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Impact of disk milling on corn stover pretreated at commercial scale

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HIGHLIGHTS

- Dilute acid pretreated corn stover was obtained from a commercial plant.
- Disk milling enhanced sugar yields of dilute acid pretreated samples.
- Cutting and internal and external fibrillation were achieved by milling.
- Enzymes were more effective than milling in increasing xylose yields.
- Dilute acid pretreatment condition affected final sugar yields.

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ABSTRACT

In cellulosic biofuel production, chemical pretreatment performed at laboratory or pilot scale, followed by mechanical refining, has been demonstrated to be effective to increase feedstock enzyme digestibility. To take the combined pretreatment process one step closer to commercialization, disk milling was performed with commercially pretreated corn stover. Dilute acid pretreated samples with combined severity factors (cSF) of 0.09 (DA09) and 0.43 (DA43) were obtained from a commercial plant. Effects of pretreatment conditions (DA09 and DA43), milling cycles (0, 3, 9, and 15) and enzyme dosages (7.8, 15.6 and 31.2 mg cellulase/g dry biomass) were evaluated. Milling improved glucose yields by 0.7 to 1.2-fold. Higher enzyme dosages enhanced sugar yields. Milling was more effective to improve glucose yields, while enzyme dosage was more effective to improve xylose yields. However, dilute acid pretreatment condition was the most important factor to increase final sugar yields compared to milling cycles and enzyme dosages.

1. Introduction

In cellulosic biofuel production, pretreatment is necessary to disrupt biomass cell walls and increase enzyme accessibility. Combined pretreatments that leverage synergistic effects of different pretreatment modalities (e.g., mechanical, chemical or biological) have gained attention to maximize pretreatment effectiveness. Among various types of combined pretreatments, chemical pretreatment followed by mechanical refining is a promising method to reduce biomass recalcitrance (Kim et al., 2016a; Park et al., 2016). The combined process is adapted from the pulp and paper industry; mechanical refining (milling) in particular is used widely in commercial scale processes with capacities of ~1500 dry tons biomass per day. When chemical pretreatment is combined with mechanical refining, higher sugar yields can be achieved than chemical pretreatment or mechanical refining alone. Jones et al. (2013) pretreated hardwood chips with green liquor (Na_2CO_3 and Na_2S) and analyzed sugar yield improvement by calculating sugar yields before and after mechanical refining. In most cases, mechanical refining increased sugar yields. In the two step pretreatment process, refining brought about the largest increase in final sugar yields for samples that had moderate sugar yields (60%) after green liquor pretreatment. However, mechanical refining could not overcome biomass recalcitrance when unrefined samples had low sugar yields (Jones et al., 2013). Combined pretreatments increased sugar yields as well as enabled reduction of chemical pretreatment severity without sacrificing sugar yields. Hot water pretreatment with disk milling led to more than 1.4-fold higher glucose yields compared to dilute acid pretreatment alone (Kim et al., 2016b).

Chemical pretreatment followed by mechanical pretreatment also enables reduction of enzyme dosage or shortening of hydrolysis time. Hot water pretreatment of rice straw followed by disk milling resulted in 1.2, 1.5 and 1.3-fold higher glucose, xylose

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and arabinose yields, respectively, with cellulase dosage of 5 FPU (filter paper unit)/g rice straw compared to disk milling alone with cellulase dosage of 20 FPU/g sample. As a result, enzyme costs could be reduced 19 to 67% for the combined pretreatment (Hideno et al., 2012). Similar results were observed from Koo et al. (2011) in processing of hardwood chips pretreated with green liquor and ground using a Papirindustriens Forskningsinstitutt (PFI) mill. Combined pretreatment with enzyme dosage of 5 FPU achieved 71.8% sugar yield, which was higher than chemical pretreatment alone with 10 FPU enzyme dosage (51.5% sugar yield).

In previous studies with pretreatment conducted at laboratory or pilot scale before applying mechanical refining, combined pretreatments showed promise toward achieving cost effective ethanol production (Kim et al., 2016a; Park et al., 2016). To take the combined pretreatment one step closer to commercialization, corn stover pretreated at a commercial cellulosic plant with different dilute acid conditions was used to demonstrate the effects of disk milling on sugar yields, especially the effects of number of disk milling cycles and enzyme dosages after milling were tested.

2. Materials and methods

2.1. Feedstock

Two sets of commercially pretreated corn stover with solid contents of 10% were obtained. Samples were collected after dilute acid pretreatment with harsh and mild conditions for milling and hydrolysis (Fig. 1). The harsh dilute acid pretreatment had a combined severity factor (cSF) value of 0.43 (DA43). The mild dilute acid pretreatment had a cSF value of 0.09 (DA09). The cSF values with an incorporated acidity function were calculated as described in Chum et al. (1990).

$$\mathrm{cSF} = \log R' = \log R_0 - pH,$$

$$R_0 = t \cdot \exp\{(T - T_R)/14.75\},$$

where t is reaction time (min), T is target temperature (°C), T_R is reference temperature (100 °C) and pH is that of the pretreated liquor. Heating and cooling times were incorporated in the reaction time (t) as described in Kim et al. (2016b).

