Bioresource Technology 164 (2014) 128–135

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effect of pelleting process variables on physical properties and sugar yields of ammonia fiber expansion pretreated corn stover

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HIGHLIGHTS

- Durability of pelletized AFEX stover was >97.5% for all but preheated pellets.
- Bulk density was in the range of 588–634 kg/m^3 after pelleting AFEX stover.
- Die speed had no effect on glucose and xylose yields of AFEX stover pellets.
- Heating or larger grind size for pelleting lowered or did not affect sugar yield.
- The highest quality pellets used 4 mm AFEX stover, 60 Hz die speed and no heating.

ARTICLE INFO

Article history:
Received 9 November 2013
Received in revised form 30 January 2014
Accepted 1 February 2014
Available online 11 February 2014

Keywords:
Pelletization
Densification
Ammonia fiber expansion (AFEX)
Corn stover
Enzymatic hydrolysis

ABSTRACT

Pelletization process variables, including grind size (4, 6 mm), die speed (40, 50, 60 Hz), and preheating (none, 70 °C), were evaluated to understand their effect on pellet quality attributes and sugar yields of ammonia fiber expansion (AFEX) pretreated biomass. The bulk density of the pelletized AFEX corn stover was three to six times greater compared to untreated and AFEX-treated corn stover. Also, the durability of the pelletized AFEX corn stover was >97.5% for all pelletization conditions studied except for preheated pellets. Die speed had no effect on enzymatic hydrolysis sugar yields of pellets. Pellets produced with preheating or a larger grind size (6 mm) had similar or lower sugar yields. Pellets generated with 4 mm AFEX-treated corn stover, a 60 Hz die speed, and no preheating resulted in pellets with similar or greater density, durability, and sugar yields compared to other pelletization conditions.

1. Introduction

Interest in renewable energy resources has increased in recent years because of rising energy demand and fuel costs, environmental and national security concerns, and finite supplies of fossil fuels. Lignocellulosic biomass is a promising energy source, because it is available in large quantities that do not conflict with food production and may contribute to environmental sustainability (Demirbas, 2009). Primary sources of lignocellulosic biomass include agricultural residues, energy crops, forest products, and municipal solid waste.

1.1. Densification

Low bulk density of lignocellulosic biomass is a factor limiting its use as a feedstock, because it negatively affects storage and transportation (Eranki et al., 2011; Tumuluru et al., 2011), and directly influences costs throughout the supply system (Sokhansanj and Fenton, 2006). Increasing bulk density through a variety of available densification processes can increase unit density by about 10-fold (Tumuluru et al., 2010c). The different biomass densification technologies are (a) pellet mills, (b) balers, (c) briquette presses, (d) screw presses, and (e) agglomerators. These technologies can convert biomass, such as woody and herbaceous biomass, into densified products for fuel applications (Tumuluru et al., 2011). The densified products produced using these technologies will have improved bulk density, handling, conveyance efficiency, feedstock uniformity, and compositional quality, as well as conformance to specifications for conversion technologies and the supply system (Tumuluru et al., 2011). Among these

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http://dx.doi.org/10.1016/j.biortech.2014.02.005

technologies, pelleting and briquetting have been used for many years to produce densified biomass for fuel applications.

Process variables that typically impact the pelleting or briquetting process are particle size, preheating temperature, and die rotational speed (Tumuluru et al., 2011). Die speed impacts the material retention time in a pellet mill or an extruder, which further influences the viscosity of the biomass, die pressure and temperature of the resulting pellet (Rolfe et al., 2001; Tumuluru et al., 2011). Particle size of the feedstock influences the binding phenomena as smaller size particles have more surface area or contact area and facilitate better binding. Preconditioning biomass by preheating it prior to densification can affect both the chemical composition and the mechanical preprocessing attributes, thereby changing the way the feedstock responds during densification and improving the overall quality of the pellets (Bhattacharya et al., 1989; Tumuluru et al., 2010c). Preheating in the presence of moisture plays an important role as it softens some of the natural binders like starch, lignin, and protein in the biomass prior to pelletization and helps to produce more durable pellets.

1.2. Biomass conversion

Biomass pellets from a pellet mill are suitable for biochemical, thermochemical, and co-firing applications (Tumuluru et al., 2011). For biochemical conversion processes, pretreatment of lignocellulosic biomass is necessary to improve enzyme accessibility to cellulose and hemicellulose thus increasing product yields and reducing costs (Himmel et al., 2007). There is limited information on the effects of pelleting on pretreatment efficiency and biochemical conversion of biomass. Sugar yields have been reported to increase following pelleting of switchgrass when samples were treated by soaking in aqueous ammonia (Rijal et al., 2012), and pelleting of mixed feedstocks did not affect sugar yields and hydrolysis kinetics following ionic liquid pretreatment (Shi et al., 2013). In addition, corn stover pellets were not more recalcitrant to dilute-acid pretreatment compared with un-pelleted corn stover, and even enhanced ethanol yields (Ray et al., 2013). Theerarattananoon et al. (2012) is one of the only studies to investigate pelleting process variables on conversion of dilute-acid pretreated biomass, and observed that glucan content of pretreated biomass and enzymatic conversion of cellulose (ECC) was positively affected by die thickness, but die thickness negatively affected xylan content of pretreated solids. Mill screen size had the opposite trends for glucan and xylan content of pretreated solids, and had no significant effect on ECC.

