



Novel micronized woody biomass process for production of cost-effective clean fermentable sugars

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ABSTRACT

Thermo-chemical pretreatments of biomass typically result in environmental impacts from water use and emission. The degradation byproducts in the resulting sugars can be inhibitory to the activities of enzymes and yeasts. The results of this study showed that combining existing commercial comminution technology can reduce total energy consumption with improved saccharification yield while eliminating chemical use. Impact mill was found to be the most efficient milling for size reduction of forest residual chips from ca. 2 mm to a specific value below 100 μm . The further micronization effectively disrupted the recalcitrance of the woody biomass and produced the highly saccharifiable substrates for downstream processing. In addition, extrusion can be integrated into a clean cellulosic sugar process for further fibrillation in place of the conventional mixing processing. The highest energy efficiency was observed on the impact-milled samples with 0.515 kg sugars kWh^{-1} .

1. Introduction

Woody biomass is a lignocellulosic material with abundance and renewability and has been considered as a potential feedstock for second-generation biofuel production. As with all cellulosic sugar-based biofuel processes, the first step is to expose the cellulose macromolecules to enzymes to enhance digestibility using a pretreatment process. Many pretreatment methods have been developed to disrupt the structure and morphology of woody biomass (Haghighi Mood et al., 2013; Chen et al., 2018; Liu et al., 2018). Wood particle characteristics, such as cellulose crystallinity and particle size, have been found to enhance digestibility of lignocellulosics with a variety of pretreatment methods (Barakat et al., 2014). However, the pretreatment efficacy of woody biomass is heavily dependent on the controlling mechanisms.

Among all the pretreatment methods, bio- and thermo-chemical methods rely upon exposing cellulose by delignification and removal of the hemicelluloses. These methods include bisulfite, dilute acid, and alkaline pretreatment processes. The active agents released from the acids and bisulfites may break the ether bonds that link hemicelluloses and lignin with cellulose through hydrolysis, thereby improving accessibility of cellulose to enzymes and making conversion more amenable (Sjostrom 1993). However, these severe pretreatment methods not only chemically modify the native cellulose structures but

also result in substances in the resulting sugars that are detrimental to downstream biological processing (Klinke et al., 2004; Maurya et al., 2015). Pretreatment severity affects the degree to which monosaccharides are degraded into fermentation inhibitors such as acetic acid, furfural, and 5-hydroxymethylfurfural (Maurya et al., 2015; Boussaid et al., 1999). In some cases, a separate detoxification or washing process is required to recover enzyme activity.

In contrast to the chemical pretreatments, mechanical size reduction employs grinding equipment to reduce coarse woody biomass into fine particles beneficial to enzyme accessibility. Mechanical pulverization of lignocellulose may improve cellulose accessibility and increase efficiency in producing fermentable sugars without inhibitor formation. In addition, a fully mechanical pretreatment process would facilitate a depot-style production of fermentable sugars for fermentation or catalytic conversion to chemical intermediates. However, mechanical size reduction of wood particles and disruption of the fiber cell ultrastructures is an energy-intensive process most typically powered by electricity.

In recent decades, various mechanical milling machines such as ball, disc, hammer, knife, roll, and attrition mills, have been employed for size reduction of wood biomass (Barakat et al., 2014). Among mechanical pretreatments disc milling resulted in the highest specific energy consumption with 200–400 kWh t^{-1} , followed by the hammer mill

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with 90–130 kWh t⁻¹ and finally the knife mill with 80–120 kWh t⁻¹ to produce final particle sizes of 1–2 mm (Schell and Harwood, 1994). The other study (Repellin et al., 2010) also found that mechanically grinding spruce and beech at a given 500 µm screen size consumed electrical energy of 750 kWh t⁻¹ and 850 kWh t⁻¹, respectively. For many processes, efficient mechanical size reduction can help promote biomass preparation for efficient biofuel production. However, to achieve a satisfactory sugar yield, the existing single pulverizing equipment undoubtedly would require extreme grinding conditions accompanied by high energy consumption. This high electrical energy consumption would hamper the economic and technical feasibility of the mechanical pretreatment process, presenting a major challenge for scaling-up to commercial operations.

Extrusion is a mechanical process widely used in the polymer and food processing fields. The high-shearing force and pressure in twin-screw configurations assist in rapid heat generation and effective mixing, thereby facilitating short residence times. The mechanical pulverization and fibrillation from the extrusion process can be expected to afford enhanced interactions between enzymes and wood particles, promote efficient water diffusion, and increase the accessibility of cellulose to enzymatic attacks (Zheng et al., 2014). Extrusion practiced in a continuous processing mode with high solid concentrations could play a partial role in reducing overall energy consumption and may show some advantages over other mechanical pretreatments, while achieving high continuous throughput and good compatibility with other equipment in a biorefinery.

While emphasizing the overall equipment efficiency and scalability of the overall process for industrial biomass conversion, there is still a lack of supporting information as to using softwood forest residuals for producing second generation biofuels. While a single milling operation is difficult in achieving a suitable performance, a carefully selected combination of different milling technologies may reduce overall energy consumption and enhance economic viability. Our previous research works concluded that the multistep milling process could decrease the amount of energy consumption and significantly improve net energy use in mechanical pretreatment (Liu et al., 2017; Gu et al., 2018).). However, opportunities exist to further explore mechanical pretreatment processes to reduce energy consumption using a combination of industrial milling equipment for particle size reduction for cellulosic biofuel production.

The objective of this research is to explore the feasibility of pilot-scale processes for mechanical pretreatments as a pulverization strategy to micronize woody biomass into highly hydrolysable substrates. Initially, the stepwise pulverization process of woody biomass is developed to determine a desirable particle size from a trade-off between energy consumption and total sugar yield. The study investigated the effects of pilot scale coarse milling, fine milling/amorphization, and extrusion fibrillation on particle morphology, cellulose crystallinity, sugar yield, and energy consumption. Different micronizing mechanisms of hammer, impact, and tandem-ring mills were used to disrupt lignocellulosic structures and reduce particle size resulting in different energy consumption levels. The specific research objectives were to: (1) develop a grinding scheme to achieve desirable material characteristics with acceptable energy consumption levels; (2) evaluate the effects of extrusion fibrillation on the sugar yields and energy consumption in the course of cellulose hydrolysis. Therefore, it would be of practical importance to ensure the efficient utilization of the current equipment for addressing a technically feasible pretreatment process with forest residuals for biofuel production.

2. Materials and methods

2.1. Mechanical pretreatment for particle size reduction

Douglas-fir (*Pseudotsuga menziesii*) chips obtained from a local sawmill (Vaagen Brothers Lumber Inc., Colville, WA) were used in this

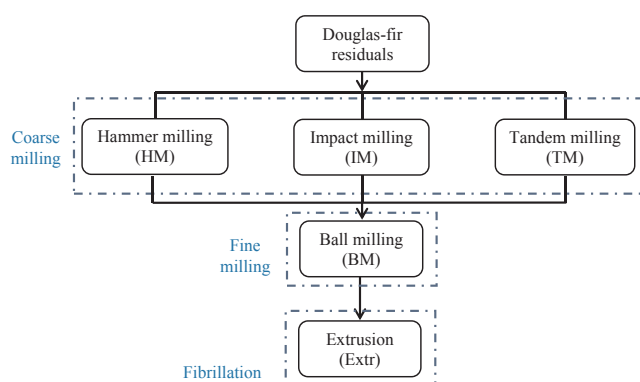


Fig. 1. Flow diagram of stepwise pulverization process on woody biomass.

study. The wood chips with the initial moisture content of 51% (wet weight base) were further separated using a vibrating screen (Model 580, Serial 4095-76, Black Clawson, Everett, WA) with 25.4 mm aperture size. The chip samples were subsequently stored in an environmental moisture-conditioned room with equilibrium moisture content (EMC) of 12% before hammer mill pretreatment. In addition, Douglas-fir grindings with a geometric mean diameter of 13.42 mm and a moisture content of 10% were reduced into particles with geometric mean of 1.29 mm for impact milling (IM) and tandem milling (TM) pretreatments.

