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Comparison of different mechanical refining technologies on the enzymatic digestibility of low severity acid pretreated corn stover ☆

Xiaowen Chen [a,*], Erik Kuhn [a], Wei Wang [b], Sunkyu Park [c], Keith Flanegan [d], Olev Trass [e], Lisette Tenlep [f], Ling Tao [a], Melvin Tucker [a]

[a] *National Bioenergy Center, National Renewable Energy Laboratory, 15013 Denver West Parkway, Golden, CO 80401, United States*
[b] *National Bioscience Center, National Renewable Energy Laboratory, 15013 Denver West Parkway, Golden, CO 80401, United States*
[c] *Department of Forest Biomaterials, North Carolina State University, 2820 Faucette Drive, Campus Box 8005, Raleigh, NC 27695, United States*
[d] *IdeaCHEM, Inc., 710 Fairview St., Rapid City, SD 57701, United States*
[e] *Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College St, Toronto, ON M5S 3E5, Canada*
[f] *Biomethodes – OptaFuel, 5516 Industrial Park Rd, Norton, VA 24273, United States*

HIGHLIGHTS

- We compared five different mechanical refining technologies on digestibility of PCS.
- We studied the effect of refining severity on digestibility of PCS.
- We explored the particle size and morphological change of biomass after MR.
- We did a pilot scale trial with Szego mill showing the possibility for scaling up.

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ABSTRACT

The effect of mechanical refining on the enzymatic digestibility of pretreated corn stover (PCS) was investigated. Low severity, dilute sulfuric acid PCS was subjected to mechanical refining using a bench-scale food processor blender, a PFI mill, a 12-inch laboratory disk refiner, and a 25 mm co-rotating twin-screw extruder. Glucose yields from enzymatic hydrolysis were improved by 10–15% after blending and disk refining, while PFI refining and twin-screw extrusion showed a glucose yield improvement of 16–20%. A pilot scale refining test using a Szego mill was performed and showed approximately 10% improvements in biomass digestibility. This suggests the possibility to scale up a mechanical refining technique to obtain similar enzymatic digestibility glucose yield enhancement as achieved by PFI milling and extrusion technologies. Proposed mechanisms of each mechanical refining technology are presented and reasons for improvements in biomass digestibility are discussed in this paper.

1. Introduction

A mechanical, particle-size reducing unit operation is necessary for woody biomass prior to chemical or biochemical processing in a forest biorefinery (Barakat et al., 2013; Zhu and Pan, 2010). Mechanical particle-size reduction has also been applied to woody biomass after thermal-chemical pretreatment to improve enzymatic digestibility of cellulose (Koo et al., 2011; Wang et al., 2002; Zhang et al., 2012; Zhu et al., 2011; Zhu and Pan, 2010). Compared to mechanical refining prior to pretreatment, post-pretreatment refining requires less milling energy because a portion of the wood mass is solubilized and the wood structure is softened during pretreatment (Barakat et al., 2013; Zhu and Pan, 2010). However, the application of mechanical refining to herbaceous biomass has been thought unnecessary for the following reasons: (1) herbaceous biomass requires less processing and energy to achieve a desired size reduction, (2) mechanical size reduction could be achieved during harvesting (Zhu et al., 2011), (3) and size-reduction can be achieved by chemical pretreatment and steam explosion. Therefore, the application of mechanical refining to pretreated corn stover has not been widely studied.

In addition to particle size, mechanical refining can affect other physical properties of biomass. It has been found that mechanical refining could decrease cellulose crystallinity and increase accessible specific surface area of cellulose (Barakat et al., 2013).

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* Corresponding author. Tel.: +1 303 384 6828; fax: +1 303 384 6877.
E-mail address: Xiaowen.Chen@nrel.gov (X. Chen).

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Mechanical refining has three mechanisms that alter the physical properties of pulp fiber: cutting, which reduces fiber length; shearing, which fibrillates fibers on their exterior surface; and compression, which crushes and fibrillates fibers internally (Hartman, 1984). Each of the three mechanisms occur simultaneously during mechanical refining, but to a different extent depending on refining technology (Hartman, 1984).

In a recent study the effects of mechanical refining on particle morphology were divided into two classes. Class I refers to biomass fibers being cut, separated, and fragmented. External fibrillation occurs in Class I refining, producing hair-like micofibrils on the fiber surface increasing the external surface area. Class II refining causes the cross-links between microfibrils to break apart, especially when compression-induced internal fibrillation occurs (Leu and Zhu, 2013). The study concluded that Class I mechanical refining of untreated biomass provides up to 20% enzymatic hydrolysis glucose yield improvement by increasing external surface area. The effect of Class I refining on the enzymatic digestibility of moderately-to-highly severe, dilute-acid pretreated biomass is negligible because the effect that external fibrillation has on untreated biomass can be achieved by chemical modification during dilute-acid pretreatment. Class II refining increases the enzymatic digestibility of untreated and dilute-acid pretreated biomass by increasing the surface area of cellulose that is accessible to enzymes, as well as by reducing cellulose crystallinity (Leu and Zhu, 2013). Ball milling is a typical Class II refining procedure.

