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Combined effect of pelleting and pretreatment on enzymatic hydrolysis of switchgrass

Binod Rijal, C. Igathinathane, Bishnu Karki, Manlu Yu, Scott W. Pryor *

Department of Agricultural and Biosystems Engineering, North Dakota State University, 1221 Albrecht Blvd., Fargo, ND 58102, USA

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ABSTRACT

Switchgrass was pelleted to evaluate the effect of densification on acidic and alkaline pretreatment efficacy. Bulk density and durability of pellets were 724 kg/m^3 and 95%, respectively. Ground switchgrass (D_{90} = 21.7 mm) was further ground to a fine power (D_{90} = 0.5 mm) in the pellet mill prior to densification. This grinding increased enzymatic hydrolyzate glucose yields of non-pretreated materials by 210%. Pelleting had no adverse impact on dilute acid pretreatment efficacy. Grinding and pelleting increased hydrolyzate glucose yields of switchgrass pretreated by soaking in aqueous ammonia (SAA) by 37%. Xylose yields from SAA-pretreated switchgrass pellets were 42% higher than those from the original biomass. Increases in sugar yields from SAA-pretreated pelleted biomass are attributed to grinding and heating of biomass during the pelleting process. Potential transportation, storage, and handling benefits of biomass pelleting may be achieved without negatively affecting the downstream processing steps of pretreatment or enzymatic hydrolysis.

1. Introduction

Low densities of biomass feedstocks and the associated handling, transportation, and storage costs are major impediments to the utilization of biomass for biofuel production. The densification of biomass into uniform pellets could be one method to reduce these challenges. Pelleting increases the biomass density by almost ten-fold (Tumuluru et al., 2011) thereby facilitating easy handling and decreasing transportation and storage costs (Hess et al., 2007). Significant work has been done to study the densification characteristics of agricultural and woody biomass (Kaliyan and Morey, 2010; Larsson et al., 2008; Mani et al., 2006).

The recalcitrant structure of lignocellulosic biomass is another challenge to commercial bioethanol production. Expensive pretreatment is needed for disruption of cell wall structures to make polysaccharides more accessible to enzymes for effective hydrolysis. Several cost-effective methods including dilute acid (DA) and soaking in aqueous ammonia (SAA) pretreatments have been developed to disrupt plant cell wall structures for efficient bioconversion of lignocellulosic biomass to fermentable carbohydrates (Tao et al., 2011; Wyman et al., 2011).

Chemical pretreatment of biomass increases the biomass surface area and pore volume through delignification, hemicellulose solubilization, and reduction in cellulose crystallinity (McMillan, 1994). Acid treatments are more effective for hemicellulose solubilization while alkaline pretreatments are more effective for lignin solubilization with minimal losses of cellulose and hemicellulose. Physical pretreatments of biomass reduce particle size and cellulose crystallinity in order to increase the specific surface area and reduce the degree of polymerization. Extrusion has been shown to be an effective pretreatment for many forms of biomass (Karunanithy and Muthukumarappan, 2010; Karunanithy et al., 2008), although in one case it negatively affected sugar release from soybean hulls (Lamsal et al., 2010). This effect was attributed to the interaction of lignin with carbohydrate fibers during extrusion.

Pelleting increases biomass bulk density through mechanical and thermal processing similar to extrusion (Larsson et al., 2008). High temperatures generated in the pelleting process soften lignin and enable it to act as a binder to form durable pellets (Kaliyan and Morey, 2010). Because lignin content and distribution has a strong influence on biomass recalcitrance, alteration of lignin during densification could impact pretreatment efficacy or hydrolysis yields. Theerarattananoon et al. (2011) showed that changes in pelleting parameters can influence sugar yields.

Literature on the impact of pelleting under different pelleting and pretreatment conditions of biomass feedstock is limited; the present study used switchgrass as a model substrate to investigate the interaction of densification and pretreatment. Switchgrass is a promising feedstock for bioethanol production (Hu et al., 2010; Isci et al., 2008) because of its high productivity, suitability for growth

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* Corresponding author. Address: Department of Agricultural and Biosystems Engineering (Dept 7620), North Dakota State University, 1221 Albrecht Blvd., Fargo, ND 58102, USA. Tel.: +1 701 231 7274; fax: +1 701 231 1008.
E-mail address: scott.pryor@ndsu.edu (S.W. Pryor).

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on marginal land, and environmental benefits (McLaughlin et al., 1999). DA and SAA pretreatments are effective methods to increase hydrolysis yields from switchgrass (Isci et al., 2008; Tao et al., 2011; Wyman et al., 2011). They were used as model pretreatments to demonstrate interaction between densification for acidic and alkaline pretreatments. The overall objective of this study was to evaluate the impact of pelleting on the efficacy of acidic and alkaline pretreatments for enhancing enzymatic hydrolysis of switchgrass.

