step for biofuel production from cellulosic biomass (including dedicated energy crops such as big bluestem, forest residues, and agricultural residues) through biochemical pathway. Biomass size reduction is usually performed on a mill with a sieve installed to control the size of the produced particles. The literature has studies that consistently showed that energy consumption in biomass size reduction increased greatly when smaller sieve sizes were installed. Nevertheless, these studies were either not for biofuel production purpose or did not include the biochemical conversion of produced particles to fermentable sugar. The absence of in-depth knowledge about effects of sieve size throughout the biochemical conversion of cellulosic biomass makes it difficult for decision makers to decide what sieve size should be used for biomass size reduction in order to minimize energy consumption on mills, maximize cellulose recovery rate after pretreatment, and maximize the enzymatic hydrolysis efficiency. The objective of this work is to close this gap by generating new knowledge on effects of sieve size in these three processes: size reduction, pretreatment, and enzymatic hydrolysis, using big bluestem biomass. Results show that a larger sieve size saved energy in biomass size reduction on a knife mill. Moreover, big bluestem particles produced with a larger sieve size achieved higher cellulose recovery rate after pretreatment, higher enzymatic hydrolysis efficiency, and higher total sugar yield.

Keywords: Big bluestem, cellulosic biofuel, energy consumption, enzymatic hydrolysis, pretreatment, sieve size

8.1 Introduction

There is a growing need to find alternatives to petroleum-based liquid transportation fuels [1,2]. Recognized as promising alternatives are biofuels produced from cellulosic biomass

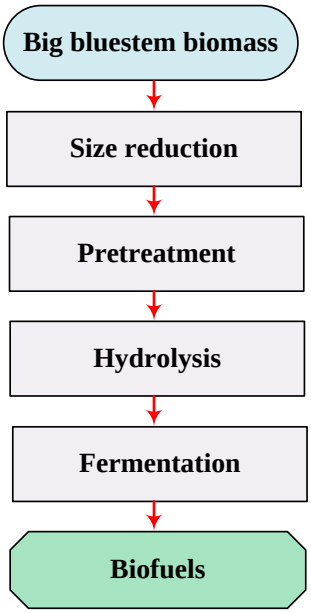
(including dedicated energy crops such as big bluestem, forest residues, and agricultural residues) [3-5]. Using cellulosic biomass as the feedstock for biofuel production is advantageous because of its low cost, abundance, and sustainability [6]. An investigation in 2005 jointly supported by the U.S. Department of Energy and Department of Agriculture shows that land resources in the U.S. are sufficient to sustain production of enough cellulosic biomass (about 1 billion dry tons) annually to replace 30% or more of the nation's current consumption of liquid transportation fuels [3-5].

Conversion of dedicated energy crops such as big bluestem into biofuels offers both economic and environmental benefits [7]. Big bluestem is a dominant grass in the tallgrass prairies of North America, and comprises up to 80% of the prairie biomass in the Midwest grassland in the United States [8,9]. Big bluestem biomass can be converted into ethanol biofuels through biochemical pathway. Figure 8.1 illustrates the major steps in the conversion. First, size reduction of big bluestem biomass is necessary because current conversion technologies cannot efficiently convert whole stems of big bluestem biomass into ethanol biofuels [10,11]. The biomass size reduction is usually conducted on a knife mill [12] or hammer mill [13-16] to produce particles with sizes from 0.1 to 10 mm [17]. Second, pretreatment can break the lignin seal, disrupt the crystalline structure of cellulose in the biomass, and increase its surface area to make cellulose more accessible to enzymatic hydrolysis. Hydrolysis breaks cellulose into its component sugars (glucoses) that are convertible to ethanol by fermentation [6]. It is known that fermentable sugar yield in hydrolysis is approximately propositional to the biofuel yield in fermentation [18]

Mills for biomass size reduction are equipped with sieves to control the size of the produced particles. The size of the openings on a sieve is known as sieve size. During size reduction,

particles smaller than sieve size would pass through, and particles larger than sieve size would be recirculated and continue being milled. Studies about effects of sieve size on energy consumption in biomass size reduction have been reported in the literature. It has been consistently observed that energy consumption in biomass size reduction increased greatly when smaller sieve sizes were used [19-21]. Nevertheless, these studies were either not for biofuel production purpose or did not include biochemical conversion of produced particles to fermentable sugar. Many other reported studies included biomass biochemical conversion to ethanol biofuels with biomass particles produced by size reduction, but did not cover energy consumption in biomass size reduction [22-24].

Figure 8.1 Major steps in biochemical conversion of big bluestem biomass into biofuels



The absence of in-depth knowledge about effects of sieve size throughout biochemical conversion of cellulosic biomass makes it difficult for decision makers to decide what sieve size should be used in biomass size reduction in order to minimize energy consumption on mills, maximize cellulose recovery rate after pretreatment, and maximize enzymatic hydrolysis

efficiency. The objective of this work is to close this gap by generating new knowledge on effects of sieve size in these three processes: size reduction, pretreatment, and enzymatic hydrolysis, using big bluestem biomass.

8.2 Material and methods

8.2.1 Material

The material used in this study was big bluestem biomass harvested from the United States Department of Agriculture Plant Material Center (Manhattan, KS, USA). Entire plants except the root were used. Moisture content of the big bluestem biomass was 5%. Biomass moisture content was determined by following the laboratory analytical procedures developed by National Renewable Energy Laboratory [25]. Chemical compositions of cellulose, hemicellulose, and lignin are listed in Table 8.1 [26].

Table 8.1 Chemical compositions (% dry weight basis) of big bluestem

Component	Percentage
Cellulose	35.9 (0.4)
Hemicellulose	25.4 (0.5)
Lignin	24.0 (0.7)

Chemical compositions are means with standard deviations in brackets

8.2.2 Biomass size reduction

The experimental setup for size reduction is illustrated in Figure 8.2. A knife mill (SM 2000, Retsch GmbH, Haan, Germany) was used. It was powered by a 1.5 kW electric motor. The milling chamber of the mill is pictured in Figure 8.3. The knife mill is equipped with three knives (95 × 35 mm) on the rotor and four cutting bars mounted on the inside wall of the milling chamber. Big bluestem biomass was cut and sheared into particles between the knives and the

cutting bars. A sieve (145 × 98 mm) was installed at the bottom of the milling chamber. Four sieve sizes (1, 2, 4, and 8 mm) were used in this study. Figure 8.4 shows a 4 mm sieve as an example.

Figure 8.2 Experimental setup

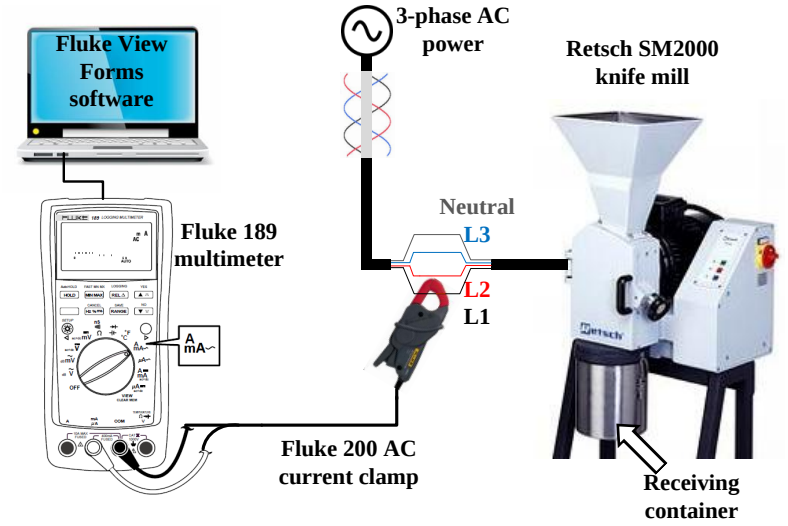


Figure 8.3 Milling chamber of the knife mill

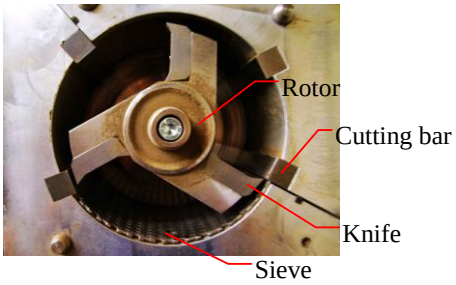
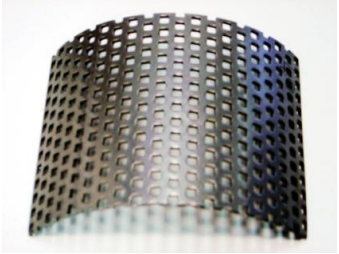


Figure 8.4 Sieve used on the knife mill (sieve size = 4 mm)



Before starting any size reduction test, the knife mill was run for 10 seconds without loading any biomass to avoid current spikes. After that, ten big bluestem stalks were loaded into the milling chamber (one stalk is about 50 cm long and 0.5-1 cm wide). During the milling process, more big bluestem stalks were loaded into the milling chamber manually by a mill operator at a rate at which the milling chamber was kept full. For one size reduction test, the total amount of big bluestem loaded was 400 grams. The mill was turned off after 10 seconds when all the 400 grams of biomass was loaded into the milling chamber. After each test, the weight of the big bluestem particles collected from the receiving container was measured. Not all the 400 grams of biomass could be collected from the receiving container, because there was still biomass retained in the milling chamber when the mill was turned off. Between two consecutive size reduction tests, the milling chamber was opened to remove any biomass left there, and the chamber was cleaned with compressed air.

8.2.3 Sugar conversion

In this study, big bluestem sugar conversion was consisted of dilute sulfuric acid pretreatment and enzymatic hydrolysis. In dilute sulfuric acid pretreatment, 10 grams (dry weight) of big bluestem particles produced with each sieve size and 200 mL of 2% (w/v) sulfuric acid were loaded in a 600 mL glass liner of a Parr pressure reactor (4760A, Parr Instrument Co., Moline, IL, USA). Pretreatment time was 30 min, and pretreatment temperature was 140 ℃.

After pretreatment, big bluestem particles were washed with 50-60°C distilled water using a suction filtration system with P4 grade filter paper (Fisher Scientific Inc., Waltham, MA, USA) to conduct solid-liquid separation. The solid biomass after filtration was carefully collected from the filter paper using a stainless steel micro spatula. The dry weight of the collected solid biomass was measured, then a small portion of the solid biomass was used for biomass

composition analysis, and the rest was used for subsequent enzymatic hydrolysis. The liquid after filtration was removed, which included dissolved sugars, acid residues, and inhibitors (substances that could decrease enzymes' ability to break cellulose into glucose) formed during pretreatment.

Enzymatic hydrolysis was carried out in eight 125-mL flasks in a water bath shaker (C76, New Brunswick Scientific, Edison, NJ, USA) at 50°C for 48 h. The agitation speed of the water bath shaker was 110 rpm. There were two flasks containing big bluestem particles produced with each of the four sieve sizes. Each flask contained 50 mL of hydrolysis slurry. The slurry was consisted of 4% (w/v) biomass on dry weight base, sodium acetate buffer (50 mM, pH = 4.8), and 0.02% (w/v) sodium azide to prevent microbial growth during hydrolysis. Accellerase 1500^{TM} enzyme complex (Danisco USA, Inc., Rochester, NY, USA) was used. The enzyme loaded was 0.5 mL for each gram of dry biomass.

8.3 Measurement and statistical analysis

8.3.1 Energy consumption in biomass size reduction

Energy consumption in biomass size reduction was the electricity consumed by the electric motor of the knife mill. As illustrated in Figure 8.2, electric current to the motor was measured using a Fluke 200 AC current clamp connected to a Fluke 189 multimeter (Fluke Corp., Everett, WA, USA). The 3-phase AC power supply in this study was in a Y configuration with four wires (3 phases: L1, L2, L3, and neutral). Electric current readings were collected by software (FlukeView Forms Basic, Fluke Corp., Everett, WA, USA) with a sampling rate of two readings per second. Data acquisition began after the initial ten stems of big bluestem were loaded into the milling chamber, and stopped until the mill was turned off.

8.3.2 Biomass composition

Carbohydrates (cellulose and hemicellulose) and lignin make up a major portion of cellulosic biomass. Cellulose can be converted to fermentable sugar (glucose) in enzymatic hydrolysis. Hemicellulose is also a sugar component; however, almost all of the hemicellulose will be decomposed by dilute sulfuric acid pretreatment. Lignin contains no sugar [27]. Biomass composition analysis is needed for the analyses described in Section 8.3.4.

In this study, biomass composition after pretreatment was determined according to the laboratory analytical procedures developed by National Renewable Energy Laboratory [28]. The biomass collected for composition analysis was dried in an oven (Isotemp 500 Series, Fisher Scientific Inc., Waltham, MA, USA) at 40°C for 48 h. About 0.3 g of oven-dried biomass sample was soaked in 72% sulfuric acid at 30 ℃ for 1 h with constant stirring. Then, the biomass sample was diluted to a 4% acid solution and heated at 120 ℃ for another 1 h. After heating, the liquid and solid parts of the biomass sample were separated by suction filtration. The liquid part was adjusted to pH neutral by adding calcium carbonate, then the cellulose and hemicellulose contents in the liquid part were measured by a high-performance liquid chromatography (HPLC) system (more information about the HPLC system will be provided in Section 8.3.3), and the acid-soluble lignin content in the liquid was measured by a UV-visible spectrophotometer (BioMate 3, Thermo Electron Corp., Madison, WI, USA). The solid part was dried and combusted. The weight difference between the dry solid and combustion residue was reported as acid-insoluble lignin. The sum of the acid-soluble and acid-insoluble lignin contents was the total lignin content. Two duplications for each biomass sample were prepared for measurement. Biomass composition reported in this study was the weight percentage of cellulose, hemicellulose, and lignin.

8.3.3 Sugar content

After 48 h of enzymatic hydrolysis, sugar contents in the biomass samples were determined by analyzing the supernatant from the hydrolysis slurry using an HPLC system (Shimadzu, Kyoto, Japan). HPLC is an analytical tool for separating and quantifying components in complex liquid mixtures. The HPLC system was equipped with an RCM-monosaccharide column (300 × 7.8 mm; Phenomenex, Torrence, CA, USA) and a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan). The column was eluted with double distilled water at a flow rate of 0.6 mL/minute, and the temperature of the column was maintained at 80 ℃.

8.3.4 Statistical analysis

Chemical compositions in Tables 8.1 and 8.3 are reported as means with standard deviations in brackets. Multiple comparisons using one-way analysis of variance (ANOVA) were conducted using Minitab software (Version 16, Minitab Inc., State College, PA, USA) to determine if there were significant differences between the means.