The extent that Class I and Class II physical effects are observed depend on the type of biomass refined, if the biomass is untreated or pretreated, and the processing equipment used to refine the biomass. Zhu et al. have shown mild improvements in cellulose enzymatic hydrolysis yields by disk-refining SPORL treated softwood (Zhu et al., 2010), while Koo et al. have shown significant cellulose enzymatic hydrolysis yield improvements on “green liquor” pretreated hardwood (Koo et al., 2011). Dibble et al. showed an insignificant improvement in enzymatic hydrolysis yields of cellulose on dilute-acid pretreated corn stover processed in a blender (Dibble et al., 2011). Therefore, current study investigates the effects of four different mechanical refining technologies on dilute-acid pretreated corn stover. Material from a single dilute-acid pretreatment condition was prepared at mild severity. The enzymatic hydrolysis glucose yield after processing through one of the four mechanical refining technologies was compared to the enzymatic digestibility of un-refined, dilute-acid pretreated solids.

It is pertinent to maintain enzymatic hydrolysis yield improvements at the pilot, demonstration and commercial scales from mechanical refining technologies that have been tested at the laboratory and bench scales. Lab scale refining, for example, using a PFI milling has shown to significantly improve the digestibility (Chen et al., 2012b; Koo et al., 2011) of pretreated corn stover and hardwood. However, a PFI mill is a batch, laboratory refiner that may be difficult, and economically unfavorable to scale up. In order to obtain equivalent enzymatic hydrolysis enhancement observed from material refined in a PFI mill, enzymatic hydrolysis of PCS passed through a Szego mill was performed. A Szego mill is a continuous, wet mill currently used at commercial scale. The Szego mill, developed by researchers at the University of Toronto, Ontario, Canada, is a planetary ring-roller mill shown in Fig. 1 (Trass and Gandolfi, 1990). It consists of a stationary, cylindrical grinding surface known as a stator. Inside the stator a number of helically grooved rollers that are flexibly suspended between upper and lower flanges connected to a central drive shaft rotate. The material is fed by gravity or pumped into the top of the mill and is discharged out the bottom. During refining, biomass is subjected to crushing and shearing forces generated by the radial acceleration of the rollers, and by the high velocity gradients between the roller ridges and the stator, respectively. Material is transported through the mill by the roller grooves. This transporting action is particularly important with materials that do not readily flow by gravity, such as pastes and sticky materials. PCS slurries fall in this category. Szego mills are available in industrial sizes capable of throughputs up to 10 t/h. In this study, a pilot scale Szego mill was used to produce mechanically refined PCS. The Szego milled material was enzymatically hydrolyzed and the results compared to that of the lab and bench scales. To our knowledge this is the first published study that investigated scaling up refining technology for the purpose of increasing enzymatic hydrolysis yields of PCS while comparing the pilot or demonstration scale results to the lab and bench scale results.

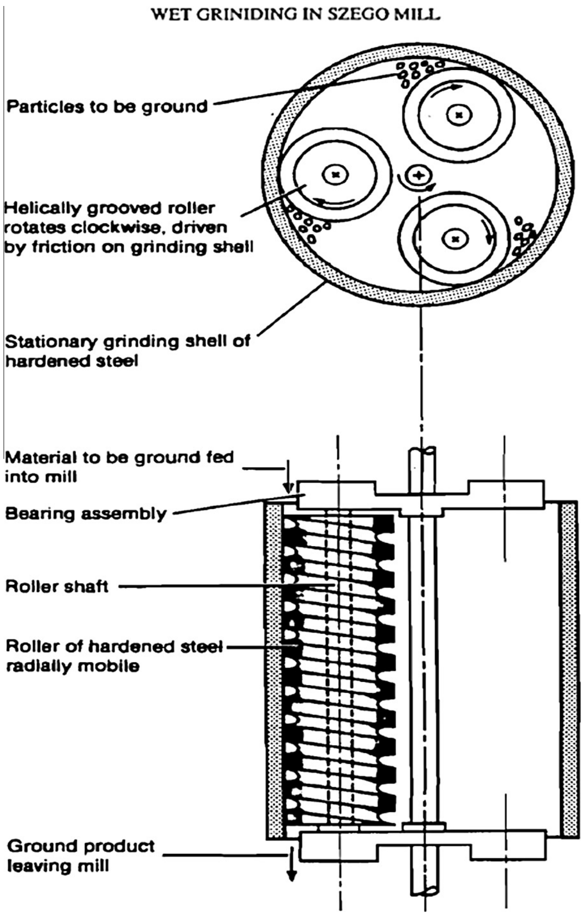

Fig. 1. Schematic diagram of a Szego mill.