2. Methods

2.1. Materials

Sunburst switchgrass (*Panicum virgatum* L.) was harvested in the fall of 2008 and obtained from North Dakota State University's (NDSU) Central Grassland Research and Extension Center in Streeter, ND. The switchgrass was ground in a model 915 hammermill (CrustBuster/Speedking, Inc.; Dodge City, KS, USA) fitted with an 8-mm sieve. This original ground material at 5% moisture content (dry basis (db)) was stored in a sealed plastic bag at room temperature. Reagent grade glucose and xylose were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ, USA). The enzymes, NS50013 (endo/exo-cellulase, activity: 77.0 filter paper units (FPU)/mL), Novozyme 188 (β-glucosidase, activity: 500.5 cellobiase units (CBU)/mL), and Cellic HTec (endo-xylanase, 10,596 IU/mL) were provided by Novozymes North America, Inc. (Franklinton, NC, USA). The cellulose and cellobiase activities were determined using the method described by Ghose (1984) while xylanase activity of Cellic HTec was determined as described by Bailey et al. (1992).

2.2. Pellet production

Ground switchgrass was pelleted using a Buskirk Engineering pellet mill (PM 810; North Ossian, IN) in the NDSU Biomass Feedstock Processing Laboratory at the USDA-ARS Northern Great Plains Research Laboratory (Mandan, ND). The pellet mill had a 7.5-kW motor and a capacity to produce 180 kg of biomass pellets per hour. The 200-mm (diameter) × 38-mm (thickness) machinated plate die had holes 6.3 mm in diameter. The moisture content of the original biomass was adjusted to 12% db prior to feeding into the pellet mill. Additionally, biomass in the hopper was wetted lightly using a spray bottle to aid pelleting. The original switchgrass was ground to a fine powder within the pellet mill prior to being forced through the die to form pellets. Samples of this powdered material were used to distinguish impacts of fine grinding and subsequent pelleting on pretreatment efficacy. No external binders were added while pelleting. The pellets (4 kg) were collected and stored in plastic bags at room temperature. Glucose and xylose yields of the original, powdered, and pelleted biomass were compared for three pretreatments (non-pretreated, DA-pretreated, and SAA-pretreated) to evaluate the interaction of pelleting and pretreatments.

2.3. Bulk density and durability of pellets

Bulk density of pellets was determined using ASABE Standard S269.4 (ASABE, 2007a). Durability is defined as the ability of densified biomass to remain intact when handled and was determined using ASABE Standard S269.4 (ASABE, 2007a). Pellet samples were prepared by sieving through a 4-mm aperture sieve to remove fines. Triplicate 500-g samples of sieved pellets were used for testing. After 10 min of tumbling at 50 rpm, the samples were removed and sieved again using a 4-mm sieve. Durability was calculated as the ratio of total sieved pellet mass after tumbling to mass prior to tumbling.

2.4. Determination of particle size and size distribution of original and powdered biomass

Original switchgrass (milled through 8-mm mesh screen) and powdered biomass (ground in pellet mill between the rollers and die prior to compression and pellet formation) were used as control materials. The machine vision approach as reported by Igathinathane et al. (2012) was used to determine the size distribution of particulate material. A flatbed scanner was used to obtain digital images of milled and powdered biomass. A user-coded ImageJ plugin was used for analyzing the digital images for the size and size distribution of these particulate materials. The particle size distribution (PSD) was analyzed based on the length and $\sum$volume approach (Igathinathane et al., 2012).

2.5. DA pretreatment

DA pretreatment was conducted in a 1-L Parr pressure reactor (4600 Series, Parr Instrument Company; Moline, IL, USA). Biomass samples in original, powdered, and pelleted forms (50 g (db)) were mixed with dilute sulfuric acid (1.5% (w/w)) at a solid loading rate of 10% (w/v). The samples were treated at 140 °C for 20 min. The time to reach the desired temperature (140 °C) was approximately 60 min for all samples and the resulting pressure was 275 kPa. After holding for 20 min at the set temperature, the reaction vessel was immediately transferred to an ice-water bath until the inside pressure was equalized to that of the atmosphere. Pellets were completely disintegrated during pretreatment. The DA-pretreated biomass was washed with distilled water (3 L) in a vacuum-assisted Buchner funnel through Whatman #41 filter paper (20–25 μm pore size). Samples of supernatant were collected to analyze individual sugars via high performance liquid chromatography (HPLC). The solids remaining after filtration were weighed and stored in a sealed plastic bag at 4 °C. A fraction of pretreated and washed biomass was used for determining moisture content and chemical composition.

2.6. SAA pretreatment

Switchgrass samples in each of the three biomass forms were pretreated by SAA using 15% ammonium hydroxide (EMD Chemicals Inc., Gibbstown, NJ, USA). The pretreatment was performed in screw-capped Pyrex bottles (1-L) at a solid-to-liquid ratio of 1:6 at 40 °C for 24 h without agitation. Biomass pellets were completely disintegrated during pretreatment. The pretreated biomass was washed with distilled water (3 L) in a vacuum-assisted Buchner funnel through Whatman #41 filter paper (20–25 μm pore size). The washed solids were collected and stored at 4 °C. The solid recovery of pretreated biomass was calculated by drying a small fraction of wet solids overnight in a convection oven at 105 °C.

2.7. Composition analysis

Compositional analysis was performed for non-pretreated, DA-pretreated, and SAA-pretreated switchgrass in the original, powdered, and pelleted forms. Carbohydrate and lignin (acid-soluble and acid-insoluble) contents were calculated using National Renewable Energy Laboratory (NREL) Chemical Analysis and Testing Standard Procedures (Sluiter et al., 2008b). The extractives were removed from the untreated biomass following the NREL Chemical Analysis and Testing Procedures (Sluiter et al., 2008a). Each analysis was performed in triplicate for all samples.