Bioresource Technology Reports 8 (2019) 100301

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.journals.elsevier.com/bioresource-technology-reports

Measuring the influence of biomass preprocessing methods on the bioconversion efficiency of miscanthus giganteus and sugarcane bagasse

Zewei Miao[a,b,1,2], Stefan Bauer[c,1], Ana B. Ibáñez[c], Tony E. Grift[a,b,*]

[a] Energy Biosciences Institute, University of Illinois at Urbana-Champaign, 1206 West Gregory Drive, Urbana, IL 61801-3838, USA
[b] Department of Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, 1304 West Pennsylvania Avenue, Urbana, IL 61801, USA
[c] Energy Biosciences Institute, University of California at Berkeley, 2151 Berkeley Way, MC 5230, Berkeley, CA 94720-5230, USA

ARTICLE INFO

Keywords:
Bioenergy
Biomass
Bioconversion
Pretreatment
Glucose release

ABSTRACT

Miscanthus and sugarcane bagasse were preprocessed into various forms. To measure their conversion efficiency, these forms were pretreated using dilute acid in a microwave oven, with subsequent enzymatic digestion. The results showed that in the case of miscanthus, comminution had a positive effect on conversion efficiency, but pelletization to 6.35 mm pellets did not, nor did biomass compression to 756 MPa. In addition, no significant difference was found in conversion efficiency between material obtained from a chopper harvester, and material made from full stems, cut into 10 mm long particles. In the case of sugarcane bagasse, comminution had a negative effect on conversion efficiency, but pelletization had a positive effect. Finally, a 50:50 w/w blend of miscanthus and sugarcane bagasse had a conversion efficiency that was not significantly different from its miscanthus origin material, but it did have a significantly higher conversion efficiency compared to its sugarcane bagasse origin material.

1. Introduction

The main processes in the production chain of second generation liquid biofuels comprise biomass production/collection, mechanical preprocessing, storage, pretreatment, conversion, and biofuel distribution. Since these processes are typically geographically distributed, logistics operations such as transportation, transloading, and materials handling must take place among them. At present, the majority of mechanical preprocessing research has been conducted without considering the performance of the preprocessed material in pretreatment and bioconversion.

The costs of feedstock production/collection, mechanical preprocessing, storage, and logistics operations account for 40–50% of the total cost of liquid biofuel production (Miao et al., 2013a, 2012; Perlack et al., 2005; Perlack and Stokes, 2011). The methods of mechanical processing, storage, and logistics operations are determined by feedstock type and associated physical properties, purpose of use, biofuel plant size and bioconversion technology (Mani et al., 2004; Miao et al., 2013a). The design of preprocessing technologies must take into account not only its own efficiencies in terms of energy, time and costs, but also consider the conversion efficiency of the feedstock forms it produces. For instance, for liquid biofuel production, current pretreatment and hydrothermal/biochemical conversion require herbaceous feedstock particles with an average size smaller than 9.53 mm of geometric length (Humbird et al., 2011). To realize this size range, milling machines with discharge classifiers including mesh screens, diaphragms, as well as cyclone classifiers, are common. For direct combustion of biomass however, pellets, briquettes, cubes, chopped loose particles or even whole bales may be feasible, although typically, comminuted particles with a size ranging from 12.7 to 50.8 mm are used (Miao et al., 2011, 2012, 2014b). To realize the latter size range, single rotation fractionation mechanisms such as forage choppers, wood chippers, shredders, crushers, and juicing machines are employed (Miao et al., 2013a). The single rotation fractionation mechanism is reported to have a higher energy efficiency than multiple rotation milling machines, but it fails to produce particles of very small size. Earlier research has shown that the biomass conversion efficiency of non-pretreated corn stover and miscanthus is inversely proportional to particle size; the enzymatic hydrolysis glucose yields of small particles (< 0.15 mm for corn stover and < 0.08 mm for miscanthus) were two

* Corresponding author at: Department of Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, 1304 West Pennsylvania Avenue, Urbana, IL 61801, USA.

E-mail address: grift@illinois.edu (T.E. Grift).

[1] Authors contributed equally to the paper.

[2] Present address: Monsanto Company, 800 N Lindbergh Blvd, Creve Coeur, MO 63167, USA.

https://doi.org/10.1016/j.biteb.2019.100301
Received 23 March 2019; Received in revised form 4 August 2019; Accepted 6 August 2019
Available online 27 August 2019
2589-014X/

to four times higher than those of larger particles, being 0.08–0.50 mm for corn stover and < 6.00 mm for miscanthus (Chundawat et al., 2007; Khullar et al., 2013). Alas, although the energy output through conversion efficiency seems proportional to particle size, so is the energy input requirement for comminution. For example, to comminute miscanthus giganteus particles to an average particle size of 70 µm using a commercial hammer mill, 100% of the particles' inherent heating value (PIHV) was consumed (Miao et al., 2011, 2013a). Hence, an optimum in terms of net energy yield must exist.

In the case of biomass treated with the ammonia fiber explosion (AFEX) process, size reduction and washing of corn stover feedstock enhanced the effectiveness of pretreatment and improved the hydrolysis glucan conversion by 15–20% (Chundawat et al., 2007). In addition, glucose yields of miscanthus digestion increased by 19–23% as the particle size was decreased from 6.0 mm to 0.08 mm among pretreatment alternatives including hot water, dilute acid and dilute ammonium hydroxide (Khullar et al., 2013). Since this article did not report how much energy was consumed to produce fine particles, the net energy efficiency of these processes could not be traced.