2. Methods

2.1. Materials

2.1.1. Material used in all sections except 3.1.3 and 3.3

Whole corn stover (Pioneer variety 33A14) was harvested in 2002 and tub ground at the Kramer farm in Wray, Colorado, and further milled at National Renewable Energy Laboratory (NREL) through a Mitts & Merrill rotary knife mill (model 10 × 12) to pass a ¼-inch screen. The chemical composition of original Kramer corn stover 33A14 is shown in Table 1.

2.1.2. Material used in Section 3.1.3

Due to a limited supply of Kramer 33A14 corn stover, an additional batch of corn stover supplied by Idaho National Laboratory (INL) was used in the refining severity study (Section 3.1.3) as well

Table 1
Solids composition analysis of original corn stover.

Sample	% Total Ash	% Sucrose	% Lignin	% Glucan	% Xylan	% Galactan	% Arabinan	% Acetyl
Kramer	4.6	4.0	12.3	34.0	22.0	1.6	3.1	2.9
INL	5.7	0.5	16.0	35.7	21.3	1.6	3.4	2.6

as in the pilot trial study using Szego milling (Section 3.3). The chemical composition of each INL corn stover batch is shown in Table 1. The INL corn stover was knife milled at NREL through a ½-inch rejection screen.

2.2. Acid impregnation

2.2.1. Bench-scale impregnation in a recirculating atmospheric pressure impregnator

Approximately 10 kg of air dried corn stover (~94% solids) was loaded in a Hastelloy C-276 wire mesh (40 mesh screen) basket and soaked in 120 L of warm (40–50 °C) 0.5 wt% sulfuric acid solution for 2 h and then drained to approximately 20 wt% total solids (TS) content. The acid impregnated feedstock was then dewatered to approximately 60 wt% TS by pressing in a piston type hydraulic dewatering press.

2.2.2. Pilot-scale deacetylation and acid impregnation in a dynamic impregnator (DI)

Deacetylation and acid impregnation were carried out in the NREL pilot scale DI to produce the required amount of PCS for the Szego milling study, which operates continuously and requires feed material at the kilogram scale. Milled corn stover, INL lot #2, was deacetylated and acid impregnated using the NREL DI, a jacket heated, 2000-L horizontal paddle mixer.

A 100 kg aliquot of corn stover (93% solids) was deacetylated at 8 wt% TS in a 0.4 wt% NaOH solution at 80 °C, and mixed at 15 rpm for 2 h in the NREL DI. The deacetylated biomass was allowed to settle for 15 min, and dewatered by pressurizing the vessel to 20 psi and filtering by discharging through two 6" screens. The drained biomass was washed with 1000 L of 45 °C water, and dewatered as described previously. The final pH of the washed material was ~8.5. The wet, deacetylated corn stover was acid impregnated with dilute sulfuric acid in the NREL DI.

Acid impregnation of deacetylated corn stover was performed by estimating the amount of biomass remaining in the paddle mixer based on previous experiments where ~20% of the dry feedstock was extracted by deacetylation and washing. Based on the gravimetric solids contents, the amount of acid and water required for impregnation was calculated to achieve ~8 wt% solids slurry at 0.8 wt% sulfuric acid concentration. The water and acid were batched in a 200-L mix tank and sprayed onto the wet solids in the vessel mixing at 20 rpm. The washed and deacetylated solids were impregnated at 45 °C for 1 h, drained as described above, removed from the paddle mixer, then dewatered using a compression screw dewatering press to approximately 50 wt% solids.

2.3. Steam explosion pretreatment

2.3.1. Bench-scale steam gun pretreatment

The steam explosion pretreatments were carried out in a 4-liter NREL Digester (Steam explosion reactor). The Digester was loaded with 800.0 g (~480 g dry) of acid impregnated and pressed feedstock, quickly heated (about 5–15 s) via direct steam injection to 150 °C, with pretreatments residence times of 15 and 20 min. Based on the impregnation acid concentration of 0.5 wt% and initial solids loading of 60 wt%, the acid loading was approximately 3.4 mg H_2SO_4/g of dry biomass.

2.3.2. Continuous pretreatment in a pilot-scale horizontal reactor

Pilot scale pretreatments were carried out on deacetylated and acid impregnated corn stover in a Metso 1-ton/d continuous horizontal screw reactor at 152 °C, 0.8 wt% H_2SO_4, and a nominal residence time of 10 min. The feed rate was 25–30 kg corn stover on a dry basis. The acid impregnated corn stover was fed through a plug screw feeder where the wet corn stover was further dewatered from 48 wt% to 60 wt% solids. The acid loading in the reactor was approximately 8 mg H_2SO_4/g of dry biomass.