8.4 Results and discussion

8.4.1 Effects of sieve size on energy consumption in biomass size reduction

For big bluestem size reduction by knife milling, the data acquisition software recorded the average current (I_{AVE}). The voltage (V) was 208 V. The energy consumed in each size reduction test (t seconds) (P) was calculated as follows:

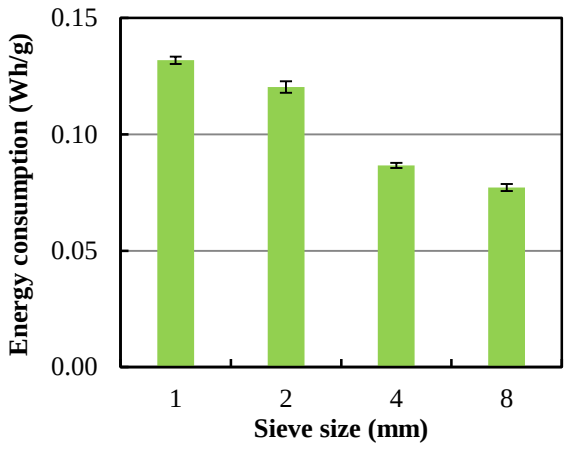
$$P = \frac{\sqrt{3} \cdot I_{AVE} \cdot V \cdot t}{3600} (Wh) \quad (1)$$

Dividing P by the weight (w) of the big bluestem particles collected after the test would give energy consumption (E) per unit weight:

$$E = \frac{P}{w}(Wh/g) \quad (2)$$

Figure 8.5 shows that sieve size had significant effects on energy consumption in size reduction of big bluestem. Energy consumption decreased greatly as sieve size increased. Energy consumption was as high as 0.13 and 0.12 Wh/g for sieve sizes of 1 and 2 mm, respectively. When using 4 and 8 mm sieve sizes, energy consumption decreased to 0.09 and 0.08 Wh/g, respectively.

Figure 8.5 Effects of sieve size on energy consumption in size reduction of big bluestem



In the literature, there are no reports on energy consumption in size reduction of big bluestem. There are some reports on energy consumption about size reduction of other types of herbaceous biomass using Retsch® SM 2000 knife mill. Table 8.2 summarizes energy consumption data when using different sieve sizes on the knife mill. As indicated in the table, using smaller sieve size would consume more energy to produce the same amount of biomass particles. It is noticed that, in previous studies reported by Miao et al. [19,20], herbaceous biomass loaded into the knife mill was biomass segments prepared by a chopping machine before knife milling. However, other studies in Table 8.2 used whole stem of herbaceous biomass as input materials to the knife mill.

Table 8.2 Energy consumption in biomass size reduction using Retsch SM2000 knife mill

Biomass material	Moisture content (% dry weight basis)	Sieve size (mm)	Energy consumption (Wh/g)	Reference
Big bluestem (whole stems)	5	1	0.13	This study
		2	0.12	
		4	0.09	
		8	0.07	
Miscanthus (segments)	7-10	1	0.28	[19,20]
		2	0.10	
		4	0.06	
		8	0.04	
Switchgrass (segments)	7-10	1	0.27	[19,20]
		2	0.12	
		4	0.06	
		8	0.03	
Wheat straw (whole stems)	12	1	0.16	[29]
		2	0.12	
		8	0.06	
Sorghum stalk (whole stems)	9	1.5	0.09	[30]
		8	0.04	
Kochia (whole stems)	10	1.5	0.07	[30]
		8	0.02	

8.4.2 Effects of sieve size on cellulose recovery rate after pretreatment

Table 8.3 lists big bluestem chemical compositions of big bluestem after pretreatment. It is noticed that chemical compositions of particles produced with different sieve sizes are approximately the same. Biomass weight loss in pretreatment (L) (%) was calculated as follows:

$$L\ (\%) = \frac{W_{BP} - W_{AP}}{W_{BP}} \times 100\% \quad (3)$$

Where W_{BP} (g) and W_{AP} (g) are the dry weight of biomass before and after pretreatment, respectively.

Table 8.3 Chemical compositions (% dry weight basis) of big bluestem after pretreatment

Component	Sieve size (mm)			
	1	2	4	8
Cellulose	56.4 (1.2)	58.6 (0.9)	58.4 (0.5)	58.9 (0.6)
Hemicellulose	5.7 (0.6)	5.2 (0.3)	4.9 (0.7)	4.6 (0.2)
Lignin	28.6 (0.7)	28.0 (1.3)	29.8 (0.2)	29.6 (1.0)

Chemical compositions are means with standard deviations in brackets

Figure 8.6 shows that there was more biomass weight loss in pretreatment for particles produced with a smaller sieve size. The weight loss in pretreatment was primarily caused by the decomposition of hemicellulose. The main objective of dilute sulfuric acid pretreatment is to decompose hemicellulose to acid soluble products (i.e. xylose). This will cause cellulose become more accessible to enzymes in enzymatic hydrolysis [31]. However, a side effect of pretreatment is that a small amount of cellulose may be degraded to hydroxymethyl-furfural (HMF) [31]. HMF is soluble in the pretreatment liquid and will be separated from the solid biomass after pretreatment. Only the solid biomass collected after pretreatment goes into enzymatic hydrolysis. The degradation of cellulose to HMF results in some cellulose loss and leads to a decrease total sugar yield [32].

Cellulose recovery rate after pretreatment (R_P) is used to evaluate how much cellulose can be recovered in the pretreatment. It is calculated as the ratio of the cellulose weight after pretreatment to the cellulose weight before pretreatment:

$$R_P\ (\%) = \frac{C_{AP}}{C_{BP}} \times (1 - L) \tag{4}$$

Where C_{AP} (%) is the cellulose content in the biomass after pretreatment, C_{BP} (%) is the cellulose content in the biomass before pretreatment, and L (%) is the biomass weight loss in pretreatment. These two cellulose contents were obtained by biomass composition analysis. A higher R_P means

that there was less cellulose loss during pretreatment. Figure 8.7 shows that cellulose recovery rate after pretreatment was higher for big bluestem particles produced with a larger sieve size.

Figure 8.6 Effects of sieve size on biomass weight loss in pretreatment

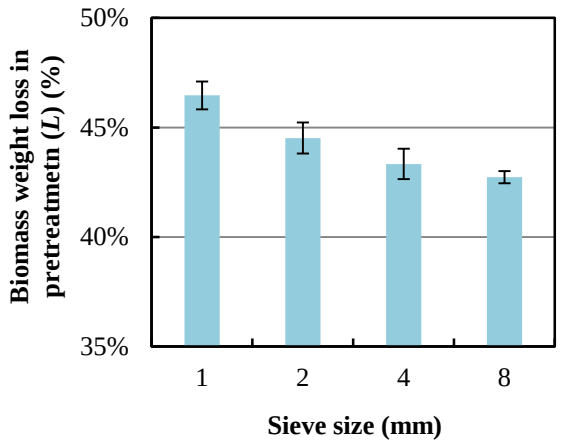
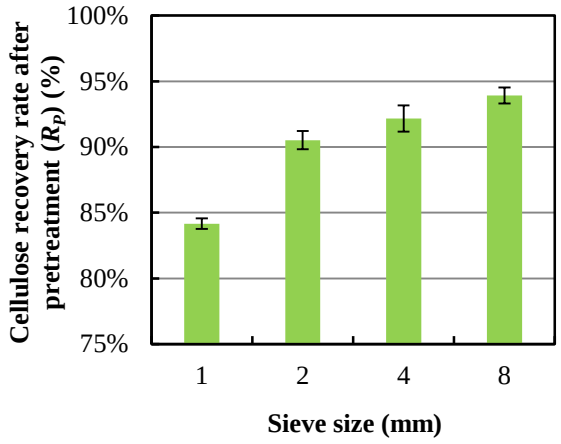


Figure 8.7 Effects of sieve size on cellulose recovery rate after pretreatment



A study conducted by Ballesteros et al. [33] showed the same trend. They used softwood chips of three size levels (2-5, 5-8, 8-12 mm) treated with steam-explosion pretreatment. They observed that chip size had a significant influence on cellulose recovery rate after pretreatment. As chip size increased, cellulose recovery rate after pretreatment increased.

8.4.3 Effects of sieve size on enzymatic hydrolysis efficiency

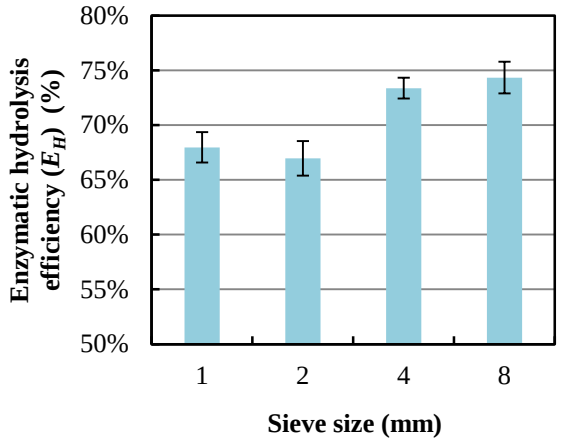
Enzymatic hydrolysis efficiency (E_H) is expressed in terms of the percentage of cellulose converted to glucose by enzymatic hydrolysis, and can be calculated using the following equation:

$$E_H\ (\%) = \frac{c \times V}{1.11 \times W_H \times C_{AP}} \times 100\% \quad (5)$$

Where c (g/L) is the concentration of glucose in the flask slurry after 48 h hydrolysis, V (L) is the total volume of the slurry, W_H (g) is the dry weight of the biomass loaded into the flask, and C_{AP} (%) is the cellulose content in the biomass before hydrolysis (after pretreatment). Factor 1.11 is the cellulose-to-glucose conversion factor and reflects the weight gain in converting cellulose to glucose in hydrolysis [34].

As shown in Figure 8.8, big bluestem particles produced with 4 or 8 mm sieve sizes had higher enzymatic hydrolysis efficiency than the particles produced with 1 or 2 mm sieve sizes. The difference in enzymatic hydrolysis efficiency between particles produced with 4 and 8 mm or between those produced with 1 and 2 mm sieve sizes was insignificant. One possible explanation given by Sarkar et al. [16] is that fine biomass particles may cause the generation of clumps during enzymatic hydrolysis. Theerarattananoon et al. [35] reported similar results. Three types of biomass materials (big bluestem, corn stover, and wheat straw) were processed on a hammer mill with sieve sizes of 3.2 and 6.5 mm. Particles produced with sieve size of 6.5 mm had higher enzymatic hydrolysis efficiency than those produced with sieve size of 3.2 mm. In their experiments, there was a pelleting process (the agglomeration of small particles into firm, uniformly shaped granules by means of mechanical processes) between size reduction and pretreatment.

Figure 8.8 Effects of sieve size on enzymatic hydrolysis efficiency



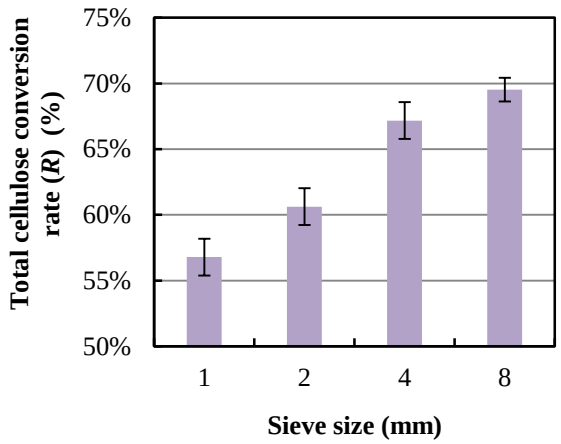
8.4.4 Effects of sieve size on total cellulose conversion rate and total sugar yield

Total cellulose conversion rate (R_T) is used to evaluate the overall efficiency of pretreatment and enzymatic hydrolysis in converting cellulose to glucose. It is the percentage of cellulose in unpretreated biomass that is converted to glucose after enzymatic hydrolysis. It is the product of enzymatic hydrolysis efficiency (E_H) and cellulose recovery rate after pretreatment (R_P):

$$R_T\ (\%) = \frac{E_H \times R_P}{100\%} \tag{6}$$

As shown in Figure 8.9, big bluestem particles produced with larger sieve sizes achieved higher total cellulose conversion rate. Nearly 70% of the cellulose in particles produced with the 8 mm sieve size was converted to glucose, which was about 20% higher than that produced with the 1 mm sieve size.

Figure 8.9 Effects of sieve size on total cellulose conversion rate



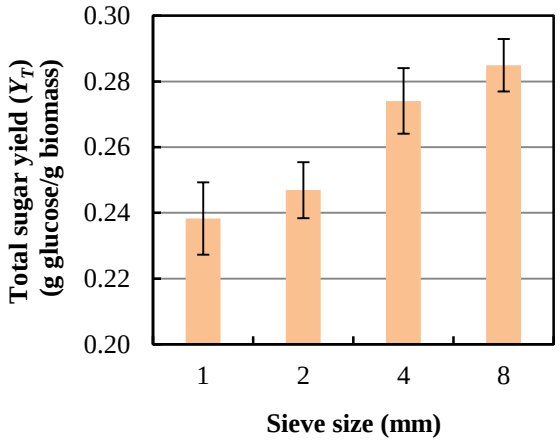
In this study, total sugar yield (Y_T) measures how much glucose a unit dry weight of biomass (before pretreatment) can yield through biochemical conversion. Its calculation is as follows:

$$Y_T \text{ (g glucose/g biomass)} = \frac{c \times V \times W_{AP}}{W_H \times W_{BP}} \quad (7)$$

Where c (g/L) is the concentration of glucose in the flask slurry after 48 h hydrolysis, V (L) is the total volume of the slurry, W_{AP} (g) is the dry weight of biomass after pretreatment, W_H (g) is the dry weight of the biomass loaded into the flask, and W_{BP} (g) is the dry weight of biomass before pretreatment.

Figure 8.10 shows total sugar yield results. As sieve size used in size reduction increased, total sugar yield of the produced particles increased. Big bluestem particles produced with 8 mm sieve size yielded 20% more sugar than those produced with 1 mm sieve size.

Figure 8.10 Effects of sieve size on total sugar yield



8.5 Conclusions

This paper presents the first effort of investigating effects of sieve size used in biomass size reduction throughout the biochemical conversion of big bluestem to fermentable sugar. Major conclusions are:

1. Energy consumption in biomass size reduction increased greatly as sieve size became smaller (from 8 to 1 mm).
2. Big bluestem particles produced with larger sieve sizes (4 and 8 mm) had higher cellulose recovery rate after pretreatment than those produced with smaller sieve sizes (1 and 2 mm).
3. Big bluestem particles produced with larger sieve sizes (4 and 8 mm) had higher enzymatic hydrolysis efficiency, higher total cellulose conversion rate, and higher total sugar yield than those produced with smaller sieve sizes (1 and 2 mm).