Mechanical pretreatments on wood chips were conducted according to the stepwise pulverization process as shown in Fig. 1. The first step of HM, IM and TM was used for coarse milling of forest residues into different initial particle sizes, followed by fine milling of ball milling (BM) to disrupt the wood structure, and finally applied by fibrillation of extrusion processing. The samples for each milling were re-equilibrated in sealed plastic bags for further treatments.

- Hammer milling** – Wood chips were ground with a hammer mill (Prater, 14.9 kW) using a 1.29-mm screen size. A 50 kg sample of the Douglas-fir wood chips was manually fed into the hammer mill at a feed rate of 17.5 kg/min. After milling, the resulting particles were sieved with a Ro-Tap Shaker (The W. S. Tyler Company, Cleveland, Ohio) for 10 min to analyze the size distribution.
- Impact milling** – Wood chips with a geometric mean of 1.29 mm were pulverized by an impact mill (Zhenzhou Tianyuan Environmental Protection Machinery Co. Ltd., China) with 30 min milling time to a nominal 2 mm wood particle.
- Tandem ring milling** – Wood chips with geometric mean of 1.29 mm were pulverized by a tandem ring mill (HV-70Av), developed by Akita Prefectural University (Takahashi et al., 2014). This novel vibratory mill was equipped with the twin motors with 100 kW each. The loading chip amount in each chamber was 48 kg in trials and the milling time was 30 min.
- Ball milling** – The wood powders resulting from HM, IM and TM were further micronized using a large planetary ball mill (Across International, PQN 20L) with 5-liter capacity stainless steel containers and mixed steel balls of $\phi 5$ mm, $\phi 10$ mm, and $\phi 20$ mm for four times of 30, 60, 90, 120 min, respectively. The jars were loaded with a ball-to-material ratio of 18.3:1. The rotation speed of the disc was maintained at 270 rpm with one rotation direction. Five types of materials for each milled material were generated by these treatments: not ball-milled materials (BM0), ball milled for 30 min (BM30), and ball milled for 60 min (BM60), ball milled for 90 min (BM90), and ball milled for 120 min (BM120), total types of the materials are $3 \times 5 = 15$.

2.2. Extrusion refining (Extr)

The extrusion treatment was designed to reduce the severity of ball milling requirements. The ball-milled samples were continuously fed to the twin-screw extruder (C. W. Brabender, TSE 20/40, length/diameter of screws, L/D = 30). Extrusion was conducted at 50 rpm with a discharging rate of approximately 20 g per minute. The moisture content of the ball-milled samples was first adjusted to be 55% on a wet weight basis by adding additional buffer solution. The prepared samples were subsequently fed to the co-rotated twin-screw extruder for extrusion fibrillation. Three screw configurations (2 kneading and 1 reverse screw elements represented by *2m-1r*, 1 kneading and 3 reverse screw elements represented by *1m-3r*, and 3 kneading and 1 reverse screw elements represented by *3m-1r*, respectively) and three processing temperatures (50, 130, 150 °C) were employed to influence the flowability of the wood powders and the torque level in order to access the utility of extrusion in improving the digestibility of the ball-milled wood powders.

2.3. Specific energy consumption (SEC) measurement

The energy consumption of the milling process was measured by a Fluke 1735 Power Logger (Fluke Corporation, Everett, WA). The electricity data of current, voltage, and power factor as well as derived active power and active energy were recorded during the milling processing. The electricity consumption from the empty run (without material charge) preceding and trailing the milling was also collected to obtain a baseline for net specific energy consumption calculation, the power above which was assumed as being consumed on breaking down the particles in contrast to running the mill itself. The total specific energy consumption was calculated by integrating the area under the active power demand curve for the total milling time used in the process. The net specific energy consumption, E_p (kWh kg⁻¹), was calculated by the following equation:

$$E_p = \int_0^t (P_t - P_0) dt / m \quad (1)$$

where P_t , P_0 , t and m are the power consumption at time t , the average power consumed under empty run, and the mass charge (kg wood) to be pulverized, respectively.

The specific mechanical energy (SME) from extrusion is calculated by the equation (Godavarti et al., 1997):

$$SME = \left(\frac{\text{kWh}}{\text{kg}} \right) = \frac{(\text{Torque} - \text{Friction Torque}) \times N \times 0.00164}{200 \times 250 \times m_f} \quad (2)$$

where N , 200, 250, 5.6, and m_f are the screw speed (RPM), maximum allowable torque, maximum allowable screw speed, power of the drive motor at a rated screw speed of 250 rpm, and mass flow rate (kg s⁻¹), respectively.

2.4. Characterization

2.4.1. Morphology

Morphological analysis of samples was performed through SEM (FEI Quanta 200F, field emission gun, high vacuum, ETD detectors, FEI Company, Hillsboro, Oregon, USA) after sputter coating with gold.

2.4.2. Particle size and distribution

The milled wood samples were first dispersed in distilled water at a 0.5 wt% loadings and were further sonicated for 10 min with 20% amplitude (Branson, Switzerland) to break particle agglomerates. The median particle size and distribution of the sonicated wood powders were immediately analyzed using a laser diffraction particle size analyzer with a Hydro LV wet sample dispersion unit (Mastersizer 3000, Malvern instrument, UK).

2.5. X-ray diffraction analysis

An X-ray diffractometer (XRD) (Miniflex 600, Rigaku, Japan) equipped with a Cu K α ($\lambda = 0.154$ nm) radiation source was used to perform XRD analysis of the samples at 40 kV and 15 mA in the range of 10–40° at the scanning speed of 1° per min and step size 0.005. Each sample was measured at least two times for accuracy.

The crystallinity index (CrI) of the samples was calculated using the Segal equation in the following form (Segal et al., 1959):

$$CrI = \frac{I_{002} - I_{am}}{I_{002}}$$

where I_{002} and I_{am} are the intensity of the 002 peak at $2\theta = 22.4^\circ$ and the intensity of the amorphous portion of cellulose at 2θ close to 18.5° .

2.6. Enzymatic hydrolysis

The prepared samples were hydrolyzed with Cellic CTec2 and HTec2, complementarily provided by Novozymes North America (Franklinton, NC). The CTec2 cellulase activity was 150 FPU mL⁻¹. Specifically, the hydrolysis suspension was prepared by adding the 0.5 g oven dry sample, 5 wt% of the 9:1 CTec and HTec mixture based on the dry wood, and 100 μ L of 2% sodium azide solution into a 20 mL glass scintillation vial, filling 0.1 M sodium citrate buffer solution to 10 mL, and then adjusting pH to 4.8 using a NaOH solution. The prepared samples were hydrolyzed for 72 h on an orbital shaker at a shaking frequency of 180 rpm inside an incubator at 50 °C. At the end of the hydrolysis, the vials were placed into the boiling water for 15 min to deactivate the enzymes. Six replicates were carried out for the pre-treatment samples (2 replicates/run and 3 runs for each sample).

2.7. Sugar analysis

The compositional analysis of the samples was conducted according to a two-step acid hydrolysis. A 300 ± 10.0 mg oven-dried raw sample was loaded in a 100 mL pressure tube and incubated with 3.00 $\pm$ 0.01 mL of 72% sulfuric acid at $30 \pm 3^\circ\text{C}$ for 1 h and stirred every 5–10 min. Then, 84.00 $\pm$ 0.04 mL of water was added into the mixture and autoclaved for 1 h at 121 °C.