2.4. Mechanical refining

2.4.1. PFI milling

A PFI mill is a laboratory scale refiner used to beat pulp fiber. It can cause fibrillation, which generates smaller diameter fibers and fines. Approximately 30 g of PCS is placed in the PFI mill and rotation of the rotor causes the pretreated slurry to be thrown against the wall of the mill housing (bedplate) by centrifugal forces. As the slurry forms a smooth film against the bedplate the fibers are subjected to impact by the rotating bars of the rotor. The shearing and compression forces produced by the impacts of the rotor bars cause intra-fiber bond breaking, external fibrillation and fiber cutting. Steam gun pretreated corn stover (Kramer 33A14) was subjected to PFI refining at 20 wt% total solids at several different revolution counts (2000, 4000, 6000, 8000, and 10,000 total revolutions). The refining conditions can be found in Table 2. The refining experiments were conducted in Dr. Sunkyu Park's laboratories at North Carolina State University.

2.4.2. Mechanical size reduction by a food processor

Mechanical size reduction of PCS was carried out using a Hamilton Beach food processor blender (Washington, NC). The PCS slurry was diluted to 10–15% solids and subjected to approximately 3 min of shear in the food processor. The blending was paused every 30 s to allow scraping of material from the walls back into the processing chamber. Switches on the food processor control panel allowed for the selection of two different speeds allowing two energy levels to be studied.

2.4.3. Extrusion

Extrusion was carried out by Blue Sugars Corporation (formerly KL Energy Corporation) in Rapid City, SD, on dilute-acid pretreated corn stover produced in the NREL steam gun. A bench top twin screw (25 mm element diameter) extruder was operated at 50 °C, 500 RPM, and feed rates of 100 as-is g/min and about 200 as-is g/min, which was the maximum input rate for that particular screw design, speed, and feedstock. A proprietary screw configuration was developed for biomass conveying and pretreatment.

2.4.4. Disk refining

Bench scale disk refining was carried out using NREL's 12-inch laboratory disk refiner (Sprout Waldren Koppers model 12″) equipped with type D2A506NH plates on the stationary disk and type 18034ANH plates on the rotating disk. The clearance between

Table 2
Effects of mechanical refining on volume weighted mean particle size of PCS slurries mechanically refined by a bench-scale PFI mill, a twin screw extruder, a 12-inch disk refiner, and a food processor blender.

Sample ID	Refining methods	Refining conditions	Volume weighted mean particle size
Control	Control	N/A	270.7
P-1	PFI	2k revolutions	95.5
P-2	PFI	4k revolutions	86.7
P-3	PFI	8k revolutions	83.7
E-1	Extruder	100 g/min	71.2
E-2	Extruder	Max flow	85.5
D-1	Disk refining	0″	139.3
D-2	Disk refining	0.003″	163.8
D-3	Disk refining	0.006″	150.2
B-1	Food processor/ blending	Level 1 (low)	192.3
B-2	Food processor/ blending	Level 2 (high)	171.5

the rotating and stationary disks was varied between zero (plates barely touching) to 0.006 inches.

2.4.5. Szego milling

The Szego SM-160 mill features a 160 mm inside diameter heavy walled chamber, with three 160 mm long and 60 mm diameter steel rollers, and helical grooves containing 5 mm ridges and 5 mm grooves with a 10 mm pitch. The refining of PCS with the Szego Mill was performed at a rotational speed of 1160 rpm. The PCS was diluted with deionized water at a 1:1 weight ratio to facilitate feeding. The biomass was fed at a rate of 108 kg/h. Water addition was needed after each pass in order to make it possible to feed through the mill multiple limes because the finer solids in the mill absorbed water. The first pass was run in four lots, ~2 kg original material in each, totaling 8.4 kg, with 8.7 kg water added. Subsequent passes were run together.

2.5. Enzymatic hydrolysis

Enzymatic digestions of washed (5× by centrifugation) pretreated residues from the pretreatment experiments were performed in 125 ml Erlenmeyer shake flasks at 1% cellulose loading (approximately 2 wt% solids loadings), 50 °C, and 130 rpm. Novozymes Cellic® CTec2 cellulase enzyme preparation was added at the level of 20 mg protein (15 FPU) per gram of cellulose. The total volumes of the saccharification slurries after adding enzyme and buffer was 50 ml. Samples of the slurries were taken at 24, 48, 96, and 168 h, with glucose and xylose release measured by HPLC. The enzymatic hydrolysis procedure is adopted from NREL enzymatic hydrolysis protocol (NREL Technical Report NREL/TP-510-42629).

2.6. Chemical compositional analysis and yield calculations

Pretreatment slurries, enzymatic saccharification liquors, and fermentation samples were analyzed using HPLC according to standard NREL laboratory analytical procedures (LAPs) (Sluiter et al., 2008a). Solid residues were also analyzed in accordance with the published NREL LAPs (Sluiter et al., 2008b). Sugar yields from high solids enzymatic hydrolysis were calculated using the equations developed by (Zhu et al., 2011).