Increasing initial wood chip size and a high moisture content had a positive effect on the recovery of glucose during steam explosion, because lignin was more easily removed from larger particles with a high moisture content (Cullis et al., 2004). Pelletization did not cause significant carbohydrate loss and did not restrict the pretreatment and enzymatic hydrolysis for softwood feedstock (Kumar et al., 2012). In addition, a single steam pretreatment facilitated both pelletization and subsequent enzymatic hydrolysis without a further pretreatment step (Kumar et al., 2012). Fiber swelling caused by increased biomass porosity had a significant influence on the efficiency of enzymatic hydrolysis, but fiber size reduction had an insignificant influence on enzymatic hydrolysis (Ju et al., 2013). However, the materials used in their study merely contained 0% and 2% lignin, while lignocellulosic feedstock, including hardwood/softwood stems, agricultural residues, and perennial herbaceous energy crops, usually feature a lignin content of about 10–40% dry basis. Because lignin restricts the swelling effect and minimizes the enzyme accessibility to substrates (Ju et al., 2013), their conclusions might not be applicable to lignocellulosic feedstock.

The objective of this research was to evaluate the effects of 1) particle size, 2) compression level, and 3) blending, on biomass conversion efficiency. To allow for objective comparisons, the pretreatment method for all materials was identical, being a dilute acid treatment, applied in a microwave oven. As discussed, the literature regarding the influence of particle size on conversion efficiency is scant. Regarding the influence of compression level, research has shown that, even under extreme pressure, no damage to cell walls was observed, implying no degradation in conversion efficiency (Miao et al., 2013b) but no experiments to that effect were conducted. The reported research is also limited in terms of the potential benefits of blending biomass, although some have been observed (Humbird et al., 2011).

2. Materials and methods

This research comprises experiments in which the influence of mechanical preprocessing on bioconversion efficiency was assessed for miscanthus giganteus (*Miscanthus × giganteus*, Poaceae/Gramineae) and sugarcane bagasse (Saccharum) in nine forms. A partial factorial experimental design was employed with treatments including five preprocessing methods for miscanthus, two preprocessing methods for sugarcane bagasse, and one blending treatment combining miscanthus and sugarcane bagasse. Miscanthus samples were collected from two sources. Approximately 30 kg of miscanthus whole stemmed plants were manually harvested from a 2-year old miscanthus stand located at Parkland Community College, Champaign, IL, (Lat: 40.129358; Lon: −88.292258). In addition, approximately 100 kg of miscanthus material was harvested from a field planted in the spring of 2009, at the Energy Farm of University of Illinois at Urbana-Champaign (Lat: 40.065838; Lon: −88.208445). This material was obtained in chopped form, output by a forage chopper harvester (John Deere 5830, Deere & Company, Moline, IL, USA), fitted with a Kemper 330 head (Kemper GmbH, Stadtlohn, Germany). The miscanthus plants consisted of approximately 75–90% stem material and 10–25% sheath and leaf material. After harvesting, the manually harvested stems were placed in an indoor storage bucket for air-drying. The miscanthus chips were distributed on a concrete floor allowing air-drying for four days until the sample moisture content of miscanthus reached an equilibrium of 11.5%, and subsequently bagged and sealed.

About 500 kg of freshly-produced sugarcane bagasse samples were collected from a commercial sugar mill located at Patoutville, Jeanerette, Louisiana. The exact location where the sugarcane was grown is unknown. The sugarcane bagasse material had an original moisture content of > 50%. Before mechanical preprocessing, the bagasse was air dried on a concrete floor for ten days, until the material reached an equilibrium moisture content of approximately 15%, after which the material was bagged and sealed.

2.1. Mechanical preprocessing

Fig. 1 shows schematically how the two base materials miscanthus giganteus (MxG) and sugarcane bagasse (SB) were processed into nine different forms. Images of the material forms can be found in the

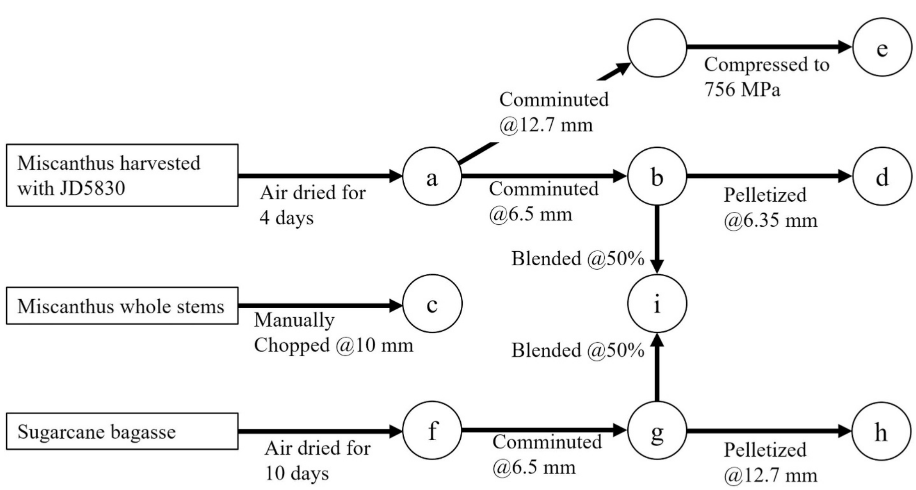

Fig. 1. Schematic overview of the preprocessing methods producing material forms used in the conversion experiments. Note that the empty circle indicates an intermediate material that was not used in the experiments.

Supplementary data section.