2.2. Disk milling

Before performing disk milling, dilute acid pretreated corn stover was hand squeezed and adjusted to 20% solid contents. The squeezed liquid was discarded and only pretreated solid was used for disk milling and hydrolysis. Wet samples (150 g) were fed into the disk mill (Quaker City grinding mill model 4E, Straub Co., Philadelphia, PA). The mill has one stationary and one rotating disk with zero clearance (minimal gap). The output speed of the mill was 89 rpm. Samples were ground 3, 9 and 15 times consecutively with three replications. Disk milling electrical energy usage was recorded by an electricity usage monitor (Kill A Watt® P4480, P3 International, New York, NY).

2.3. Hydrolysis

Following disk milling, enzyme hydrolysis was conducted at 12% solids content with 6 g dry biomass and three replications. Samples with known moisture content, which were determined as described in Ehrman (1994), were added to preweighed tubes. To each tube, 2.5 mL of sodium citrate buffer (pH 4.2, 1 M) was added. Per gram of dry biomass, 7.8, 15.6 and 31.2 mg of cellulase protein received from DSM Biobased Products & Services (Elgin, IL) was added, which is a proprietary cocktail of thermophilic enzymes. Deionized water was added to bring the sample to 12% solids content. An enzyme blank flask was prepared consisting of all reaction constituents except substrate.

Hydrolysis was performed on all prepared samples with enzyme blank flasks in a water bath (Gyromax 939XL, Amerex Instruments, Inc., Lafayette, CA) set at 62 °C and 75 rpm. Aliquot samples (1 ml) were taken at 0, 4, 8, 24, 48 and 72 h for glucose and xylose determinations. Each sample was centrifuged at 10,000 rpm (9300g) (Model 5415 D, Brinkmann-Eppendorf, Hamburg, Germany) and the supernatant was analyzed using HPLC. The glucose concentration in the enzyme blank flask was subtracted from the glucose concentrations in the hydrolysis samples. To determine enzyme digestibility, sugar concentration at 0 h was subtracted from the sugar concentration at each sampling time. Sugar yields were calculated as follows:

$$\mathit{Sugar\,recovery\,yield} = \frac{\mathit{sugar\,conc.\,in\,hydrolysate} - \mathit{sugar\,conc.\,at}\ 0\,\mathrm{h}\ \mathit{hydrolysate}}{\mathit{sugar\,conc.\,in\,milled\,samples} - \mathit{sugar\,conc.\,at}\ 0\,\mathrm{h}\ \mathit{hydrolysate}}$$

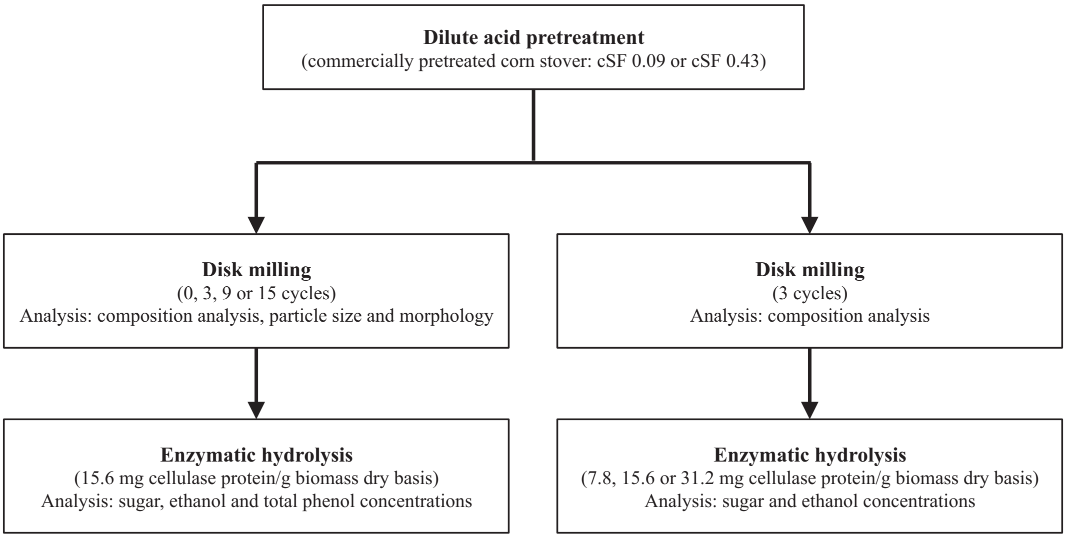

Fig. 1. Process diagram.