The monomeric sugar concentrations before and after hydrolysis were measured by an ionic chromatography system (Dionex ICS-3000), equipped with an ED40 electrochemical detector and AS40 autosampler (Dionex Corp.). The 10 μ L of sample solutions were taken and then injected into the system to quantify the concentrations of monosugars. In the system the guard columns (CarboPac PA20 4 $\times$ 50 mm) and analytical columns (IonPac AS11-HC, 4 $\times$ 250 mm) were used to separate sugars in the solution. The degassed E-pure water, 50 mM and 200 mM NaOH solution was taken as the eluent mobile phase at a flow rate of 0.5 mL/min. The detector was maintained at a pH of 10.4 and the autosampler was used for continuous running. Owing to the co-elution of xylose and mannose, the two sugars were reported as one value as xylan/mannan or xylose/mannose. Six replicates were carried out for each pretreated sample. Analysis of variance (ANOVA) with the Fisher's LSD test were performed using SPSS 22 (IBM Corporation, NY, USA) to test the significance of treatments. All significant levels are set at 0.05. The results were reported as their means $\pm$ SD.

Enzymatic hydrolysis efficiency was represented by the obtained carbohydrate conversion and calculated as follows:

$$\text{Carbohydrate conversion (\%)} = \frac{\text{Amount of sugar released in hydrolysate}}{\text{Amount of sugar in raw material}} \times 100 \quad (3)$$

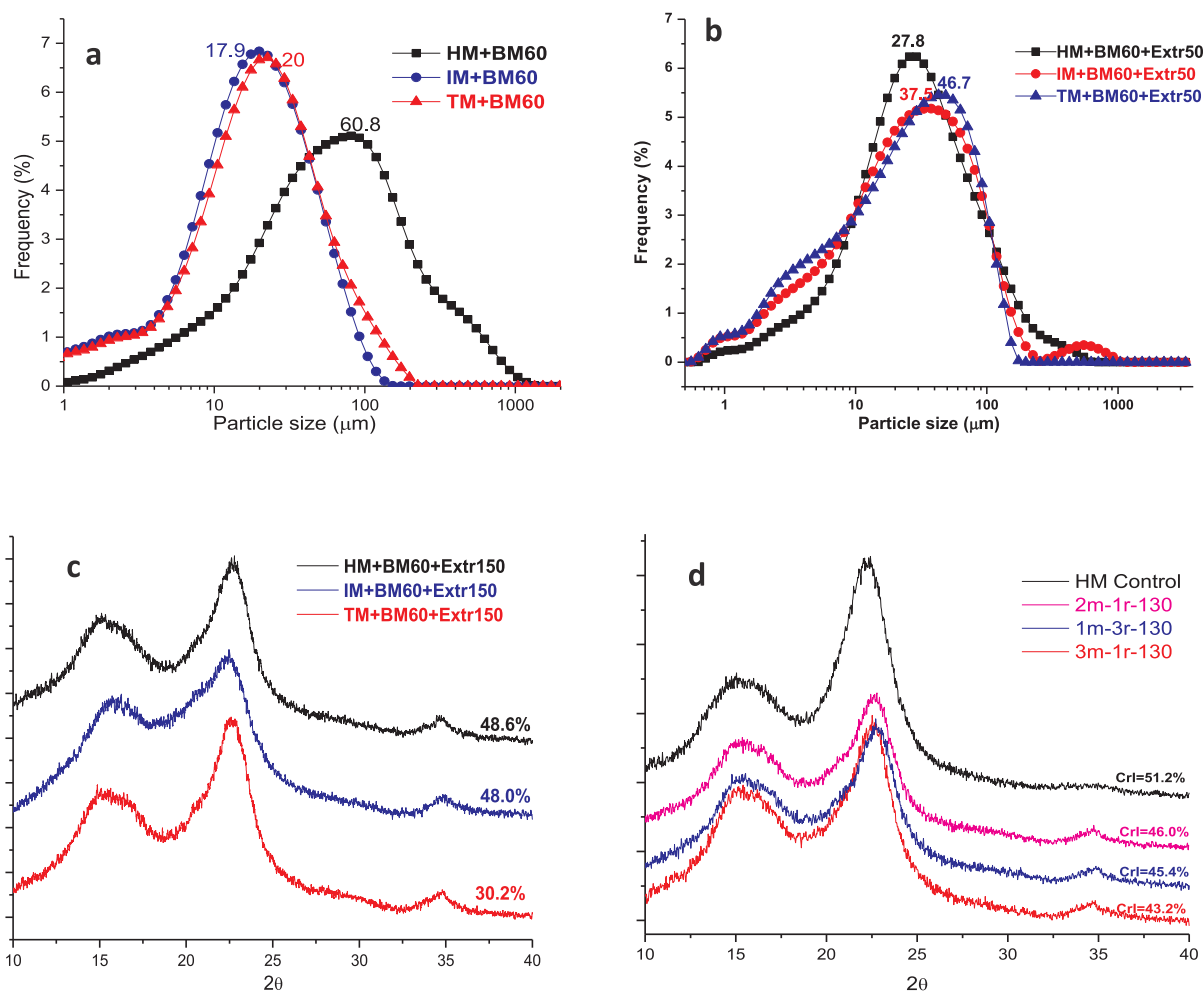


Fig. 2. Particle sizes of milled and extruded samples.

3. Results and discussion

3.1. Particle morphology

3.1.1. Mechanical milling

Effective separation and fractionation of the different lignocellulosic biomass components is critical to mechanical pretreatment. Particle morphologies from different milling processes correlate to different specific surface areas and hence may influence the accessibility of cellulose by enzymes. The hammer, impact and tandem mills employ different grinding mechanisms that generate different particle morphologies.

Their different particle morphology was observed using scanning electron microscopy (SEM). The hammer and impact mills generated larger fibers or fiber bundles, with the relatively smooth surface for hammer milled samples, while the tandem ring-milled samples appeared more flake-like, roundish and much rougher on surfaces. The larger fiber bundles in the hammer milled samples may originate from the repeated crushing of the mounted hammers, the collision with the chamber walls, and the inter-particle impacts. On the other hand, the tandem mill appeared to significantly pulverize wood fibers, contributing to consistent and strong impacts on the cell walls. Whatever the exact mechanism, this vibratory milling produced very short fiber bundles with increased surface roughness. Our previous studies indicated that the fracture modes of the cell walls differed by milling method and duration (Jiang et al., 2016, 2017). Consistent with the research of others (Vaidya et al., 2016), the microscopic observations

here confirmed that the fiber cell walls were completely delaminated into fiber fragments with narrow size distribution using a tandem mill.

Ball milling was adopted for further size reduction of the hammer milled materials. Morphological analysis of ball milled samples revealed a significant influence on the fiber morphology. During 30 min of ball milling the diameter of the fibers remains practically unchanged, however, with 60 min of milling, the average fiber length decreased. For the impact and tandem milled samples, 60 min of ball milling deconstructed the fibers and generated small particles with micro-scaled dimensions. The primary impact of the ball milling appears to be in splitting and delamination of the large fiber bundles along and across the fiber direction. The aspect ratio and circular form of impact and tandem milled samples clearly indicated this action. The 60 min of ball milling, effectively destroyed the fibrous structure and resulted in the formation of amorphous microparticles. Recent studies have also shown that the ball milling can effectively break chemical bonds between lignin and hemicellulose while decreasing particle size (Liu et al., 2017). However, this milling method is energy-intensive compared to the other milling processes.

3.1.2. Extrusion refining

Extrusion processes use mixing and shear forces to generate heat. When conducted on a wood slurry, this action may enable moisture to penetrate particles and alter particle morphology. The effect of extrusion on the morphology of the milled samples was seen from SEM images. After extrusion processing, the samples appeared more chapped and cracked with coarser and porous structures. Specifically, the wood

structure of hammer-milled samples was disintegrated into a ribbon and network of sponge-like morphology. The wood particles from impact- and tandem-milled samples showed smaller fiber agglomeration resulting in large roundish structures with coarse surfaces.