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Chapter 9 - Relationship between Biomass Particle Size and Enzymatic Hydrolysis Sugar Yield Using Two Sugar Yield Definitions

Paper title:

A consistency mapping for the effects on enzymatic hydrolysis sugar yield using two sugar yield definitions in cellulosic biofuel manufacturing

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Abstract

Two different sugar yield definitions (cellulose-based and biomass-based) were used in reported studies investigating the relationship between biomass particle size and enzymatic hydrolysis sugar yield. It is noticed that these reported relationships are not consistent if sugar yield is defined differently. The literature does not contain any reports on the effects of sugar yield definition on the relationship between biomass particle size and enzymatic hydrolysis sugar yield. This paper presents a consistency mapping to show under what conditions the relationships are consistent (or inconsistent) when these two definitions are used. The application of this mapping is illustrated via an experimental study with poplar wood biomass on the relationship between biomass particle size and enzymatic hydrolysis sugar yield using both sugar yield definitions. The application of this mapping is also illustrated via data reported in the literature. Not limited to particle size, this mapping is applicable to investigations of the relationships between a variety of parameters (biomass type, pretreatment condition, etc.) and enzymatic hydrolysis sugar yield.

Keywords: Biofuel; cellulosic biomass; enzymatic hydrolysis; particle size; sugar yield

9.1 Introduction

Biofuels have been recognized as promising alternatives to petroleum-based liquid transportation fuels [1-3]. Cellulosic biomass can be converted into biofuels through biochemical pathway. Before biochemical conversion, cellulosic biomass has to go through a size reduction step to make it easier to handle and to make the biofuel production process more efficient [4]. Cellulosic biomass biochemical conversion consists of two major processes. First, biomass particles produced by size reduction are depolymerized to fermentable sugars through

pretreatment and enzymatic hydrolysis. Second, the fermentable sugars are converted into biofuel (ethanol) through fermentation [5].

Cellulosic biomass ethanol yield is highly dependent on the cellulose conversion rate during enzymatic hydrolysis [6]. Extensive research has been conducted to enhance the digestibility of cellulosic biomass in order to increase the enzymatic hydrolysis sugar yield [7,8]. Cellulosic biomass consists of mainly three different polymers, namely cellulose, hemicelluloses, and lignin. Cellulose is trapped in the shield formed by lignin and hemicelluloses [7,9,10].

The size of particles produced after biomass size reduction (referred as particle size in the following content) is an important input parameter affecting enzymatic hydrolysis sugar yield [11,12]. The literature contains many studies investigating the relationship between particle size and sugar yield. However, the reported relationships are inconsistent. As shown in Table 9.1, many publications reported that smaller biomass particles had higher enzymatic hydrolysis sugar yield than larger biomass particles. However, there are also publications that did not support such a relationship.

Table 9.1 Reported relationship between particle size and sugar yield.

Biomass material	Smaller particles produced higher sugar yield	Sugar yield definition	Reference
Douglas fir	Yes	Cellulose based	[13]
Douglas fir	Yes	Cellulose based	[14]
Corn stover	Yes	Cellulose based	[15]
Red oak	Yes	Biomass based	[16]
Spruce wood	Yes	Cellulose based	[17]
Lodgepole pine	Yes	Cellulose based	[18]
Switchgrass	No	Biomass based	[19]
Corn stover	No	Biomass based	[20]
Wheat straw	No	Biomass based	[21]

It was found that two different sugar yield definitions were used in the related publications. One definition is cellulose-based sugar yield, and calculated as the percentage of cellulose in biomass converted to fermentable sugar (glucose) by enzymatic hydrolysis. The other definition is biomass-based sugar yield, and calculated as the ratio of the glucose produced by enzymatic hydrolysis to the initial dry weight of the biomass. In this paper, these two definitions are so called for the purpose of easy comparison and discussion. These concepts might be called differently elsewhere.

It is interesting to note that, when cellulose-based sugar yield definition was used, all (except one) publications reported the relationship that smaller biomass particles had a higher sugar yield. In the three publications that did not support such a relationship [19-21], biomass-based sugar yield definition was used. Furthermore, the literature does not contain any reports on the effects of sugar yield definition on the relationship between biomass particle size and enzymatic hydrolysis sugar yield.

This paper presents a consistency mapping to show under what conditions the relationships are consistent (or inconsistent) when these two definitions are used. The application of this mapping is then illustrated via an experimental study with poplar wood biomass the relationship between biomass particle size and enzymatic hydrolysis sugar yield using both sugar yield definitions. The application of this mapping is also illustrated via data reported in the literature.

9.2 Development of the consistency mapping

9.2.1 Two sugar yield definitions

9.2.1.1 Cellulose-based sugar yield

Cellulose-based sugar yield was used to evaluate the efficiency of enzymatic hydrolysis. It is expressed in terms of the percentage of cellulose converted to fermentable sugar (glucose), and calculated by the following equation:

$$\text{Cellulose-based sugar yield}(\%) = \frac{c \times V}{1.11 \times m \times C_b} \times 100\% \quad (1)$$

where c is the concentration (g/L) of glucose in the hydrolysis slurry, V (L) is the total volume of the slurry, m (g) is the dry weight of the biomass loaded into the hydrolysis flask, and C_b (%) is the cellulose content in the biomass before hydrolysis. The factor 1.11 is the cellulose-to-glucose conversion factor, which reflects the weight gained in converting cellulose to glucose in hydrolysis.

9.2.1.2 Biomass-based sugar yield

Biomass-based sugar yield evaluates the glucose yield (g) per unit dry weight of biomass loaded into the hydrolysis process. It is calculated by the following equation:

$$\text{Biomass-based sugar yield}(\text{g glucose/g dry biomass}) = \frac{c \times V}{m} \quad (2)$$

where c is the concentration (g/L) of glucose in the hydrolysis slurry, V (L) is the total volume of the slurry, and m (g) is the dry weight of the biomass loaded into the hydrolysis process.

9.2.2 Derivation of the consistency mapping

The formulae for sugar yield calculation using the abovementioned two definitions involve two variables. One is glucose concentration c (g/L) in two samples under comparison after hydrolysis (c_1 and c_2), and the other is cellulose content C_b (%) in the two samples before

hydrolysis (C_{b1} and C_{b2}). To simplify the derivation, the sample with a higher glucose concentration is subscripted as "1".

Whether the relationships between particle size and sugar yield using the two sugar yield definitions are consistent or not is determined by the relative values of x and y. Where, "x" is the difference in glucose concentration, and calculated as $x=[(c_1-c_2)/c_2]\times 100\%, x>0$, and "$y$" is the difference in cellulose content, and calculated as $y=[(C_{b1}-C_{b2})/C_{b2}]\times 100\%, y>-1$. The statement that the relationships using these two definitions are consistent is equivalent to the following inequality:

$$\left(\frac{c_1\times V_1}{1.11\times m_1\times C_{b1}}\times 100\%-\frac{c_2\times V_2}{1.11\times m_2\times C_{b2}}\times 100\%\right)\cdot\left(\frac{c_1\times V_1}{m_1}-\frac{c_2\times V_2}{m_2}\right)>0 \quad (3)$$

where, $m_1=m_2=m,$ and $V_1=V_2=V.$ Taking $c_1=(1+x)\cdot c_2,$ and $C_{b1}=(1+y)\cdot C_{b2}$ into the inequality gives

$$\left(\frac{(1+x)\cdot c_2\times V}{1.11\times m\cdot(1+y)\cdot C_{b2}}\times 100\%-\frac{c_2\times V}{1.11\times m\times C_{b2}}\times 100\%\right)\cdot\left(\frac{(1+x)\cdot c_2\times V}{m}-\frac{c_2\times V}{m}\right)>0 \quad (4)$$

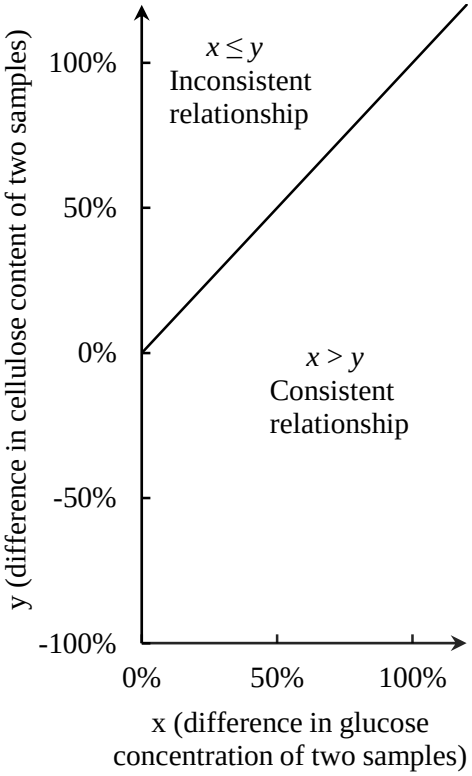
Inequality (6) is reduced to

$$\frac{V^2\times {c_2}^2}{1.11\times m^2\times C_{b2}}\cdot\frac{x\cdot(x-y)}{(1+y)}>0 \quad (5)$$

Because $x > 0$, $1 + y > 0$, and others are positive constants, the solution to the inequality is $x > y$. To summarize, if $x > y$, the relationships are consistent; on the other hand, if $x \leq y$, the relationships are inconsistent. The above derivation is based on the scenario that $x > 0$. In the special scenario that $x = 0$, it is easy to find out that if $y = 0$, the relationships are consistent; if $y \neq 0$, the relationships are inconsistent.

A consistency mapping (x > 0), as shown in Figure 9.1, is developed to show under what conditions the relationships between particle size and sugar yield using these two sugar yield definitions are consistent (or inconsistent).

Figure 9.1 Consistency mapping.



9.3 Experimental study to illustrate the application of the mapping

9.3.1 Material and methods

9.3.1.1 Material

Poplar wood chips were purchased from Petco Animal Supplies, Inc. (Manhattan, KS, USA). The moisture content of the wood chips was 7.1%. The wood chips were placed in sealed

Ziploc® bags and stored at room temperature before size reduction by mills. Table 9.2 lists the chemical composition of the wood chips.

Table 9.2 Chemical composition of poplar wood chips.

Component	Percentage on dry weight basis
Cellulose	41.1 ± 0.4
Hemicellulose	22.9 ± 0.3
Lignin	24.0 ± 0.7
Ash	2.9 ± 0.1

9.3.1.2 Biomass size reduction

Two types of mills were used for size reduction of poplar wood chips: a knife mill (Model SM 2000, Retsch, GmbH, Haan, Germany) and a hammer mill (Model No. 5, Meadows Mills, Inc., North Wilkesboro, NC, USA). Sieves of two sieve sizes (with openings of 1 and 4 mm on the sieves) were used in both mills to produce poplar biomass particles with two levels (− and +) of particle size. Wood chips remained in the milling chamber until they were small enough to pass through the openings on the sieve. After milling, particles were collected and kept in sealed Ziploc® bags and stored in a refrigerator at 4 ℃ until further processing. Table 9.3 lists the experimental conditions in biomass size reduction.

Table 9.3 Particle size levels and size reduction conditions.

Condition No.	Particle size level	Mill type	Sieve size (mm)
1	−	Knife	1
2	+	Knife	4
3	−	Hammer	1
4	+	Hammer	4

9.3.1.3 Biomass extraction

The purpose of biomass extraction is to remove extractives from wood particles produced by mills because these extractives could potentially interfere with subsequent analysis. The two-step extraction process was conducted by following National Renewable Energy Laboratory procedure (NREL/TP-510-42619) [22]. In the first step, distilled water was used (for 24 h) to remove water-soluble extractives. In the second step, ethyl alcohol (190 proof) was used (for 24 h) to remove alcohol-soluble extractives. After biomass extraction, wood particles were dried in an oven at 40 ℃ for 24 h and stored in individual self-seal sample bags.

9.3.1.4 Biomass pretreatment

Dilute sulfuric acid pretreatment was employed in this study. Ten grams of extractive-free biomass particles and 200 mL of 2% (w/v) sulfuric acid were loaded in the 600-mL vessel of a Parr pressure reactor (Model 4760A, Parr Instrument Co., Moline, IL, USA), and treated at 140 ℃ for 30 min.

The pretreated biomass particles were washed with hot distilled water using a centrifugal (Model Marathon 2100, Thermo International Equipment Co., Needham, MA, USA) to remove dissolved sugars, acid residues, and inhibitors (substances that would decrease enzymes' ability to depolymerize cellulose to glucose [23]) formed during pretreatment. The rotation speed of the centrifugal was 4,000 rpm. Each biomass sample was washed three times, and each time lasted for 15 min. The solid biomass after centrifugal was carefully collected. For each test condition, a small portion of the collected solid biomass was used for chemical composition analysis, and the rest was used for subsequent enzymatic hydrolysis.

9.3.1.5 Enzymatic hydrolysis

Enzymatic hydrolysis was carried out in eight 125-mL flasks in a water bath shaker (Model C76, New Brunswick Scientific, Edison, NJ, USA) with agitation speed of 110 rpm at 50°C for 48 h. There were two flasks containing biomass particles collected under each of the four size reduction conditions. Each flask contained 50 mL of hydrolysis slurry. The slurry consisted of 5% (w/v) biomass on dry weight base, sodium acetate buffer (50 mM, pH = 4.8), and 0.02% (w/v) sodium azide to prevent microbial growth during hydrolysis. Accellerase 1500^{TM} enzyme complex (Danisco USA, Inc., Rochester, NY, USA) was used. The enzyme loaded was 1 mL for each gram of dry biomass.

After hydrolysis for 48 h, 0.1 mL of the hydrolysis slurry was withdrawn from each flask, and mixed with 0.9 mL of double distilled water in a 1.5-mL micro-centrifuge tube. The caped tubes were placed into boiling water for 15 min to deactivate the enzyme. Afterwards, the tubes were centrifuged at 10,000 rpm for 15 min to separate supernatant liquid from solid biomass residues using a micro-centrifuge (Model RS-102, Revolutionary Science, Shafer, MN, USA). Supernatant liquid from each tube was filtered through a 0.2-μm hydrophilic PTFE syringe filter (EMD Millipore, Billerica, MA, USA). Filtered supernatant liquid was kept in 1.5-mL autosampler vials at 4 ℃ in a refrigerator before sugar concentration measurement.

9.3.2 Measurement procedures

9.3.2.1 Moisture content and dry weight

Biomass moisture content was measured by following National Renewable Energy Laboratory procedure (NREL/TP-510-42621) [24]. About 2.5 g of biomass was placed in an aluminum weighing dish and dried in an oven at 105 ℃ for 24 h. The loss in weight of the biomass after oven drying was recorded. Moisture content was calculated as follows:

$$\text{Moisture content } (MC)\,(\%) = \frac{\text{Loss in weight}}{\text{Weight of biomass before drying}} \times 100\% \quad (6)$$

Knowing the moisture content, dry weight could be calculated as follows:

$$\text{Dry weight (g)} = (1 - \text{MC}) \times \text{weight of biomass with moisture} \quad (7)$$

Biomass weight reported in this study is dry weight.

