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Influence of Feedstock Particle Size on Lignocellulose Conversion—A Review

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Abstract Feedstock particle sizing can impact the economics of cellulosic ethanol commercialization through its effects on conversion yield and energy cost. Past studies demonstrated that particle size influences biomass enzyme digestibility to a limited extent. Physical size reduction was able to increase conversion rates to maximum of ≈50%, whereas chemical modification achieved conversions of >70% regardless of biomass particle size. This suggests that (1) mechanical pretreatment by itself is insufficient to attain economically feasible biomass conversion, and, therefore, (2) necessary particle sizing needs to be determined in the context of thermochemical pretreatment employed for lignocellulose conversion. Studies of thermochemical pretreatments that have taken into account particle size as a factor have exhibited a wide range of maximal sizes (i.e., particle sizes below which no increase in pretreatment effectiveness, measured in terms of the enzymatic conversion resulting from the pretreatment, were observed) from <0.15 to 50 mm. Maximal sizes as defined above were dependent on the pretreatment employed, with maximal size range decreasing as follows: steam explosion>liquid hot water>dilute acid and base pretreatments. Maximal sizes also appeared dependent on feedstock, with herbaceous or grassy biomass exhibiting lower maximal size range (<3 mm) than woody biomass (>3 mm). Such trends, considered alongside the intensive energy requirement of size reduction processes, warrant a more systematic study of particle size effects across different pretreatment technologies and feedstock, as a requisite for optimizing the feedstock supply system.

Keywords Cellulosic ethanol · Lignocellulose conversion · Biomass particle size · Biomass recalcitrance

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Introduction

It has been proposed that up to a trillion kilogram of biomass can be produced in the U.S. to help meet automotive fuel requirements under a carbon constrained future [1]. Conversion of this biomass to liquid fuels is projected to displace 30% of current petroleum usage or about 2.3×10^8 m^3 (60 billion gallons) per year. In 2008 alone, the US produced 3.4×10^7 m^3 (9 billion gallons) of ethanol, almost entirely from corn. The scale of such an undertaking requires technological and economic breakthroughs, of which feedstock cost is a critical factor [2].

At the Energy Bioscience Institute, University of Illinois, a systems engineering approach has been taken to optimize biomass supply. This involves integrating the different tasks in the biomass supply chain to find the best schemes among technologies that exist or are being developed (Fig. 1). Decision-making in regards to feedstock preprocessing needs to be guided by biomass conversion requirements; or more precisely, what form of the feedstock is desirable as it crosses the interface in Fig. 1 from supply to conversion.

Although there are many physical characteristics possibly relevant to preprocessing of biomass, this review will focus primarily on particle sizing for the following reasons. First, size reduction is rendered by mechanical means (e.g., cutting, shredding, attrition milling, and compression), making it convenient for incorporation into harvest or postharvest. Among physical characteristics, particle size is most straightforward to modify at the supply stage, and possibly the least modified by subsequent chemical treatments [3, 4]. Second, comminution or size reduction is an energy intensive process, with potentially significant feedstock cost implications. Third and last, particle size influences decisions on biomass storage and transportation (e.g., bulk density is a function of particle size).

Two major platforms have been proposed for biomass conversion: thermochemical and biological [5]. Sizing has implications on both types of processes; however, this review will consider only biochemical conversion of biomass to ethanol , which is among the technologies very near commercialization [2]. Consequently, feedstock preprocessing other than particle sizing, such as liquefaction and pelletizing [6], which are more relevant to the thermochemical platform, were not given consideration. Although

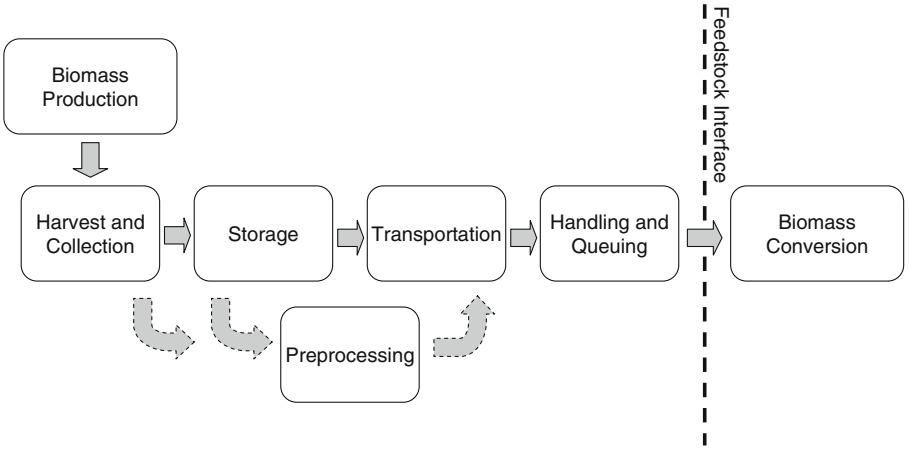

Fig. 1 Schematic of feedstock supply system showing its interface with the conversion side, adapted from Foust et al. [5]

densification (e.g., pelletizing and briquetting) is an attractive solution to the problem of storage and transport, no study has considered its feasibility from the bioconversion standpoint, probably because its economic viability is hampered by a consequent comminution step just before conversion. In the following discussions, we use the term cellulose hydrolysis or digestibility interchangeably to mean sugar production, and cellulose conversion to either sugar or ethanol, in which case we specify the conversion product.

