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Reduced pretreatment severity and enzyme loading enabled through switchgrass pelleting

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ABSTRACT

This study investigated the effectiveness of low severity alkaline pretreatment conditions and reduced cellulase and hemicellulase loadings on glucose and xylose yields from pelleted switchgrass. Pelleting improved the efficacy of soaking in aqueous ammonia pretreatment enabling milder pretreatment and lower enzyme loadings to obtain high sugar yields. Although 80% glucose yields could be achieved with cellulase alone, hemicellulase addition improved yields from pelleted switchgrass to above 90% at cellulase loadings as low as 10 FPU g^{-1} glucan. Similar efficacy was found with higher cellulase loadings and reduced hemicellulase loading. Xylose yields greater than 80% were achieved with cellulase loadings greater than 14 FPU and 200 XU g^{-1} glucan. This study demonstrated that biomass pelleting can enable biorefineries to use less severe pretreatment conditions and reduced enzyme loadings while maintaining high hydrolysis yields. Models were developed to quantify the range of cellulase and hemicellulase loadings to achieve chosen glucose and xylose yields.

1. Introduction

Consistent supply of low-cost biomass feedstocks is vital for establishing cellulose-based bioindustries. Due to low biomass bulk densities, transportation energy requirements to grow and deliver bulk lignocellulosic biomass to a biorefinery are about 7–26% of total energy needs compared to just 3–5% for grains and oilseeds [1,2]. The use of baled or loose biomass not only burdens transportation systems but also leads to substantial challenges for unloading, stacking, storing, and moving considerable amounts of biomass at the biorefinery. The high transportation, handling and storage costs for feedstock at industrial scale may contribute to the delayed development of the cellulosic biofuel industry.

Densification of biomass into pellets could be beneficial for both supply systems and the biorefinery itself. Pelleting increases biomass density by about 7–10 times and may improve the economics of cellulosic ethanol production by facilitating improvements for storage, transportation and handling systems. Previous studies suggest that transportation cost savings for pelleted biomass might be offset by pelleting cost [3,4]. However, those studies only considered the cost of transportation and did not account for potential processing synergies of pelleting and pretreatment for biochemical conversion. Even though biomass densification is not a new practice, there is a research gap to understand the interaction between densification and pretreatment efficacy for biochemical conversion.

The majority of projected production cost of cellulosic ethanol is related to releasing sugars from cellulose and

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hemicellulose by the combined effect of pretreatment and enzymatic hydrolysis. The pretreatment and hydrolysis processes are considered the highest costs with pretreatment accounting for 20–30% of total process costs by itself [5,6]. Pretreatment at lower temperatures or shorter residence time reduces the associated energy cost and may reduce the formation of degradation products which inhibit enzyme performance. Therefore several studies focused on optimizing enzyme mixtures for enzymatic hydrolysis following mild pretreatment [7–9]. Soaking in aqueous ammonia (SAA) is an example of an alkaline pretreatment that has been extensively examined for use with diverse cellulosic biomass on both laboratory and pilot scales [10–13]. SAA removes or modifies lignin with less impact on hemicellulose; hydrolysis yields therefore improve when cellulases are supplemented with hemicellulases during enzymatic hydrolysis for SAA-pretreated biomass.

Pelleting increases biomass bulk density through mechanical and thermal processing. High temperatures during the pelleting process lead to melting of lignin and subsequent solidification to form durable pellets [14,15]. However, limited studies examined the impact of pelleting on biomass pretreatment and bioconversion. Theerarattananoon et al. [16] showed that pelleted biomass facilitates conversion of cellulose more than unpelleted biomass. Guragain et al. [17] showed better hydrolysis of alkali-pretreated pelleted biomass without affecting the quality of sugars for fermentation. Other studies [18,19] confirmed that pelleting biomass did not have any negative impact on the efficacy of dilute acid pretreatment despite the effective biomass particle size increase inherent during densification. Moreover, Rijal et al. [19] reported that pelleting improved the efficacy of SAA-pretreated switchgrass and suggested that pelleting biomass might allow reduction in pretreatment severity or enzyme loadings. Such improvements have been documented using hydrothermal and alkaline pretreatments [20].

Literature on the impact of pelleting on different pretreatment conditions and enzymatic hydrolysis yield is still limited and reduced severity pretreatments and reduced enzyme loadings in conjunction with pelleting have received little attention [19,20]. However, lower enzyme loadings are essential to reducing overall processing costs and may be an undocumented benefit of biomass pelleting. The primary objective of this study was to demonstrate the effectiveness of reduced severity SAA pretreatment conditions and reduced enzyme loadings for hydrolysis of pelleted switchgrass. A reduced severity pretreatment was chosen and a response surface methodology approach was used to quantify interaction between cellulase and hemicellulase enzyme loadings on hydrolysis yields of pretreated pelleted switchgrass.

2. Materials and methods

2.1. Raw material

Sunburst switchgrass (*Panicum virgatum* L.), harvested at full maturity in the fall of 2008, was collected from the Central Grassland Research and Extension Center in Streeter, ND (46° 39′ 19″ N 099° 21′ 14″ W). Switchgrass was air dried to a moisture content of 5% and was ground with a hammermill (Model 915, CrustBuster/Speedking, Inc.; Dodge City, KS, USA) using an 8-mm sieve. Sieved switchgrass was stored in a sealed plastic bag at room temperature until use.

2.2. Pellet production

Pellets were prepared 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) as previously described [19]. Briefly, the pellet mill had a 7.5-kW motor, which had capacity to produce 180 kg of biomass pellets per hour. The moisture content of the biomass was adjusted to 12% prior to feeding into the pellet mill and no external binder was added for making pellets. The original switchgrass is ground to a fine powder within the pellet mill before entering the plate die (200-mm diameter × 38-mm thickness with 6.3 mm holes) to produce pellets. The pellets were stored in sealed plastic bags at room temperature. Glucose and xylose yields of the original and pelleted biomass were compared for non-pretreated and SAA-pretreated conditions to evaluate pelleting and pretreatment interactions.

2.3. Carbohydrate and lignin determinations

SAA-pretreated and non-pretreated switchgrass in the original and pelleted forms were analyzed to determine carbohydrate and lignin contents (acid-soluble and acid-insoluble) following the National Renewable Energy Laboratory (NREL) Chemical Analysis and Testing Standard Procedures [21]. The extractives were removed from the non-pretreated biomass following NREL protocols [22] before carbohydrate and lignin determination. All compositional analysis was performed in duplicate.

2.4. Soaking in aqueous ammonia (SAA) pretreatment

Ground switchgrass and switchgrass pellets were pretreated by soaking in aqueous ammonia with 15% ammonium hydroxide. Pretreatment was performed in 1-L screw-capped Pyrex bottles; ground switchgrass or switchgrass pellets with a dry weight of 100 g were mixed with 600 mL of 15% aqueous ammonia. No grinding of pelleted material was required as preliminary work showed that pellets deteriorate during SAA pretreatment. The pretreatment bottles were placed in a preheated incubator at 40 °C or 60 °C. Once the mixture reached the desired temperature, they were incubated for 6 h without agitation. The pretreated solids were separated by filtering through Whatman # 41 filter paper (20–25 μm pore size) using a vacuum filtration unit. The solids were washed with distilled water (~4 L) to neutralize the pH, weighed, and stored in sealed plastic bags at 4 °C until use in enzymatic hydrolysis experiments. The moisture content and solid recovery of pretreated biomass were determined in triplicate by drying a small portion of wet solids (~2 g) overnight in a convection oven at 105 °C. A portion of pretreated wet solids (~20 g) was also dried at room temperature for compositional analysis.