9.3.2.2 Chemical composition

The chemical composition of biomass (wood chips before size reduction or biomass particles collected after pretreatment) was measured by following the National Renewable Energy Laboratory procedure (NREL/TP-510-42618) [25]. Two duplications for each test condition were employed. Structural carbohydrates in biomass were reported as the percentages of cellulose and hemicellulose. Lignin, the major non-carbohydrate component, was reported as the percentage of the sum of acid-insoluble and acid-soluble lignin. The percentage of ash content was also reported.

9.3.2.3 Sugar concentration

Sugar concentration was measured using high performance liquid chromatography (HPLC). The HPLC (Shimadzu, Kyoto, Japan) system was equipped with an RPM-monosaccharide column (300 × 7.8 mm; Phenomenex, Torrence, CA, USA) and a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan). The mobile phase was 0.6 mL/min of degassed double-distilled water, and the column oven temperature was 80 ℃.

9.3.3 Experimental results

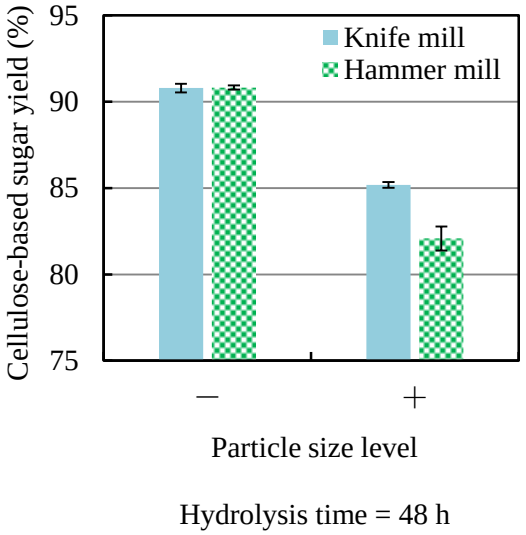
9.3.3.1 Cellulose-based sugar yield

In order to calculate cellulose-based sugar yield, the content of cellulose in biomass samples before hydrolysis were acquired through chemical composition analysis and are listed in Table 9.4 together with other chemical components. It can be seen that cellulose contents for the two particle size levels are approximately the same.

Table 9.4 Chemical composition (percentage on dry weight basis) for biomass particles before hydrolysis.

Condition No.	Particle size level	Mill type	Cellulose	Hemicellulose	Lignin	Ash
1	−	Knife	62.9 ± 1.1	4.2 ± 0.1	30.7 ± 0.2	1.6 ± 0.1
2	+	Knife	62.8 ± 0.1	4.5 ± 0.1	31.0 ± 0.2	1.6 ± 0.2
3	−	Hammer	64.1 ± 0.1	4.6 ± 0.1	29.3 ± 0.1	1.6 ± 0.1
4	+	Hammer	63.2 ± 0.7	4.4 ± 0.1	31.9 ± 0.7	1.3 ± 0.1

Figure 9.2 Relationship between particle size and cellulose-based sugar yield in this study.



Hydrolysis time = 48 h

The relationship between particle size and cellulose-based sugar yield is shown in Figure 9.2. Smaller biomass particles had a higher sugar yield than larger particles, for both knife milling and hammer milling methods. This can be interpreted as that cellulose in smaller biomass particles were more efficiently hydrolyzed into glucose by enzymes in hydrolysis.

Mooney et al. [13] hydrolyzed Douglas fir woody biomass of two particle size levels. Their results showed that cellulose-based sugar yield of smaller particles was 24% higher than that of larger particles after 72-h hydrolysis (Figure 9.3). The same trend was also reported by Zhu et al. [17] using a shorter hydrolysis time (12 h) to convert spruce woody biomass of four particle size levels (Figure 9.4). This trend was also reported for herbaceous biomass. As an example, Zeng et al. [15] milled corn stover and separated milled particles into two particle size levels. They found that when using cellulose-based sugar yield definition, smaller particles produced higher yield (Figure 9.5).

Figure 9.3 Relationship between particle size and cellulose-based sugar yield reported by Mooney et al. [13].

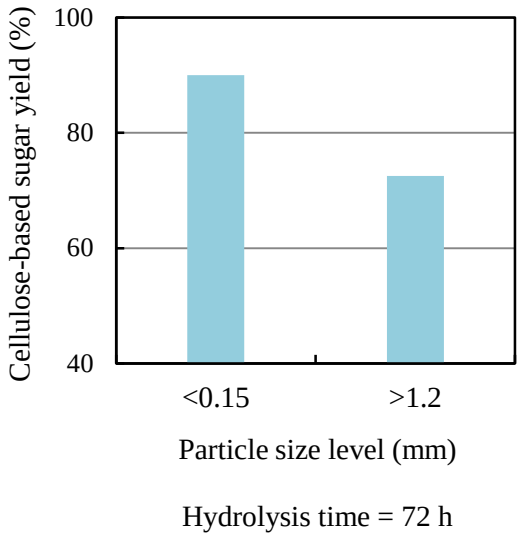


Figure 9.4 Relationship between particle size and cellulose-based sugar yield reported by Zhu et al. [17].

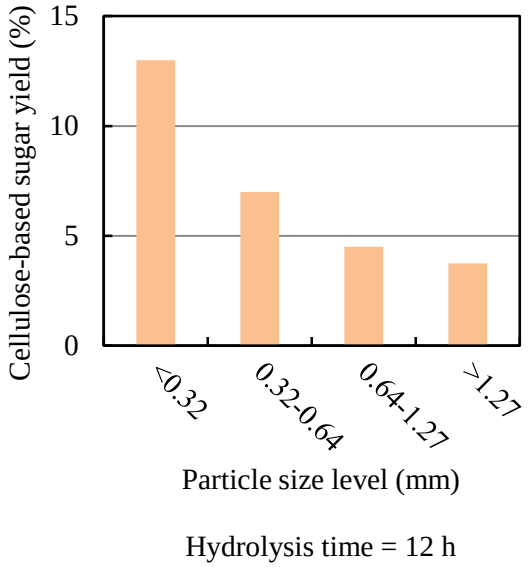
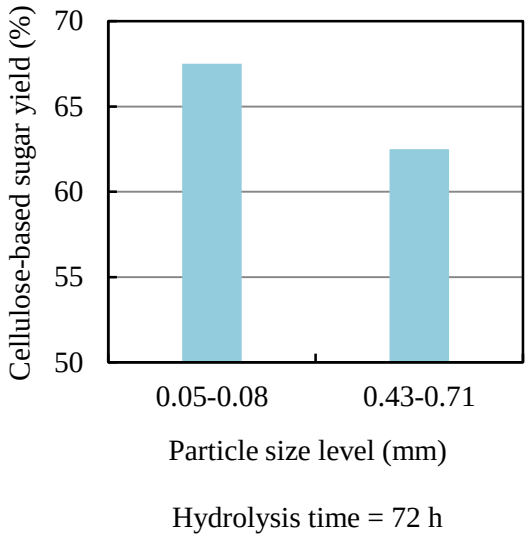


Figure 9.5 Relationship between particle size and cellulose-based sugar yield reported by Zeng et al. [15].

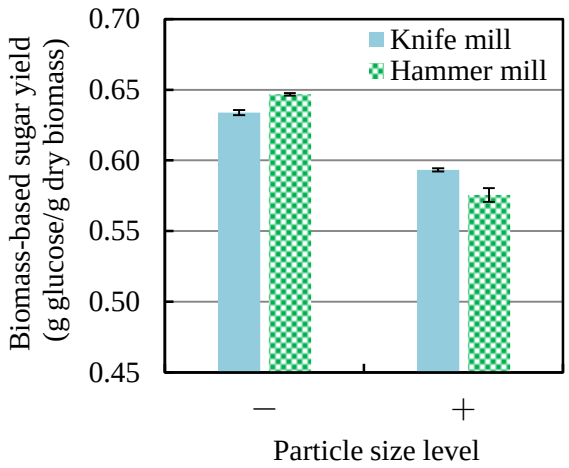


9.3.3.2 Biomass-based sugar yield

Figure 9.6 shows the relationship between particle size and biomass-based sugar yield. For both knife milling and hammer milling methods, smaller biomass particles have a higher sugar yield than larger biomass particles. Dasari and Benson [16] reported a similar trend for red-oak (Figure 9.7). Smaller particles had a higher sugar yield than larger particles.

Not all related publications support this relationship. Zhang et al. [21] found that larger wheat straw particles milled using a 2-mm sieve had higher cellulose-based sugar yield than smaller particles milled using a 1-mm sieve (Figure 9.8). It is noted that, in this work, before pretreatment, a pelleting process was employed to agglomerate milled biomass particles into pellets. Kaar and Holtzapple [20] found that cellulose-based sugar yield of smaller corn stover particles was lower than that of larger particles (Figure 9.9). Chang et al. [19] found that, though switchgrass particles with particle size of 0.40-0.84 mm had 18% higher cellulose-based sugar yield than particles with particle size of 0.84-2 mm, reducing particle size below 0.4 mm did not increase sugar yield (Figure 9.10).

Figure 9.6 Relationship between particle size and biomass-based sugar yield in this study.



Hydrolysis time = 48 h

Figure 9.7 Relationship between particle size and biomass-based sugar yield reported by Dasari and Benson [16].

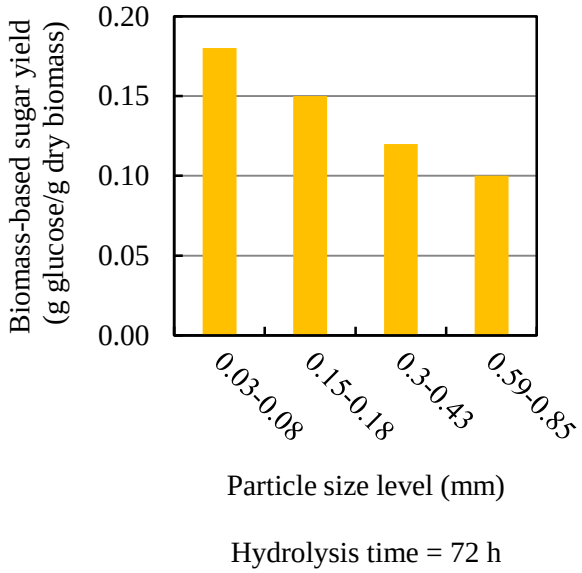


Figure 9.8 Relationship between particle size and biomass-based sugar yield reported by Zhang et al. [21].

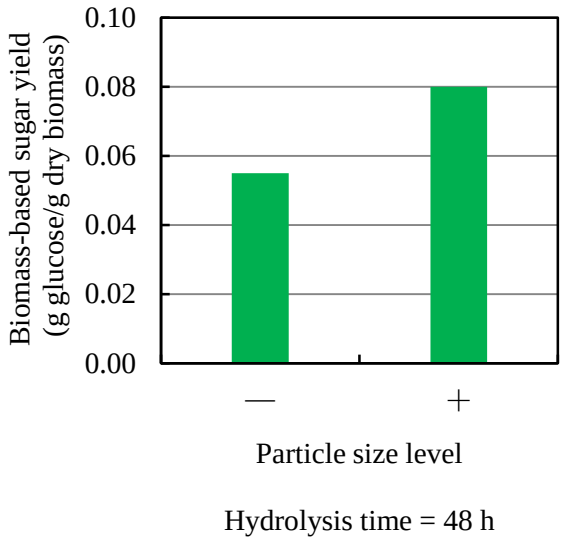


Figure 9.9 Relationship between particle size and biomass-based sugar yield reported by Kaar and Holtzapple [20].

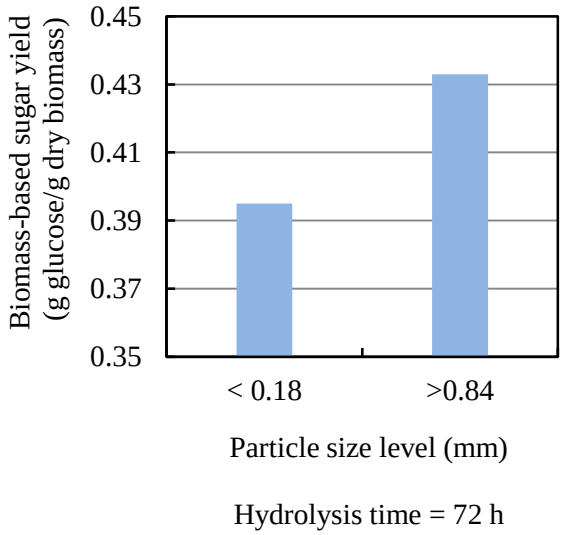
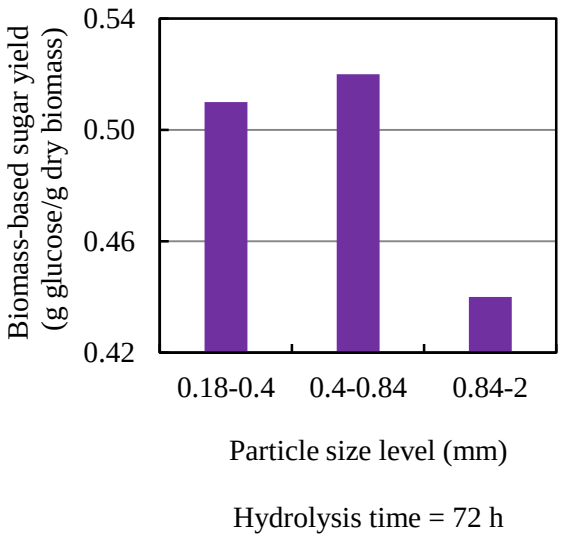


Figure 9.10 Relationship between particle size and biomass-based sugar yield reported by Chang et al. [19].



9.4 Application of the consistency mapping

9.4.1 Illustration using data from the experimental study

In the present experimental study, the required values to apply the consistency mapping are calculated as x = 6.84% and y = 0.24% for the small and large particles produced by knife milling; x = 12.37% and y = 1.40% for the small and large particles produced by hammer milling (values were calculated using the means of the two duplicated tests). Since $x > y$, the relationships between particle size and sugar yield using the two sugar yield definitions are consistent.