2.7. Particle size analysis

The particle size distributions of biomass samples were measured using laser diffraction on a Mastersizer 2000 with the Hydro 2000G module (Malvern Instruments) over the range from 0.02 to 2000 μm in a recirculating liquid suspension. For the analysis, 0.05–0.2 g of each cellulose sample was dispersed in water in a 15-mL centrifuge tube. Thereafter, individual dispersed samples were vortex mixed and transferred to the Hydro 2000G module that contained 0.8–1.0 L of deionized water ($n_r = 1.33$ at 20 °C), with a stirrer setting of 600 RPM and a pump setting of 1250 RPM. After 30 s delay, three, 15 s readings (30 s apart) of the circulating samples were acquired and averaged. The volume weighted mean value was used to represent the mean particle diameter (MPD). Each sample was run in triplicate and MPD are shown as the average of the triplicates.

2.8. SEM imaging

Pretreated corn stover samples were analyzed by Scanning Electron Microscope (SEM). Samples were first air-dried to 93%, and then sputter coated with gold. The SEM images were taken by a FEI Quanta 600i Environmental SEM (eSEM) at Metallurgical & Materials Engineering Department, Colorado School of Mines (Golden, CO). The environmental SEM is equipped with a tungsten cathode and a PGT energy dispersive spectrometer. The eSEM was operated under high vacuum mode with 30 kV of accelerating voltage and a probe current of 2 uA. The images were produced at a magnification of 4000×.

3. Results and discussion

3.1. Mechanical refining of low severity dilute acid pretreated corn stover

3.1.1. Effect of mechanical refining on particle size reduction

Fig. 2 shows the effects of mechanical refining on particle size distribution of PCS using various bench scale refiner mills. The corn stover (Pioneer variety 33A14) was pretreated at 150 °C, 0.5 wt% H_2SO_4 at 60% initial solids (acid loading of 3.4 mg H_2SO_4 per gram of O.D. biomass) for 15 min in the NREL Digester with a calculated pretreatment severity of 2.65 (Schell et al., 2003). The pH before pretreatment was measured at 1.8, and with steam condensation during the pretreatment increased to 2.2, giving a combined severity of 0.65 based on the severity calculation formula (Chum et al., 1990).

The PCS slurries were mechanically refined using a PFI mill (Fig. 2a), a 1-inch twin screw extruder (Fig. 2b), a food processor blender (Fig. 2c), and a 12-inch disk refiner (Fig. 2d). Mechanical refining of the pretreated slurry with increasing energy input causes a decrease in measured particle size and significant changes in particle size distributions (Barakat et al., 2013). The PFI mill and twin screw extruder showed significant effects on decreasing the particle sizes found in the distribution. The bench scale 12-inch refiner showed an intermediate effect on particle size reduction. The food processor blender showed the least effect on decreasing particle size of particles in the slurries.

Table 2 shows that the volume weighted mean particle size of the PCS control is approximately 270 μm. The use of a PFI mill and a twin screw extruder reduced the particle size distribution into the range of 80–100 μm. A lower particle size distribution occurred with higher refining energy input. As an example, increasing the PFI refining total revolutions or decreasing the mass flow rate into the extruder shifted the particle size distribution to smaller particles. Disk refining reduced the particle size distribution into the range of 140–150 μm particles. The non-linear relationship between disk refiner gap and particle size distribution indicates the refining effects of the disk refiner are heterogeneous. The food processor blender shows the least refining effect, where particle sizes are in the range of 170–200 μm. It should be noted