1.3. AFEX pretreatment

Ammonia fiber expansion (AFEX) is a promising pretreatment that involves treating biomass with ammonia under increased temperature and pressure followed by a rapid release of pressure resulting in physical and chemical alterations to the biomass (Balan et al., 2009). AFEX pretreatment causes cellulose decrystallization (Gollapalli et al., 2002), altered lignin structure, increased surface area accessible to enzymes (Sulbarán-De-Ferrer et al., 2003), and only partial degradation of hemicellulose and lignin, which are not removed into a separate liquid stream (Chundawat et al., 2011). AFEX pretreatment has increased glucan and xylan conversions and ethanol yields for a variety of feedstocks including switchgrass, corn stover, and bagasse (Balan et al., 2009; Teymouri et al., 2005). Recently Campbell et al. (2013) published a paper on development of methods for AFEX pretreatment to establish the technical feasibility of the packed bed AFEX process with an emphasis on understanding the effectiveness of this process on sugar yields of AFEX-treated corn stover and wheat straw and the impact of AFEX pretreatment on pellet physical properties. The authors indicated that high-quality pellets in terms of density and durability can be produced; for material that was 20% moisture, bulk density approached that of corn grain and durability was 99%, which exceeds the standard durability (97.5%) set for handling and transportation of pellets (BSI, 2010). Bals et al. (2013) went a step further and did enzymatic hydrolysis of pelletized AFEX-treated corn stover at high solid loadings. In both of the previously mentioned studies the effects of different pelletization process conditions on the quality of pellets and sugar yields are not thoroughly investigated. Also, our literature review indicated that there are no published data available on quality and sugar yields of AFEX pellets produced with different pelleting conditions.

The overall objective of this study was to understand the effect of AFEX pretreatment and pelleting process variables on the quality of pellets and sugar yields. Tumuluru et al. (2011), in their review on biomass densification for producing a uniform feedstock commodity for bioenergy application, identified process variables (die temperature, pressure, and die geometry), feedstock variables (moisture content and particle size and shape), and biomass compositional properties (protein, fat, cellulose, hemicellulose, and lignin) that play major roles in the quality of the densified biomass. In the present study, process variables like grind size, die speed, and preheating were selected to understand their impact on quality and bioconversion. The specific objectives of this study were to determine the impact of grind size (4 mm, 6 mm), die speed (40, 50, 60 Hz), and preheating (none, 70 °C) on physical properties of pellets (unit, bulk and tapped density; and durability) and sugar yields (glucose and xylose) from enzymatic hydrolysis.

2. Methods

2.1. Feedstock

Corn stover (*Zea mays* L.) used in this study was harvested and baled in Boone, Iowa, USA in Fall 2011, and stored on pallets under a tarp in Idaho Falls, Idaho, USA for approximately 3 months. The material was ground with a Vermeer BG480 grinder (Vermeer Corporation, Pella, IA, USA), passed through a 25.4 mm (1.0 in.) screen and dried using a rotary drier (~93 °C; SD75-22 Dryer System, Baker–Rullman, Watertown, WI, USA). Untreated corn stover was subsequently milled to 4 mm and 6 mm with a Thomas Model 4 Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) for subsequent analyses. Chemical composition of the untreated corn stover was determined in duplicate using National Renewable Energy Laboratory (NREL) Laboratory Analytical Procedures (LAP) (Sluiter et al., 2010). Fig. 1 indicates the different experiments performed on the untreated, AFEX pretreated, and AFEX pretreated and pelletized corn stover.

2.2. AFEX pretreatment

Ammonia fiber expansion (AFEX) pretreatment of the 25.4 mm grind corn stover was completed using an ammonia loading of 1 kg ammonia to 1 kg of dry biomass, 90–110 °C, a 30 min residence time, and 40% biomass moisture content, by following the method described by Campbell et al. (2013). After the AFEX treatment was completed, the sample was dried at 45 °C overnight. AFEX-treated corn stover was ground to 4 mm and 6 mm with a Thomas Model 4 Wiley mill for subsequent analysis and pelletization.

2.3. Experimental plan

The experiment was designed to investigate the effects of pelletization speed, feedstock particle size and preheating on physical and biochemical properties of AFEX-treated corn stover.

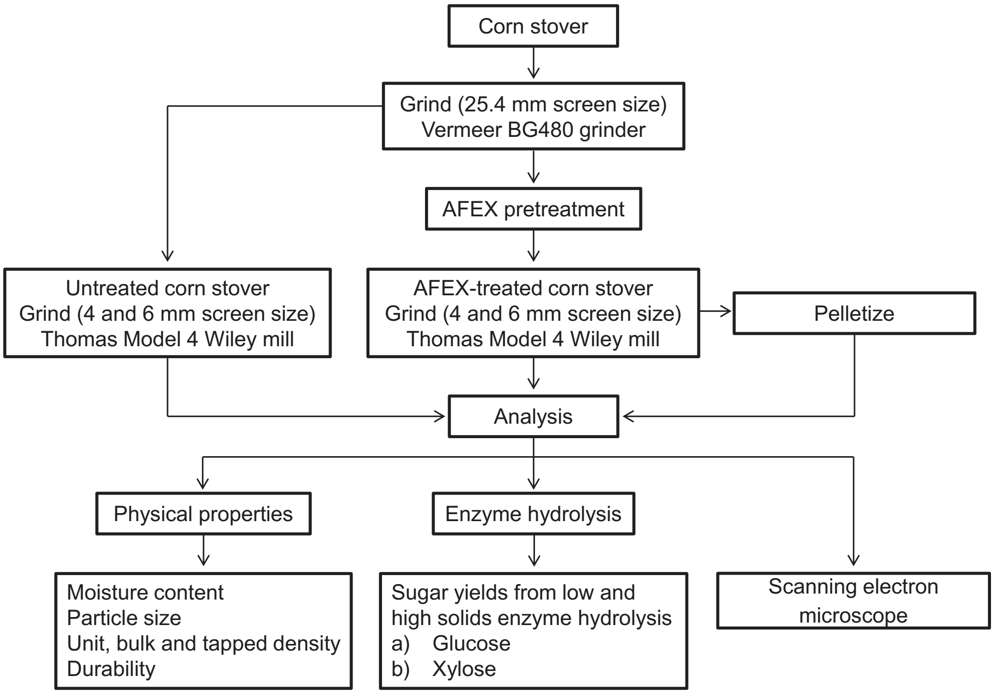

Fig. 1. Flow diagram of the different experiments conducted for untreated, AFEX-treated, and AFEX-pelletized corn stover.

