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Enhancement in enzymatic hydrolysis by mechanical refining for pretreated hardwood lignocellulosics

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HIGHLIGHTS

- Mechanical refining improved the enzymatic hydrolysis sugar recovery.
- The increase in sugar recovery correlated with the water retention value.
- Mechanical refiners with different mechanisms affected the biomass digestibility differently.
- A maximum in absolute enzymatic hydrolysis improvement due to refining was observed.
- The maximum occurred at conditions that produced intermediate hydrolysis conversions.

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ABSTRACT

This study investigated the effectiveness of mechanical refining to overcome the biomass recalcitrance barrier. Laboratory scale refining was conducted via PFI mill and valley beater refiners using green liquor and Kraft hardwood pulps. A strong positive correlation was determined between sugar recovery and water retention value. Refining produced significant improvements in enzymatic hydrolysis yield relative to unrefined substrates (e.g., sugar recovery increase from 67% to 90%, for 15% lignin Kraft pulp). A maximum absolute enzymatic hydrolysis improvement with refining was observed at enzymatic hydrolysis conditions that produced intermediate conversion levels. For a 91% target sugar conversion, PFI refining at 4000 revolutions allowed for a 32% reduction in enzyme charge for 15% lignin content hardwood Kraft pulp and 96 h hydrolysis time, compared to the unrefined material.

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1. Introduction

The natural recalcitrance of plant biomass feedstock to resist the breakdown of its fibers to structural carbohydrates by enzymatic hydrolysis is a major issue in the commercialization of cellulosic biofuels (Zhao et al., 2012). These natural recalcitrant properties include the waxy outer skin of the plant body, dense packing of the fiber bundles, thick fiber wall, degree of lignification, and structural complexity of the cell wall (Himmel et al., 2007). All of these issues, combined with the difficulty for soluble cellulase enzyme proteins to act on insoluble cellulose, make the processing of lignocellulosic biomass very difficult and reduces the subsequent enzymatic hydrolysis efficiency. It has been recognized that a pretreatment operation is critical for increasing the lignocellulosic biomass enzymatic digestibility by opening up the biomass structure to increase the cellulose accessibility to enzymes (Zhao et al., 2012; Zheng et al., 2009). Unfortunately, severe pretreatment conditions of high temperature and chemical charge are needed to achieve high sugar conversions, which generate lower pretreatment yields and higher fermentation inhibiting compound concentrations. It has been determined that the overall saccharification efficiency is governed by three basic processes: (1) substrate accessibility of cellulose—component removal and size reduction, (2) substrate and cellulase reactivity—limited by inhibitors, and (3) reaction conditions—optimizing and controlling pH, temp, etc. (Leu and Zhu, 2012).

The combination of chemical pretreatment followed by a mechanical refining post-treatment operation provides an opportunity to generate improved fiber characteristics for enzyme digestibility while attempting to reduce the pretreatment severity and inhibitory compounds generated. It is widely known that the high temperature and pressures experienced during pretreatment can generate carbohydrate- and lignin-derived degradation products, "inhibitors" (Klinke et al., 2004). More severe pretreatment conditions generate higher concentrations of these compounds;

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such as acetic acid, furfural, and phenolic compounds; that can reduce the performance of enzymatic hydrolysis and fermentation. Mechanical refining has been used in the pulp and paper industry to separate chemically pulped fibers after pulping and also to improve papermaking properties (Smook, 1982). Papermaking properties are improved with mechanical refining by externally fibrillating the exterior wall of the fiber and by delaminating the interior cell wall of the fiber to increase swelling and flexibility. In some cases, refining offers some cutting of longer fibers to provide a more uniform sheet formation (Annergren and Hagen, 2009).

It is expected that the shearing action in mechanical refining will allow for more internal delamination and surface fibrillation, which would disrupt the crystalline structure of the cellulose micro-fibrils, increase the accessible specific area and allow for a more efficient biomass digestibility. In addition to an expected increase in enzymatic hydrolysis yield, refining may also have the potential to allow for a less severe pretreatment. This reduction in severity can reduce the operation costs associated with pretreatment, as well as reduce the amount of by-products formed that would inhibit fermentation efficiency.

The three dominant refining mechanisms of (i) external fibrillation – creating fibrils on fiber surface; (ii) internal delamination – fiber swelling and loosening of internal structures; and (iii) fiber cutting – fiber shortening due to shearing action, can be observed to different extents depending on the refining apparatus and conditions (Kerekes, 2005). Understanding which of these mechanisms drives the optimum scenario for enzyme hydrolysis improvement, in addition to key target fiber properties changed by refining, will allow for the most effective use of refining as a post-treatment in biofuels production.

Mechanical refining has been shown to have a positive effect on the enzymatic digestibility of dilute acid pretreated corn stover (PCS) (Chen et al., 2012b; Tao et al., 2012), green liquor (GL) pretreated hardwood (Koo et al., 2011) and loblolly pine (Wu et al., 2012), sulfite pretreated softwood (Zhu et al., 2010), and recovered office printing paper (Chen et al., 2012a). It has also been shown that micro- and nano-scale mechanical refining of hardwood (Endo, 2010) and softwood (Hoeger et al., 2013) fibers that have not been thermo-chemically pretreated are especially effective in lignocellulose cell wall breakdown in order to improve the substrate enzymatic digestibility.