Overview of Lignocellulose Structure and Enzyme Digestibility

The relationship between physical or structural characteristics of the biomass feedstock and susceptibility to enzyme hydrolysis has been reviewed extensively [7, 8]; some features relevant to subsequent discussions are recapped briefly. Lignocellulose has three basic components: cellulose, non-cellulosic carbohydrates (predominantly hemicellulose), and lignin (Fig. 2). The cellulose component is organized into elementary fibrils, an aggregate of 36 glucan chains in a 3.0×5.5 nm hexagonal projection [9]. This geometry, arising from sheets of β-1,4 glucan chains stacked on top of each other, gives cellulose its crystalline characteristic. Surrounding cellulose elementary fibrils are hemicellulose components that form a gel matrix by bonding with cellulose and other hemicellulose molecules via noncovalent and covalent bonds [10]. Lignin is considered a hydrophobic filler, its biosynthesis occurring late during plant fiber synthesis, thereby displacing water and providing a matrix that overlies and encrusts the cellulose fibrils [10]. The extensive covalent crosslinking of lignin with other polysaccharides (primarily hemicellulose) limits accessibility to carbohydrases (polysaccharide degrading enzymes) and prevents extraction of polysaccharides by neutral aqueous solvents [10].

A number of different physical characteristics associated with lignocellulose structure have been implicated in biomass resistance to enzyme hydrolysis. Among these are crystallinity (measured as crystallinity index (CI) or the ratio of crystalline to amorphous

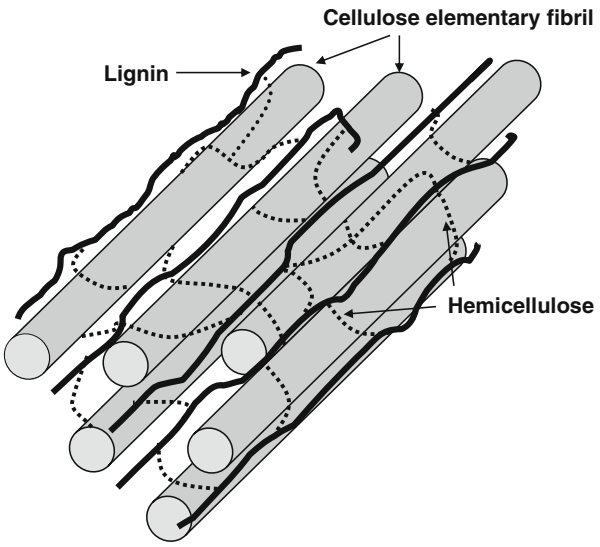

Fig. 2 Structure and organization of lignocellulose

composition), degree of polymerization (DP) or length of glucan chain, specific surface area, accessible surface area (or pore volume), and particle size. Studies have often shown conflicting results as to the extent of the influence of each of these physical properties on biomass recalcitrance. Plausible reasons cited for the inconsistencies are interactions between properties due to common underlying structure and chemistry, and dependence to overall environment in which measurement is made (i.e., whether lignocellulose complex or isolated cellulose fibril) [7]. For example, CI and DP measurements can be affected by noncellulose components, and therefore more applicable to cellulose elementary fibril than to lignocellulose cell wall matrix [11, 12]. Surface area measurements (especially enzyme accessible area) are relevant to the lignocellulose complex as evidenced by numerous studies demonstrating a strong correlation between this property and enzyme digestibility [12–14]. However, it is very common and unavoidable that processes that change one property also affect others [7]. Physical or chemical treatments that change surface area could potentially change other properties such as CI [15]. In this regard, lignin and hemicellulose removal, which are strong predictors of enzyme digestibility [11], would be expected to also alter accessible surface area. Finally, while lignocellulose physical structure plays a role in enzyme hydrolysis susceptibility, enzyme characteristics are just as important [8].

Feedstock Size Reduction—Energy Requirement

Biomass particle size has implication in mass and heat transfer processes. Size can be reduced through the process of comminution. For lignocellulose, the starting materials are typically presized using some convenient method such as shredding for herbaceous biomass or chipping for wood. The material then goes through a final sizing suitable for subsequent processing, wherein the most commonly employed mill types are the hammer, knife, and disk or attrition mill [16]. Because comminution of biomass (especially at the final stage of sizing) is an energy intensive process that produces an essentially low-value product [16], there has been interest to identify the most efficient milling processes. Working with wood and herbaceous materials (moisture content, or m.c. of 4% to 7%), Cadoche and Lopez [17] reported greater energy consumption with the hammer mill for hardwood and straw, but not for corn stover (knife mill consumed 20 kWh/t, whereas hammer mill consumed 9.6 kWh/t, for a 7× reduction). Hardwood had an order of magnitude higher energy requirement compared to either straw or corn stover. The main conclusion drawn was that energy consumption can be limited to 30 kWh/t, as long as the final particle size is kept between 3 to 6 mm (screen opening size). What limited the study was the lack of information on the size distribution of the products.