AFEX-treated corn stover was pelletized under three die speeds (40, 50, 60 Hz), two grind sizes (4, 6 mm), and with or without 70 °C preheating.

2.4. Pelleting process

Fig. 2 depicts the flat-die pellet mill used for pelleting AFEX-treated corn stover at different conditions (Tumuluru, 2013; Tumuluru, 2014). A laboratory-scale ECO-10 (Colorado Mill Equipment, Cañon City, CO, USA) flat-die pellet mill with a rotating die, stationary roller shaft, and 10 HP motor was used for the present pelletization studies. The rotational speed of the die at a maximum of 60 Hz was 1750 rpm. A hopper with a screw feeder uniformly fed biomass to the pellet mill. Both the hopper and feeder are provided with flexible heating tape and *J*-type thermocouples and controllers to preheat the biomass both in the hopper and the feeder at a constant temperature. The die of the pellet mill is provided with a variable frequency drive (Altivar model 71 variable-frequency AC motor driver for 10 HP, 480 V/3 P) to control the rotational speed of the die.

Approximately 3 kg of AFEX-treated corn stover that was size reduced to a 4 or 6 mm screen size was used for the pelleting studies. This size-reduced material was mixed in a ribbon blender (RB 500, Colorado Mill Equipment, Cañon City, CO, USA), to adjust the moisture levels to 26% (w/w, wet basis). The moisture-adjusted corn stover was stored for 48 h in a cold storage unit set at about

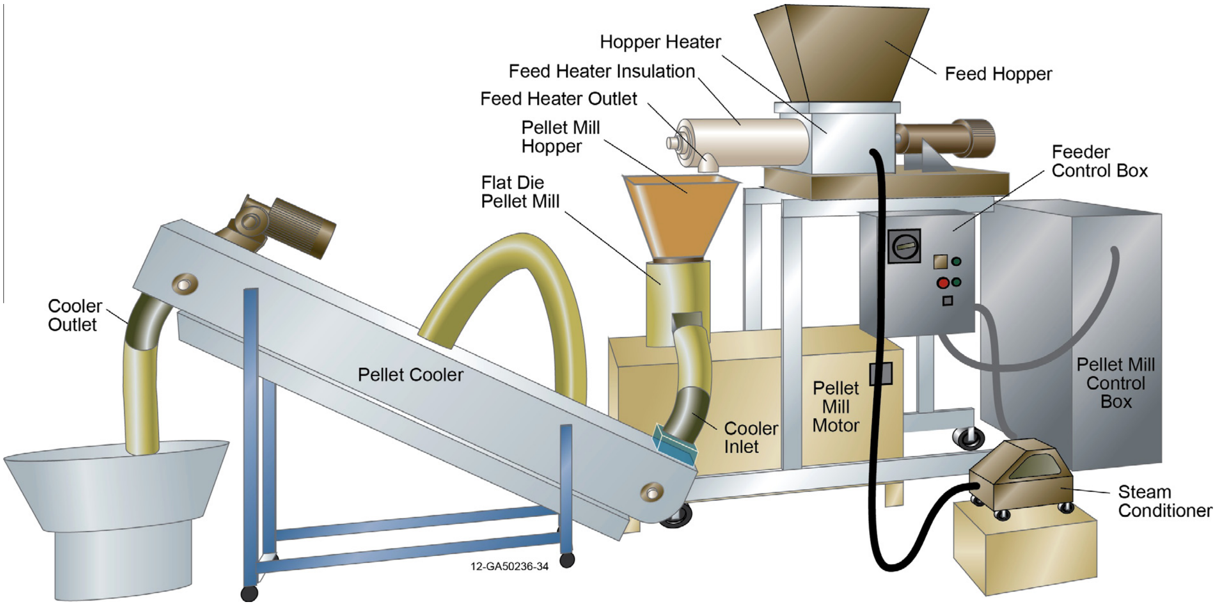

Fig. 2. Flat-die pellet mill used in the present study (adapted from Tumuluru (2014)).

4 °C for moisture equilibration. The material was then loaded to the feeder hopper of the pellet mill, where it was preheated to 70 °C before pelletization. The pellets produced were passed through a horizontal cooler to reduce the moisture content. The cooled pellets produced still had moisture too high to store the samples without degradation; therefore, samples were dried in a conventional oven at 40 °C for 7 h to bring the pellet moisture content to about 9%. At this point the physical properties of the pellets including unit, bulk, and tapped density and durability were measured. Pellets were dried additionally at 45 °C until they had between 4% and 6% moisture content (w/w, 105 °C wet basis), and then scanning electron microscopy and enzymatic hydrolysis assays were performed.

2.5. Physical properties

2.5.1. Particle-size distribution

A digital image processing system (CAMSIZER®, Horiba Instruments Inc., Irvine, CA, USA), equipped with two digital cameras, was used to determine particle morphology (or characteristics) for duplicate samples of 4 mm and 6 mm untreated and AFEX-treated corn stover according to methods described by Ray et al. (2013).