9.4.2 Illustration using data from study reported in the literature

Applications of the consistency mapping can also be illustrated using the data published in the literature. A study conducted by Ballesteros et al. [26] was employed as an example. The authors studied the sugar yield of softwood biomass of two levels of particle size. The reported sugar yield was cellulose-based. From the data listed in Table 9.5, the values needed to apply the consistency mapping are calculated as x = 6.16% and y = 12.79%. Since x < y, the relationships between particle size and sugar yield using two sugar yield definitions are inconsistent.

Table 9.5 Data reported by Ballesteros et al. [26].

	Small particle size	**Large particle size**
Particle size level (mm)	2-5	5-8
Cellulose-based sugar yield (%)	36	34
Biomass-based sugar yield (g glucose/g dry biomass)[a]	0.14	0.15
Sugar concentration (g/L)[a]	2.76	2.93
Cellulose content (%)	34.4	38.8

[a]Data obtained through calculation based on data provided by Ballesteros et al. [26]

9.5 Conclusions

This paper develops a consistency mapping for the effects on enzymatic hydrolysis sugar yield using two sugar yield definitions. The application of this mapping is illustrated via an experimental study with poplar wood biomass on the relationship between biomass particle size and enzymatic hydrolysis sugar yield. Under the experimental conditions in this study, smaller particles had a higher sugar yield. This relationship remained consistent using both sugar yield definitions. This mapping is not limited to investigations on the relationship between particle size and sugar yield. It is applicable to studying relationships between a variety of parameters (such as biomass type, pretreatment condition, etc.) and sugar yield.

Acknowledgements

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Chapter 10 - Relationship between Biomass Particle Size and Total Sugar Yield of Dilute Acid Pretreatment and Enzymatic Hydrolysis

Paper title:

Diluted acid pretreatment and enzymatic hydrolysis of woody biomass for biofuel manufacturing: effects of particle size on sugar conversion

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Abstract

Biofuels derived from cellulosic biomass offer a promising alternative to petroleum-based liquid transportation fuels. Cellulosic biomass can be converted into biofuels through biochemical pathway. This pathway consists of two major conversions: sugar conversion and ethanol conversion. Sugar yield in sugar conversion is critical to the cost effectiveness of biofuel manufacturing, because it is approximately proportional to the ethanol biofuel yield. Cellulosic biomass sugar conversion consists of pretreatment and enzymatic hydrolysis. Biomass particle size is an important factor affecting sugar yield. The literature contains many studies investigating the relationship between particle size and sugar yield. Many studies focused only on the sugar yield in enzymatic hydrolysis, and failed to take into account the biomass weight loss during pretreatment. This weight loss results in a loss of the amount of potential sugar (cellulose), which continues going into enzymatic hydrolysis. Without considering this loss, cellulosic biomass with a higher enzymatic hydrolysis sugar yield may end up with a lower total sugar yield through sugar conversion. The present study aims to address this issue by investigating the effects of biomass particle size using total sugar yield, a parameter considering both the biomass weight loss in pretreatment and the sugar yield in enzymatic hydrolysis.

Keywords: Biofuel, cellulosic biomass, enzymatic hydrolysis, particle size, pretreatment, sugar yield

10.1 Introduction

There is a growing need to find alternatives to petroleum, a depleting non-renewable resource for liquid transportation fuels [1]. Biofuels produced from cellulosic biomass (forest and agricultural residues and dedicated energy crops) have been recognized as promising alternatives to petroleum-based transportation liquid fuels [2-5]. Using cellulosic biomass as the

feedstock is advantageous because of its low cost, abundance, and sustainability [6]. The United States has the resource to produce over 1 billion dry tons of biomass. This amount of biomass is sufficient to produce 90 billion gallons of liquid fuels that can replace about 30% of the nation's current annual consumption of petroleum-based transportation fuels [3,4]. Among which, more than 80% is cellulosic biomass including about 320 million dry tons of woody biomass [3,4].

Cellulosic biomass consists of mainly three different polymers, namely cellulose, hemicelluloses, and lignin. Cellulose is trapped in the shield formed by highly associated lignin and hemicellulose [7-9]. This shield largely protects cellulose from enzymatic hydrolysis degradation [10-12]. Cellulosic biomass can be converted into biofuels through biochemical pathway. This pathway consists of two major conversions. The first one is sugar conversion, which converts cellulose to fermentable sugar (mainly glucose) by pretreatment and hydrolysis. The second one is ethanol conversion, which converts fermentable sugar to ethanol biofuel by fermentation and ethanol recovery [13-16].

The link between these two conversions is the sugar yield after sugar conversion. This yield is approximately propositional to the biofuel yield through the entire pathway [17]. Extensive research has been conducted to enhance the digestibility of cellulosic biomass in order to increase the sugar yield through sugar conversion [7,18].

Effective sugar conversion greatly relies on the structural properties of the biomass feedstock. Particle size is one of the most important properties [19-21]. The literature contains many studies investigating the effects of particle size on sugar yield. Many studies focused only on the sugar yield in enzymatic hydrolysis [22-28]. However, those reported studies failed to take into account the biomass weight loss during pretreatment. This weight loss results in a loss of the amount of potential sugar (cellulose), which continues going into the following enzymatic

hydrolysis. Without considering this loss, cellulosic biomass with a higher sugar yield in enzymatic hydrolysis may end up with a lower total sugar yield through sugar conversion. The present study aims to address this issue by investigating the effects of biomass particle size using total sugar yield. This parameter considers both the biomass weight loss in pretreatment and sugar yield in enzymatic hydrolysis. Experimental results of enzymatic hydrolysis sugar yield, total sugar yield, biomass weight loss in pretreatment, and cellulose recovery rate were reported.

10.2 Experimental conditions and procedures

10.2.1 Biomass material and size reduction

Poplar wood chips were purchased from Petco Animal Supplies, Inc. (Manhattan, KS, USA). The moisture content of the wood chips was 7.1%, and the size of the wood chips was approximately 5-12 mm. The wood chips were placed in sealed Ziploc® bags and stored at room temperature before size reduction. Size reduction of woody biomass is necessary because large size woody biomass cannot be converted to biofuels efficiently by current conversion technologies [29-31].

Figure 10.1 Retsch Model SM 2000 knife mill.

The wood chips were milled into particles using a knife mill (Model SM 2000, Retsch, GmbH, Haan, Germany), as shown in Figure 10.1. It was equipped with a three-phase 1.5-kW electric motor. The rotation speed of the motor was 1,720 rpm. Figure 10.2 shows the milling chamber of the knife mill. Three knives (95 mm long and 35 mm wide) were mounted on the rotor inside the milling chamber. Four cutting bars were mounted on the inside wall of the milling chamber. The gap between a knife and a cutting bar was 3 mm. A sieve (145 mm long and 98 mm wide) was mounted at the bottom of the milling chamber. Sieves of two sieve sizes (1 and 4 mm), as shown in Figure 10.3, were used to produce small and large particles. Wood chips remained in the milling chamber until they were small enough to pass through the openings on the sieve.

Figure 10.2 Milling chamber of knife mill.

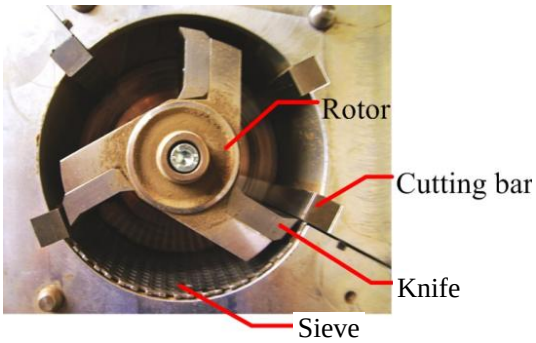
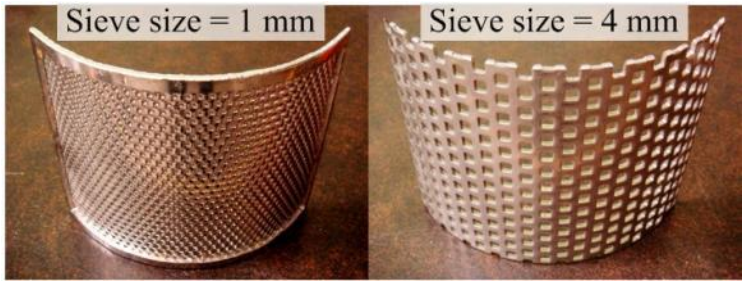


Figure 10.3 Sieves used in knife mill.



10.2.2 Sugar conversion

Dilute sulfuric acid pretreatment method was applied as the first step of sugar conversion. Ten grams (dry weight) of poplar wood particles and 200 mL of 2% (w/v) sulfuric acid were loaded in the 600 mL vessel of a Parr pressure reactor (Model 4760A, Parr Instrument Co., Moline, IL, USA). Pretreatment time was 30 min, and pretreatment temperature was 140 ℃.

Poplar wood particles after pretreatment were washed with 70-80 ℃ distilled water using a centrifuge (Model Marathon 2100, Thermo International Equipment Co., Needham, MA, USA) to conduct solid-liquid separation. The solid biomass after centrifugation was carefully collected using a stainless steel micro spatula. The dry weight of the collected solid biomass was measured, then a small portion of the solid biomass was used for biomass composition analysis, and the rest was used for subsequent enzymatic hydrolysis. The liquid part was removed, which included dissolved sugars, acid residues, and inhibitors (substances that would decrease enzymes' ability to depolymerize cellulose to glucose [32]) formed during pretreatment.

Enzymatic hydrolysis was carried out in four 125-mL flasks in a water bath shaker (Model C76, New Brunswick Scientific, Edison, NJ, USA) with agitation speed of 110 rpm at 50 ℃ for 48 h. There were two flasks containing either large or small particles. Each flask contained 50 mL of hydrolysis slurry. The slurry consisted of 5% (w/v) biomass on dry weight base, sodium acetate buffer (50 mM, pH = 4.8), and 0.02% (w/v) sodium azide to prevent microbial growth during hydrolysis. Accellerase 1500^{TM} enzyme complex (Danisco USA, Inc., Rochester, NY, USA) was used. The enzyme loaded was 1 mL for each gram of dry biomass.

10.3 Measurement procedures

10.3.1 Moisture content and biomass dry weight

Biomass moisture content (*MC*) was measured by following the National Renewable Energy Laboratory procedure (NREL/TP-510-42621) [33]. About 2.5 g of biomass was placed in an aluminum weighing dish and dried in an oven at 105 ℃ for 24 h. The loss in weight of the biomass after oven drying was recorded. Moisture content was calculated as follows:

$$\text{Moisture content } (MC)\,(\%) = \frac{\text{Loss in weight}}{\text{Weight of biomass before drying}} \times 100\% \quad (1)$$

Knowing the moisture content, biomass dry weight could be calculated as follows:

$$\text{Dry weight (g)} = (1 - MC) \times \text{weight of biomass with moisture} \quad (2)$$

Biomass weight reported in this study is dry weight.

10.3.2 Biomass composition

Biomass composition before and after pretreatment was measured by following the National Renewable Energy Laboratory procedure (NREL/TP-510-42618) [34]. Two duplications for small and large particles were employed. Biomass composition reported in this study is the weight of cellulose, hemicellulose, and lignin based on 10 g of biomass before pretreatment. Cellulose, hemicellulose, and lignin are the three major compositions in cellulosic biomass. Cellulose is the potential sugar, which can be converted to fermentable sugar (glucose) in enzymatic hydrolysis. Hemicellulose is also a sugar component; however, almost all of the hemicellulose will be degraded by dilute sulfuric acid pretreatment. Lignin contains no sugar and cannot be digested by enzymes [35].

10.3.3 Glucose concentration

Glucose concentration in the hydrolysis slurry was measured using a high performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan). HPLC can identify and quantify individual components of a liquid mixture. The HPLC system was equipped with an RPM-monosaccharide column (300 × 7.8 mm; Phenomenex, Torrence, CA, USA) and a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan).

10.4 Evaluation parameters

10.4.1 Biomass weight loss in pretreatment and cellulose recovery rate

After measuring the dry weight of biomass before and after pretreatment (W_{BP} and W_{AP}) by the procedure presented in Section 9.2.1, biomass weight loss in pretreatment (L) (%) was calculated as follows:

$$L\ (\%) = \frac{W_{BP} - W_{AP}}{W_{BP}} \times 100\% \tag{3}$$

Cellulose recovery rate (R) is used to look into the weight loss from the aspect that how much cellulose can be recovered after pretreatment. It is calculated as the ratio of the cellulose weight after pretreatment to the cellulose weight before pretreatment:

$$R\,(\%) = \frac{C_{AP}}{C_{BP}} \times 100\% \tag{4}$$

where C_{AP} (g) is the cellulose weight after pretreatment, and C_{BP} (g) is the cellulose weight before pretreatment. These two cellulose weights were measured by biomass composition analysis introduced in Section 9.3.2. A higher R means less loss potential sugar (cellulose) in pretreatment.

10.4.2 Enzymatic hydrolysis sugar yield

Enzymatic hydrolysis sugar yield (Y_H) evaluates the glucose yield (g) per unit dry weight of biomass in enzymatic hydrolysis. It is calculated by the following equation:

$$Y_H \text{ (g glucose/ g dry biomass)} = \frac{c \times V}{W_H} \tag{5}$$

where c is the concentration (g/L) of glucose in the slurry detected by HPLC, V (L) is the total volume of the hydrolysis slurry, W_H (g) is the dry weight of the biomass loaded into the hydrolysis flask.

10.4.3 Total sugar yield

Total sugar yield (Y_T) provides a straightforward interpretation about how much glucose a unit dry weight of biomass (before pretreatment) can yield through sugar conversion. It considers both the biomass weight loss (including potential sugar loss) in pretreatment and the sugar yield in enzymatic hydrolysis. It is calculated as follows:

$$Y_{BP} \text{ (g glucose/g biomass)} = \frac{c \times V \times W_{AP}}{W_H \times W_{BP}} \tag{6}$$

10.5 Results and discussion

Figure 10.4 shows the effects of biomass particle size on enzymatic hydrolysis sugar yield. Small particles had a higher enzymatic hydrolysis sugar yield (Y_H) than that of the large particles. This result is consistent with those reported in the literature; for example, Dasari and Berson [22] reported a similar effect for red oak woody biomass (Figure 10.5).

Result of the effects of biomass particle size on total sugar yield (Y_T) is shown in Figure 10.6. Although large particles had a lower enzymatic hydrolysis sugar yield (Y_H), the total sugar yield (Y_T) through sugar conversion was higher than that of small particles. Large particles

surpassing small particles in total sugar yield (Y_T) is mainly attributed to the less weight loss in pretreatment comparing with small particles as shown in Figure 10.7.

Figure 10.4 Effects of biomass particle size on enzymatic hydrolysis sugar yield (Y_H).