Himmel et al. [16] quantified the particle size distribution resulting from comminution of four types of biomass (aspen chips, wheat straw, corn stover, and corn cobs, having 6%, 4%, 7%, and 7% m.c., respectively) using hammer, knife, and attrition mills. The hammer and attrition mills produced greater amounts of fines (passing through 0.25 mm opening) than the knife mill, while it had the least specific energy consumption among the three (specific energy discounted for no-load energy requirement). In the case of aspen chips, using 1/16″ (1.59 mm) rejection mill screen, the attrition mill consumed >200 kWh/t; whereas the hammer and knife mill consumed between 100 and 150 kWh/t. With a 1/4″ (6.35 mm) rejection screen, the hammer mill specific energy consumption reduced to ≈100 kWh/t, but the knife mill reduced to <30 kWh/t. The type of biomass is another huge factor, as evident in the energy consumption of milling wheat straw: ≈50 kWh/t and <10 kWh/t for hammer and knife mill, respectively, using the 1/16″ rejection screen.

Schell and Harwood [18] investigated the pilot-scale milling of high moisture (≈60% m.c.) wood (hybrid poplar) chips, employing both hammer mill and disk mill. The use of high-moisture starting material has implications in the need for pre-drying the material (additional energy cost) and the associated issues of milling moist wood chips such as low production rates, excessive heat buildup, screen blinding, and equipment damage [18]. The study concluded that disk mill produced smaller particles (>80% *w*/*w* below 2.5 mm) compared to hammer mill (>80% *w*/*w* below 3.5 mm) but required higher energy (range of 134 to 669 kWh/t, depending on the type and operating mode) than the hammer mill (range of 88 to 112 kWh/t). The energy consumed was discounted for the idling load but included the cooling fan and energy consumed for steam generation in the case of the pressurized disk mill. The energy consumption was generally higher than previously reported for dried wood (e.g., [16, 17]), indicative of the higher energy requirement for comminution of high-moisture biomass.

Recent studies have focused considerably on hammer mill. Mani et al. [19] subjected four biomass feedstocks (wheat and barley straws, corn stover, and switchgrass) to hammer milling with three screen openings (3.2, 1.6, and 0.8 mm). After accounting for moisture content and grind size, specific energy requirement was highest for switchgrass, followed by wheat and barley straws, and least for corn stover. Differences in the energy requirements of these different plant materials were likely due to structural differences in their cellulosic fibers, although this has not been investigated by the authors in detail. In the case of 1.6 mm screen opening, at the initial m.c. between 6.2% and 8.3%, the specific energy requirement was measured to be 51.8, 37.0, 37.9, and 14.8 KWh/t for switchgrass, wheat straw, barley straw, and corn stover, respectively. Except for barley straw, increasing the m.c. of feedstocks uniformly to 12% increased the power consumption (in the case of 1.6 mm screen opening, specific energy requirement increased by 13%, 18%, and 34% for switchgrass, wheat straw, and corn stover, respectively).

Utilizing similar feedstocks (m.c.=9%), Bitra et al. [20] did extensive characterization of particle size distribution obtained by the sieving method. They studied the energy consumption involved in hammer milling to an opening size of 3.2 mm, using different milling speeds and hammer configurations. In general, speed neither affected the product particle size nor the effective specific energy (defined as the energy required above the no-load requirement) substantially. Total specific energy was not different for the three feedstocks (100 to 125 MJ/mg, equivalently 28 to 35 kWh/t, at the lowest speed setting), but when normalized to size reduction (i.e., total specific energy per unit size reduction), wheat straw had a higher energy requirement than corn stover and switchgrass, both of which had identical values. This was in contrast to the findings of Mani et al. [19], suggestive of milling performance being not only dependent on the equipment (e.g., size and configuration) and mode of operation (e.g., feedrate, material m.c., and starting size), but also on the method of evaluation (i.e., direct mechanical energy measurement as opposed to electrical power measurement).

Combining pilot-scale hammer mills and separators, Esteban and Carrasco [21] investigated an optimum strategy to pulverize wood biomass (pine chips, pine bark and poplar chips, having m.c. from 10% to 15%). In their study, the particle size target was set by a more stringent requirement of pulverized fuel combustion (i.e., 95% *w*/*w* below 1 mm and minimum 12% *w*/*w* below 0.125 mm). Except for the case of poplar chips, they found the use of a single hammer mill (single pass) inadequate for attaining the target particle size. On the other hand, use of a two-stage hammer milling without separation in between resulted in excessive blinding of screens in the second hammer mill. The best product particle size distribution was attained by a two-stage hammer milling, with dynamic

separation in between to remove particles already meeting the size requirement. The specific energy consumption (total requirement without discounting for no-load) did not vary substantially among processes, but varied substantially among biomass types (85, 118, and 20 kWh/t for poplar chips, pine chips and pine bark, respectively). A significant (27% to 50%) increase in bulk density was reported with pulverization.