2.5.2. Moisture content

The moisture content of pellets was measured in triplicate by drying samples in a 105 °C convection oven for 24 h following the ASABE (2003) procedure. The samples were weighed before and after drying, and the moisture content was expressed on a wet basis.

2.5.3. Density

2.5.3.1. Bulk and tapped density for untreated and AFEX-treated corn stover. A container with a volume of 261 mL was used to determine loose and tapped bulk density of untreated and AFEX-treated corn stover for three replicates. For loose bulk density, the container was filled until overflowing, excess material was removed by striking a straight edge across the top, and the weight of the material in the container was recorded. The container was dropped 25 times from 15 cm, filled to the top with material, and weighed to determine tapped bulk density.

2.5.3.2. Unit, bulk, and tapped density for AFEX pellets. Unit density was determined by measuring the mass of the individual pellets and dividing it by its volume (Tumuluru et al., 2010a,b). Loose and tapped bulk densities were done in triplicate for untreated, AFEX-treated, and AFEX-treated and pelletized corn stover according to ASABE (2007). For loose bulk density, the pellets were poured slowly into the standard size container until it was overflowing. The excess material was removed by striking a straight edge across the top. The weight of the material in the container was recorded. For tapped density, the loosely filled container was tapped on the laboratory bench and then the container was filled to the top and weighed. Loose and tapped bulk densities were calculated by dividing the mass over the container volume.

2.5.4. Durability

Durability of pellets was determined using a four-compartment, pellet-durability tester (Seedburo Equipment Co., Des Plaines, IL, USA). A sample of pellets to be tested was sieved on the appropriate sieve to remove fines. Approximately 500 g of sieved pellets were placed in each compartment of the tumbler. After tumbling for 10 min, the sample was removed and sieved using a 4.0 mm screen to remove the fines. Pellet durability was calculated by the following equation (ASABE, 2007):

$$\text{Durability} = \frac{\text{Mass of pellets after tumbling}}{\text{Mass of pellets before tumbling}} \times 100 \tag{1}$$

2.6. Scanning electron microscopy

Scanning electron microscopy was performed with a Quanta 650 FEG (FEI, Hillsboro, OR, USA) to collect micrographs of 4 mm untreated corn stover; 4 mm AFEX-treated corn stover; and 4 mm, 40 Hz AFEX-treated corn stover pellets.

2.7. Biochemical conversion

2.7.1. Low solids enzymatic hydrolysis for untreated and AFEX-treated stover and AFEX pellets

Enzymatic saccharification of untreated corn stover, AFEX-treated corn stover, and each pellet experiment was conducted in triplicate following LAPs developed by NREL (Selig et al., 2008) at 1.5% solid loading (w/v, on a dry weight basis). The hydrolysis mixture contained 7.5 μL of xylanase enzyme (Accellerase XY, Genencor, Palo Alto, CA, USA) and 30 filter paper units of cellulose enzyme (Accellerase 1500, Genencor, Palo Alto, CA, USA) per gram biomass on a dry weight basis. Sodium azide was added to prevent microbial growth. The mixture was hydrolyzed at 50 °C rotating at 200 rpm for 168 h. For sugars analysis, 1.5 mL aliquots were taken at 48 h and 1.5 mL to 5 mL aliquots were taken at 168 h, filtered through a 0.2 μm filter, and analyzed using high-performance liquid chromatography (HPLC). A blank test was included that contained enzyme and all inputs except biomass. Hydrolysis yields were calculated according to Selig et al. (2008) using the composition of the untreated corn stover described in Section 2.1 and HPLC sugar analysis of the samples taken during the hydrolysis.

2.7.2. High solids enzymatic hydrolysis for AFEX pellets

Enzymatic hydrolysis experiments were conducted in duplicate at 20% solid loading (w/v, on dry weight basis). Tetracycline and cycloheximide were added to the mixture at 40 mg/L and 30 mg/L, respectively, to prevent microbial growth. Citric acid buffer was used to adjust the pH to about 5. Commercial enzymes provided by Novozymes (Bagsvaerd, Denmark), CTec3 and HTec3, were each added at 10 mg protein per g glucan available in the biomass. Protein concentration was determined as total nitrogen less ammonia nitrogen multiplied by 6.25, which is a common conversion factor for nitrogen-to-protein calculations (Sluiter et al., 2010). The hydrolysis was carried out for 72 h in an incubator shaker at 50 °C. Agitation was at 200 rpm. A blank test was also conducted that contained enzyme and all inputs except biomass.

After 72 h of hydrolysis, a 10-mL sample was taken from the hydrolysate slurries for HPLC analysis and density measurement. Density measurement was performed in duplicate by weighing the filtered hydrolysates in 1-mL volumetric flasks on an analytical balance to the nearest 0.1 mg. Hydrolysis yields were calculated using the density method as described in Zhu et al. (2011) using the composition of the untreated corn stover described in Section 2.1.

2.8. Statistical analysis

Statistical analysis was completed using R 2.14.0 (R Development Core Team, 2011). All data were analyzed with one-way ANOVA and post hoc Tukey's honestly significant difference tests using the R functions ANOVA and pairw.test, except tapped bulk density of pellets. Tukey's tests were completed only if the one-way ANOVA was significant at $p < 0.05$. Data transformations were done if datasets did not meet the assumptions related to homogeneity or normality of residuals. Residuals of tapped bulk density for pellet treatments did not meet the assumptions of normality even when data transformations were applied; therefore, a Brunner–Dette Monk rank-based permutation test was used, because it does not require meeting assumptions related to normality.