The surface morphologies showed greater disruption and loosely spherical and porous structures as extrusion temperature increased. When the temperature reached 150 °C, the individual particles were less than 100 µm in diameter and less regular fibrous structures than those processed at 50 or 130 °C. The fibrillated samples were morphologically finer and evened compared to the other samples at lower temperatures.

3.2. Particle size and crystallinity

3.2.1. Mechanical milling

Particle size reduction increases specific surface area (SSA) of lignocellulosic biomass and is useful in increasing hydrolysis efficiency. The study found that particle size reduction from 590–850 to 33–75 µm nearly doubled sugar conversion rates (Dasari and Berson, 2007). Conversion rate increased up to 70–90% for median particle sizes less than 40 µm (Hoeger et al., 2013). Particle size distributions of hammer, impact, and tandem milled Douglas-fir chips are shown in Fig. 2a. The median particle diameters of the impact and tandem milled samples were about 30–35 µm, while the hammer mill produced particles of ca. 120 µm. Subsequent ball milling on the hammer milled material resulted in further changes in particle size (Fig. 2b). Hammer milling for 30 and 60 min decreased the median particle size from 117 to 83.3 and 60.8 µm, respectively. With 90 and 120 min of ball milling, the particle size decreased to 22.3 and 19.2 µm, respectively.

In addition to the changes in particle size, 60 min of ball milling decreased the crystallinity index (CrI) significantly for the hammer, impact, and tandem milled wood powders (Fig. 2c and d). Different initial milling treatments resulted in different amorphization levels. Specifically, CrI levels of 56.2%, 44.1%, and 30.3% were found for hammer, impact, and tandem milled samples, respectively. Following an additional 60 min ball milling, the CrI of the hammer milled particles was only slightly reduced to 51.2%, however the CrI of the tandem and impact milled materials were found to decrease to 11.9 and 10.8%. In addition to the decreased peak intensities, the 2θ shift indicates progressively more amorphous structures in the cellulose.

3.2.2. Extrusion fibrillation and CrI

Fig. 3a and b show the changes of the median sizes after extrusion. Particle agglomeration was observed for the impact and tandem milled samples with an increase of 17–20 µm in median size after extrusion. However, the median diameter of the hammer milled particles decreased in size by 32.2 µm with the same extrusion process. The larger feeding particle size and fibrillated nature of the hammer milled particles may have been more amenable to further disintegration during extrusion. Collectively, these results are consistent with those of other studies indicating that extrusion is not a typical size reduction process (Karunanithy and Muthukumarappan, 2011; Zhang et al., 2012; Um et al. 2013). The aggregated particles of the impact and tandem milled material appeared to offer more friction and resistance for screw rotation and may have affected flowability of materials resulting in a long residence time. This was observed with an increased torque that may result in effectively utilized shear forces to disintegrate and flake off the cell walls of the lignocellulosic biomass by overcoming recalcitrance of the lignin component.

The crystallinity of the extruded samples as measured by XRD is depicted in Fig. 3c for samples extruded at 150 °C using a 45 wt% loading. The process slightly decreased the CrI from 51.2% to 48.6%. When comparing the effect of extrusion on material produced using the different milling methods, hammer milled samples revealed less change in crystallinity as compared to the other two milled samples. This XRD data demonstrates that extrusion fibrillation slightly increased the CrI as comparison to the previously milled samples. However, it is known

that the reduction in CrI depends not only on the initial crystallinity, but also on the structural feature of materials including the supramolecular organizational structure, the degree of polymerization of cellulose as well as the process parameters (such as temperature, number of passes, screw configuration, etc) (Haghighi Mood et al., 2013). Therefore, the increase in crystallinity might be attributed to the recrystallizing of amorphous regions by the molecular relaxation from the combine heat and moisture during extrusion processing.

Although disrupting crystallinity may enhance the enzymatic saccharification of biomass, it has been indicated that increasing SSA is more effective for improving enzymatic accessibility (Zhang et al., 2012). Other reports have also shown that increasing SSA is crucial for enhancing biomass digestibility (Lamsal et al., 2010; Silva et al., 2013). At the same time, increased temperatures in the presence of moisture will soften the wood cell wall and plasticize the lignocellulosic biomass during the extrusion process. This disruption of the surface structures allows nano-sized fibrils (i.e. less than 100 nm in diameter) to protrude, increase SSA, and ultimately enhance sugar recovery.

Twin screws extruders have limited clearance between the screws and the barrel. The co-rotation of the twin screws resulted in high-shear forces and intensive mixing. Combining forward, reverse, and kneading screw elements in a twin-screw extruder may impart different shear forces on the feedstock in the barrel. As observed in Fig. 3b and d, the differences in fiber diameter and crystallinity of extruded samples may be attributed to different screw configurations used during extrusion processing. The resulting higher pulverization forces from reverse screw elements may cause the accumulation and compression of the feedstock and effectively liberate microfibrils from the cell wall. More reverse screw elements will increase the residence time as well as impart a pulverizing effect. Furthermore, more kneading screw elements offer more intensive mixing and more uniformly distribute the moisture. Moisture content plays a role in thermal softening of the biomass and the viscosity and flowability of the material. The other study (Zheng et al., 2016) also found differences in the glucose yield among different screw configurations.

3.3. Enzymatic sugar yields

3.3.1. Chemical compositions

Table 1 presents the chemical compositions of three milled samples. It indicates that the chemical composition did not substantially change after the different milling treatments. The result is consistent with a previous report that mechanical milling pretreatment had less modification on chemical composition (Liu et al., 2017).

3.3.2. Sugar yields after mechanical milling

The significant change in morphology and structure of milled woody biomass aims at improving the rate and extent of enzymatic hydrolysis. The effects of the different milling methods on carbohydrate conversion are presented in Table 2 as both total sugar yield (g sugar/kg wood) and energy efficiency (kg sugars/kWh). The total energy used is the sum of the serial milling stages; e.g. HM + BM. The difference in morphology and structures suggested different saccharification efficiency of woody biomass. Among the three different milling processing alone (HM, IM, TM), the tandem milled samples resulted in the highest carbohydrate conversion and the highest total sugar yield. We previously noted that this process resulted in strong pulverization impacts on the wood particles and disrupted the cell walls producing microfibrils with accessible new surfaces. Creating new surfaces and fiber fragments of the woody biomass may have enhanced the enzyme adsorption on substrate and the interactions between enzyme and substrate during enzymatic hydrolysis. Additionally, the porous and cracked surface morphology would be easily accessible by enzymes.

With additional ball milling, the efficiency of the enzymatic hydrolysis was further improved. After 1 h of ball milling the conversion of saccharides increased up to 80% from 20.5% for untreated wheat