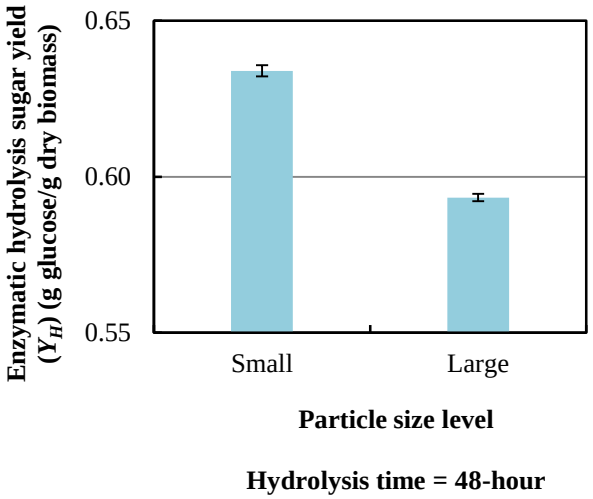


Figure 10.5 Effects of biomass particle size on enzymatic hydrolysis sugar yield (Y_H) reported by Dasari and Berson [22].

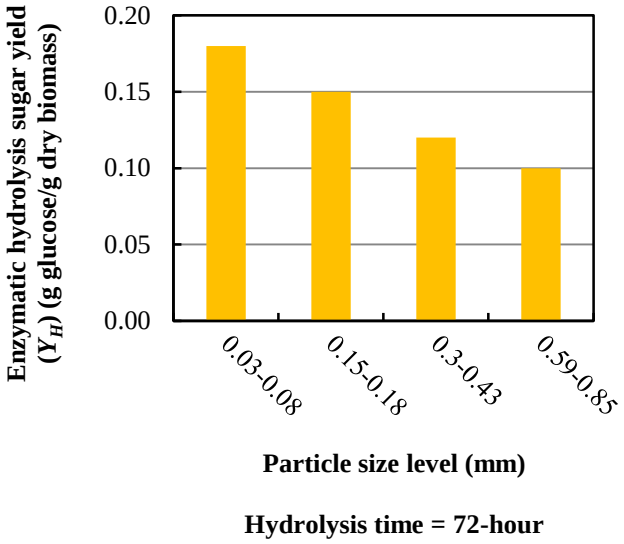


Figure 10.6 Effects of biomass particle size on total sugar yield (Y_T).

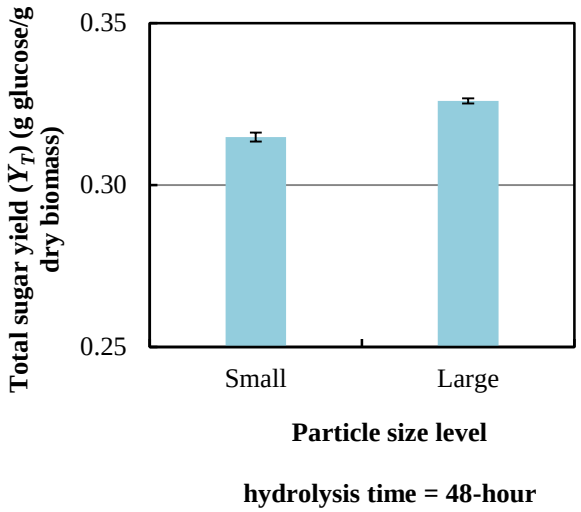


Figure 10.7 Effects of biomass particle size on biomass weight loss in pretreatment (L).

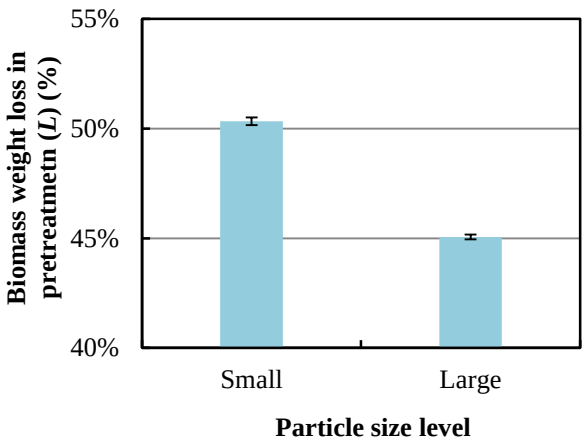


Table 10.1 compares biomass composition before and after pretreatment. The major weight loss is the degradation of hemicellulose. The primary objective of dilute sulfuric acid pretreatment method is to break down the shield formed by highly associated lignin and hemicellulose by decomposing hemicellulose to acid soluble products (i.e. xylose); so that cellulose can be released and becomes more accessible to enzymes in enzymatic hydrolysis [36]. However, a side effect is that a small amount of cellulose may be degraded to hydroxymethyl-

furfural (HMF) [36]. HMF is soluble in the pretreatment liquid but only the solid biomass after pretreatment goes into enzymatic hydrolysis. The degradation of cellulose to HMF results in a potential sugar (cellulose) loss and leads to decreasing total sugar yield [37].

Table 10.1 Biomass composition before and after pretreatment (based on 10 g of biomass before pretreatment).

Condition	Particle size level	Composition (g)		
		Cellulose	Hemicellulose	Lignin
Before pretreatment	Small and large	4.40	2.47	2.58
After pretreatment	Small	3.60	0.21	1.52
	Large	3.71	0.25	1.70

Figure 10.8 Effects of biomass particle size on cellulose recovery rate (*R*).

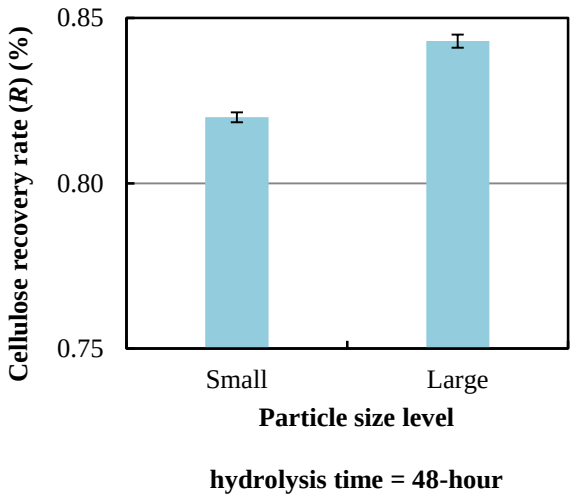


Figure 10.8 shows the effects of biomass particle size on cellulose recovery rate (*R*). This rate presents how much cellulose is recovered without degradation in pretreatment. As it is indicated, large particles have a higher cellulose recovery rate (*R*) than small particles. In another way, for the same dry weight of small and large particles before pretreatment, more potential sugar (cellulose) in large particles continued to enzymatic hydrolysis without degradation in

pretreatment. Enzymatic hydrolysis sugar yield is calculated based on the weight of biomass before hydrolysis without considering the previous weight loss in pretreatment. As it is discussed in the present work, this loss cannot be neglected and that is why large particle had a lower enzymatic hydrolysis sugar yield, but eventually a higher total sugar yield than small particles.

10.6 Concluding remarks

Under the experimental condition of the present study, main conclusions are drawn as follows:

1. Small particles have higher enzymatic hydrolysis sugar yield (Y_H), but lower total sugar yield (Y_T) than large particles through sugar conversion of dilute acid pretreatment and enzymatic hydrolysis.
2. Large particles have less biomass weight loss in dilute acid pretreatment (L).
3. Large particles have higher cellulose recovery rate in pretreatment (R)

This study raised an interesting fact that a higher enzymatic hydrolysis sugar yield from small particles could be reversed by a lower cellulose recovery rate in pretreatment, and resulted in a lower total sugar yield than large particles. In the future, expended size ranges and levels of particles will be used to conduct the similar test to investigate the big map of the relationship between biomass particle size and total sugar yield.

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Chapter 11 - Effects of Cutting Orientation in Woody Biomass Size Reduction on Enzymatic Hydrolysis Sugar Yield

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Effects of cutting orientation in woody biomass size reduction on enzymatic hydrolysis sugar yield

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Abstract

Size reduction is an indispensable process in biofuel manufacturing from woody biomass; however, the connection between size reduction and the subsequent enzymatic hydrolysis process was not well established. Little knowledge was available regarding which parameters in size reduction would influence enzymatic hydrolysis sugar yield, or what modification on biomass structural features during size reduction would be beneficial to achieve higher enzymatic hydrolysis sugar yield. The objective of this study was to obtain an understanding on how cutting orientation affects the enzymatic hydrolysis sugar yield of produced wood particles for biofuel manufacturing. This study employed a metal cutting (milling) machine to produce wood particles from three cutting orientations, and demonstrated that cutting orientation had significant effects on enzymatic hydrolysis sugar yield of wood particles. Particles produced by size reduction from the best cutting orientation (i.e. the orientation corresponding to the highest sugar yield) had large enzyme accessible area and low crystallinity. Particle size alone did not determine enzymatic hydrolysis sugar yield.

Keywords: Biofuel, crystallinity, enzymatic hydrolysis, orientation, particle size, size reduction, surface area

11.1 Introduction

Cellulosic biomass (woody, herbaceous, and generally inedible portions of plant matter) is an ideal source of manufacturing renewable liquid transportation fuels such as bioethanol. Producing bioethanol from cellulosic biomass can reduce greenhouse gas emissions, and does not compete with food or feed production [1-3]. Bioethanol is the most widely used biofuel, which has been blended to gasoline at ratio up to 85% [4]. Bioethanol can be readily used in current generation vehicles and distributed through the existing infrastructure without (or with

slight) modifications [5]. An investigation in 2005 jointly supported by the U.S. Department of Energy and Department of Agriculture shows that land resources in the U.S. are sufficient to sustainably produce over 1 billion dry tons cellulosic biomass (including about 320 million dry tons of woody biomass) annually to replace 30% or more of the nation's current consumption of liquid transportation fuels [1,2,6].

Woody biomass (e.g. hardwood, softwood, and woody shrub) has strong structure and high lignin content, making it very recalcitrant to the microbial bioconversion into bioethanol [7]. The size of woody biomass needs to be reduced from logs or chunks down to particles with fiber bundles for efficient enzymatic hydrolysis [7,8]. Without size reduction, woody biomass cannot be converted to bioethanol efficiently with current technologies [9-11]. Size reduction of woody biomass usually involves two steps. The first step is chipping [12,13]. Machines available for chipping include disk, drum, and V-drum chippers [14-16]. The second step is milling to comminute wood chips into small particles. This step is usually conducted on ball mills [17], knife mills [18] or hammer mills [19,20].

It is generally accepted that size reduction can disrupt crystallinity and increase surface area of cellulosic biomass, rendering the biomass more amenable to enzymatic hydrolysis [21-23]. However, the understanding about the mechanism is inadequate. Size reduction equipment is usually considered as a "black-box", where interactions between biomass and comminuting media (such as balls, knives, or hammers) are not clear [24]. The connection between size reduction and enzymatic hydrolysis was not well-established [25]. Little knowledge was available regarding which parameters in size reduction would influence the enzymatic hydrolysis sugar yield, or what modification on biomass structural features during size reduction would be helpful to achieve high enzymatic hydrolysis sugar yield.

Wood is an anisotropic material, when processed on size reduction equipment, different relative positions between the travelling direction of the cutting edge and the wood stem direction will generate wood chips that are cut from different orientations. The objective of this study is to obtain an understanding on how cutting orientation affects enzymatic hydrolysis sugar yield of produced wood particles for biofuel manufacturing. This study employed a metal cutting (milling) machine to produce wood particles from three cutting orientations. Dilute sulfuric acid pretreatment was used to process produced wood particles before enzymatic hydrolysis. Particle size, crystallinity, and enzyme accessible surface area of particles cut from different orientations were measured, and their relationships with enzymatic hydrolysis sugar yield were also discussed.

11.2 Methods

11.2.1 Material

Poplar wood was used in this investigation. Wood discs with thickness of about 3 inch were first debarked and cut into squares, so that they could be securely held by fixtures on the size reduction equipment. The moisture content of the poplar wood was 5%. Poplar wood chemical compositions are listed in Table 11.1. Moisture content and chemical composition were determined by following laboratory analytical procedures developed by the National Renewable Energy Laboratory (NREL) [26,27].

Table 11.1 Chemical compositions (% dry weight basis) of poplar wood

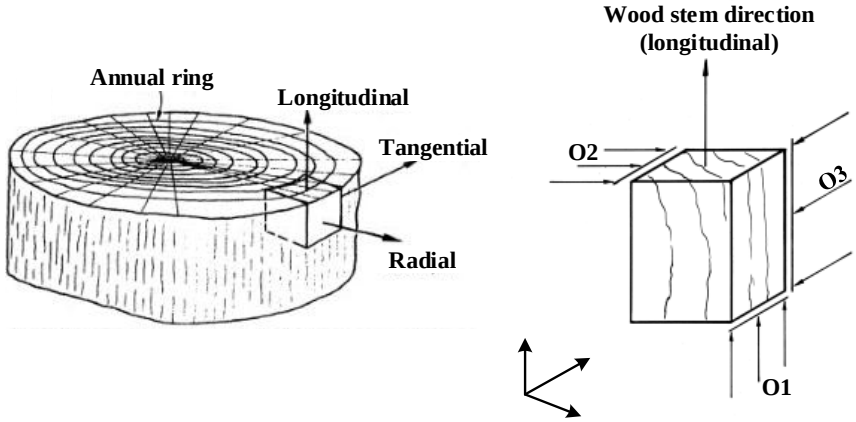
Component	Percentage
Cellulose	53.8 (0.1)
Hemicellulose	15.0 (0.4)
Lignin	25.5 (0.2)
Ash	0.6 (0.1)

Chemical compositions are means with standard deviations in brackets

11.2.2 Size reduction

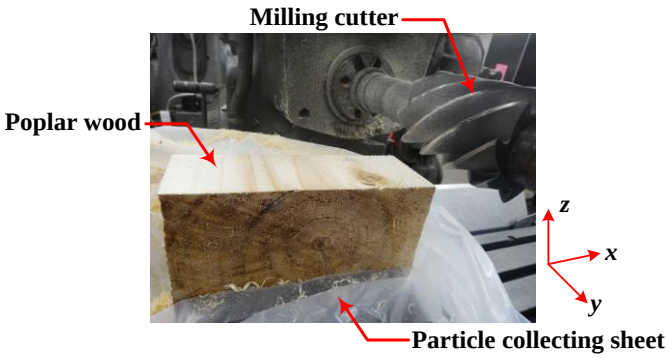
Three cutting orientations (O1, O2 and O3), as illustrated in Figure 11.1, were employed to process poplar wood size reduction. These orientations are determined by three directions of wood. The longitudinal direction is parallel to the wood stem direction, the radial direction is perpendicular to both wood annual rings and the wood stem direction, and the tangential direction is tangent to wood annual rings. O1 defines the surfaces parallel to the longitudinal and radial directions; O2 defines the surfaces parallel to the radial and tangential directions; and O3 defines the surfaces parallel to the longitudinal and tangential directions. The arrows in Figure 11.1 show the travelling directions of the cutting edge. Poplar wood size reduction was processed as illustrated in Figure 11.1 to produce wood particles from three cutting orientations.