The PCS study exhibited increased glucan and xylan conversions after a laboratory scale refining process (PFI mill with 4000 revolutions), increasing the glucose yield to over 90% compared to the unrefined control at 70% (Chen et al., 2012b). PFI refining (8000 revolutions) after green liquor pretreatment of hardwood allowed about a 50% decrease in enzyme charge at the same hydrolysis conversion of a non-refined sample, highlighting the ability of refining to reduce the required enzyme charge (Koo et al., 2011). In the same study, the refining showed an increase in sugar conversion of GL hardwood from 51% to 72% at the same enzyme dosage. Softwood has been found to be very recalcitrant to enzymatic hydrolysis, but PFI refining at 9000 revolutions increased the sugar conversion from 41% to around 55–60% and was further increased to 78% when mechanical refining was used in combination with oxygen delignifcation (Wu et al., 2012). The sulfite pretreatment on softwood combined with disk milling proved to have cellulose saccharification efficiencies of over 90% in 48 h at a cellulase loading of 15 FPU/g-substrate, an increase from 77% without refining (Zhu et al., 2010). Recovered office printing paper showed an average percent increase of sugar conversion with 2–8 FPU/g-substrate of approximately 10% with PFI refining at 5000 revolutions (Chen et al., 2012a). In that study, the combination of ash removal from the recovered paper and refining resulted in enzyme conversions of 97%. All of these reports indicate the effectiveness of mechanical refining for improving the biomass enzymatic hydrolysis sugar conversion. However, each of these studies has different refining processes and none of them compare two or more refining methods within a single study.

The objective of this study is to determine if the post-treatment of mechanical refining improves the enzymatic hydrolysis sugar conversion efficiency in green liquor and Kraft pretreated hardwood pulps. This was accomplished by mechanically refining pulps using two types of refining laboratory equipment at different refining severities and subsequently hydrolyzing the pulps with enzymes. The results showed improvements in enzymatic hydrolysis due to refining that were a maximum at moderate enzymatic hydrolysis conditions of time and enzyme dosage. It was also observed that the different refining actions achieved by using different refining apparatuses, made a difference in the enzymatic hydrolysis improvement.

2. Methods

2.1. Hardwood biomass characterization

All pulps were made using a mixture of various species of southern hardwood chips. Compositional analyses of the hardwood chips and the various pulps were measured using a modified version of the NREL standard procedure (Sluiter et al., 2008). A quantity of 0.1 g OD powder of wood (40 mesh) was hydrolyzed with 72% H_2SO_4 for 2 h. The hydrolyzed biomass was diluted to 3% H_2SO_4 and autoclaved at 12 °C for 1.5 h. The hydrolysate was filtered and oven-dried to determine the insoluble solids, and the filtrate was collected for the determination of acid soluble lignin (UV–Vis (Lambda XLS, Perkin Elmer, Waltham, MA, USA) at 205 nm and 110 Abs coefficient) and carbohydrate content analysis using HPLC (filtered with 0.2 μm syringe filter).

2.2. Biomass pretreatment procedure

The green liquor pretreatment of hardwood chips (GL) was carried out in a 7-liter M&K Batch Digester (MK Systems, Inc., Peabody, MA) with 800 OD grams of chips (Jin et al., 2010). The chips were cooked with green liquor (Na_2CO_3 and Na_2S) at a fixed alkali charge of 16% total titratable alkali (TTA) as Na_2O to the target H Factor of 800 at a maximum temperature of 160 °C. The liquor to wood ratio was 4:1 and the sulfidity (based on TTA) was 25%. This pulp was used for the increasing PFI and valley beater refining severity experiments.

Kraft pulping is commonly used in the pulp and paper industry and is known for its efficient delignification of wood chips to make high quality pulps for papermaking. Changing the cooking strength by varying the H-factor (400, 600, 700, 1000), and white liquor (NaOH and Na_2S) chemical charge (10, 12, 13, 15% TTA) at the same temperature (160 °C), liquor to wood ratio (4:1), and sulfidity (25%) generated a spectrum of similar Kraft pulps with different lignin contents. These pulps will be used to understand how lignin content changes the effect of mechanical refining on enzymatic hydrolysis.

After pulping, the chips were washed thoroughly with tap water, centrifuged (Fletcher, Sharples Corp, Philadelphia, PA, USA), and the yield of insoluble solids was determined by total weight and the moisture content. The chips were then disintegrated using a disk refiner (148-2, Bauer, Springfield, OH, USA) at 0.13 mm (0.005 in.) gap and then screened with a laboratory flat screen (Custom, NCSU, Raleigh, NC, USA) using a 0.20 mm (0.008 in.) screen plate. The rejects were refined with a disk gap of 0.03 mm (0.001 in.) and then added back to the accepts from the first screen. The pulp was then centrifuged and fluffed for further