- Material a comprised chopped stems of MxG, obtained directly from a John Deere 5830 forage harvester that were air dried for four days until it attained an equilibrium moisture content of 11.5%.
- Material b was obtained by grinding material a with a hammer mill (W-8-H, Schutte-Buffalo Hammer Mill LLC, Buffalo, NY, USA) through a milling screen with aperture size of 6.35 mm.
- To obtain a material with a highly consistent particle size, material c was obtained by manually cutting whole stems of miscanthus into 10 mm long chips using a paper trimmer.
- Material d was obtained by pelletizing ground MxG biomass (material b) with a commercial roller-die pellet mill with a 304.8 mm diameter × 38.1 mm thick die plate (PM1230, Buskirk Engineering, Ossian, IN, USA), producing pellets with a diameter of 6.35 mm. During this process, water was sprayed onto the biomass, because the machine could not pelletize material with a moisture content lower than < 20–25%.
- To obtain material e, 230 g of material a was comminuted through a screen size of 12.7 mm, and subsequently compressed into a single disc-shaped "pie". The uncompressed material was placed between two anvils, and compressed to a maximum applied pressure of 756 MPa. The resulting miscanthus "pie" had a diameter of 160.7 mm, and a height of 11.8 mm. During compression, the material reached an in-mold density of over 1700 kg m^{-3}, but after two months, the sample rebounded to a density of approximately 650 kg m^{-3}. The extreme compression was achieved using a Southwark-Emery Universal Testing Machine, one of the largest in the United States, with a loading capacity of 13 MN (Miao et al., 2013b).
- Material f consisted of SB material that was allowed to dry in air for ten days, at which point it reached an equilibrium moisture content of approximately 15%.
- Material g consisted of material f that was ground with the same machines as material b, using the same screen size aperture of 6.35 mm.
- Material h consisted of material g, which was pelletized using a custom-made laboratory-scale hydraulic compressor that produced cylindrical pellets with a diameter of 12.7 mm at a pressure of 189 MPa, resulting in a mass of approximately 1.5–2 g dry matter per pellet, (Miao et al., 2015)
- Material i consisted of a 50:50 w/w blend of MxG and SB comminuted through a screen with an aperture size of 6.35 mm (materials b and g).

Table 1 shows the nine material forms along with their measured properties. Samples a, b, c, f, g, i (grayed out) are loose materials, whereas samples d, e, h are pelletized/compressed.

2.2. Measurement of material bulk density, Angle Of Repose and size distribution

Tap bulk densities of biomass particles were measured with an 18.8 L (5-gal) bucket following ASAE S269.5 (ASAE, 2012), whereas the Angle Of Repose was measured with a Mark 5 AOR tester (Powder Research Ltd., Harrogate North Yorkshire, UK; (Miao et al., 2014a)). To determine the material size distribution, following ANSI/ASAE Standards S319.4 (ANSI/ASAE, 2008), approximately 100 g of comminuted miscanthus and sugarcane bagasse particles (materials b, g) were sieved for 10 min with a Ro-Tap Shaker (Model D-4325, Dual Manufacturing Co. Inc., Chicago, IL, USA) using US sieve numbers 1/2, 1/4, 7, 12, 16, 18, 20, 50 and 80. After sieving, the biomass retained on each sieve was weighed and the mass concentration calculated. The particle size distribution analysis was replicated three times for each sample. The geometric mean length of biomass particles and its standard deviation were calculated using equations 1 and 2 of ANSI/ASAE Standard S424.1 (ANSI/ASAE, 2007). Detailed information regarding the determination of particle geometrical mean length and particle size distribution was reported (Miao et al., 2011).

2.3. Energy consumption of mechanical preprocessing

The specific energy consumption for comminution of miscanthus and sugarcane, while producing materials b and g, was measured using a clamp-on power meter, attached to the Schutte-Buffalo hammer mill (CW120/121, Yokogawa M&C Corporation, MA). The net energy consumption was calculated by subtracting the idle energy consumption of the machine.

During pelletization with the lab-scaled mechanical hydraulic compressor while producing material h, the energy consumption for compression was measured by integrating the applied force derived from a pressure transducer (PX603-5KG5 V, Omega Engineering Inc., Stemford, CT) through the displacement of a piston, which was measured using a linear potentiometer (LP801-300, Omega Engineering Inc., Stemford, CT), (Miao et al., 2015). The energy consumption during the high pressure (up to 756 MPa) compression while producing material e was measured by integrating the applied force, derived from an integral load cell through the displacement of the compression platen. The integral load cell was traceable to the National Institute of Standards and Technology (NIST). Detailed information about the specific energy consumption measurements and calculations was reported in (Miao et al., 2011), (Miao et al., 2013b), and (Miao et al., 2015).

2.4. Biomass pretreatment

Biomass was ground using a ZM200 ultra centrifugal mill (Retsch, Haan, Germany) with a 0.08 mm sieve screen. An Ethos EZ microwave (Milestone Inc., Shelton, CT) equipped with a closed 100 mL PTFE reaction vessel was used for the dilute acid pretreatment of biomass. The vessel contained the equivalent of 5 g dry and ground biomass, 45 g of 1% (w/w) sulfuric acid and a stir bar. The concentration of sulfuric acid used to give the final 1% concentration was adjusted based on the actual moisture content of the biomass determined using a model HB43-S halogen moisture balance (Mettler Toledo, Columbus, OH, USA). As an example, if the moisture content was 10%, 5.56 g of biomass and 44.44 g of a 1.013% sulfuric acid was used. The vessel was closed and the mixture stirred at 30 °C for 30 min. The temperature was then increased over 6 min to 180 °C and held for 2 min. The mixture was cooled in an ice bath to 30 °C, the vessel was opened and the contents removed. This pretreatment procedure was performed in quadruplicate for every biomass under study and the pretreated biomass of the four pretreatments was combined. The biomass was collected after centrifugation (5000*g*), suspended in water and separated again by centrifugation (5000*g*). This water washing step was repeated until wash phases had reached pH 5 (indicator paper). Excess water was removed by manually compressing the biomass between paper towels.

2.5. Cellulose analysis

An aliquot of the pretreated and washed biomass was dried at 105 °C for 16 h and 5 mg were incubated in triplicate in a 2 mL Sarstedt vial with 50 μL of 72% sulfuric acid at room temperature for 1 h. During that time, the suspension was vortexed every 15 min. After the addition of 1.40 mL of water and a vortexing step, the suspension was autoclaved in the closed vial for 1 h, cooled to room temperature, mixed and filtered (0.2 μm). A sugar recovery standard containing glucose, xylose and arabinose containing the same sulfuric acid concentration was co-autoclaved with the samples and used to estimate the degradation of glucose during the autoclave step. The released glucose was measured by HPLC and converted into cellulose content by a conversion factor of 0.9. This simplified cellulose measurement is possible since in Miscanthus and switchgrass glucose is predominantly released from

Table 1
Physical properties, energy consumption for mechanical processing, and conversion efficiencies of various biomass forms. MxG = Miscanthus, SB = Sugarcane Bagasse. Grayed out rows represent loose materials (samples a, b, c, f, g, i) whereas samples d, e, and h represent compressed materials.