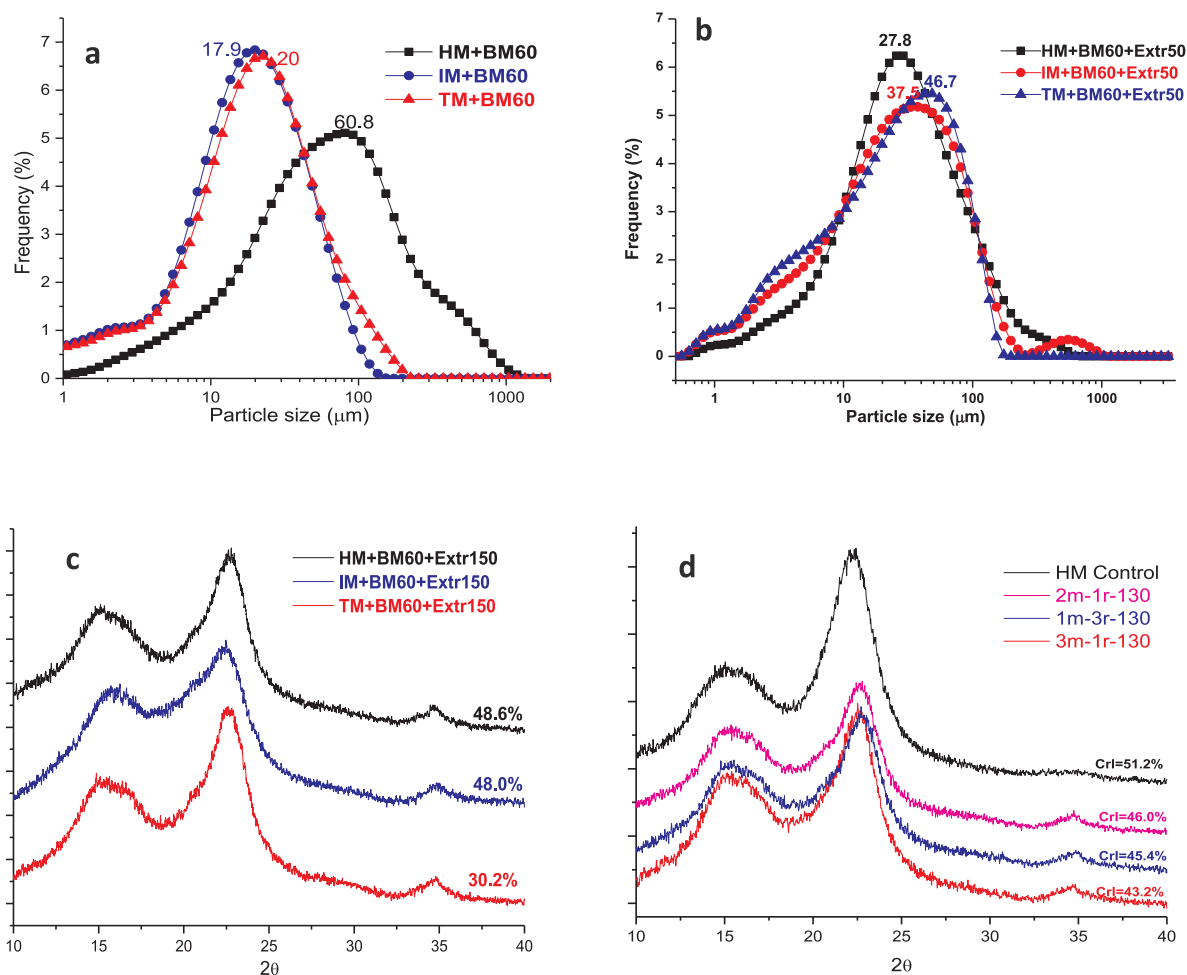


Fig. 3. Crystallinity of extruded samples and ones with different screw configurations.

Table 1

Chemical compositions of the milled samples.

Samples	Glucan (%)	Galactan (%)	Xylan/Mannan (%)	Arabinan (%)
IM	38.6	2.9	13.2	1.2
TM	40.7	2.7	12.7	1.0
HM	41.1	3.1	13.7	1.2

straw (Silva et al., 2012). BM for 2 h was sufficient to completely convert the cellulose into an accessible amorphous form (Buaban et al., 2010). BM could increase the total hydrolysis yield from 70 to 82% (Mais et al., 2002). As presented in Table 2, after 2 h of ball milling hammer milled samples increased the total sugar yield nearly 4 fold over the control samples. Ball milling of the IM and TM materials increased carbohydrate conversion by 40 and 50%. This result may be attributed to the smaller particle sizes achieved in ball milling. Small particles from bundle separation and breakage of fibers resulted in increased SSA and enhanced enzymatic saccharification (Sangseethong et al., 1998; Dasari and Berson, 2007). Although it is effective in disintegrating recalcitrance of woody biomass, it is worthy to note that ball milling is an energy-intensive processing and may become a limiting factor for micronizing larger particles at a pilot scale.

3.3.3. Sugar yield after extrusion fibrillation

Glucose and xylose yields for hammer milled samples after extrusion fibrillation processing at 150 °C are shown in Fig. 4. The conversion efficiency of holocellulose increased with extrusion fibrillation for

Table 2

Comparison of sugar yield from different mechanical comminution.

Mechanical milling	Glucan conversion (%)	Xyl/mannan conversion (%)	Total sugar yield (g/kg wood)	Energy efficiency (kg sugars/kWh)
HM + BM				
HM Control	9.6	2.4	40.1	0.155
30	13.0	6.1	59.2	0.155 + 0.073
60	19.7	10.7	107.7	0.155 + 0.259
90	37.2	21.8	201.3	0.155 + 0.619
120	43.1	22.7	233.5	0.155 + 0.743
IM + BM				
IM Control	17.7	7.8	87.9	0.121
30	53.6	28.3	288.2	0.121 + 0.277
60	71.0	37.1	380.4	0.121 + 0.394
90	80.0	37.3	425.0	0.121 + 0.453
120	87.8	38.8	455.3	0.121 + 0.494
TM + BM				
TM Control	35.8	13.8	187.3	0.181
30	62.8	22.5	316.1	0.181 + 0.123
60	79.6	30.5	403.8	0.181 + 0.207
90	92.0	37.0	469.1	0.181 + 0.271
120	95.7	43.1	494.5	0.181 + 0.295

low ball milling times. In the case of hammer milled samples, extrusion fibrillation increased glucose yield from 13% to about 26% for 30 min balling time, however the effectiveness appears to decrease with increased ball milling. Slightly increased glucose yield from extrusion processing was also evident for the impact milled samples that were

