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Effects of reduced severity ammonia pretreatment on pelleted corn stover

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A B S T R A C T

Biomass densification impacts pretreatment efficacy and subsequent biochemical conversion to biofuels and other biobased chemicals. Pelleted corn stover was used to evaluate the soaking in aqueous ammonia (SAA) pretreatment efficacy at high solid loadings with reductions ammonia concentration, temperature, and pretreatment time. Pretreatment resulted in 70% greater delignification of stover pellets than loose stover. Glucose yields from enzymatic hydrolysis were 49% higher for the pelleted stover and yields did not change with a twofold increase in pretreatment solid loadings. Hydrolysis yields were modeled as a function of pretreatment conditions and the developed model predicted a maximum 24-h hydrolysis glucose yield of 96% at a pretreatment temperature of 60 °C, for 4 h, with 18% ammonia. Pretreatment severity can still be reduced while maintaining 90% or higher yields with different combinations of temperature, time, and ammonia concentration. Temperature was the most important pretreatment parameter within the design space for achieving high glucose yields. FTIR and SEM analysis of SAA-pretreated corn stover pellets illustrated that the pelleting process increases pretreatment efficacy by modifying the biomass structure, disrupting the lignin-hemicellulose linkages, and partially removing lignin. Using pelleted biomass can reduce the required severity of SAA-pretreatment while still producing glucose yields above 90%. Using pelleted corn stover as a biorefinery feedstock has potential to lower pretreatment costs in addition to the improved handling and transportation.

1. Introduction

Pretreatment of cellulosic biomass is a required step to reduce its natural recalcitrance for biological processing to produce biofuels or biobased chemicals. However, pretreatment adds significant cost constraints due to pretreatment process conditions such as high temperatures, long pretreatment times, and high chemical loadings. Low biomass bulk density also limits pretreatment solid loadings. Obtaining high yields with the reduction of pretreatment severity will reduce the economic input to achieve low-cost conversion of cellulosic biomass and increase the viability of cellulosic bioindustries.

Densification such as pelleting increases biomass bulk density and therefore facilitates cost reductions for handling, storage, and transportation for cellulosic biorefineries (Eranki et al., 2011; Tumuluru et al., 2011). Moreover, biomass form and structure impacts subsequent pretreatment and hydrolysis processes. Therefore, understanding the inherent link and potential synergies of densification and pretreatment processes are crucial.

Despite pellet durability, biomass pelleting does not negatively effect hydrolysis yields following acid, alkaline, or ionic liquid pretreatments (Ray et al., 2013; Rijal et al., 2012; Shi et al., 2013). Further, pelleting has been shown to improve biomass conversion using both hydrothermal and alkaline pretreatments (Li et al., 2014). Guragain et al. (2013) also showed significantly higher sugar yield and productivity from enzymatic hydrolysis of alkali-pretreated pelleted biomass compared to unpelleted biomass. Pelleting has also been shown to increase sugar yields from switchgrass pretreated by soaking in aqueous ammonia (SAA) (Nahar and Pryor, 2014; Rijal et al., 2012). This effect was primarily attributed to effects from particle size reduction occurring within the pellet mill prior to pellet formation (Rijal et al., 2012).

Nahar and Pryor (2014) found that a less severe SAA-pretreatment (40 °C for 6 h) and lower enzyme loading (10 FPU/g glucan) were still effective for maintaining high hydrolysis yields from pelleted switchgrass. Although the lower-severity pretreatment in that study showed promising results, there are additional ways to reduce pretreatment severity and cost. For SAA pretreatment, temperature, pretreatment time, and ammonia concentration, could be further reduced when processing densified biomass.

Improving process economics by a combination of a high-solid pretreatment followed by high-solid hydrolysis will increase sugar yield while decreasing capital costs (Roche et al., 2009). Although high-solids enzymatic hydrolysis has been addressed in the literature (Hodge et al., 2008; Jørgensen et al., 2007; Roche et al., 2009), data for high-solid pretreatment is more limited (Modenbach and Nokes, 2012). However,

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there is potential for increasing pretreatment solid loadings with pelleted biomass, which would have significant impacts on reactor sizing and associated energy and chemical costs. Therefore, it is important to consider the reduced severity conditions for effective SAA pretreatment technologies along with higher solid loadings pretreatment and incorporate modified technologies into the economic and environmental models to quantify the proper benefits of pellets beyond improved logistical handling of biomass.

The objective of this study was to quantify the effect of SAA pretreatment parameters (solid loading, temperature, time, and ammonia concentration) on the digestibility of pelleted corn stover. Pelleted corn stover was selected as model feedstock and a series of SAA pretreatments were conducted based on a face-centered central composite design involving three process variables: temperature, time, and ammonia concentration. Fourier Transform Infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and Scanning Electron Microscopic (SEM) analysis were used to compare the structure of the constituents that occur during the pelleting and pretreatment process. The efficacy of pretreatment severity was evaluated by measuring 24-h glucose yields from enzymatic hydrolysis of the pretreated pelleted corn stover.