Material		Preprocessing	Bulk density (kg DM m^{-3})	Angle Of Repose (deg)	Geom. mean length (mm)	Cumulative energy cons. kJ kg^{-1} DM (PIHV)	Percentage of total glucose present after pretreatment and enzymatic digestion for 48 h
a	MxG	JD 5830 chopper harvester, dried 4 days	83.6±4.4	51.1±2.3	5.1±1.6	8.9 (0.05%)††	74.2±1.2
b	MxG	a, comminuted @6.35 mm	89.3±0.76	46.3±2.7	0.9±2.1	171.8±50.7 (1.06%)	82.3±0.8
c	MxG	Manually chopped into 10 mm chips	112.4±6.5	47.4±1.9	5.3±1.4		72.4±0.9
d	MxG	b, pelletized through 304.8-mm diameter × 38.1-mm die (pellet diameter 6.35 mm)	616.7±26.1	26.9±1.7‡		436.5 (2.7%)††	83.4±0.4
e	MxG	a, comminuted @12.7 mm, compressed to 756 MPa	650			242.5 (1.5%)	76.8±1.2
f	SB	Air dried for 10 days	67.3±2	49.8±4.9	3.6±2.4		78.8±1.2
g	SB	f, comminuted @6.35 mm	92.7±5.4	46.2±0.7	0.6±2.2	162.2±22.8 (0.93%)	72.2±0.2
h	SB	g, pelletized @12.7mm, 189 MPa	572.6±2.7			209 (1.19%)	77.1±0.5
i	MxG + SB	b + g, blended in 1:1 (w/w) ratio	91.0±3.1†	46.3±1.7	0.7±2.1	167.0±34.0 (0.99%)	81.3±0.4

†Mean of bulk densities of samples b and h;
‡Angle Of Repose of sample h was expected to be similar to that of sample d;
††Cited from (Ward, Cunney, & McNulty, 1985) for silage harvest and (Zhang, Investigations on power consumption, pelleting temperature, pellet quality, and sugar yield in pelleting of cellulosic biomass., 2013) for wheat straw pelletization, respectively;

cellulose.

2.6. Enzymatic digestion

In a 150 mL Erlenmeyer flask, 25 mL of citrate buffer (0.1 M, pH 4.8), 2 mL of 0.5% sodium azide, 30 FPU/(g cellulose) cellulase (from *T. reesei* ATCC 26921, Sigma), 20 U/(g cellulose) β-glucosidase (Novozyme 188, Sigma) was added to the equivalent of pretreated biomass that contained 1.00 g of cellulose. Water was added up to a final total liquid volume of 50 mL, accounting for water originating from the enzyme preparations and the moisture content from the pretreated biomass. Erlenmeyer flasks including content were closed with a rubber stopper and incubated in an orbital shaker (200 rpm) at 50 °C for 6 days. Before adding the enzymes, flasks and contents were pre-incubated for 30 min. Addition of the enzymes triggered the start of the digestion. At each time point, 0.1 mL of the suspension was removed and centrifuged. The clear supernatant was analyzed via HPLC for glucose.

2.7. High pressure liquid chromatography

For the analysis of glucose, samples were injected onto a Rezex RFQ fast acid H+ column (100 × 7.8 mm, Phenomenex) including an HPX-87H guard column (30 × 7.8 mm, BioRad) operated at 55 °C. Compounds were eluted with a mobile phase of 0.01 N sulfuric acid at a flow rate of 1.0 mL/min and detected by a refractive index detector. Quantification was done by external calibration with glucose standard solutions of known concentration.

3. Results and discussion

To characterize the materials used in the pretreatment/conversion process, the particle size distribution was determined for loose materials (samples a, b, c, f, g) except for the blended material (sample i) (Fig. 2). The particle distributions were distinctly related to the mechanical preprocessing procedure. In particular, the particle size distribution of unground sugarcane bagasse was wider than those of the remaining samples. The means and standard deviations of the geometric mean lengths of the materials in mm are shown in Table 1, column 6.

Table 1, columns 4, and 5 show the bulk density in kg DM m^{-3}, Angle Of Repose (AOR) in degree of the material forms. Note that in Table 1, the grayed out rows represent loose materials (samples a, b, c, f, g, i) whereas samples d, e, and h represent pelletized/compressed materials. It is clear that the compressed materials have a much higher bulk density ranging from 572.6 to 650 kg DM m^{-3} than the loose materials' which range from 67.3 to 112.4 kg DM m^{-3}. The bulk density of the biomass disc that was compressed to 756 MPa (sample e) reached an in-mold density of over 1700 kg DM m^{-3} during compression, but rebounded to 650 kg DM m^{-3} after two months. Since in this case, there was only one sample, no error is indicated.

The Angle Of Repose (AOR) was determined for all loose materials (samples a, b, c, f, g, i) as well as for pellets generated with the commercial pelletizer (sample d). It is clear that the AOR of miscanthus pellets (sample d) was significantly lower than those of all other samples. The AOR of unground sugarcane bagasse (sample f) was higher than that of bagasse particles milled through a milling screen with an aperture size of 6.35 mm (sample g). Overall, the results confirmed the expected trend, being that the AOR is proportional to particle size. The largest geometric mean lengths were seen for miscanthus particles produced with the forage chopper harvester (sample a) and for manually chopped miscanthus (sample c).