3. Results and discussion

3.1. Effects of AFEX pretreatment and pelletization on physical properties

3.1.1. AFEX pretreatment

Geometric mean particle size was significantly smaller for 4 mm compared to 6 mm untreated corn stover at the 16th, 50th and 84th percentile, and these differences were also present for AFEX-treated stover except for at the 16th percentile (Table 1). In addition, AFEX-treated corn stover had a significantly smaller mean particle size than untreated corn stover for both 4 mm and 6 mm grind sizes indicating the AFEX-treated corn stover lost its tenacious nature and became more brittle and friable. Loose and tapped bulk densities were significantly greater for 4 mm untreated stover compared to 6 mm untreated stover, and after AFEX pretreatment bulk density was about 1.7 times greater for both grind sizes (Table 1).

3.1.2. Pelletization

3.1.2.1. Moisture content. Moisture content of pellets measured immediately after pelletization was in the range of 19–22% (w/w, wet basis), which was too high for storage without degradation of the biomass. After drying at 40 °C for 7 h the final moisture content of the pellets was about 9% (w/w, wet basis). At this moisture content the other physical properties such as unit, bulk, and tapped density and durability were measured.

3.1.2.2. Unit, bulk and tapped density. The methods used to measure bulk density were slightly different between raw and pelleted material and, with this considered, the bulk density of the pelletized AFEX corn stover was three to six times greater compared to untreated and AFEX-treated corn stover. Bulk density of AFEX pellets (588–634 kg/m^3) was slightly higher than values reported by Campbell et al. (2013) for untreated corn stover (444 kg/m^3) and AFEX-treated corn stover (505–575 kg/m^3) likely because the pellets in Campbell et al. (2013) were produced with 25.4 mm material. These values are close to the bulk density of corn grain (700 kg/m^3), but are only approximately half of the bulk density of coal (1346 kg/m^3). Greater bulk density of AFEX pellets compared to raw corn stover and untreated corn stover pellets can have benefits for transportation with fewer trucks or railcars necessary to transport the same weight of material. These potential benefits are important to consider in analyses of supply chain logistics such as the exploration of Regional Biomass Preprocessing Depots in Eranki et al. (2011). Pellets generated under all conditions had similar loose and tapped bulk density with the only significant difference of note that 4 mm, 60 Hz pellets had greater loose bulk density and unit density compared to 4 mm, 50 Hz pellets (Table 2). Lowering die speed increases the residence time of the material in the die and results in moisture flash-off when pellets exit the die. This flash-off of moisture can result in expanded products with lower density. The data indicated that even though grind size of the feed material had significant impacts on mean particle sizes (Table 1) it did not have any significant impact on the pellet densities, but changing the die rotational speed did influence the densities. The observations were consistent with respect to preheating; the change in grind size did not have a significant effect on the densities of preheated materials.

3.1.2.3. Durability. Pellets generated with 4 mm AFEX-treated corn stover without heat had 99% durability, which exceeds the standard durability (97.5%) set for handling and transportation of

Table 1
Particle size parameters from digital image analysis (mean (absolute value of the difference between duplicates); $n = 2$) and bulk density (mean (1 SD); $n = 3$) for untreated and AFEX-treated corn stover. Upper-case letters indicate significant differences using post hoc Tukey's tests ($p < 0.05$).

Treatment	D_{16}[a] (mm)	D_{50} (mm)	D_{84} (mm)	Loose bulk density (kg/m^3)	Tapped bulk density (kg/m^3)
4 mm untreated	0.24 (0.00)^{A}	0.50 (0.01)^{A}	1.00 (0.01)^{A}	120.46 (2.33)^{A}	152.99 (0.46)^{A}
6 mm untreated	0.33 (0.00)^{B}	0.81 (0.00)^{B}	1.65 (0.01)^{B}	99.17 (1.35)^{B}	123.71 (1.51)^{B}
4 mm AFEX	0.20 (0.01)^{C}	0.43 (0.02)^{C}	0.91 (0.02)^{C}	198.84 (5.57)^{C}	252.93 (2.36)^{C}
6 mm AFEX	0.22 (0.01)^{C}	0.61 (0.03)^{D}	1.35 (0.06)^{D}	170.42 (3.95)^{D}	206.07 (3.31)^{D}
ANOVA p-value	4.5E-5	3.2E-5	1.5E-5	2.6E-9	6.8E-12

[a] D is the geometric mean particle size for the 16th, 50th, and 84th percentiles.

Table 2
Pelleting process parameters and physical properties for pellets generated under each experimental set of conditions (mean (1 SD)). Upper-case letters indicate significant differences using post hoc Tukey's tests ($p < 0.05$).

Expt. #	Operating parameters			Physical properties			
	Grind size (mm)	Die speed (Hz)	Preheating (°C)	Unit density[a] (kg/m^3)	Loose bulk density[b] (kg/m^3)	Tapped bulk density[b] (kg/m^3)	Durability index[c]
1	4	40	None	1109.57 (122.90)^{AB}	613.82 (9.70)^{AB}	678.80 (14.40)	99.22 (0.20)^{A}
2	4	50	None	1049.82 (48.07)^{A}	588.06 (18.81)^{A}	657.05 (17.05)	99.00 (0.07)^{A}
3	4	60	None	1188.41 (56.19)^{BC}	630.65 (3.84)^{B}	699.79 (9.44)	99.11 (0.04)^{A}
4	6	60	None	1267.38 (44.65)^{C}	633.98 (13.85)^{B}	699.27 (12.85)	98.06 (0.05)^{B}
5	4	60	70	1201.96 (61.58)^{BC}	603.68 (20.08)^{AB}	673.81 (23.26)	96.75 (0.14)^{C}
6	6	60	70	1217.14 (101.23)^{C}	606.97 (17.76)^{AB}	681.14 (12.70)	97.42 (0.04)^{D}
		ANOVA p-value		1.40E-06	0.024	0.11[d]	<2.2E-16

[a] $n = 9$, unit density values were raised to the third power before a one-way ANOVA was performed.