2. Materials and methods

2.1. Raw material

Corn (*Zea mays* L.) stover (stalks and leaves) was collected from a USDA-ARS research field (46°48′ 38.51″N 100°54′ 52.53″W) in Mandan, North Dakota, USA. Corn stover was air dried (10% moisture content, dry basis) and ground in a Wiley Mill with a 6-mm sieve. Sieved corn stover was stored in a sealed plastic bag at room temperature until use.

2.2. Pellet production

Dried biomass was ground using a hammer mill and 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 in Mandan, ND. The biomass was mixed with distilled water to 12% moisture before feeding into the pellet mill. No external binder was added for making pellets. The original corn stover 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 standard 1/4″ pellets. Pellets were stored in sealed plastic bags at room temperature.

2.3. Pretreatment

Corn stover pellets were pretreated by soaking in aqueous ammonia. Two sets of SAA pretreatment studies were conducted to identify: 1) the impact of solid loading on pretreatment efficacy and 2) the effect of low-severity pretreatment on hydrolysis yield using different temperatures, times, and ammonium hydroxide concentrations during pretreatment.

Pretreatments were performed in 2-L screw-capped Pyrex bottles. The pretreatment bottles with aqueous ammonia were preheated in a water bath, and corn stover pellets were mixed with the liquid to achieve the chosen solid loadings. Pretreatment temperature was monitored with a thermometer inserted into the bottle and incubated without agitation for a time period according to the experimental design. No grinding of pelleted material is required as preliminary work showed that pellets deteriorate during SAA pretreatment.

The SAA pretreated slurry was filtered through Whatman # 41 filter paper (20–25 μm pore size) using a vacuum filtration unit. SAA-pretreated solids were washed with distilled water to neutralize the pH, weighed, and stored in sealed plastic bags at 4 °C for subsequent enzymatic hydrolysis experiments. Samples were taken to determine ultra-structural changes due to pretreatment. 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 (~40 g) was also dried at room temperature for physical and chemical characterization.

2.4. Enzymes

Pretreated solids were used for enzymatic hydrolysis to determine the impact of pelleting and low severity pretreatment on glucose yields. Cellulase (NS50013), β-glucosidase (Novo188), and hemicellulase (Cellic HTec) enzymes were used for hydrolysis. All enzymes were provided by Novozymes North America, Inc. (Franklinton, NC, USA). The cellulase activity of NS50013 and β-glucosidase activity of Novo 188, as determined by Ghose (1987), were 77.0 filter paper units (FPU)/mL and 500 cellobiase units (CBU)/mL, respectively. Xylanase activity of Cellic HTec was 10,600 xylanase units (XU)/mL as determined by Bailey et al. (1992).

2.5. Enzymatic hydrolysis

Enzymatic hydrolysis of pretreated solids was performed to measure the digestibility of the pretreated substrate. The glucose concentration in the pretreated solid residues was used to determine yields (% of theoretical) after enzymatic hydrolysis for 24 h. All hydrolysis experiments were carried out in triplicate at 1% (w/v) glucan loading in 125-mL Erlenmeyer flasks. Pretreated biomass was mixed with 50-mM sodium citrate buffer (pH 4.8) supplemented with enzymes according to the experimental design. Sodium azide was also added at 0.04% to prevent microbial contamination during hydrolysis. Samples were incubated at 50 °C and 130 rpm for 72 h in a water bath shaker (MaxQ 7000, Thermo Scientific; Dubuque, IA, USA). Aliquots (1 mL) were taken at 24 h intervals and immediately centrifuged at 13,226 × *g* for 5 min (Galaxy 16 Micro-centrifuge, VWR International, Bristol, CT, USA). The supernatant was filtered through a 0.2-μm nylon filter (Pall Corporation; West Chester, PA, USA) after centrifugation, and stored at −20 °C until sugar analysis by HPLC.

2.6. High performance liquid chromatography (HPLC) analysis

Hydrolysis samples were analyzed by HPLC (Waters Corporation; Milford, MA), equipped with a Bio-Rad Aminex HPX-87P column (Bio-Rad Laboratories; Hercules, CA), and a refractive index (RI) detector (model 2414, Waters Corporation). The sugars from the injected sample (20 μL) were eluted with 18 mΩ NANOpure water at a flow rate of 0.6 mL/min. The sugars were quantified with column and detector temperatures of 85 °C and 50 °C, respectively. Glucose and xylose were quantified using 4-point external standard curves.

2.7. Experimental design and statistical analysis

2.7.1. Testing solid loadings for pretreatment

The effects of higher solid loadings for corn stover pellets was tested using SAA pretreatment with 15% aqueous ammonia at 60 °C for 4 h at 10, 15 and 20% solid loadings. Loose corn stover and corn stover pellets at a 10% solid loadings were used as controls. Low bulk density of loose stover prevented pretreatment at higher solid loadings. Biomass was hydrolyzed by cellulase and hemicellulase at 25 FPU/g glucan and 500 XU/g glucan, respectively. β-glucosidase was added using a 1:1 ratio of cellulase (FPU): β-glucosidase (CBU).

2.7.2. Response surface model (RSM) for pretreatment

Response surface methodology (RSM) was used to investigate the effects of pretreatment temperature, time, and ammonia concentration on hydrolysis yields. A face-centered design was used for the three