From the above discussion, and as summarized in Table 1, the required energy to mill herbaceous biomass to some acceptable fineness (<2 mm screen) is ≤50 kWh/t. To put this in perspective, the upper limit of 50 kWh/t is equivalent to 2% of the energy that can be recovered from the ethanol produced (using process yield assumptions reported by Aden et al. [22]). It takes at least twice this value to achieve a similar size reduction for woody biomass (Table 1). The need to improve the efficiency of milling, thus improving the overall process energy cost, is therefore compelling. Holtzapple et al. [23] investigated alternative methods of particle size reduction. In particular, explosive depressurization, which involved subjecting wood chips (nominally 25.4×6.4 mm) to high pressure steam (22.4 atm, 219°C), then releasing pressure instantaneously, resulted in a size distribution of 50% passing through 20 mesh (0.85 mm sieve opening). The authors found that the steam explosion method required only 60% of

Table 1 Energy requirements for biomass comminution of various feedstocks

Biomass	Mill type	Size description[a]	Specific energy (kWh/t)	Reference
Wood				
Hardwoods	Knife, hammer	1.6 mm	130	[17]
Aspen	Attrition	1.6 mm	>200	[16]
	Hammer	1.6 mm	100-150	[16]
	Knife	1.6 mm	100-150	[16]
Poplar	Hammer	<1 mm (95%)	85	[21]
Pine	Hammer	<1 mm (95%)	118	[21]
Pine bark	Hammer	<1 mm (95%)	20	[21]
Hybrid poplar (high moisture)	Attrition	<2.5 mm (>80%)	>130	[18]
	Hammer	<3.5 mm (>80%)	88–112	[18]
Herbaceous				
Straw	Knife	1.6 mm	7.5	[17]
	Knife	1.6 mm	<10	[16]
	Hammer	1.6 mm	42	[17]
	Hammer	1.6 mm	50	[16]
	Hammer	1.6 mm	37	[19]
	Hammer	3.2 mm	28–35	[20]
Corn stover	Knife	3.2 mm	20	[17]
	Hammer	1.6 mm	14	[17]
	Hammer	1.6 mm	15	[19]
	Hammer	3.2 mm	28–35	[20]
Switchgrass	Hammer	1.6 mm	52	[19]
	Hammer	3.2 mm	28–35	[20]

[a] Sizes are screen openings, except when indicated with a percentage (representing weight percent of material below the stated size cutoff within a size distribution)

[b] Except for reference [20] and [21], energy values reported are discounted by no-load requirement

the energy used by attrition mill for achieving the same increase in specific surface area. The use of chemical pretreatment prior to milling has also been shown to improve milling efficiency, as was the case of wood disk milling in which sulfite pretreatment resulted in two folds increase of volumetric surface area using the same amount of milling energy [24].

Particle Size Influence on Cellulose Digestibility

Numerous studies have investigated the influence of particle size on cellulose digestibility, and several have reported no significant correlation, leading some to suggest that particle size is a weak predictor of susceptibility to enzyme hydrolysis [7, 11]. For instance, hydrolysis rates were similar for microcrystalline cellulose having average particle sizes of 0.038 and 0.090 mm [25] among crystalline (type I) cellulose separated by different mesh sizes (0.037 to 0.149 mm) [26] and among cotton linters having average particle sizes of 0.017 to 0.032 mm [12]. In contrast, it was reported that ball milling (15 min) of cotton linters improved digestibility [27], and compression milling of model cellulosic substrates (absorbent cotton, Avicel and Solka Floc) increased hydrolysis rates in proportion to the number of milling passes [28]. Because ball milling was reported to improve digestibility of crystalline components [29], and compression milling was shown to decrease CI and increase accessible surface area (to water and iodine) of the cellulose samples [28], particle size was not directly implicated in either case.

These studies have been done on pure cellulose, not on lignocellulose where the structural components (lignin and hemicellulose) and their organization are expected to play major roles [8]. Ball milling of poplar wood resulted in decreased CI and increased digestibility by enzyme hydrolysis [11]. It should be noted that samples were ball milled up to 8 days to observe the improvement in digestibility. Gharpuray et al. [15] found that ball milling up to 24 h did not change CI, and the maximum conversion achieved with enzyme hydrolysis only improved slightly with longer milling time (although still four times higher compared to unmilled samples). In either case, where chemical pretreatment was not employed, cellulose conversion was limited to less than 50%; in contrast, chemical treatment to remove lignin or hemicellulose achieved >80% cellulose conversion [11]. Rivers and Emert [30–32] investigated the effect of mechanical treatment on various lignocellulosic materials, expressly relating the treatment to particle size reduction. Hydrolysis of cardboard and newspaper (subjected to both dry and wet attrition milling), with average particle size ranges of 0.099 to 0.406 mm and 0.097 to 0.356 mm, respectively, did not result in conversion yields that correlate with particle size [31]. Similarly, bagasse conversion did not correlate with average particle size, although it increased with greater percentage of fines (particles passing through 0.053 mm screen); whereas, rice straw conversion (after subjecting to the same milling procedures) exhibited no correlation either to average particle size or percentage of fines [32]. Dry milling produced smaller average particle sizes and higher percentages of fines (by as much as factor of 10) in a wide range of cellulosic materials, but the difference did not translate to higher conversion to glucose or ethanol [30]. This suggests the importance of total accessible surface area, and depending on the material that is comminuted, the particle size may or may not directly relate to the surface accessible area (e.g., authors noted an increase in enzyme hydrolysis with purified and modified cellulose, but not with native ones).