Figure 11.1 Illustration of three cutting orientations used in size reduction



As shown in Figure 11.2, poplar wood size reduction was conducted on a milling machine (Model No. 2, Brown & Sharpe Manufacturing. Co., Providence, RI, USA) typically used for metal cutting. The cutting tool used was a high-speed steel slab milling cutter. The diameter of the cutter was 4 inch, and the length of the cutter was 6 inch. The cutter had a helical angle of 45 °and a rake angle of 10 °.

Figure 11.2 Poplar wood size reduction



From each cutting orientation, three groups of poplar wood particles were obtained at three levels of depth of cut: 0.4, 0.25, and 0.1 inch. Feedrate was kept constant as 6.75 inch·min^{-1}. Tool rotation speed was kept constant as 635 rev·min^{-1}. In total, nine groups of wood particles were produced under different size reduction conditions, as listed in Table 11.2. Particles were cut off from a poplar wood block and fell onto the particle collecting sheet underneath. About 200 g of particles under each size reduction condition was collected and saved in Ziploc® bags. These bags were sealed and stored in a refrigerator at 4°C until further processing.

Table 11.2 Poplar wood size reduction conditions

Condition	Cutting orientation	Depth of cut (inch)
1	1	0.4
2	1	0.25
3	1	0.1
4	2	0.4
5	2	0.25
6	2	0.1
7	3	0.4
8	3	0.25
9	3	0.1

The size of wood particles produced by size reduction under different conditions was determined by following the American Society of Agricultural and Biological Engineers (ASABE) standard (ANSI/ASAE S424.1) [28] using a screen shaker (Model RO-TAP® 8" RX-29, W.S. Tyler Industrial Group, Mentor, OH, USA).

11.2.3 Pretreatment

Dilute sulfuric acid pretreatment was employed in this investigation. One gram of wood particles produced under each size reduction condition and 10 mL of 1% (w/w) sulfuric acid were loaded in the 15-mL stainless steel tube reactor (Swagelok, WA, USA), and treated at 195 ℃ for 30 min.

11.2.4 Enzymatic hydrolysis and sugar yield measurement

Enzymatic hydrolysis was performed in nine 250-mL flasks containing 100 mL hydrolysis slurry. The slurry consisted of 2% (w/v) biomass on dry weight base, sodium acetate buffer (50 mM, pH = 4.8). Enzymes used in this study were Cellic® CTec2 produced by Novozymes North America (Franklinton, NC, USA). The enzyme loaded was 0.5 mL for each gram of dry biomass. Enzymatic hydrolysis was carried out at 50 ℃ for 72 hr.

Triplicate samples of hydrolysis slurry were taken from each flask after 72 hr of hydrolysis. All samples were first centrifuged, and then followed by filtration through a 0.45μm membrane prior to sugar measurement. Sugar measurement was performed by following NREL laboratory analytical procedure (NREL/TP-510-42623) [29] using a Perkin Elmer high performance liquid chromatography (HPLC) system (Perkin Elmer Inc., Waltham, MA, USA) equipped with an Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA, USA).

Sugar yield in this study was defined as the glucose concentration in the hydrolysis slurry. Reported sugar yield results were means with stand deviations based on sugar measurement of the triplicate samples of hydrolysis slurry taken from each flask. Each sample was measured once. The means and standard deviations were calculated using Origin Pro 8 software (OriginLab Corp., Northampton, MA, USA).

11.2.5 Morphology observation

A Hitachi scanning electron microscope (SEM) (Model S-3500N, Hitachi High Technologies America, Inc., Dallas, TX, USA) was used to study morphology of wood particles produced by size reduction from different orientations. It was operated at an accelerating voltage of 20 kV. Quartz PCI image acquisition and archiving software (Quartz Imaging Corp., Vancouver, BC, Canada) was used. Samples were mounted on specimen stubs using double-coated tapes. Samples were then sputter coated with Au/Pd in order to prevent charging on the surface. Au/Pd coating was performed in the presence of ambient air using a Denton vacuum sputter coater (Model Desk II, Denton Vacuum, LLC, Moorestown, NJ, USA).

11.2.6 Surface area

Enzyme accessible surface area in cellulosic biomass is an essential structural feature influencing enzymatic hydrolysis efficiency [30]. Simon's stain is a useful method for estimating enzyme accessible surface area in cellulosic biomass [31,32].

Simon's stain consists of a mixture of a blue and an orange dye. The blue dye has small molecular size and low affinity towards cellulose. The orange dye has large molecular size and much stronger affinity towards cellulose. If cellulose is shielded by hemicellulose and lignin, and pores on fiber wall are small, the small-molecular-sized blue dye can penetrate in but the large-molecular-sized orange dye cannot. Cellulose then adsorbs only the blue dye. On the other hand, when shield on cellulose is disrupted, and pores on fiber wall are large enough for the orange dye to penetrate, cellulose adsorbs the orange dye preferentially because of the orange dye's stronger affinity for cellulose [31-33]. It is also known that cellulosic biomass with larger pore size is more amenable to enzymes in enzymatic hydrolysis, because it has more available surface area for enzymes to access [34]. It is indicated that cellulosic biomass sample with higher orange dye adsorption has more available surface area for enzymes in hydrolysis [32,33].

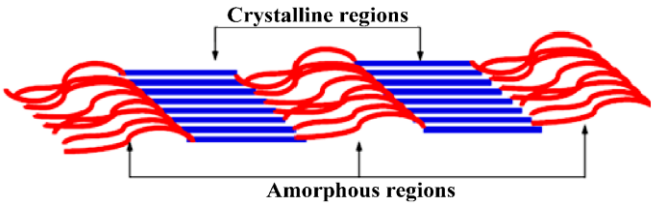
The Simons' stain of all samples was performed as described in the literature [33]. Orange dye was filtered by a 10K Amicon membrane (EMD Millipore Corp., Billerica, MA, USA) under pressure of 35 psi. The purpose of the filtration is to remove the small particles in the orange dye.

11.2.7 Crystallinity

As shown in Figure 11.3, cellulose consists of crystalline regions and amorphous regions. Crystallinity is determined as the percentage of crystalline regions in cellulose and expressed as crystallinity index (CI). The crystallinity of wood particles was measured by an X-ray

diffractometer (Model MiniFlex II, Rigaku Americas Corp., The Woodlands, TX, USA). The X-ray source was a ceramic X-ray tube with Cu anode. Operating power was 30 kV and 15 mA (450 W). X-ray diffraction patterns of samples were recorded at room temperature with a scan range from 10 ° to 45 °. The step size of the scan was 0.05 °. Crystallinity index (CI) was calculated using the Segal method [35] as the height ratio between the intensity of the crystalline peak ($I_{002}-I_{AM}$) and total intensity (I_{002}) after subtracting the background signal measured without biomass sample. For each size reduction condition, three particles were randomly picked for CI measurement.

Figure 11.3 Crystalline and amorphous regions in cellulose



11.3 Results and discussions

11.3.1 Characterization of wood particles

11.3.1.1 Particle size

Pictures of wood particles produced by size reduction were shown in Figure 11.4. Particle size was expressed in terms of geometric mean length and standard deviation and the data are listed in Table 11.3. Particle size calculation was based on the assumption that particles are logarithmic normally distributed. It can be seen that, within each of the three orientations, geometric mean length of particles decreased as the depth of cut decreased. Generally, particles produced from O1 had larger geometric mean length than particles obtained from O2 and O3.

This is associated with the fact that, when cutting from O1, the traveling direction of the cutting edge was parallel to the wood stem direction. In this way, long wood fibers were torn off and resulted in long and curved particles as shown in Figure 11.4. In contrast, particles produced from O2 and O3 were much smaller than those obtained from O1. When cutting from O2 and O3, there was an angle between the traveling direction of the cutting edge and the wood stem direction. As a result, long wood fibers were cut into small pieces, and much finer particles with shorter wood fibers were produced. Geometric mean lengths and standard deviations of particles produced from O2 and O3 with the same depth of cut were similar. With 0.1 inch depth of cut, particles cut from O2 and O3 had the two smallest geometric mean lengths, which were less than 1 mm.

Figure 11.4 Wood particles produced by size reduction

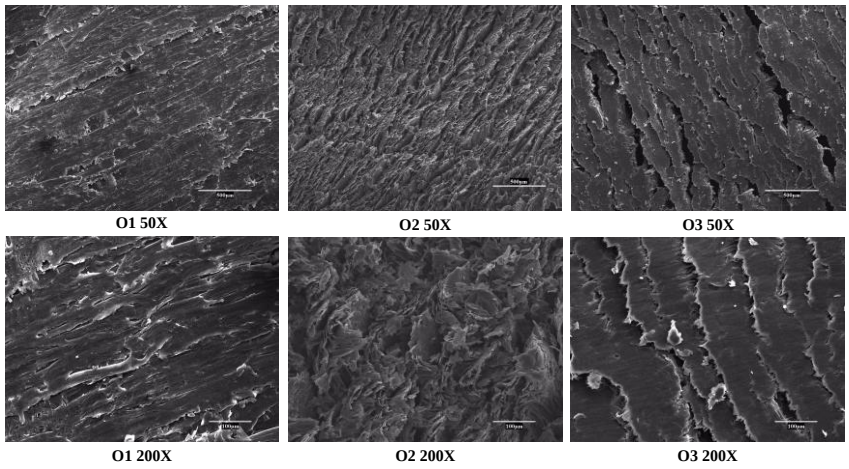
Table 11.3 Geometric mean length and standard deviation of particles produced by size reduction

Size reduction condition (orientation-depth of cut inch)	Geometric mean length (mm)	Standard deviation (mm)
O1-0.4	10.65	3.64
O1-0.25	10.41	3.48
O1-0.1	4.69	1.39
O2-0.4	2.07	0.75
O2-0.25	1.10	0.47
O2-0.1	0.61	0.18
O3-0.4	2.32	0.64
O3-0.25	1.03	0.54
O3-0.1	0.35	0.10

11.3.1.2 Morphology

Figure 11.5 shows SEM images of wood particles cut from three orientations. These particles were produced with 0.4 inch depth of cut. It can be seen that the particle cut from O1 had smooth surfaces. Particle cut from O2 had carpet-like wrinkled surface with disordered wood fibers. Particle cut from O3 was characterized by scale-like surfaces with wood fibers much shorter than those in O1.

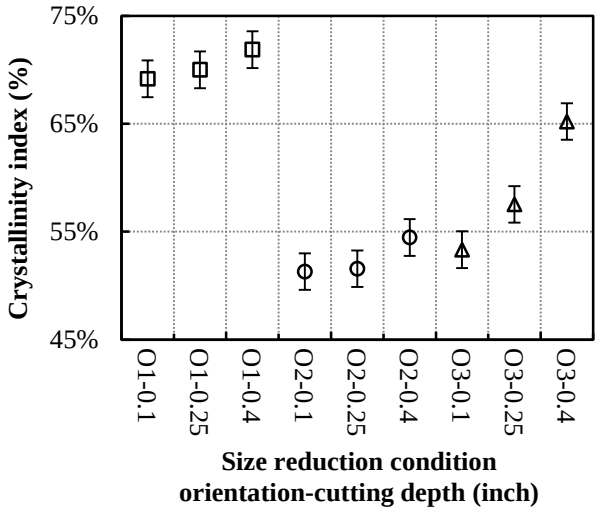
Figure 11.5 SEM images of wood particles cut from three orientations



11.3.1.3 Crystallinity

It is known that intensive mechanical action in size reduction is able to cause distortion or destruction to the original crystalline structure in cellulosic biomass; thus, to reduce the crystallinity of biomass that undergoes size reduction [36]. Results on crystallinity of wood particles cut under different size reduction conditions are shown in Figure 11.6. Generally, particles cut from O1 had high crystallinity, and particles cut from O2 had low crystallinity. It is hypothesized that cutting wood from O2, where the edge of the cutting tool was traveling perpendicular to the wood stem direction, would bring more severe distortion or destruction to the original crystalline structure in wood. In addition, one trend was noticed that, within the same orientation, crystallinity of particles increased as the depth of cut used to produce them increased. However, this trend was not conclusive for particles cut from O1 and O2 because of the overlaps in the confidential intervals (significant level $\alpha = 0.05$).

Figure 11.6 Crystallinity of wood particles produced by size reduction



11.3.1.4 Surface area

Total dye adsorption and orange dye adsorption of dilute acid pretreated wood particles measured using Simons' stain technique are shown in Figures 11.7 and 11.8, respectively. The total dye adsorption can be used to represent the total surface area of wood particles, while the orange dye can indicate the amount of available surface area that can be accessed by enzymes in hydrolysis. Figure 11.7 shows that particles cut from O2 with 0.25 and 0.1 inch depth of cut ranked top two in total dye adsorption, and particles cut from O3 with 0.1 inch had the least total dye adsorption. While, amounts of total dye adsorption for particles cut under the other size reduction conditions were approximately the same. The top two groups of particles (O2-0.1 and O2-0.25) had more than 80 to 90% higher total dye adsorption than other particles. Similar rankings were observed for amounts of orange dye adsorption as shown in Figure 11.8. Again, particles cut from O2 with 0.25 and 0.1 inch depth of cut had the highest two orange dye adsorption, about 60 to 80% higher than the other particles.