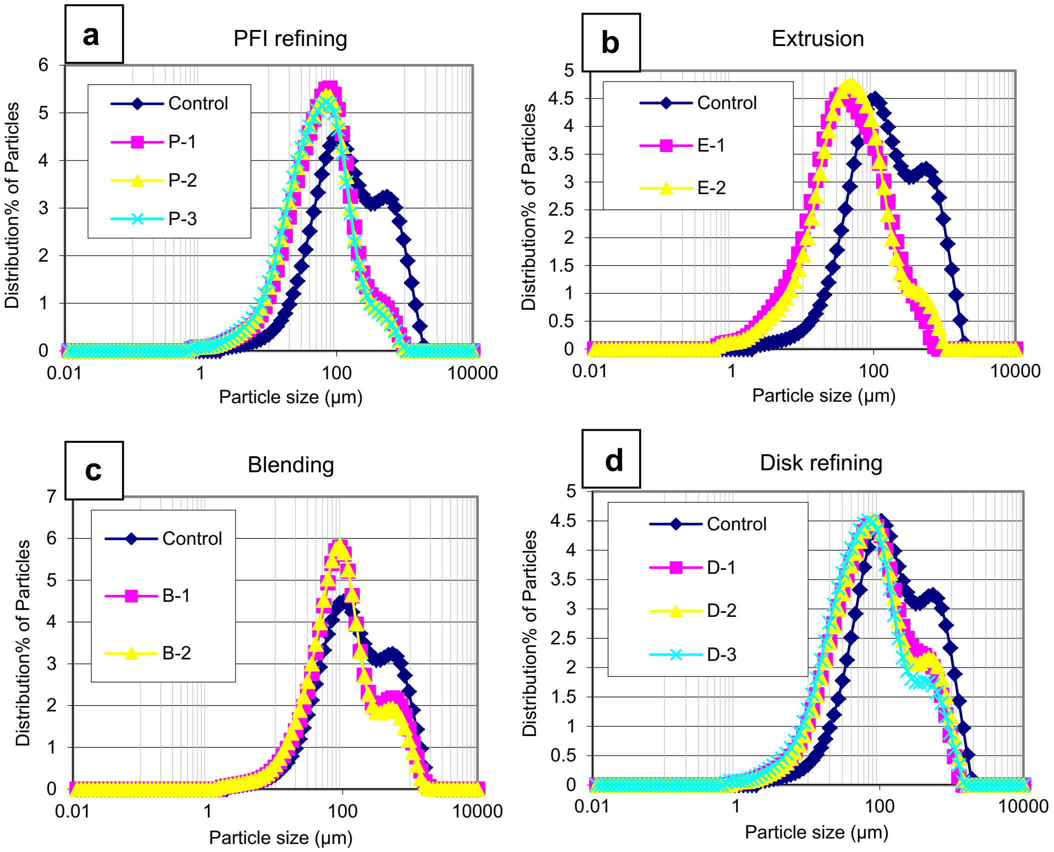

Fig. 2. Volume wt-% versus particle size distribution before and after mechanical refining. Corn stover (Pioneer variety 33A14) pretreated in the NREL Digester at 150 °C, 0.5 wt% H_2SO_4, 15 min was mechanically refined by a PFI mill (Panel a), a twin screw extruder (Panel b), a food processor blender (Panel c), and a 12-inch disk refiner (Panel d). Refining conditions for each sample are described in Table 2.

that two general peaks of distribution at lower and higher particle sizes are found in the particle size distribution for all samples tested. The control sample displays the largest peak area for the two peaks and the PFI refined and the extruded PCS slurries displayed the smallest area for the larger particle size peak shifting area to the lower particle size peak.

Particle size reduction can be concluded as a result of Class I refining (Leu and Zhu, 2013). In addition to particle size reduction, mechanical refining also led to morphological changes in PCS. SEM images shows that extrusion caused some cell wall breakup on the substrate, while PFI refining caused significant cracking and ruptures on the fiber wall (See supplementary material), indicating Class 2 refining occurs in extruded and PFI milled substrates to some degree. However, similar Class 2 refining effect is not observed on the substrates subjected to blending and disk refining.

3.1.2. Effect of various mechanical refining techniques on enzymatic hydrolysis

After pretreatment and mechanical refining, the corn stover slurries were extensively washed and subjected to enzymatic hydrolysis at a cellulose loading of 1 wt%, with the CTec2 enzyme loading of 20 mg protein per gram (15 FPU) of cellulose. The chemical composition of the PCS in this experiment is shown in Table 3.

As shown in Table 3, approximately 14% of the xylan is found in the PCS solids, which is consistent with a mild, low severity pretreatment. Fig. 3 shows the glucose and the xylose yields of refined solids after 48 h (Panel A) and 168 h (Panel B) of digestion. After 168 h of enzymatic hydrolysis, mechanical refining has improved glucose and xylose yields in all cases. The highest glucose yield improvements were found using the twin screw extruder at two feeding rates and using the PFI mill at 8000 revolutions of the rotor. The glucose yields in these three cases improved by ~20% compared to non-refined controls, while xylose yields improved by ~10–14%. The significant yield improvement indicates a good agreement of fiber morphological changes. Pretreated slurry samples refined with a PFI mill show increasing glucose and xylose yields with increasing refining energy input, as measured by the number of revolutions of the rotor. However, increasing the number of revolutions to 6000 resulted only in a 4% and 3% increase in the glucose and xylose yields, respectively. The minor increase in sugar yields indicates that a techno-economic analysis is necessary to justify whether tripling the refining energy will reduce ethanol production cost by increasing the overall sugar yield going into fermentation by ~7%. The PFI mill cannot be scaled up directly, so the Szego mill is investigated as a commercially available mill that may mimic the effects of the strictly bench-scale PFI mill.

Disk milling shows promise by increasing the enzymatic hydrolysis glucose yields when the gap or clearance between the disks is reduced. Reducing the disk gap actually increases the specific refining energy input by increasing the refining residence time. When disk clearances are reduced to "0" (plates barely touching), enzymatic hydrolysis glucose yields are improved by 15% (up to ~78%), and xylose yields are improved by 9% (up to ~53%) at the low severity pretreatment conditions.

Refining the low severity pretreated slurry using a food processor blender does improve the digestibility slightly when compared to the control, but it is much less of an improvement compared to the other refining techniques. Glucose yield improves by 10% and xylose yield improves by 7% using the level 1 setting of the food processor blender. This result is contradictory to the findings by

Table 3
Solids composition analysis of acid PCS.