In contrast, other studies found that reducing particle size had a positive effect on cellulose conversion. Cotton gin trash experienced higher in vitro dry matter digestibility (mimicking digestion by ruminants) when ground using a knife-type mill fitted with 0.5, 1.0, and 2.0 mm screen sizes [33]. Oilseed meals from sunflower and palm kernel exhibited ≈100% higher cellulose conversions when particle sizes were reduced from <0.5 mm (screen opening size) to a size distribution of 0.02–0.20 mm by freeze-milling [34]. After screening Red Oak dust to obtain four size fractions, the smallest size fraction (0.033 to 0.075 mm) yielded 50% and 55% more glucose than the largest size fraction (0.590 to 0.850 mm) following a 72-h hydrolysis of 10% and 13% solids slurry, respectively [35]. Corn stover saccharified with bacterial cellulase exhibited higher conversion with finer grinding, with 45% conversion achieved for the 100 and 200 mesh (0.152 and 0.075 mm) compared to just above 35% for the 8 and 10 mesh (2.0 and 1.68 mm) [36]. Disk and hammer milling of spruce chips resulted in an increase of cellulose conversion after 72-h enzyme hydrolysis. The increase was strongly dependent on volumetric surface area, with conversion rate increasing from 2% to 20% as the surface area increased by an order of magnitude [24]. Pulped softwood (mainly Kraft pulped Douglas fir) exhibited correlation of particle size with conversion, with refined particles containing 20% fines (passing through 0.15 mm screen) achieving 90% conversion as against 70% conversion for coarse fraction (retained on 1.20 mm screen) after 72-h cellulose hydrolysis [37]. The difference in the conversion rate (especially during initial hydrolysis) was largely attributed to the contribution of fines, which were observed to adsorb enzymes more readily and be hydrolyzed preferentially [37]. This agreed with the findings of Jackson et al. [38], who observed that fines in bleached softwood Kraft pulp were preferentially consumed during enzymatic hydrolysis. A possible reason is the higher pore volume in fines, as supported by Laivins and Scallan [39], who demonstrated linear correlation between fines content and pore volume.

With a few exceptions, numerous studies (summarized in Table 2) demonstrated that particle size affects lignocellulose conversion, with smaller particle size positively correlating with higher conversion. Rivers and Emert [30–32], whose results showed no definite correlation, may have introduced confounding factors by using different processes to achieve size reduction in their samples. For instance, although wet attrition milling did not reduce the particle size as much as did dry attrition milling, fiber swelling expectedly occurred. This effect may have been similar to the mechanical refining of pulp, which resulted in better accessibility to enzymes without change in fiber size [40]. Although Rivers and Emert [30] also reported an increase in fines especially with dry attrition milling, this did not translate to better conversion yields. These results were in contrast to softwood Kraft pulp, likely because softwood Kraft pulp already has reduced lignin, explaining its higher conversion (>70% even for the coarsest particle size) [37]. Lignin removal overshadowed influence of particle size, as shown by comparison of delignified softwood pulp with untreated samples or samples treated only for fiber swelling [4]. With lignin removed, cellulose conversion increased proportionately with amount of fines present in the sample. Thus, while evidence showed significant effect of particle size on lignocellulose conversion, without chemical pretreatment to alter lignin or hemicellulose content, the conversion rates were limited to <50%; whereas chemical modification achieved conversions of >70% regardless of particle size effect [4, 11, 15]. This implies that (1) mechanical pretreatment by itself is insufficient to effect realistic lignocellulose conversion yields, and (2) assessment of the effect of particle size on conversion to ethanol cannot be made independent of the necessary thermochemical pretreatment.

Table 2 Effect of particle size reduction on the enzymatic conversion of various cellulosic materials

Material	Particle size range[a]	Effect of size reduction on conversion	Reference
Purified cellulose			
Crystalline cellulose	0.038 and 0.090 mm (average)	No difference	[25]
	0.037 to 0.149 mm (opening)	No difference	[26]
Cotton linters	0.017 to 0.032 mm (average)	No difference	[12]
	Ball milled	50% increase in digestibility (organic matter weight loss)	[27]
Cotton and crystalline cellulose	Compression milled	>5× increase in initial rate (reducing sugar production)	[28]
Lignocellulose			
Poplar wood	Ball milled	8× increase in sugar (glucose and xylose) yield (72 hr)	[11]
Kraft pulped Douglas fir	<0.150 mm (20% *w*/*w*) to >1.20 mm (opening)	30% increase in glucose yield (72 h)	[37]
Spruce wood	<0.127 to >1.814 mm (opening)	1.5× increase in glucose yield (72 h)	[24]
Red oak dust	0.033 to 0.850 mm (opening)	50% increase in yield	[35]
Wheat straw	Ball milled	4× increase in reducing sugar yield (8 hr)	[15]
Bagasse	0.224 to 0.443 mm (average)	No difference[b]	[32]
Rice straw	0.107 to 0.465 mm (average)	No difference	[32]
Cardboard	0.099 to 0.406 mm (average)	No difference	[31]
Newspaper	0.097 to 0.356 mm (average)	No difference	[31]
Corn stover	0.075 to 0.152 mm, 1.68 to 2.0 mm (opening)	15% increase in glucose yield (24 hr)	[36]
Cotton gin trash	0.5 to 2.0 mm (opening)	40% increase in digestibility (IVDMD[c])	[33]
Oilseed meals	freeze milled	2x increase in neutral sugar yield (72 h)	[34]

[a] Particle size fractions are indicated as either "opening" (for screen size retaining the fraction) or "average" (for average particle size). In cases where sizes were not reported or reported in a format difficult to tabulate, the mode of size reduction is reported instead

[b] 7× increase in glucose yield (48 h) reported with 7× increase in fines (percent *w*/*w* passing through 53 μm screen)

[c] IVDMD (in vitro dry matter digestibility): an in vitro method for measuring digestibility in ruminant digestion