3.1. Energy consumption of mechanical preprocessing

Table 1, column 7 shows the cumulative energy consumption for all material forms except for the manually cut miscanthus material. The specific energy consumption is expressed in kJ kg^{-1} DM, but to put these numbers in perspective, they are also expressed as a Percentage of the Inherent Heating Value (PIHV) of the material, which is 17.744 MJ kg^{-1} for miscanthus (Collura et al., 2006) and 15.9 MJ kg^{-1} (value for carbohydrate biomass) for sugarcane bagasse. The data confirms that the preprocessing energy costs are proportional to the number of actions; the lowest energy consumption is for sample a, where the miscanthus material was obtained directly from the forage chopper harvester at 0.05 PIHV. The same material was comminuted in sample b, which increased the energy costs to 1.06 PIHV. This comminuted material was pelletized with a ring-die pellet mill in sample d,

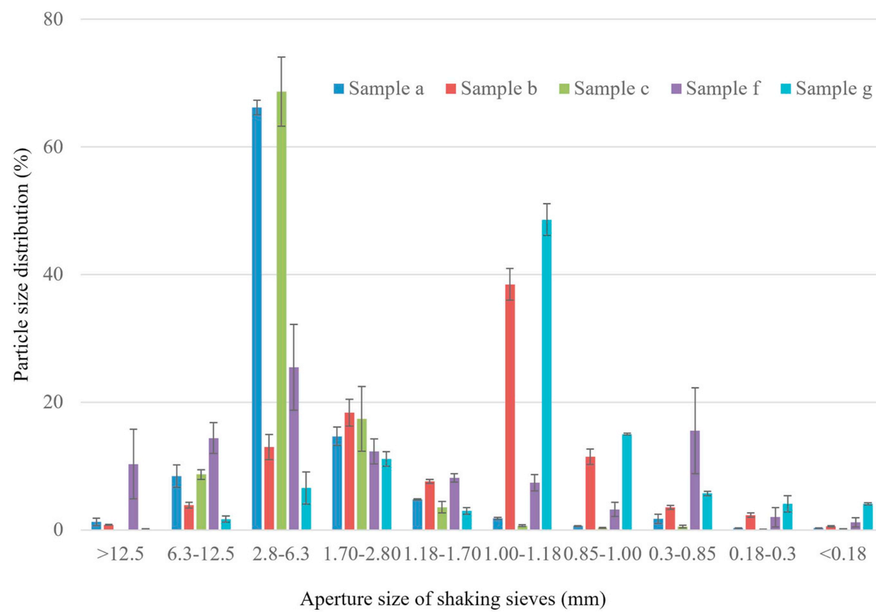

Fig. 2. Particle size distribution of miscanthus and sugarcane bagasse produced with the mechanical preprocessing procedures defined in Fig. 1.

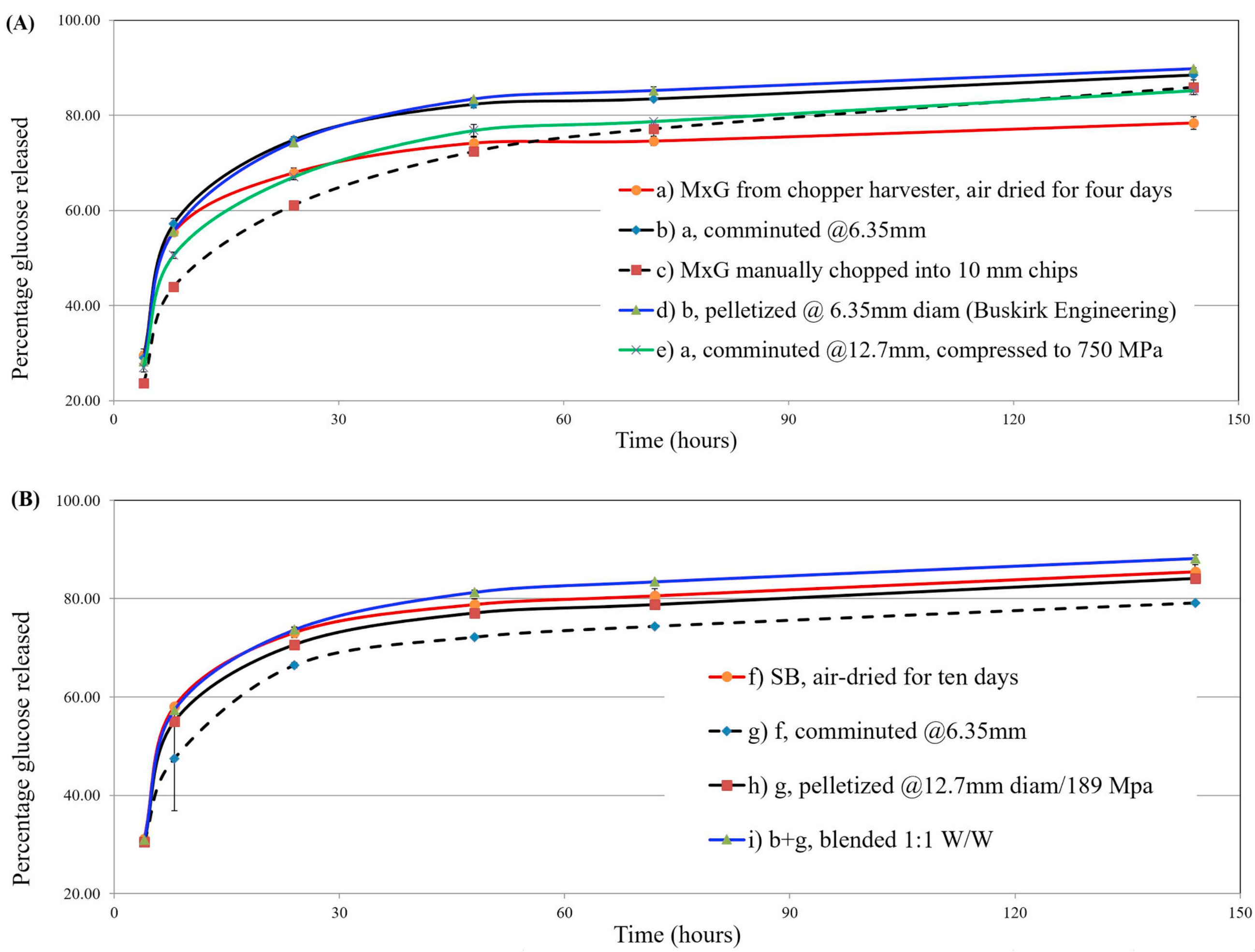

Fig. 3. Bioconvertibility of biomass forms produced with the mechanical preprocessing procedures defined in Fig. 1. The conversion experiments were conducted in three repetitions. (A) Shows the percentage of glucose released for all miscanthus giganteus (MxG) samples and (B) shows the same for sugarcane bagasse (SB) materials, including the blended material (i). Note that the error bar at 8 h for material g (sugarcane bagasse ground through a 6.35 mm screen) is quite large, due to a failed conversion, yielding an outlier.