[b] $n = 3$.

[c] $n = 4$.

[d] Statistical results are from a Brunner–Dette Monk rank-based permutation test, not a one-way ANOVA.

pellets (Table 2; BSI, 2010). These durability values corroborate the findings of Campbell et al. (2013). Compared to raw pellets reported for corn stover and other feedstocks (Ray et al., 2013; Rijal et al., 2012; Theerarattananoon et al., 2012), AFEX pellets had higher durability. Pellets generated using 6 mm AFEX-treated corn stover with a 60 Hz die speed without preheating had slightly lower durability, but still above the 97.5% standard; however, the 4 mm and 6 mm, 60 Hz pellets with preheating had durability less than this that corresponds to their lower moisture contents (<9% after 40 °C drying).

Overall, using a higher die speed did not have a negative effect on bulk density or durability of AFEX pellets. Pellets produced with a 60 Hz die speed and a larger grind size of AFEX-treated stover had similar density, but decreased durability. Preheating of pellets further reduced the durability, but had no effect on the density of the pellets. The highest quality AFEX pellets with the same or significantly greater density and durability compared to other pellet treatments were produced using a 4 mm grind size and a 60 Hz die speed, and the use of a higher die speed (60 Hz) is preferable as it increases the throughput of the pellet mill. Payne (1978) concluded that medium or fine-ground materials are desirable in pelleting because they have greater surface area for moisture addition during steam conditioning, which increases starch gelatinization and promotes binding. However, very small particles of <2 mm can lead to jamming of pellet mills and affect production capacity.

3.2. Scanning electron microscopy (SEM)

AFEX pretreatment and pelleting can have an effect on corn stover cell morphology. Chundawat et al. (2011) reported that the middle lamella and outer secondary cell wall were locations affected by AFEX pretreatment and are areas enzymes can access cell structures (Donohoe et al., 2009). Cell structure of AFEX-treated corn stover had slight deformations evident in the micrographs of the cell wall (Supplemental Fig. 1a and b). Micrographs of the epidermis and underlying cell structure of AFEX-treated stover indicated similar patterns (Supplemental Fig. 1c). Pelleting of AFEX-treated corn stover caused severe disruptions of cell structure apparent in the micrographs of the epidermis and underlying cells of pelletized AFEX stover (Supplemental Fig. 1d). Similarly, tracheary elements in untreated corn stover (Supplemental Fig. 1e) had more remaining structure than in pelletized AFEX stover (Supplemental Fig. 1f). In general, it was difficult to locate remaining cell structures in pellets to image with SEM. Much of the pellet appeared similar to SEM images of corn stover pellets in Kaliyan and Morey (2010) and Ray et al. (2013).

3.3. Effects of AFEX pretreatment and pelleting on sugar yields

3.3.1. AFEX pretreatment

Chemical composition of untreated corn stover before AFEX pretreatment was: glucan 35.8%, xylan 18.7%, galactan 1.6%, arabinan 2.9%, mannan 1.0%, and ash 8.0%. In the present study, AFEX pretreatment significantly increased the glucose and xylose yields by four to six fold for both 4 mm and 6 mm particle sizes (Table 3). These results corroborate the findings of Teymouri et al. (2005) and Balan et al. (2009), where increases of glucan and xylan hydrolysis were observed for feedstocks such as corn stover, switchgrass, and sugar cane bagasse. Chundawat et al. (2011) proposed that the surface area accessible to enzymes increased in AFEX-treated biomass due to cleavage of lignin-carbohydrate ester linkages and relocation of hemicellulose oligomers and extractables to the surface of the outer cell wall creating a more porous structure. The SEM observations in Supplemental Fig. 1a–c indicate that the cell structure of corn stover was disrupted after AFEX pretreatment in this study; however, it is not possible to determine which areas of the cell wall were affected.

Table 3
Glucose and xylose yields (mean (1 SD); $n = 3$) at 48 h and 168 h from low solid loading enzymatic hydrolysis. Upper-case letters indicate significant differences using post hoc Tukey's tests ($p < 0.05$).

Treatment	Glucose yield (%)		Xylose yield (%)	
	48 h	168 h	48 h	168 h
4 mm untreated	17 (0)^{A}	20 (0)^{A}	10 (0)^{A}	13 (0)^{A}
6 mm untreated	16 (1)^{A}	18 (1)^{A}	9 (1)^{A}	11 (1)^{A}
4 mm AFEX	67 (2)^{BC}	72 (2)^{B}	54 (1)^{B}	58 (2)^{BCD}
6 mm AFEX	66 (1)^{BC}	72 (0)^{B}	52 (1)^{BC}	56 (2)^{BD}
4 mm, 40 Hz AFEX pellet	69 (1)^{BD}	79 (1)^{CD}	54 (1)^{BD}	59 (1)^{BC}
4 mm, 50 Hz AFEX pellet	72 (4)^{B}	79 (1)^{CD}	55 (2)^{B}	60 (1)^{C}
4 mm, 60 Hz AFEX pellet	69 (4)^{BD}	81 (1)^{C}	54 (1)^{B}	60 (0)^{C}
6 mm, 60 Hz AFEX pellet	59 (3)^{C}	74 (3)^{BD}	49 (2)^{C}	54 (2)^{D}
4 mm, 60 Hz, 70° AFEX pellet	62 (5)^{CD}	77 (1)^{D}	50 (2)^{CD}	56 (1)^{BCD}
6 mm, 60 Hz, 70° AFEX pellet	63 (3)^{CD}	72 (1)^{B}	50 (1)^{CD}	55 (1)^{D}
ANOVA *p*-value	<2.2E-16	<2.2E-16	<2.2E-16	<2.2E-16