Particle Size Effects on Thermochemical Biomass Pretreatments

To achieve economic levels of lignocellulose conversion, a thermochemical pretreatment step is deemed necessary for enzymatic hydrolysis to proceed effectively [41, 42]. Therefore, in assessing the desired feedstock particle size for lignocellulose conversion, it is essential to know the effects of particle size on thermochemical pretreatment processes. A number of pretreatments were shown to have comparable outcomes [42] and economics [43], but there is a lack of systematic assessment on how different feedstock characteristics impact across various pretreatment technologies. Studies made on particle size effect have been generally confined to a single pretreatment method, and evaluations of pretreatment

effectiveness often vary among different researchers. The closest to such a systematic study was done by Gharpuray et al. [15], who studied the effect of combining different physical preprocessing (to increase specific surface area) with different subsequent chemical treatments on the digestibility of wheat straw. Analysis of their results, in terms of relative hydrolysis extent (after 8 h) and percent change in lignin content of the feedstock, indicated none of the mechanical pretreatments improved hydrolysis extent beyond what was independently achieved by the chemical treatments. Neither were the effects of milling processes on lignin removal (with subsequent chemical treatment) consistent; milling enhanced lignin removal in peracetic acid treatment but reduced it in caustic soda treatment.

Among the leading candidates of lignocellulose pretreatment processes are dilute acid, steam explosion, ammonia fiber freeze explosion (AFEX), liquid hot water (LHW), and lime treatment [42]. In the following sections, we will consider them with respect to particle size requirement, some (e.g., dilute acid and steam explosion) more than others because they have been more extensively studied.

Dilute Acid Pretreatment

This is one of the more mature pretreatment methods, having already found commercial application, for example in furfural production [44]. Different acids have been investigated, although sulfuric acid has been most widely used [42]. The treatment involves mixing biomass with dilute concentrations of acid (≤2%, typically 0.5% to 1% *w*/*v*) and bringing the mixture to a temperature of 160–220°C. The primary objective of such a process is the breakdown of hemicellulose to oligomeric and monomeric (i.e., xylose) products [42].

Kim and Lee [45] used a diffusion model to examine the diffusivity of sulfuric acid in different biomass and showed that intraparticle diffusion can be relevant depending on the reaction time for the pretreatment (i.e., time to complete hemicellulose hydrolysis). Their experiments demonstrated that acid took three times longer to reach 70% penetration of biomass cut randomly to 1 to 2 cm compared to powdered samples (0.853 to 1.20 mm sieve size). They proposed that the critical particle size (the size above which diffusion becomes important in the pretreatment process) can be found from the square root of the ratio of diffusivity (D_e) to the kinetics rate constant of the pretreatment process (k): critical diameter$=(D_e/k)^{0.5}$. This equation did not account explicitly for the structure or composition of the cellulosic material, even though it is likely that pore volumes are affected by such factors as lignin composition or physical changes (other than size reduction) that could result from grinding. Moisture content appeared not a significant factor, as it was shown that the duration of presoaking did not affect the estimates for the diffusivity constant [45].

Critical particle sizes were calculated for four biomass feedstocks (switchgrass, straw, stover, and wood) according to this model and with D_e values obtained from [45]. Values for k, based on the simple series model for hemicellulose hydrolysis not separately accounting for hemicellulose fractions [46, 47], were obtained from literature sources [48–51]. The critical values calculated ranged from 1.0 to 3.2 mm diameter (spherical geometry) or 0.3 to 1.1 mm thickness (plate geometry) (Appendix).

Experimental studies reported slightly higher critical values than were suggested from these calculations. Singh et al. [52] showed no effect of bagasse particle size <0.5 mm on reducing sugar yield. Springer [53] showed that with high liquor to wood ratio, hardwood biomass equally responded to treatment whether the thickness was 0.38 or 2.54 mm. However, at low liquor to wood ratio, insufficient impregnation was

observed for the 2.54 mm thickness. This is supported by Yat et al. [54], who found various wood feedstocks exhibited comparable xylose formation and degradation kinetics whether prepared as <0.599 mm screenings or as 0.853 to 1.68 mm particles. Working with a pilot-scale reactor, Hsu et al. [55] demonstrated that switchgrass digestibility improved from 60% to 80% when particle size decreased from 10 to 3 mm. However, in one experiment wherein pretreated materials of different particle sizes were homogenized using a laboratory blender and then hydrolyzed, no difference in enzyme digestibility was observed [55]. This could suggest that differences in particle size exert more influence towards enzyme hydrolysis, rather than towards the efficacy of acid pretreatment.

Steam Explosion

Steam explosion is a relatively mature technology that has been used in wood pulping such as in the Masonite process [56]. It involves applying high pressure steam to biomass for a few minutes, in most cases with the addition of acid catalysts such as SO_2, and then quickly venting the steam and discharging the biomass to a bigger vessel for rapid flash cooling [42]. Because of its explosion component, it is often considered a partly mechanical process, capable of biomass disintegration [23]. On the other hand, it has been demonstrated that explosion per se, whether causing particle disintegration or not, has limited effect on the enzymatic digestibility outcome [57]. A most likely mechanism for steam explosion's effect is removal of hemicellulose [42]. This is primarily the reason for employing acid catalysts, usually SO_2, which reacts with water in the biomass interstitial spaces to form sulfuric acid that then catalyzes hemicellulose degradation [58].