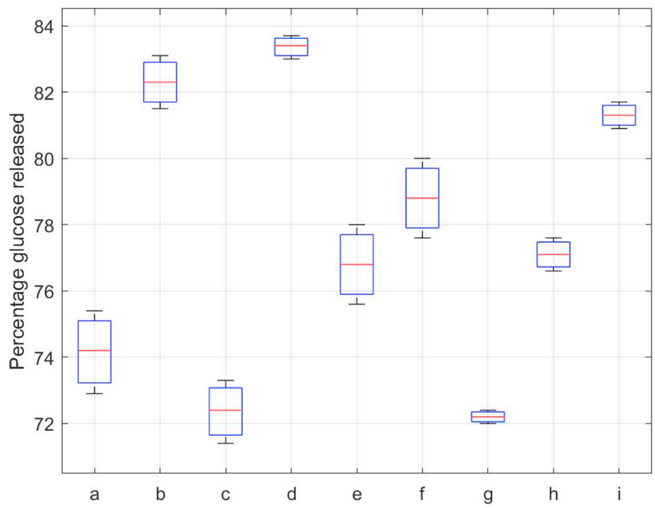

Fig. 4. Box-whisker plot of percentage glucose released of various material forms after 48 h.

which increased the energy costs further to 2.7 PIHV. Although the energy requirement for compressing miscanthus to an extreme pressure of 756 MPa (sample e), and a density of over 1700 kg m^{-3}, was a mere 242.5 kJ kg^{-1} DM (1.5 PIHV), no conclusions can be drawn as to whether the large scale of the machine was influential, since only a single sample was compressed.

3.2. Digestibility of feedstock after mechanical preprocessing

The progression of the percentage of glucose released over time of MxG materials are shown in Fig. 3A, whereas those of SB (including the blend between MxG and SB materials) are shown in Fig. 3B.

From Fig. 3A, it is clear that after 4 h, the conversion rate is high for all MxG materials, but soon after, the conversion rate decreases and, around 48 h, enters a region where the conversion rate becomes quasi-constant and slow. Although all materials except material a (obtained directly from the chopper harvester) follow a similar trend, the total percentage of glucose released is different at 48 h and ultimately at 144 h. Material a is unique in that it has a high conversion rate at the start, which seems to level off earlier than those of the other materials.

From Fig. 3B it is clear that all SB materials follow a similar trend as the MxG materials shown in Fig. 3A. Material g (SB, air dried for ten days, then comminuted through a 6.35 mm screen), levels off much earlier than the other SB materials, and attained an overall low conversion rate. Note that the error bar for material g at 8 h is large due to one of the three conversion experiments failing (no outliers were removed in the complete dataset). Across the 144 h of conversion, the blend of MxG and SB materials consistently had the highest percentage of glucose released.

Since the economically optimal time for a batch release is where the conversion rate reaches a quasi-constant value (here around 48 h), the mean and standard deviations of the biomass conversion percentage values after 48 h of digestion are shown in the last column of Table 1. In addition, Fig. 4 shows a box-whisker plot of the same data points.

To compare the conversion performance of all materials in a statistical sense, Table 2 shows a pairwise *t*-test for sample means of percentages glucose released over 4, 8, 24, 48, 72 and 144 h. Confidence levels larger than 0.05 (5%) were considered not-significant (NS).

3.2.1. Effect of comminution

To study the effect of comminution in miscanthus, material b must be compared to material a. The pairwise *t*-test showed that the overall digestibility of the comminuted material b was larger than that of its

Table 2
Paired *t*-test comparisons in conversion efficiencies of materials as defined in Fig. 1.

Material		Processing	b	c	d	e	f	g	h	i
a	MxG	JD 5830 chopper harvester, dried 4 days	a < b, p = 0.0098	**NS, p = 0.1934**	a < d, p = 0.0214	**NS, p = 0.3220**	a < f, p = 0.0016	**NS, p = 0.1419**	a < h, p = 0.0153	a < i, p = 0.0047
b	MxG	a, comminuted @6.35 mm		b > c, p = 0.0028	**NS, p = 0.3908**	b > e, p = 0.0011	**NS, p = 0.1043**	b > g, p = 0.0048	b > h, p = 0.0129	**NS, p = 0.4779**
c	MxG	Manually chopped into 10 mm chips			c < d, p = 0.0014	c < e, p = 0.0128 0.0025	c < f, p = 0.0109	**NS, p = 0.3239**	c < h, p = 0.0212	c < i, p = 0.0020
d	MxG	b, pelletized through 304.8-mm diameter × 38.1-mm die (pellet diameter 6.35 mm)				d > e, p = 0.012	**NS, p = 0.1721**	d > g, p = 0.0077	d > h, p = 0.0349	**NS, p = 0.4145**
e	MxG	a, comminuted @12.7 mm, compressed to 756 MPa					e < f, p = 0.0114	**NS, p = 0.0726**	**NS, p = 0.0561**	e < i, p = 0.0002
f	SB	Air dried for 10 days						f > g, p = 0.0028	f > h, p = 0.0016	f < i, p = 0.0475
g	SB	f, comminuted @6.35 mm							g < h, p = 0.0042	g < i, p = 0.0020
h	SB	g, pelletized @ 12.7 mm, 189 MPa								h < i, p = 0.0021
i	MxG + SB	b + g, blended in 1:1 (w/w) ratio								

NS: Not significant at the 95% confidence level.