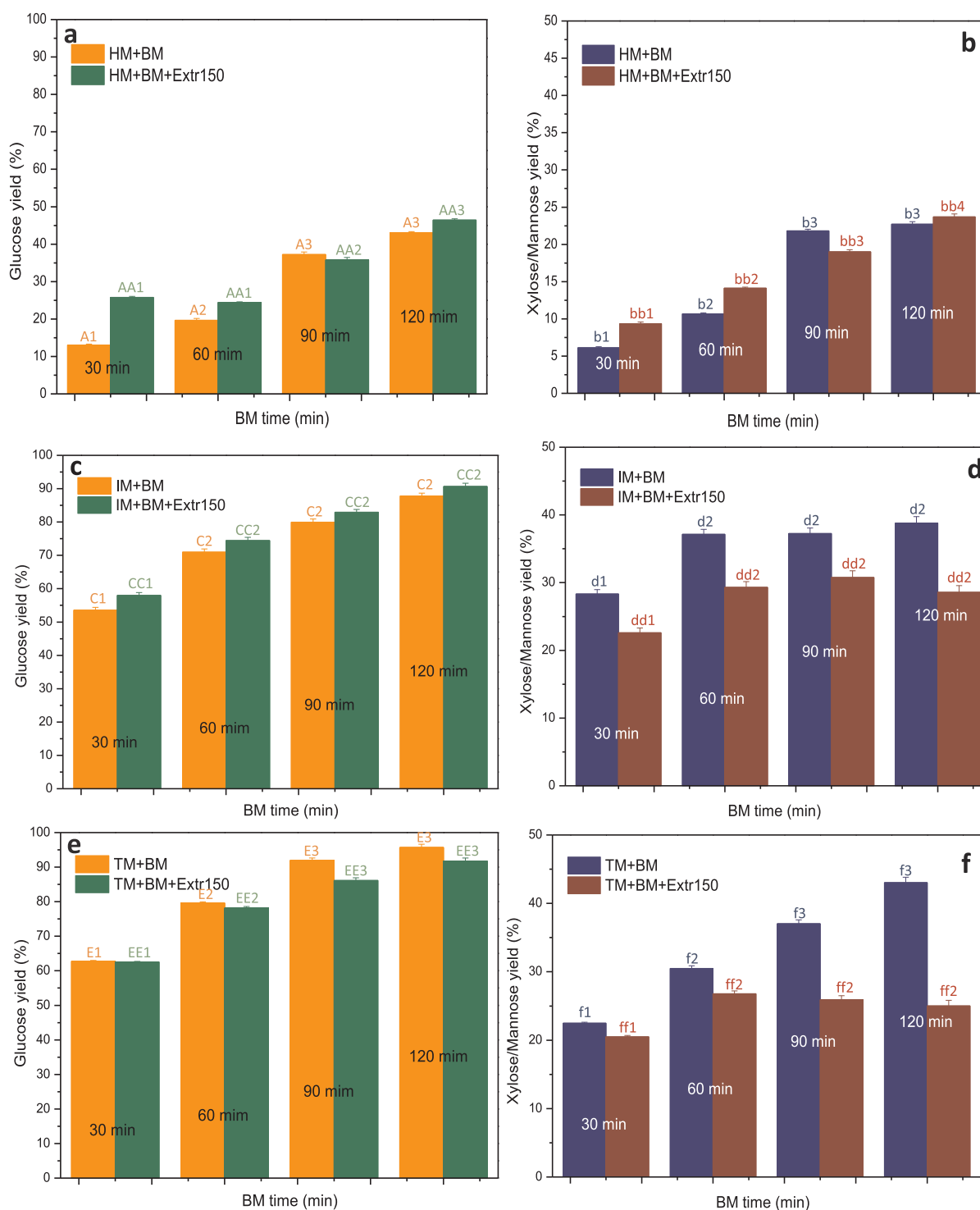


Fig. 4. Carbohydrates yield of HM, IM, and TM with different BM time. Different value with the same letter above the bars indicates the significant difference between the treatments ($p < 0.05$). Capital letters indicate compared means of glucose yield (%) and small letters indicate compared means of xylose/mannose yield (%).

extruded. However, the xylose/mannose yield exhibited decreases for the impact- and tandem-milled extruded samples. These findings may have resulted from the severe particle agglomeration from extrusion in these particle type, which may have led to decreased interactions with enzyme during hydrolysis.

The different arrangement of kneading and reverse screw elements were investigated to generate different compression and shear action on the feedstock. Table 3 shows the glucose and xylose yields after hydrolysis of hammer milled materials extruded under different screw

configuration. There was apparent approx. 7% increase in the glucose yield with increasing numbers of kneading screw elements. The more kneading screw elements may offer higher compression forces on the milled particles and make more hemicellulose exposed on the external surface, thereby improving enzyme accessibility compared to the change of the reverse screw elements. The higher compression forces of more kneading screw elements may also lower the moisture content of the samples.

Table 3

Comparison of sugar yield, moisture content, and SEC from different screw configurations for HM + BM30 materials.

Samples	Glucose (%)	Xylose/ Mannose (%)	MC after extrusion (%) wet based)	Energy consumption from extrusion (kWh/kg)
Control	13.0	6.1	8.4	N. A.
1m-3r-130	20.5	6.5	22.2	0.31
2m-1r-130	19.2	6.6	21.7	0.31
3m-1r-130	25.8	9.4	6.2	0.42

3.4. Sugar yields per unit energy consumption

The energy consumption of mechanical pretreatment is generally a major concern for the economical production of biofuels. The specific energy consumption (SEC) of different milling methods was calculated according to Eq. (1). Generally, the SEC increased with decreasing final particle size and milling time. As shown in Fig. 5a and b, different milling methods had drastic influence on SEC for different particle sizes. The hammer mill consumed approximately 20% lower SEC than the other two mills. Our previous work found that the energy consumption from HM was in the range of 0.016–0.039 kWh/kg for different particle size (Liu et al., 2016a,b). The disk mill and knife mill consumed 5 and 2–3 times more SEC than hammer mill for the same screen size and the same feedstock (Vidal et al., 2011). Compared to hammer milling, impact mills consumed more electrical energy but produced smaller particle size with lower crystallinity. Compared with impact milling the tandem mill consumed 50% higher electrical energy

to produce almost the same particle size. Although the hammer mill could pulverize Douglas-fir chips to wood particles with a lower SEC, it is not efficient for fine milling/amorphization to create surface area for enzymatic hydrolysis. To reach a well-accepted level of digestibility, the particle size must be reduced to less than 100 μm (Silva et al., 2012). Particle size smaller than 40-mesh did not significantly change the hydrolysis rate and such a fine milling of woody biomass was much more energy consuming than coarse milling (Chang et al., 1997; Phanphanich et al., 2011; Temmerman et al. 2013). The ball milling has been accepted to be an effective step to decrease the particle size and to increase the SSA simultaneously. As shown in Fig. 5c and d, energy consumption of the milled samples from fine ball milling significantly increased with the decrease of particle sizes from long ball milling time while no obvious difference in energy consumption was seen from the impact- and tandem- milled samples. Specifically, the ball milling may provide relatively efficient comminution for micronization/amorphization in one cycle pass way while for production of fine particles more cycle passes are generally needed for hammer- and impact- mills. Apparently, the repeated cycles undoubtedly resulted in dramatically higher energy demand. The disadvantage of high energy requirements from ball mill can limit its large-scale applications on economical view. However, a recent study showed that the energy consumption might drop to 0.18 kWh/kg only when a commercially available industrial-sized ball planetary mill with 20 tons of biomass per hour was applied (Kim et al., 2013). Ball mill is irrespective of moisture content of biomass, however, for hammer and knife mills there could be problems to reach micro-scaled particle sizes for biomass with more than 15% (wet basis) moisture content due to a plugged drum screen (Zhu et al., 2010). In comparison with the grinding mills extruders have advantages in