Sample	% Ash	% Lignin	% Glucan	% Xylan	% Galactan	% Arabinan	% Acetate
Control kramer	6.2	22.6	49.5	14.0	0.7	1.1	2.3
Control INL	6.7	27.3	54.2	8.5	0.0	0.4	1.5
Deacetylated INL	1.1	24.4	65.2	7.5	0.7	0.6	0.4

(Dibble et al., 2011). In the experiments performed by Dibble, NREL small 200 kg/day horizontal screw reactor and a higher severity pretreatment (158 °C, 2 wt% H_2SO_4, 5 min) was used. It is possible that the higher severity pretreatment will reduce the effect of blending since blending only provides Class I refining (Leu and Zhu, 2013). In addition, the horizontal screw reactor has a grinding effect during pretreatment, which may also produce a similar Class I size reduction, resulting in little difference in yields between the control and blended material.

All of the mechanical refining technologies investigated increased the initial enzymatic hydrolysis rates within the first 48 h of digestion (Fig. 3, Panel A). Later in the digestions, glucose yield only improves by ~3–4% between 48 h and 168 h for samples refined by the PFI mill and the twin screw extruder, while glucose yields improve by approximately 8% for the controls (Fig. 3, Panel B). Mechanical refining could shorten overall saccharification time resulting in capital investment savings.

3.1.3. Effect of refining severity on biomass digestibility with PFI refining

The effect of PFI milling was studied at successively higher total revolutions on dilute acid pretreated INL corn stover. The substrate INL corn stover was pretreated at 150 °C, 0.5 wt% acid, at an initial solids loading of 40%, with a sulfuric acid loading of 8 mg per g of biomass in NREL Digester for 20 min. Unlike the samples used in 3.1.1 and 3.1.2, the severity factor for this pretreatment is 2.77, while combined severity factor is 0.973. Therefore less residual xylan is present in the PCS solids as shown in Table 3. The enzymatic digestions at the 1% cellulose loading level were performed on washed solids after pretreatment and mechanical refining. Enzymatic digestibility increased from ~64% up to ~77% when the number of revolution increases from 0 to 8000. When refined at 2000 revolutions, the glucose yield increased by approximately 7% compared to the non-PFI milled PCS. Starting from 2000 revolutions, every increase of 2000 revolutions will increase the glucose yield by approximately 2%. Interestingly, when the number of revolutions applied to the rotor of the PFI mill was increased from 8000 to 10,000 revolutions, the enzymatic digestibility decreased from ~77% to ~70%. This decrease might be caused by collapsing pore structure which decreases enzyme accessibility even though the particle sizes may be decreased. Similar observations have also been reported by Hui et al., 2009. They found that refining with a PFI mill at less than 12,000 revolutions the total volumes for the hardwood Kraft pulp were markedly increased, while refining with a PFI mill at 12,000 revolutions caused some macro-pores (>120 Å) loss for hardwood Kraft pulp (Hui et al., 2009). They concluded that

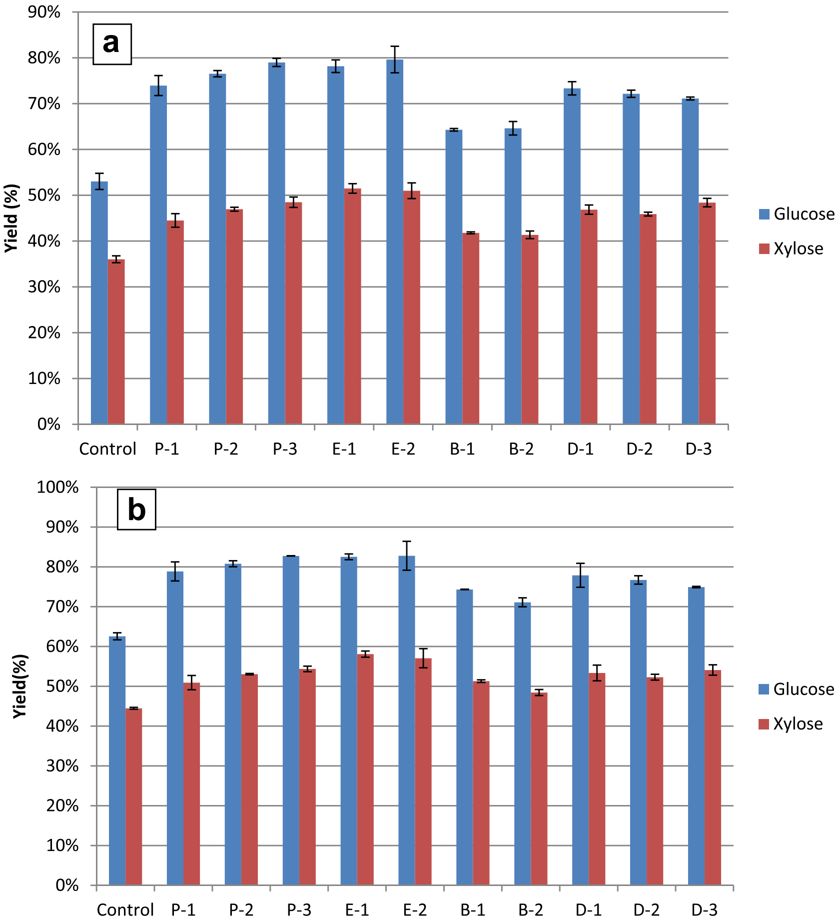

Fig. 3. Effects of refining technology on enzymatic digestibility of PCS, measured as glucose and xylose conversion yields after 48 h (Panel A) and 168 h (Panel B) of hydrolysis. Refining technology and conditions listed in Table 2.