3.3.2. Pelleting

Pelleting has also been reported to have a positive or neutral effect on sugar yields when biomass was pretreated with ionic liquid (Shi et al., 2013), with dilute acid (Ray et al., 2013; Theerarattananoon et al., 2012), or by soaking in aqueous ammonia (Rijal et al., 2012). Consistent with these results, AFEX pellets generated with 4 mm grind material and 40, 50, and 60 Hz die speeds had 6–8% greater glucose yield for low solids hydrolysis compared to the un-pelleted, 4 mm AFEX-treated corn stover at 168 h (Tukey's $p < 0.05$; Table 3); however, there was no significant difference in yield at 48 h for glucose or at 48 or 168 h for xylose (Table 3). Bals et al. (2013) investigated the effects of pelletization of AFEX-treated corn stover on enzymatic hydrolysis yields and mixing at high solid loadings. Hydrolysis yields were similar between 2 mm AFEX-treated corn stover and AFEX corn stover pelletized using 70 °C preheating, but pelleting actually enhanced sugar yields compared to 25.4 mm (1.0 in.) AFEX-treated corn stover (Bals et al., 2013). However, at 3% solids loading in the same study pelleting had slightly lower yields compared to 2 mm material. Preheated pellets in this study made with 4 mm AFEX-treated stover had significantly higher 168 h glucose yields and similar 168 h xylose yields from low solids hydrolysis compared to 4 mm AFEX-treated stover. The minor discrepancies between these studies could be due to enzymatic hydrolysis conditions or more likely pelletization parameters, which is the focus of this work.

Many mechanisms have been discussed as to why conversion yields improve or are not altered following pelletization. Bals et al. (2013) suggested that sugar yields of AFEX-treated stover improved because of decreased particle size during pelletization and increased mixing during high solid loadings with a minor negative effect of hornification. Shi et al. (2013) proposed that the pressure and heat from pelleting served as a thermochemical pretreatment. Additional mechanisms suggested by other researchers include thermal softening or plasticization of lignin during pelletization and shear from grinding, compression, and extrusion (Rijal et al., 2012; Theerarattananoon et al., 2012). SEM observations of structural changes as a result of pelletization and the severe deformation of cell walls and tracheary elements for pelleted AFEX stover (Supplemental Fig. 1d (cell walls) and 1f (tracheary element)) compared to 4 mm untreated and AFEX-treated stover (Supplemental Fig. 1a, b, and c (cell walls) and 1e (tracheary element)) shows the physical effect of a combination of the factors discussed in the literature including pressure and heating during pelletization. The surface area accessible for enzymatic attack is likely altered during pelleting because of physical disruption seen in the SEM results and chemical alterations including lignin softening or plasticization, which might be particularly important because lignin is relocalized during AFEX pretreatment (Chundawat et al., 2011). Studies of surface area and porosity of pellets produced using different pelletization conditions are underway to link trends in enzymatic hydrolysis with physical properties of pellets.

3.3.2.1. Effect of die speed. Die speed influences residence time of the biomass material in the pellet mill with material spending less time in the pellet mill at higher die speeds. Residence time can affect biomass viscosity, die pressure, product temperature, and product expansion. The higher bulk density of the 4 mm, 60 Hz pellets compared with the 50 Hz pellets was attributed to less residence time and thus less expansion of the produced pellet (Table 2). However, the expansion of the pellets does not appear to have affected the sugar yields as die speed did not affect glucose or xylose yields for low or high solids hydrolysis (Tables 3 and 4). Theerarattananoon et al. (2012) demonstrated that a larger die thickness (hole diameter × effective thickness) on a ring-die pellet mill increased enzymatic conversion to cellulose for corn stover, wheat straw, big bluestem, and sorghum stalk treated with dilute acid, but the authors are not aware of any studies on the effects of die speed on sugar yields. Based on the present results, the optimal process conditions are to operate the pellet mill at higher die speeds as it increases sample throughput of the pellet mill and reduces specific energy consumption.

3.3.2.2. Effect of grind size. A larger grind size could decrease grinding energy necessary to generate raw sample for pelletization, but can also decrease particle binding during pelletization. It is generally accepted that feed-material particle size influences the density and durability of the pellets; for example, small particles yield higher density and durability pellets (Mani et al., 2006). Pelleting significantly increased the glucose yield at 168 h for 4 mm AFEX-treated stover, but did not increase the glucose yield at 168 h for 6 mm AFEX-treated stover at low solid conditions (Table 3), and xylose yields were not altered for pellets made with either grind size (Table 3). SEM indicated that not all fibers were disrupted after pelletization (data not shown); therefore, the significantly larger particle sizes for the 6 mm AFEX-treated stover may have created pockets of cellulose inaccessible to enzymatic attack, while the hemicellulose was more accessible because it was solubilized and relocated to the cell surface. More extensive micro-characterization is necessary to understand the mechanisms behind these patterns.