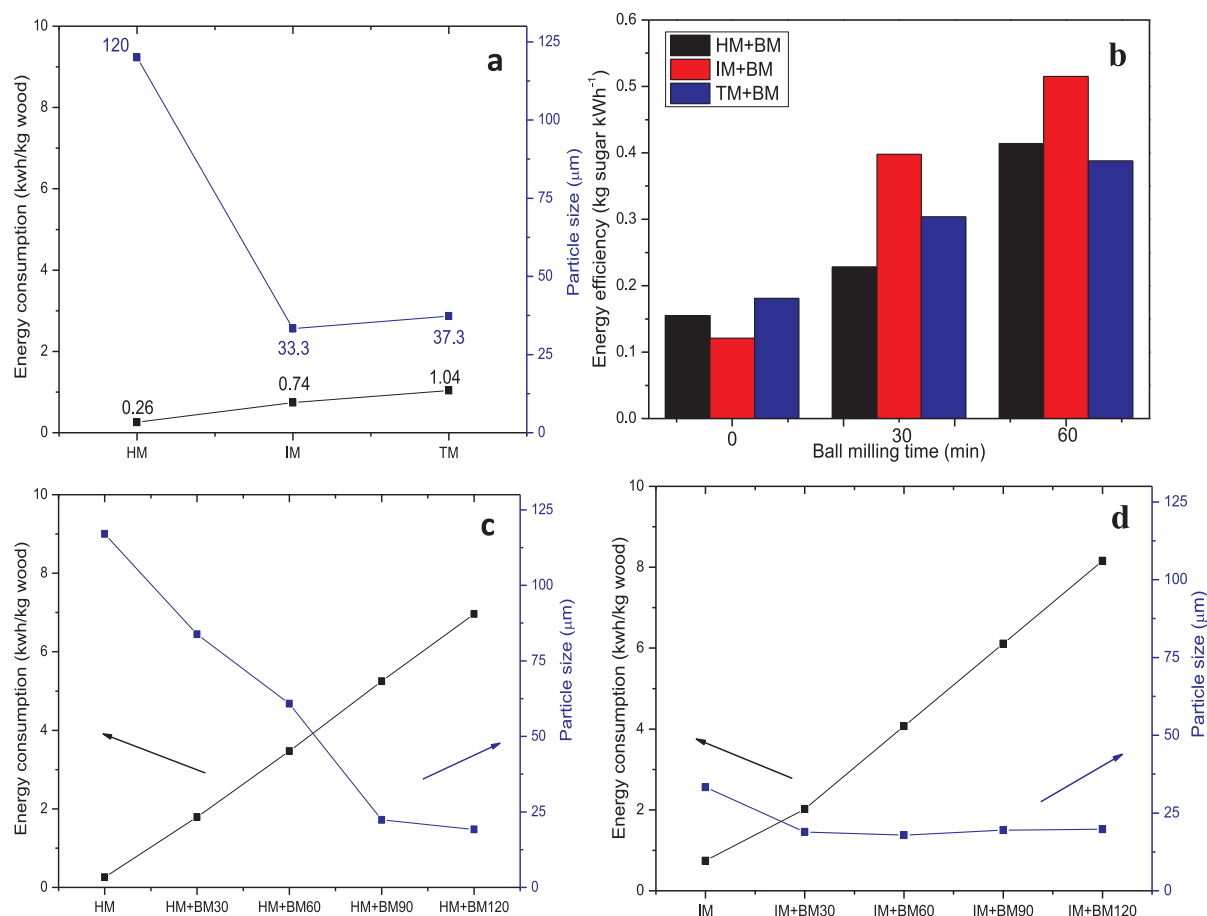


Fig. 5. Energy consumption (kWh/kg wood) and particle size of milled samples. (a) Energy consumption and particle sizes of HM, IM, TM and their different BM time; (b) Energy efficiency (kWh/kg sugar) of HM, IM, and TM after different BM time.

terms of continuous treatment, easy adjustment, large-scale applications and less specific energy consumption (less than 0.14 kWh/kg) of only 1% SEC over those of the grinding mills.

The energy efficiency (kg sugar/kWh) can be used to evaluate the performance of the mechanical milling process for producing simple sugars. The energy efficiency was calculated according to Zhu et al. (2010), that the higher energy efficiency the more effective pretreatment process. The results in Fig. 5a indicated that the energy consumption of HM, IM and TM was 0.26, 0.74, and 1.04 kWh kg⁻¹, respectively when particles were reduced from the initial size, i.e. 25.4 mm to 120 µm, 33.3 µm, and 37.3 µm, respectively. Considering 60 min ball milling time, the highest energy efficiency was 0.515 kg sugars kWh⁻¹ for impact mill and the lowest energy efficiency was 0.388 kg sugars kWh⁻¹ for tandem mill. A comparison of different milling methods indicated that glucose yields were competitive to other pretreatments developed in literature. The energy efficiency of disk milling without physicochemical co-treatment on softwood had 0.088 kg glucose kWh⁻¹ (Zhu et al., 2010). The highest energy efficiency was 0.078 kg glucose kWh⁻¹ after 60 min BM (Hideno et al., 2012). The other study reported that the highest energy efficiency was 0.046 and 0.027 kg glucose kWh⁻¹ for bagasse and straw biomass, respectively, after 10 cycles of wet disk milling on bagasse and sugarcane straw for bioethanol production (Silva et al., 2010). To be mentioned, no data on total sugar yield through different milling methods were reported in these investigations.

From the analysis above, it can be concluded that the TM had the highest SEC following the IM and finally the HM with the lowest SEC. The higher energy consumption of BM can be relieved by optimal grinding design, scaling-up, and lower instrument investment i.e. solar panel power supply compared to other mills. BM was found to be an effective mechanical treatment to reduce particle size, decrease crystallinity and increase glucose yield compared to the other millings. Results from our previous studies indicate that many materials characteristics, types of grinding machines, and type of processing influence the specific energy consumption of grinding machines (Jiang et al., 2016; 2017; Gu et al., 2018), with the initial and final particle size as one of the key parameters evaluating the energy requirement for the viability of mechanical pretreatments. The size reduction of woody biomass can be accomplished by a proper combination of chipping, grinding, and milling. The particle size of materials was usually decreased to be 10–30 mm after chipping and more than tens of microns after grinding or milling. Integrated into the conventional mixing processing, extrusion can be considered as an effective mixing step for industry. The combination of coarse and fine milling can be much more economical than one pass fine milling for large-sized feedstock. Therefore, a reasonable grinding scheme needs to be searched for the optimal material characteristics with less energy consumption at a pilot scale. In comparison of different pilot-scale milling methods, impact mill as mechanical pretreatments appeared more efficient in terms of energy-efficiency than HM and TM. The pilot-scale impact mill can be a better choice for coarse milling in the multi-step comminution while industry-based ball mill can be used for micronization/amorphization of fine milling with acceptable SEC. At the same time, extrusion as a potential mixing processing unit can be easily introduced to the production of biofuel without inducing apparent increase in SEC.

From a feasible economical perspective, multi-step grinding scheme of woody biomass should be carried out based on different milling methods. The industry-scale processing parameters and standards can be set for pilot-scale production of fine wood powders with multi-step comminution technology. Further development of combined mechanical pretreatment can be focused on optimizing combined grinding process to achieve the highest yield of sugars with less energy consumption for biofuel production. To further the study, a comparison on the techno economic analysis of the woody biomass conversion to cellulosic ethanol between our developed stepwise pulverization process and the thermochemical conversion process has been pursued for

the economic feasibility and will be published in another paper.

4. Conclusions

Combination of simple industry-based comminution technologies was developed to enhance enzymatic hydrolysis of forest residues with less total energy consumption without the involvement of any chemicals. The hammer milling appeared the most efficient coarse milling for the initial size reduction. Through initial coarse milling particle size was reduced to a specific value and then below 100 µm through further pulverization. By optimizing the combined grinding steps, a highly saccharifiable substrate can be achieved with overall low energy inputs. The findings might increase the understanding and give some insights into combination milling pretreatments for woody biomass.

The scanning electron images for particle morphology of pretreated woody biomass and figure of energy consumption vs. particle size from TM are available as a [Supplementary information \(Figs. S1–S3\)](#).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.biortech.2018.03.096>.

References

- Barakat, A., Mayer-Laigle, C., Solhy, A., Arancon, R., Vries, H., Luque, R., 2014. Mechanical pretreatments of lignocellulosic biomass: towards facile and environmentally sound technologies for biofuels production. *RSC Adv.* 4, 48109–48127.
- Boussaid, A., Robinson, J., Cai, Y., Gregg, D.J., Saddler, J.N., 1999. Fermentability of the hemicellulose derived sugars from steam exploded softwood (Douglas-Fir). *Biotechnol. Bioeng.* 64, 284–289.
- Buaban, B., Inoue, H., Yano, S., Tanapongpipat, S., Ruanglek, V., Champreda, V., Pichyangkura, R., Rengpipat, S., Eurwilaichitr, L., 2010. Bioethanol production from ball milled bagasse using an on-site produced fungal enzyme cocktail and xylose-fermenting *Pichia stipitis*. *J. Biosci. Bioeng.* 110, 18–25.
- Chang, V.S., Burr, B., Holtzapfel, M.T., 1997. Lime pretreatment of switchgrass. *Appl. Biochem. Biotechnol.* 63–65, 3–19.
- Chen, J., Adjallé, K., Barnabé, S., Perrier, M., Paris, J., 2018. Mechanical and Thermal Pretreatment Processes for Increasing Sugar Production from Woody Biomass Via Enzymatic Hydrolysis Waste Biomass Valor. doi: 10.1007/s12649-018-0217-x.
- Dasari, R.K., Berson, R.E., 2007. The effect of particle size on hydrolysis reaction rates and rheological properties in cellulosic slurries. *Appl. Biochem. Biotechnol.* 137, 289–299.
- Godavarti, S., Karwe, M.V., 1997. Determination of specific mechanical energy distribution on a twin-screw extruder. *J. Agric. Engng. Res.* 67, 277–287.
- Gu, B.-J., Wang, J., Wolcott, M.P., Ganjyal, G.M., 2018. Increased sugar yield from pre-milled Douglas-fir forest residuals with lower energy consumption by using planetary ball milling. *Bioresour. Technol.* 251, 93–98.
- Haghighi Mood, S., Hossein Golfeshan, A., Tabatabaei, M., Salehi Jozani, G., Najafi, G.H., Gholami, M., Ardjmand, M., 2013. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. *Renewable Sustainable Energy Rev.* 27, 77–93.
- Hideno, A., Inoue, H., Yanagida, T., Tsukahara, K., Endo, T., Sawayama, S., 2012. Combination of hot compressed water treatment and wet disk milling for high sugar recovery yield in enzymatic hydrolysis of rice straw. *Bioresour. Technol.* 104, 743–748.
- Hoeger, I.C., Nair, S.S., Ragauskas, A.J., Deng, Y., Rojas, O.J., Zhu, J.Y., 2013. Mechanical deconstruction of lignocellulose cell walls and their enzymatic saccharification. *Cellulose* 20, 807–818.
- Jiang, J., Wang, J., Zhang, X., Wolcott, M., 2016. Evaluation of physical structural features on influencing enzymatic hydrolysis efficiency of micronized wood. *RSC Adv.* 105, 103026–103034.
- Jiang, J., Wang, J., Zhang, X., Wolcott, M., 2017. Characterization of micronized wood and energy-size relationship in wood comminution. *Fuel Process. Technol.* 161,