There have been studies reporting that larger particle size can be beneficial for steam explosion pretreatment. Cullis et al. [59] who pretreated softwood (Douglas fir) milled to three particle sizes (<0.422 mm screenings, 1.5×1.5 cm and 5×5 cm) by SO_2 catalyzed steam explosion, observed that the largest particle size suffered less from pretreatment severity (i.e., higher solubilized carbohydrate content and lesser furan degradation products) and had the highest cellulose recovery. On the other hand, the smaller particle sizes had more hemicellulose and lignin solubilized. After subsequent refining to reduce particles to even finer size by plate milling (0.178 mm gap), the initially bigger particle size showed greater lignin removal by peroxide washing and a greater extent of enzymatic hydrolysis, which the investigators attributed to the smaller degree of lignin redeposition resulting from treatment severity.

This finding was similar for steam exploded pine (another softwood), in which the largest of three size fractions (8 to 12 mm) exhibited higher cellulose recovery and also higher solubilized hemicellulose [60]. Conversion of cellulose to glucose was only slightly higher in the larger particle size fractions than the smaller particle size fractions. However, the total recovered glucose (which also accounts for the solubilized glucose during steam explosion) was substantially higher when the largest particle size was used [60]. With a herbaceous feedstock (*Brassica carinata* residues), it was different because although cellulose recovery was still higher for the 8- to 12-mm fraction, the smaller particle size performed better during enzyme hydrolysis (100% for both fractions 5 to 8 mm and 2 to 5 mm, while 85% for 8 to 12 mm) under one of the pretreatment conditions [61]. This suggested that lignin condensation, which was critical factor in the case of softwood, was not as influential in the herbaceous feedstock. Overall, the larger particle size still gave a higher maximum glucose yield (>80%, compared to the smaller particle sizes with yields ≤70%) [61]. Steam exploded hardwood (poplar) exhibited no difference in either enzyme

digestibility or ethanol yield with simultaneous saccharification and fermentation (SSF) between two disparate particle size fractions, 2 to 5 mm and 12 to 15 mm [62], indicative that the severity effect of particle size (i.e., smaller particles enhanced lignin condensation and subsequent recalcitrance to enzyme hydrolysis) was more critical in softwood feedstock.

Other Pretreatments

Liquid hot water (LHW) is closely similar to dilute acid treatment, except that compressed water is used, thus a more neutral pH is maintained. Negro et al. [62] subjected hardwood (poplar) having two different particle size ranges (2 to 5 mm and 12 to 15 mm) to LHW pretreatment and found no difference in their enzyme digestibility and ethanol yield with SSF. Similarly, further comminution of aspen chips (starting size >3 mm) and bagasse (starting size <1.4 mm) did not result in higher cellulose conversion or ethanol yield after undergoing LHW treatment [63].

AFEX or ammonia fiber/freeze explosion involves submerging biomass in liquid ammonia, often at moderate temperatures (60°C to 100°C), then explosively releasing pressure. It is known to affect both chemical and physical characteristics of biomass, via alterations in its hemicellulose and lignin content [64]. Based on their work on corn stover, Chundawat et al. [65] demonstrated that grinding of biomass from an initial size of 0.5 to 0.85 mm down to <0.15 mm before AFEX treatment resulted in an increase of 10% to 15% higher glucan conversion (72 h) compared to grinding after AFEX treatment, but only in the case where the pretreated corn stover was washed prior to hydrolysis. The effect of washing after AFEX treatment was attributed to the removal of inhibitors (possibly lignin degradation products) which was more effective when pretreating at a smaller particle size. They further showed that grinding by itself resulted in 15% to 20% higher glucan and xylan conversion in untreated corn stover, but did not quantify the potential increase due to grinding for AFEX treated corn stover. AFEX pretreatment of corn fiber (obtained from wet-milling of corn kernels) exhibited no difference in the glucose or reducing sugars released between unground fiber and fiber ground through a 0.422-mm sieve in a Wiley mill [66], probably because lignin content is insignificant in corn fiber.

Another basic pH pretreatment is lime treatment, which involves contacting a lime-water slurry with biomass under relatively mild temperatures (ambient to 100°C) for extended periods (hours to days), but also under higher temperatures for shorter periods [42]. Chang et al. [67] showed that among five different size fractions (mesh numbers 4 to10, 10 to 20, 20 to 40, 40 to 80, and −80) obtained from switchgrass, all particle size fractions passing through 20 mesh (<0.853 mm) did not result in further increase of glucose yield after 3 day hydrolysis. Related to this, dilute alkali has also been used as a chemical pretreatment for biomass. In a study investigating the enzymatic hydrolysis of corn stover treated with dilute NaOH (0.1–1.0 N), it was found that among three grind sizes (particles passing through 2.0, 0.707, and 0.25 mm screens) there was no further benefit in grinding to a smaller particle size than what went through the 2.0 mm screen size [68].

The results of these studies on pretreatment effectiveness as a function of particle size are summarized in Table 3. The maximal particle size, defined as the size below which no further increase in pretreatment effectiveness (as judged from subsequent conversion yields) was observed, ranged from a low of <0.15 mm to a high of 50 mm. Steam explosion exhibited larger maximal particle sizes (>10 mm), dilute acid and base (AFEX, lime and dilute alkali) treatments had smaller maximal particle sizes (<3 mm), and LHW maximal particle sizes were intermediate of these two pretreatment groups.