Glucose yields were 10% greater at 48 h and 7% greater at 168 h for 4 mm, 60 Hz pellets at low solid loadings compared to 6 mm, 60 Hz pellets. This trend did not hold at high solid loadings (Tables 3 and 4) corroborating previous results from dilute-acid pretreated biomass pellets that had no significant difference between enzymatic conversion of cellulose with an increase in grind size from 3.2 mm to 6.5 mm (Theerarattananoon et al., 2012). Increasing grind size of AFEX-treated corn stover for generating 60 Hz pellets had a negative effect on xylose yield, decreasing yields by 5% at 48 h and 6% at 168 h for low solid loadings and by 4% under high solid loadings (Tables 3 and 4). It was suggested by Bals et al. (2013) that hemicellulose may react with lignin making it inaccessible for conversion, and possibly this reaction only occurs under certain pelletization conditions. In addition, the cell wall composition has been suggested to remain unchanged during pelletization with the possibility of a slight loss of hemicellulose (Bals et al., 2013; Kumar et al., 2012; Rijal et al., 2012; Theerarattananoon et al., 2012). The xylan yields reported in this study are based on the composition of the untreated material. Therefore, xylan yields from enzymatic hydrolysis may be lower if hemicellulose was lost under certain pelletization conditions.

Table 4
Glucose and xylose yields (mean (absolute value of the difference between duplicates); $n = 2$) from high solid loading enzymatic hydrolysis. Upper-case letters indicate significant differences using post hoc Tukey's tests ($p < 0.05$).

Treatment	Glucose yield (%)	Xylose yield (%)[a]
4 mm, 40 Hz AFEX pellet	82 (0)^{A}	67 (0)^{A}
4 mm, 50 Hz AFEX pellet	81 (0)^{AB}	66 (1)^{A}
4 mm, 60 Hz AFEX pellet	81 (1)^{AB}	66 (1)^{A}
6 mm, 60 Hz AFEX pellet	80 (1)^{AB}	62 (0)^{B}
4 mm, 60 Hz, 70° C AFEX pellet	79 (2)^{B}	61 (1)^{B}
6 mm, 60 Hz, 70° C AFEX pellet	80 (0)^{AB}	60 (1)^{B}
ANOVA *p*-value	0.029	7.5E-5

[a] Xylose yields were reciprocal transformed before a one-way ANOVA was performed.

3.3.2.3. Effect of preheating. Preheating was investigated because it impacts chemical composition and mechanical preprocessing attributes as discussed in Section 1.1. Preheating of 4 mm AFEX-treated stover, but not 6 mm AFEX-treated stover, significantly decreased xylose yields at 48 h in low solid loadings and under high solid conditions (Tables 3 and 4) indicating possible alteration or degradation of hemicellulose as was suggested by Bals et al. (2013). Preheating during pelletization did not significantly alter glucose yields for 4 mm or 6 mm, 60 Hz pellets for either high or low solid loadings, except for 4 mm, 60 Hz pellets in the low solid conditions at 168 h (Tables 3 and 4). The 6 mm, 60 Hz, preheated pellets had 4% lower glucose yield at 168 h compared to the 4 mm, 60 Hz, preheated pellets for low solid hydrolysis (Tukey's $p < 0.05$), but no difference was seen in the high solid hydrolysis (Tukey's $p > 0.05$). Preheating had either a neutral or negative effect on sugar yields from enzymatic hydrolysis, and decreases in yields from preheating and increased grind size were correlated to significant decreases in durability index indicating that factors important for high durability may also be important for high sugar yields. Based on the sugar yields and durability, it would only be necessary to preheat the biomass if it had another beneficial purpose such as increasing throughput or decreasing the energy requirement of pellet production.

In summary, pelleting AFEX-treated biomass increased or had no effect on the obtained sugar (glucose and xylose) yields at high (Bals et al., 2013) and low solid loadings. Die speed had no effect on the sugar yields from low or high solid loadings, but increasing the grind size of AFEX-treated corn stover prior to pelletization decreased sugar yields. Preheating during pelleting at 60 Hz had either a neutral or negative effect on sugar yields. Enzymatic hydrolysis results indicate that higher die speeds (60 Hz) could be used to increase throughput of the pellet mill, smaller grind sizes (4 mm versus 6 mm) yield more sugars, and preheating is not necessary unless it is useful to optimize other pelletization conditions.

Recent publications have demonstrated that: (1) the packed bed AFEX pretreatment of biomass is promising for use at regional depots proposed as part of an overall lignocellulosic feedstock supply chain (Campbell et al., 2013; Eranki et al., 2011), (2) pelletization of biomass from the packed bed reactors can produce quality pellets (Bals et al., 2013; Campbell et al., 2013), and (3) AFEX pellets could have advantages during mixing at high solid loadings (Bals et al., 2013). Our results further show that the conditions under which AFEX pellets are produced can affect pellet quality and bioconversion. These results, in combination, indicate that combined AFEX pretreatment with optimized pelletization may be helpful to solving logistical issues related to transportation and bioconversion.

4. Conclusion

Pelletization of AFEX-treated biomass at different grind sizes (4, 6 mm), die speeds (40, 50, 60 Hz), and preheating conditions (none, 70 °C) resulted in pellets with bulk density ranging from 588–634 kg/m^3 and durability >97.5% for all conditions except for pellets produced with preheating. Die speed had no significant effect on sugar yields, and increasing grind size or preheating had either no effect or a negative effect on sugar yield. Pellets generated with 4 mm AFEX-treated stover and 60 Hz die speed without preheating had greater or similar density, durability, and sugar yields compared to other pelletization conditions.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Acknowledgements

The authors would like to thank Chandra Nielson and Josh Videto from MBI for performing the AFEX pretreatment and the following INL colleagues for their assistance: Ian Bonner, Cynthia Breckenridge, Debra Bruhn, Karen Delezene-Briggs, Craig Conner, Rachel Emerson, Jeffrey Lacey, Sabrina Morgan, Manunya Phanphanich, Allison Ray, Tammy Trowbridge, and Neal Yancey. This research was supported by the US Department of Energy under Department of Energy Idaho Operations Office Contract No. DE-AC07-05ID14517.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.biortech.2014.02.005.

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