- 76–84.
- Karunanithy, C., Muthukumarappan, K., 2011. Optimization of switchgrass and extruder parameters for enzymatic hydrolysis using response surface methodology. *Ind. Crops Prod.* 33, 188–199.
- Klinke, H.B., Thomsen, A.B., Ahring, B.K., 2004. Inhibition of ethanol producing yeast and bacteria by degradation products produced during pretreatment of biomass. *Appl. Microbiol. Biotechnol.* 66, 10–26.
- Kim, H.J., Lee, S., Kim, J., Mitchell, R.J., Lee, J.H., 2013. Environmentally friendly pretreatment of plant biomass by planetary and attrition milling. *Bioresour. Technol.* 144, 50–56.
- Lamsal, B., Yoo, J., Brijwani, K., Alavi, S., 2010. Extrusion as a thermo-mechanical pretreatment for lignocellulosic ethanol. *Biomass Bioenergy* 34, 1703–1710.
- Liu, H., Pang, B., Zhao, Y., Lu, J., Han, Y., Wang, H., 2018. Comparative study of two different alkali-mechanical pretreatments of corn stover for bioethanol production. *Fuel* 221, 21–27.
- Liu, Y., Wang, J., Wolcott, M.P., 2016a. Assessing the specific energy consumption and physical properties of comminuted Douglas-fir chips for bioconversion. *Ind. Crops Prod.* 94, 394–400.
- Liu, Y., Wang, J., Wolcott, M.P., 2016b. Multistep process to produce fermentable sugars and lignosulfonates from softwood enzymolysis residues. *ACS Sustainable Chem. Eng.* 4, 7225–7230.
- Liu, Y., Wang, J., Wolcott, M.P., 2017. Evaluating the effect of wood ultrastructural changes from mechanical treatment on kinetics of monomeric sugars and chemicals production in acid bisulfite treatment. *Bioresour. Technol.* 226, 24–30.
- Mais, U., Esteghlalian, A.R., Saddler, J.N., Mansfield, S.D., 2002. Enhancing the enzymatic hydrolysis of cellulosic materials using simultaneous ball milling. *Appl. Biochem. Biotechnol.* 98, 815–832.
- Mauurya, D., Singla, A., Negi, S., 2015. An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. *3 Biotech.* 5, 597–609.
- Phanphanich, M., Mani, S., 2011. Impact of torrefaction on the grindability and fuel characteristics of forest biomass. *Bioresour. Technol.* 102, 1246–1253.
- Repellin, V., Govin, A., Rolland, M., Guyonnet, R., 2010. Energy requirement for fine-grinding of torrefied wood. *Biomass Bioenergy* 34, 923–930.
- Sangseethong, K., Meunier-Goddik, L., Tantasucharit, U., Liaw, E.T., Penner, M.H., 1998. Rationale for particle size effect on rates of enzymatic saccharification of micro-crystalline cellulose. *J. Food Biochem.* 22, 321–330.
- Schell, D.J., Harwood, C., 1994. Milling of lignocellulosic biomass: results of pilot-scale testing. *Appl. Biochem. Biotechnol.* 45 (46), 159–168.
- Segal, L., Creely, J., Martin, A., Conrad, C., 1959. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Text. Res. J.* 29, 786–794.
- Silva, A.A., Inoue, H., Endo, T., Yano, S., Bon, E.S., 2010. Milling pretreatment of sugarcane bagasse and straw for enzymatic hydrolysis and ethanol fermentation. *Bioresour. Technol.* 101, 7402–7409.
- Silva, G.D., Couturier, M., Berrin, J.-G., Buleon, A., Rouau, X., 2012. Effects of grinding processes on enzymatic degradation of wheat straw. *Bioresour. Technol.* 103, 192–200.
- Silva, A., Teixeira, R.S., Endo, T., Bon, E.S., Lee, S.-H., 2013. Continuous pretreatment of sugarcane bagasse at high loading in an ionic liquid using a twin-screw extruder. *Green Chem.* 15, 1991–2001.
- Sjostrom, E., 1993. *Wood Chemistry-Fundamentals and Applications*, second ed. Academic Press, pp. 4503.
- Takahashi, T., Ito, K., Ito, A., Enda, Y., Gochi, M., Mori, H., Kobayashi, J., 2014. Tandem-ring mill pulverization benefits for enzymatic saccharification of biomass. *Renewable Energy* 65, 146–151.
- Temmerman, M., Jensen, P.D., Hébert, J., 2013. Von Rittinger theory adapted to wood chip and pellet milling, in a laboratory scale hammermill. *Biomass Bioenergy* 56, 70–81.
- Um, B.-H., Choi, C.H., Oh, K.K., 2013. Chemicals effect on the enzymatic digestibility of rape straw over the thermo-mechanical pretreatment using a continuous twin screw-driven reactor (CTSR). *Bioresour. Technol.* 130, 38–44.
- Vaidya, A.A., Donaldson, L.A., Newman, R.H., Suckling, I.D., Campion, S.H., Lloyd, J.A., Murton, K.D., 2016. Micromorphological changes and mechanism associated with wet ball milling of *Pinus radiata* substrate and consequences for saccharification at low enzyme loading. *Bioresour. Technol.* 214, 132–137.
- Vidal, B., Dien, B., Ting, K., Singh, V., 2011. Influence of feedstock particle size on lignocellulose conversion-a review. *Appl. Biochem. Biotechnol.* 164, 1405–1421.
- Zhang, S., Keshwani, D.R., Xu, Y., Hanna, M.A., 2012. Alkali combined extrusion pretreatment of corn stover to enhance enzyme saccharification. *Ind. Crops Prod.* 37, 352–357.
- Zheng, J., Choo, K., Bradt, C., Lehoux, R., Rehmann, L., 2014. Enzymatic hydrolysis of steam exploded corncob residues after pretreatment in a twin-screw extruder. *Biotechnol. Rep.* 3, 99–107.
- Zheng, J., Choo, K., Rehmann, L., 2016. Xylose removal from lignocellulosic biomass via a twin-screw extruder: the effects of screw configurations and operating conditions. *Biomass Bioenergy* 88, 10–16.
- Zhu, J.Y., Pan, X., Zalesny, R.S., 2010. Pretreatment of woody biomass for biofuel production: energy efficiency, technologies, and recalcitrance. *Appl. Microbiol. Biotechnol.* 87, 847–857.