Table 3 Maximal particle sizes or biomass sizes below which no further increase in pretreatment effectiveness were observed in various studies

Pretreatment	Feedstock	Effectiveness measure	Maximal particle size[a]	Reference
Dilute acid	Switchgrass	Enzymatic hydrolysis	<3 mm	[55]
	Various woods and switchgrass	Xylose production and degradation	1.68 to 0.853 mm	[54]
	Hardwoods	Enzymatic hyrdrolysis	2.54 mm (chip thickness)	[53]
AFEX	Corn stover	Enzymatic hydrolysis	<0.15 mm	[65]
	Corn fiber	Enzymatic hydrolysis	Unground fiber	[66]
Liquid hot water	Bagasse	Enzymatic hydrolysis and SSF	<1.4 mm	[63]
	Aspen (hardwood)	Enzymatic hydrolysis and SSF	>3 mm	[63]
	poplar (hardwood)	Enzymatic hydrolysis and SSF	12 to 15 mm	[62]
Steam explosion	Douglas-fir (softwood)	Enzymatic hydrolysis	50 mm (chip thickness)	[59]
	Brassica carinata (herbaceous residue)	Overall glucose yield	8 to 12 mm	[61]
	Pine (softwood)	Overall glucose yield	8 to 12 mm	[60]
	Poplar (hardwood)	Enzymatic hydrolysis and SSF	12 to 15 mm	[62]
Lime treatment	Switchgrass	Glucose yield (3 day hydrolysis)	<0.853 mm	[67]
Dilute alkali	Corn stover	Enzymatic hydrolysis	2.0 mm	[68]

SSF simultaneous saccharification and fermentation

[a] Sizes are values of screen openings used to obtain different biomass fractions unless indicated as chip thickness

Conclusions

Evidence from literature indicated that particle size directly influences enzymatic digestibility of lignocellulose. However, the contribution of particle size was less than that of other factors, especially removal of lignin and hemicellulose. Particle size, while an indicator of surface area, may be poorly correlated with total accessible volume, which likely has a greater impact on enzyme reaction kinetics. Lignin and hemicellulose contents and their removal, on the other hand, hint at structural changes in the cellulose matrix that could aid in cellulose digestion. Physical size reduction was able to increase conversion efficiencies up to <50%; whereas chemical modification achieved conversion efficiencies of >70%, regardless of biomass particle size. This suggests that (1) mechanical pretreatment by itself is insufficient for attaining economically feasible conversion yields, and (2) assessment of particle size is dependent on the thermochemical pretreatment employed for the lignocellulose conversion process.

Among thermochemical pretreatments, dilute acid treatment has been widely studied in terms of mechanism. Theoretical critical particle sizes were obtained based on diffusion and reaction kinetics constants in the range of 1 to 3 mm diameter. This appeared in agreement with experimental data suggesting maximal particle sizes (i.e., particle sizes below which no increase in pretreatment effectiveness were observed) of <3 mm (sieve opening). Maximal sizes appeared dependent on the pretreatment employed, with the range of

maximal sizes decreasing in the order of steam explosion (highest, >10 mm), LHW (intermediate, 1 to 15 mm), and dilute acid and base pretreatments (lowest, <3 mm). This observation has implications on the selection of a pretreatment process, especially since an economic comparison of these pretreatments have not accounted for feedstock milling cost [43]. Thus, the high energy consumption of comminution as discussed in the "Feedstock Size Reduction—Energy Requirement" section could be one of the "clinchers" in the selection of a particular pretreatment process.

It also appeared that maximal particle sizes are sensitive to biomass feedstock; herbaceous and grass have maximal particle sizes <3 mm, and woody biomass generally >3 mm. Such a difference in terms of response to size reduction is significant, considering herbaceous and grassy biomass requires at least half the energy required by woody biomass to reduce to the same size (Table 1). It must be noted that these trends were inferred from different studies; and so, their generality is limited by investigators' choice of methods in assessing pretreatment effectiveness and of levels of experimental treatments (most importantly, the range of particle sizes). These trends should motivate a more systematic study of the particle size effects under different pretreatment processes, using various feedstocks.

Appendix

Critical particle sizes for dilute acid pretreatment of different biomass calculated according to the proposed model of Kim and Lee [44]. Sulfuric acid concentration=0.5% *w*/*w*; temperature=180°C. Critical thickness and diameter are for plate and spherical geometry, respectively. Values for k were obtained using the Arrhenius expression model for hemicellulose degradation (single hemicellulose fraction approximation) with parameters obtained from published sources:

$$k = A_{\mathrm{o}} c^{n} e^{(-E/RT)} \tag{1}$$

A_{o} preexponential factor (c=0)
c acid concentration (percent *w*/*w*)
n exponent parameter determined experimentally
E activation energy
R gas constant
T temperature

Critical size (mm)						
Biomass	D_e (10^{-5}cm^{2}s^{-1})	k (10^{-2} s^{-1})	$(D_e/k)^{0.5}$	Thickness	Diameter	k parameters source
Corn stover	2.27	0.80	0.53	1.06	3.19	[49]
Bagasse	1.48	5.83	0.16	0.32	0.96	[48]
Hardwood	1.24	1.69	0.27	0.54	1.63	[50]
Rice/wheat straw	9.49	7.10	0.37	0.73	2.19	[51]

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