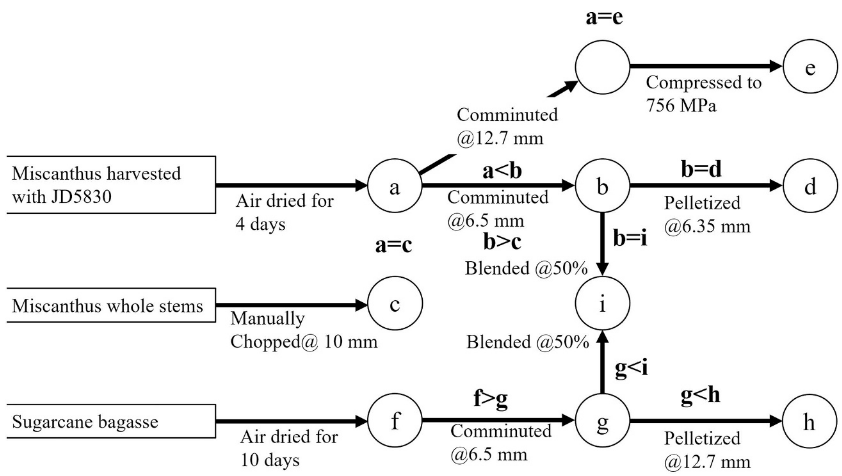

Fig. 5. Materials used in the experiments, inscribed with conversion efficiency comparisons based on a pairwise *t*-test. Note that the equal signs represent no significant differences (NS in Table 2).

origin material a (a < b, p = 0.0098). A second study of the effect of size reduction in miscanthus was made by comparing material c (full stems of miscanthus chopped to 10 mm length particles) versus material a (air dried miscanthus from the chopper harvester) and material b (material a, comminuted through a screen with an aperture size of 6.35 mm). The pairwise *t*-test shows that materials a and c were not significantly different (NS, p = 0.1934), but that the conversion efficiency of the comminuted material b was significantly higher than that of material c (10 mm length particles) as in (b > c, p = 0.0028).

The effect of comminution was also evaluated for sugarcane bagasse material, as comminuted material g was compared with its origin material f. Here the pairwise t-test indicated that the overall conversion efficiency of material g was in fact smaller than that of its origin material f (f > g, p = 0.0028). Note that both the miscanthus and sugarcane bagasse material were comminuted using the same machine, and through the same screen with an aperture size of 6.35 mm.

An earlier study reported that particle size influences biomass enzyme digestibility to a limited extent; comminution increased conversion rates to maximum of ≈50%, whereas chemical modification achieved conversions of > 70%, regardless of particle size. The results presented here show that comminution increases the conversion efficiency of miscanthus more than that of sugarcane bagasse. This effect may attribute to the smaller diameters of raw and comminuted sugarcane bagasse (Vidal et al., 2013).

3.2.2. Effect of pelletization and compression

To study the effect of pelletization on conversion efficiency in miscanthus, material b must be compared to material d. The pairwise *t*-test showed that the overall digestibility of the compressed material d was not significantly different from its origin material b (NS, p = 0.3908). Note that the pelletization from material b to d took place using a commercial pelletizer that generated pellets with a diameter of 6.35 mm at an unknown pressure.

To study the effect of pelletization on conversion efficiency in sugarcane bagasse, material g must be compared to material h. The pairwise *t*-test showed that the conversion efficiency of the pelletized material was larger than that of the origin material (g < h, p = 0.0042). Note that the pelletization from material g to h took place using an experimental pelletizer that produced pellets with a diameter of 12.7 mm after being exposed to a pressure of 189 MPa.

Finally, to study the effect of extreme compression pelletization where a biomass disc was exposed to a pressure of 756 MPa, material e must be compared to material a, which had been comminuted through a screen with an aperture size of 12.7 mm. The pairwise *t*-test showed that the conversion efficiency of the biomass disc material e was not significantly different from its origin material a (NS, p = 0.3220). Note that the in-mold density of the biomass disc was over 1700 $kg\,m^{-3}$, but after two months, it had rebounded to a density of approximately 650 $kg\,m^{-3}$.

The results as presented here are consistent with a previous study which showed that pelletization did not render corn stover more recalcitrant to dilute-acid pretreatment under low- or high-solids conditions, and even enhanced ethanol yields (Ray et al., 2013).

3.2.3. Effect of blending of biomass

The blended material i comprised a 1:1 w/w blend of miscanthus and sugarcane bagasse, both comminuted through a screen with an aperture of 6.35 mm representing material b, and g respectively. The pairwise *t*-test showed that the overall digestibility across 144 h of the blended material i was not significantly different from origin material b (miscanthus ground through a 6.35 mm screen) at p = 0.4779, but it did have a significantly higher overall conversion efficiency compared to origin material g (sugarcane bagasse ground through a 6.35 mm screen), since (g < i, p = 0.0020).

Fig. 5 shows the material origins along with the statistical comparisons based on the pairwise t-test. Note that, for brevity purposes, if two materials are not statistically significantly different instead of the NS abbreviation, they are set equal to each other using the "=" sign.

In this study, blending showed an increase in the conversion efficiency for sugarcane bagasse but not for miscanthus material. Previous literature reported that blending hybrid poplar and wheat straw resulted in more monomeric sugar recovery and less sugar degradation and, on average, 22% more sugar monomers were recovered using mixed feedstock than from either single biomass (Vera et al., 2015). More research is needed to determine why the blending effect was inconsistent between these materials.

4. Conclusions

The effect of mechanical preprocessing on the bio-conversion efficiency of miscanthus giganteus (MxG) and sugarcane bagasse (SB) was studied. Comminution had a positive effect on the efficiency of MxG, but a negative effect on that of SB. Pelletization had a positive effect on the efficiency of SB but did not yield a significant effect for MxG. Extreme compression of MxG material did not yield a significant improvement in efficiency. A 50/50 blend of MxG and SB had a higher efficiency than its SB origin material, but there was no significant efficiency difference between the blend and the MxG origin material.

Declaration of competing interest

All authors are aware of the ethics policy of the Bioresource Technology Reports Journal, declare no conflict of interest and accept responsibility for the present manuscript.