Figure 11.7 Total dye adsorption measurement of wood particles using Simons' stain

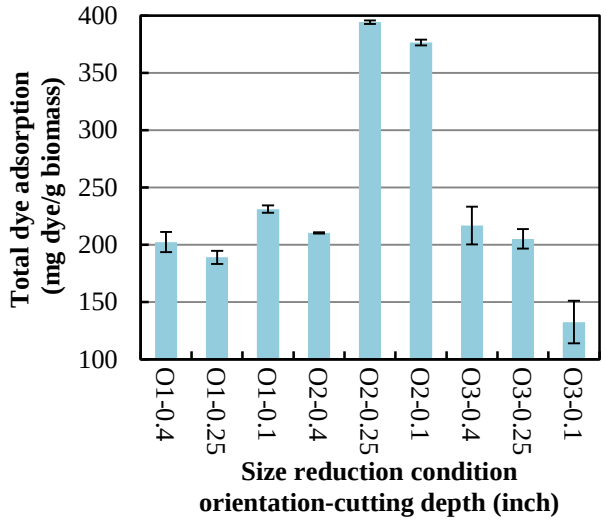
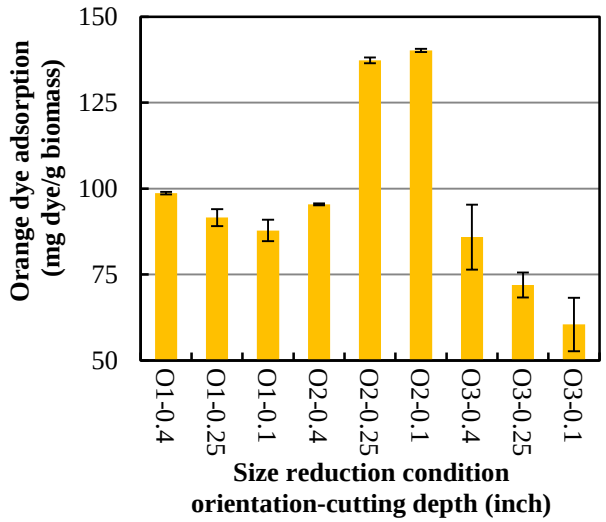


Figure 11.8 Orange dye adsorption measurement of wood particles using Simons' stain



In the literature on biofuel manufacturing, no study was found regarding effects of cutting orientation on surface area of produced particles. However, in pulp industry, where woody biomass is used as raw material to produce cellulose-based products, cutting orientation is a very important process parameter in producing wood chips for pulp production [37-41]. As found by Uhmeier and Persson [37], wood chips would undergo more severe plastic deformation when being cut with a larger angle between the travelling direction of the cutting edge and the wood stem direction. In addition, when this angle was enlarged, produced wood chips would have larger surface area [39,40].

11.3.2 Sugar yield

Sugar yield results after 72-hr of enzymatic hydrolysis are shown in Figure 11.9. For wood particles cut from O1, sugar yield increased as the depth of cut became smaller. Depth of cut used in size reduction mainly determined the thickness of produced particles. Smaller depth of cut produced thinner particles. As shown in Figure 11.4, the aspect ratio between length and thickness was very large for particles cut from O1. In another way, for the long but thin particles

obtained from O1, thinner particles were more susceptible to enzymes in hydrolysis and had higher sugar yield. Generally, wood particles cut from O2 had higher sugar yield than particles cut from the other two orientations. Particles cut from O2 with 0.25 inch depth of cut produced the highest sugar yield of 2 g/L; while, particles cut from O2 with 0.1 inch depth of cut had slightly lower sugar yield. Particles cut from O3 with 0.1, 0.25, and 0.4 inch depth of cut had approximately sugar yield of 1.3 g/L.

When sugar yield results shown in Figure 11.9 and particle size data listed in Table 11.3 are studied together, it was apparent that differences in particle size for wood particles cut under different size reduction conditions did not have a strong correlation with their sugar yield. Only for particles cut from O1, sugar yield increased as particle size became smaller. It was also noticed that the average sugar yield in O1 was the lowest among all the three cutting orientations. For particles cut from O2 and O3, sugar yield was not influenced by particle size.

Numerous studies have investigated the influence of particle size on cellulose digestibility and sugar yield in enzymatic hydrolysis. Vidal et al. have a comprehensive review on this topic [42]. With a few exceptions, majority of the studies demonstrated that particle size did affect cellulosic biomass conversion with smaller particle size positively correlating with higher sugar yield [43-46]. Other studies reported that particle size had no significant correlation with sugar yield suggesting that particle size is a weak predictor of the susceptibility to enzymatic hydrolysis [47,48].

In many cases where smaller particle size positively correlate with higher sugar yield, a statement was made as: smaller particles had larger surface area, and larger surface area produced higher sugar yield [45,49]. It would be reasonable that sugar yield should be a function of the surface area of cellulosic biomass because direct physical contact between cellulose and

enzymes is a prerequisite for conversion of cellulose to glucose [50]. However, the question is that not all the surface area can be accessed to enzymes. The generally used surface area measurement is Brunauer-Emmett-Teller (BET) method using the adsorption of gaseous nitrogen (or the adsorption of such small molecules like water) [49]. Nevertheless, for large molecules like enzymes, not all pores on cellulose are accessible [48]. Therefore, it is important to have a precise measurement of the enzyme accessible surface area since large surface area is not always associated with large enzyme accessible surface area. The Simons' stain method has been frequently used as an alternative to BET method, and can represent both total surface area and enzyme accessible area of cellulosic biomass substrate [32,51,52]. With the Simons' stain method, Ju et al. [51] demonstrated that, even though some small particles had large surface area, their enzyme accessible surface area was not necessarily large. In addition, some mechanical refining methods produced wood particles with good accessibility to enzymes without reducing particle size [53].

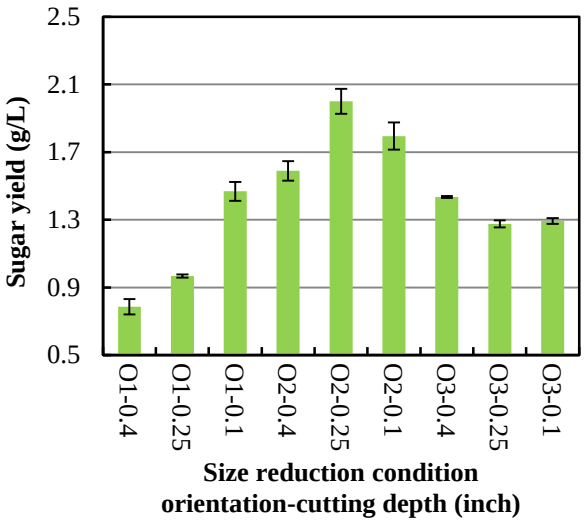
It is known that crystalline regions of cellulosic biomass are less susceptible to enzymatic hydrolysis than amorphous regions [49,54]. It is helpful to decrease the amount of crystalline regions and increase the amount of amorphous regions (that is to reduce crystallinity) in order to increase sugar yield of the hydrolysis substrates. One way to reduce crytallinity is via size reduction that brings mechanical impacts on cellulosic biomass [12,41,42]. However, size reduction methods (e.g. ball milling and disk refining) not only decrystallize cellulose and reduce crystallinity but also reduce the particle size of cellulosic biomass [12,41]. It is possible that benefits from size reduction actually attributed to a lower crystallinity could be credited to a smaller particle size. Results in this study reveal that low crystallinity was not necessarily

associated with small particle size, and high sugar yield was correlated with low crystallinity but now small particle size.

Results obtained from this study agreed with Sinitsyn et al. [48], who investigated effects of structural features of cellulosic biomass on efficiency of the enzymatic hydrolysis. Structural features in their study include particle size, total surface area, enzyme accessible surface area, crystallinity, and degree of polymerization. High hydrolysis sugar yield only correlated with large enzyme accessible surface area and low crystallinity. The other three features had little effects on the efficiency of enzymatic hydrolysis. Results in this study may serve as a good example to confirm and demonstrate the authors' previous finding that particle size would have little effects on a substrate that had been already susceptible to enzymatic hydrolysis [52]. Size reduction might be helpful for improving the sugar yield of substrates that have limited reactive enzyme accessible surface area [52].

It can be indicated, by observing the morphology of particles cut from different orientations in Figure 11.5, that size reduction from O2 not only cut long wood fibers into short segments but also brought fiber separation effect onto particles. For size reduction from the other two orientations, fiber separation effect was comparatively weak. Size reduction methods with good fiber separation effect, such as ball milling [17], disk refining [41], PFI mill refining [52], are more likely to produce particles with good susceptibility to enzymatic hydrolysis.

Figure 11.9 Sugar yield of wood particles produced by size reduction



11.4 Concluding remarks

This study demonstrates that cutting orientation in woody biomass size reduction has significant effects on the enzymatic hydrolysis sugar yield of produced particles. Three cutting orientations (O1, O2, and O3) were utilized to produce wood particles on a metal cutting (milling) machine. In general, particles cut from O2 had the highest enzymatic hydrolysis sugar yield. Cutting from O2 brought more severe deformation onto produced particles. This severe deformation could separate wood fibers and distort the original crystalline structure in particles. These effects were beneficial to opening up more enzyme accessible surface area and decreasing the crystallinity of particles. In this study, high enzymatic hydrolysis sugar yield correlated with large enzyme accessible surface area and low crystallinity. Particle size was a weak predictor of particles' susceptibility to enzymatic hydrolysis, and had little effects on a substrate that had already been susceptible to enzymatic hydrolysis.

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Chapter 12 - Conclusions and Contributions

12.1 Conclusions

In this dissertation, size reduction of cellulosic biomass for biofuel manufacturing was investigated. Metal-cutting methods (milling and turning) were utilized to separate confounding effects of particle size and biomass crystallinity. Effects of particle size and biomass crystallinity on biomass enzymatic hydrolysis sugar yield were studied independently. A knife mill was used to conduct size reduction of both woody and herbaceous biomass. Effects of sieve size on energy consumption in size reduction, cellulose recovery rate in pretreatment, and sugar yield in enzymatic hydrolysis were investigated. Furthermore, relationship between cellulosic biomass particle size and sugar yield specified by different sugar yield definitions and effects of milling orientation in size reduction of woody biomass were also studied.

Main conclusions drawn from this dissertation are:

1. Confounding effects of particle size and biomass crystallinity were separated by performing biomass size reduction on a metal-cutting (milling or turning) machine, where particle formation was well controlled. This effort made it possible to study the effects of particle size and biomass crystallinity on sugar yield independently.
2. Using a metal-cutting (turning) machine, poplar wood particles with three levels of particle size but the same biomass crystallinity were produced by changing the number of slots that cut into cylindrical wood workpieces. Experimental results showed that sugar yield increased as particle size became smaller.

3. Using the same turning machine, poplar wood particles with three levels of biomass crystallinity but the same particle size were obtained by changing the rake angle of the cutting tool. Sugar yield increased as biomass crystallinity decreased.
4. In size reduction of cellulosic biomass using a knife mill, energy consumption increased dramatically as sieve size became smaller. Particles produced with a larger sieve size on the knife mill had higher cellulose recovery rate in pretreatment, enzymatic hydrolysis efficiency, total cellulose conversion rate, and total sugar yield.
5. Relationships between particle size and enzymatic hydrolysis sugar yield could be different if different sugar yield definitions (cellulose-based or biomass-based) were used. A consistency mapping was developed to show under what conditions the relationships are consistent (or inconsistent) when these two definitions are used.
6. Cutting orientation in size reduction of woody biomass had a significant impact on sugar yield of the particles produced. It was also indicated that high sugar yield as correlated with large enzyme accessible surface area and low biomass crystallinity but not small particle size.

12.2 Contributions

Major contributions of this dissertation are:

1. This dissertation, for the first time, presents an investigation on confounding effects of particle size and biomass crystallinity by using metal-cutting (milling and turning) methods. The confounding effects were separated. Results could provide some explanations for the inconsistent results in the literature regarding the relationship between particle size and sugar yield and the relationship between biomass crystallinity and sugar yield.

2. This dissertation is the first one to study effects of sieve size throughout biomass size reduction, pretreatment, and enzymatic hydrolysis. Results obtained in this study would provide guidelines for decision makers to select sieve size used on a biomass size reduction machine to minimize the energy consumption in size reduction, maximize the cellulose recovery rate in pretreatment, and maximize the sugar yield in enzymatic hydrolysis.
3. This research, for the first time, develops a consistency mapping to show that the relationships between particle size and enzymatic hydrolysis sugar yield could be different when two different sugar yield definitions (cellulose-based and biomass-based) were used. This mapping is applicable to investigations of the relationships between a variety of parameters (biomass type, pretreatment condition, etc.) and enzymatic hydrolysis sugar yield.
4. For the first time, this research shows that cutting orientation in size reduction of woody biomass had a significant impact on sugar yield of the produced particles. Results could help to produce woody biomass particles efficiently to generate high sugar yield while save energy in size reduction.

Appendix A - Summary of measurement procedures and standards

[1] **Biomass moisture content** was measured by following NREL Laboratory Analytical Procedures (NREL/TP-510-42621) as shown on Pages 126, 151, 177, 196, and 214.

[2] **Biomass chemical composition** was measured by following NREL Laboratory Analytical Procedures (NREL/TP-510-42618) as shown on Pages 38, 60, 151, 178, and 201.

[3] **Cellulose-based sugar yield** was expressed as percentage of cellulose converted to fermentable sugar (glucose) as shown on Pages 38, 60, 109, and 161.

[4] **Biomass-based sugar yield** was expressed as glucose yield per unit dry weight of biomass loaded into the hydrolysis process as shown on Pages 109 and 175.

[5] **Glucose concentration** was measured by following NREL Laboratory Analytical Procedures (NREL/TP-510-42618) as shown on Pages 88, 131, 163, and 218.

[6] **Biomass crystallinity** was determined as percentage of crystalline material in biomass and expressed as crystallinity index (CI) and measured by x-ray diffractometer as shown on Pages 13, 59, 87, 107, and 225.

[7] **Biomass weight loss in pretreatment** was defined as percentage of dry weight loss of biomass after and before pretreatment as shown on Pages 158 and 200.

[8] **Cellulose recovery rate in pretreatment** was calculated as ratio of cellulose weight after pretreatment to the cellulose weight before pretreatment as shown on Pages 159 and 200.

[9] **Size reduction energy consumption** was electricity consumed by the electric motor of the size reduction equipment as shown on Pages 130 and 154.

[10] **Particle size distribution** was measured by following ASABE standard (ANSI/ASAE S424.1) as shown on Pages 132 and 217.

Appendix B - Publications during Ph.D. Study

Journals and transactions

[11] Zhang, M., Song, X.X., Zhang, Deines, T.W., Pei, Z.J., and Wang, D.H., 2014, "A consistency mapping for the effects on enzymatic hydrolysis sugar yield using two sugar yield definitions in cellulosic biofuel manufacturing," Renewable Energy, Vol. 62, pp. 243-248.

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Submitted and working papers

[1] Zhang, M., Song, X.X., Zhang, Ke, Pei, Z.J., and Wang, D.H., 2013, "Effects of sieve size on biochemical conversion of big bluestem biomass for biofuel production," submitted to Biomass and Bioenergy.

[2] Zhang, M., Song, X.X., Ju, X., Zhang, X., Deines, T.W., Pei, Z.J., and Wang, D.H., 2013, "Effects of cutting orientation in woody biomass size reduction on enzymatic hydrolysis sugar yield," to be submitted to Renewable Energy.

Conference papers

[1] Zhang, M., Song, X.X., Zhang, P.F., and Pei, Z.J., 2013, "Diluted acid pretreatment and enzymatic hydrolysis of woody biomass for biofuel manufacturing: effects of particle size on sugar conversion," Proceedings of the ASME 2013 International Manufacturing Science and Engineering Conference (MSEC), Madison, WI, June 10-14, 2013, MSEC 2013-1050.

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