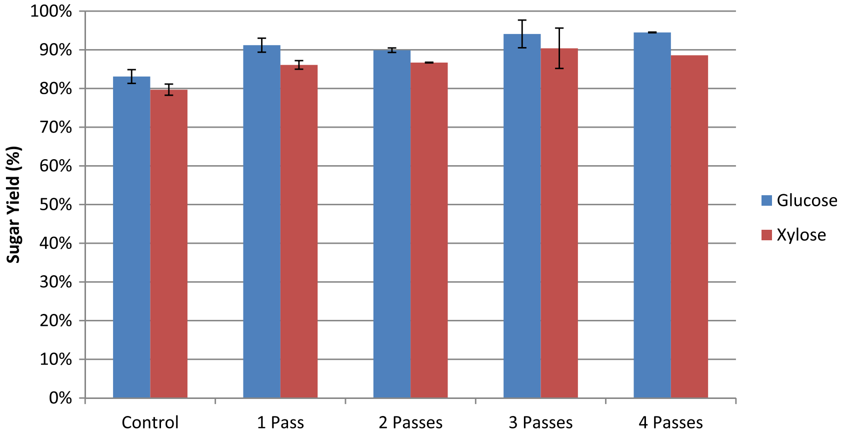

Fig. 4. Effects of Szego refining on enzymatic digestibility of PCS, measured as glucose and xylose conversion yields after 168 h of hydrolysis.

mild refining by a PFI mill (also known as beating) causes fibrillation of pulp fibers, leading to increased pore volume; however, severe beating can squeeze/destroy the pores, especially the macropores (Hui et al., 2009). In the current study, the loss of macro pores by beating for a total of 10,000 revolutions may have lowered the biomass digestibility due to the reduced enzyme accessibilities caused by the destruction of macro-pores. The accessible pore volume has been known to be a critical factor influencing enzymatic digestibility (Huang et al., 2010; Yu et al., 2011). It should be also noted that even the pretreatment severity of the PCS derived from INL corn stover as discussed in this section is higher than that of the Kramer PCS as discussed in Section 3.1.2, the digestibility of the INL PCS is much lower. The glucose yields of PFI refined INL PCS are approximately 7–10% lower than that of the Kramer PCS at the same corresponding refining energy applied, suggesting the mechanical refining effect is also influenced by biomass varieties.

3.2. Pilot-scale continuous mechanical refining with szego mill

Deacetylated INL corn stover pretreated in NREL's Metso Reactor was subjected to Szego milling. The compositional analysis shown in Table 3 shows that the continuous pretreatment reactor produces similar results as found with pretreatments in the bench scale steam explosion reactor. It is found that the deacetylation process effectively removes most of the acetyl groups from the corn stover. The PCS was passed through the Szego mill one to four times. After 1 pass, the particle size of PCS decreased from approximately 198 μm to approximately 64 μm. After 3 passes, the particle size decreased down to 56 μm. Compared to other refining technologies, Szego milling shows the significant decreases in particle sizes after refining.

Fig. 4 shows significant improvement in glucose and xylose yields during enzymatic hydrolysis following refining in the Szego mill. After 1 and 2 passes, the glucose and xylose yields are improved by 6–7%. If the PCS are refined 3+ times, the glucose and xylose yields are improved by 10–11% respectively, as final yields are approximately 95% and 90% respectively. Deacetylation process could significantly increase xylose yield by reducing xylan resistance to enzyme hydrolysis caused by the acetyl groups bounded on xylan backbone (Chen et al., 2012a). Szego milling could further enhance such effect by opening up the biomass cell wall structure and thus increasing the accessibility of xylan that originally unreachable by enzymes. The improved xylan hydrolysis led to better cellulose digestibility as a result of reducing xylan coverage on cellulose fibrils. The successful trials of Szego milling shows potential application of compression-type mechanical refining in current biorefinery process to achieve near-complete sugar hydrolysis. Future research should be focused on optimization of process parameters (feed speed, solids content, and etc.) and energy savings.

4. Conclusions

The decreased enzymatic digestibility caused by reductions in the pretreatment severity can be partially or fully recovered by applying a post-pretreatment mechanical refining step prior to enzymatic digestions. The highest enhancements in digestibility occurred using a PFI mill and a twin screw extruder as a result of Class II refining. Application of disk refiner and blender showed less enhancement in digestibility because only Class I refining occurred. Possible scaling up the PFI mill by using the Szego mill resulted in significant enhancements to the enzymatic digestibility of refined PCS.

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