Acknowledgments

This publication contains data produced under an Energy Biosciences Institute-funded award. The authors are grateful to Tim Mies, Collin Reeser, and Joshua D. Jochem for their help in sample preparation and physical property measurements. They are also grateful to Professor emeritus Dr. James Phillips who was instrumental in preparing the compressed samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2019.100301.

References

ANSI/ASAE, 2007. Standard S424.1. Method of determining and expressing particle size of chopped forage materials by screening. Am. Soc. Agric. Biol. Eng.

ANSI/ASAE, 2008. Standard S319.4. Method of determining and expressing fineness of feed materials by sieving. Am. Soc. Agric. Biol. Eng.

ASAE, 2012. Standard S269.5. Densified products for bulk handling—definitions and method. Am. Soc. Agric. Biol. Eng.

Chundawat, S.P.S., Venkatesh, B., Dale, B.E., 2007. Effect of particle size based separation of milled corn stover on AFEX pretreatment and enzymatic digestibility. Biotechnol. Bioeng. 96, 219–231. https://doi.org/10.1002/bit.21132.

Collura, S., Azambre, B., Finqueneisel, G., Zimny, T., Weber, J.V., 2006. Miscanthus × Giganteus straw and pellets as sustainable fuels: combustion and emission tests. Environ. Chem. Lett. 4, 75–78. https://doi.org/10.1007/s10311-006-0036-3.

Cullis, I.F., Saddler, J.N., Mansfield, S.D., 2004. Effect of initial moisture content and chip size on the bioconversion efficiency of softwood lignocellulosics. Biotechnol. Bioeng. 85, 413–421. https://doi.org/10.1002/bit.10905.

Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., 2011. Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol. National Renewable Energy Laboratory, Golden, CO.

Ju, X., Grego, C., Zhang, X., 2013. Specific effects of fiber size and fiber swelling on biomass substrate surface area and enzymatic digestibility. Bioresour. Technol. 144, 232–239. https://doi.org/10.1016/j.biortech.2013.06.100.

Khullar, E., Dien, B.S., Rausch, K.D., Tumbleson, M.E., Singh, V., 2013. Effect of particle size on enzymatic hydrolysis of pretreated Miscanthus. Ind. Crop. Prod. 44, 11–17. https://doi.org/10.1016/j.indcrop.2012.10.015.

Kumar, L., Tooyserkani, Z., Sokhansanj, S., Saddler, J.N., 2012. Does densification influence the steam pretreatment and enzymatic hydrolysis of softwoods to sugars? Bioresour. Technol. 121, 190–198. https://doi.org/10.1016/j.biortech.2012.06.049.

Mani, S., Tabil, L.G., Sokhansanj, S., 2004. Grinding performance and physical properties of wheat and barley straws, corn stover and switchgrass. Biomass Bioenergy 27, 339–352. https://doi.org/10.1016/j.biombioe.2004.03.007.

Miao, Z., Grift, T.E., Hansen, A.C., Ting, K.C., 2011. Energy requirement for comminution of biomass in relation to particle physical properties. Ind. Crop. Prod. 33, 504–513. https://doi.org/10.1016/j.indcrop.2010.12.016.

Miao, Z., Shastri, Y., Grift, T.E., Hansen, A.C., Ting, K.C., 2012. Lignocellulosic biomass feedstock transportation alternatives, logistics, equipment configurations, and modeling. Biofuels Bioprod. Biorefin. 6. https://doi.org/10.1002/bbb.1322.

Miao, Z., Grift, T.E., Hansen, A.C., Ting, K.C., 2013a. Energy requirement for lignocellulosic feedstock densifications in relation to particle physical properties, preheating, and binding agents. Energy and Fuels 27. https://doi.org/10.1021/ef301562k.

Miao, Z., Phillips, J.W., Grift, T.E., Mathanker, S.K., 2013b. Energy and pressure requirement for compression of Miscanthus giganteus to an extreme density. Biosyst. Eng. 114, 21–25. https://doi.org/10.1016/j.biosystemseng.2012.10.002.

Miao, Z., Grift, T.E., Hansen, A.C., Ting, K.C., 2014a. Flow performance of ground biomass in a commercial auger. Powder Technol. 267, 354–361. https://doi.org/10.1016/j.powtec.2014.07.038.

Miao, Z., Grift, T.E., Ting, K., 2014b. Size reduction and densification of lignocellulosic biomass feedstock for biopower, bioproducts, and liquid biofuel production. Encycl. Agric. Food, Biol. Eng. Second Ed. 10–13.

Miao, Z., Phillips, J.W., Grift, T.E., Mathanker, S.K., 2015. Measurement of mechanical compressive properties and densification energy requirement of miscanthus × giganteus and switchgrass. Bioenergy Res 8. https://doi.org/10.1007/s12155-014-9495-8.

Perlack, R.D., Stokes, B.J., 2011. US Billion-ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry. Oak Ridge National Laboratory, Oak Ridge, TN.

Perlack, R.D., Wright, L.L., Turhollow, A.F., Graham, R.L., Stokes, B.J., Erbach, D.C., 2005. Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility of a Billion-ton Annual Supply. (Oak Ridge, TN).

Ray, A.E., Hoover, A.N., Nagle, N., Chen, X., Gresham, G.L., 2013. Effect of pelleting on the recalcitrance and bioconversion of dilute-acid pretreated corn stover under low- and high-solids conditions. Biofuels 4, 271–284. https://doi.org/10.4155/bfs.13.14.

Vera, R.M., Bura, R., Gustafson, R., 2015. Synergistic effects of mixing hybrid poplar and wheat straw biomass for bioconversion processes. Biotechnol. Biofuels 8, 1–10. https://doi.org/10.1186/s13068-015-0414-9.

Vidal, B.C., Dien, B.S., Ting, K.C., Singh, V., 2013. Influence of feedstock particle size on lignocellulose conversion. Appl. Biochem. Biotechnol. 1405–1421. https://doi.org/10.1007/s12010-011-9221-3.