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Quantitative characterization of the impact of pulp refining on enzymatic saccharification of the alkaline pretreated corn stover

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HIGHLIGHTS

- The effect of PFI refining of corn stover on enzymatic hydrolysis was quantified.
- PFI refining could lower cellulose crystallinity and increase substrate porosity.
- Beating degree of PFI refined corn stover was linear with final total sugar yield.
- Final total sugar yield could be predicted by beating degree of refined corn stover.

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ABSTRACT

In this work, corn stover was refined by a pulp refining instrument (PFI refiner) after NaOH pretreatment under varied conditions. The quantitative characterization of the influence of PFI refining on enzymatic hydrolysis was studied, and it was proved that the enhancement of enzymatic saccharification by PFI refining of the pretreated corn stover was largely due to the significant increment of porosity of substrates and the reduction of cellulose crystallinity. Furthermore, a linear relationship between beating degree and final total sugar yields was found, and a simple way to predict the final total sugar yields by easily testing the beating degree of PFI refined corn stover was established. Therefore, this paper provided the possibility and feasibility for easily monitoring the fermentable sugar production by the simple test of beating degree, and this will be of significant importance for the monitoring and controlling of industrial production in the future.

1. Introduction

With the depleting of fossil fuels and the increasing of energy requirement around the world, more attentions have been paid on the development of renewable and sustainable energy alternatives (Ding et al., 2012). Enzymatic saccharification of lignocellulosic biomass is an environmentally benign technique for fermentable sugar production, which can be further converted to biofuels, biochemicals or biomaterials (Van Dyk and Pletschke, 2012). As known, enzymatic conversion efficiency is limited by the natural recalcitrance of complex plant structure (Himmel et al., 2007), like the presence of lignin, hemicellulose and acetyl groups, crystalline degree of cellulose, as well as the cross-linkages between hemicellulose and lignin, etc. The content of lignin and lignin distribution in plant cell wall can influence enzymatic hydrolysis as well (Mou et al., 2013a,b). Recently, it is also found that the size reduction of lignocelluloses together with the increase of surface accessibility is of critical importance for the enhancement of enzymatic saccharification (Leu and Zhu, 2013; Liu et al., 2013). Therefore, high-efficiency pretreatment of lignocelluloses is needful to remove these obstacles to the downstream processes of biomass conversion.

Biomass pretreatment can be classified as biological pretreatment, physical pretreatment, chemical pretreatment, and combined pretreatment (*e.g.* physicochemical pretreatment). Generally, combined pretreatment approaches like Ammonia Fiber/Freeze Explosion (AFEX) (Agbor et al., 2011), Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose (SPORL) (Zhu et al., 2009), and Alkaline Twin-Screw Extrusion (ATSE) pretreatment (Liu et al., 2013) are

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more effective for the improvement of enzymatic saccharification. Among of the combined pretreatment methods, chemical pretreatment with the supplement of post-refining is also considered as a high-efficiency pretreatment technology. For example, disc refining has been applied after the sulfite pretreatment to ameliorate sugar yield of woody biomass (Zhu et al., 2009). The mechanical refining of PFI mill (a laboratory pulp refining instrument) boosted ethanol yield and lowered the selling price of ethanol in the modified dilute acid pretreatment (Chen et al., 2012). However, the influence of mechanical refining of lignocelluloses on enzymatic saccharification is still not fully understood, and it is unknown how exactly the mechanical refining of the pretreated biomass links to the final sugar yields.

Mechanical refining, particularly for the disc refiner, has been generally applied in the pulp and papermaking industry to modify the fiber properties (*i.e.* decrease fiber length, reduce fiber coarseness of long fiber fraction and cell wall thickness, increase internal fibrillation/cell wall delamination, generate fines and increase specific surface area of fibers and fines) for the improvement of fiber bonding potential and paper strength, as well as the development of the fiber's absorbency and porosity (Li et al., 2011a,b). In a refiner, the substrate suspension is forced through a gap between two surfaces (at least one surface has bars with sharp edges) moving quickly relative to each other. In comparison to the large-scale disc refiner, PFI refiner is a standardized laboratory device (standards of ISO52642/2, 2002; QB/T1463, 1992; TAPPI T248, 2000), and it is usually used to simulate disc refiner, albeit its refining differs from disc refiners. A PFI refiner mainly consists of a bar roll and a roll house. During the process of the biomass PFI refining, internal fibrillation, external fibrillation, fiber cutting and swelling, as well as the generation of fines will take place (Chakraborty et al., 2007). In comparison to industrial disc refiner, PFI mill produces lower external fibrillation, lower fiber shortening and higher internal fibrillation. This is because of the fact that PFI refiner imposes a higher proportion of compressive forces to shear forces (Kerekes, 2005).

It has been known that mechanical refining of biomass after chemical pretreatment can improve enzyme accessibility or lowering the enzyme dosage by developing the reactive surface area of the pretreated substrate (Chen et al., 2013; Koo et al., 2011; Miura et al., 2012). However, how exactly the refining degree relates to enzymatic conversion is not completely known. On the other hand, it has not been exploited that what parameter of the refined biomass can be easily used in practice to accurately predict the final sugar yields. The refining degree of a PFI mill can be evaluated by revolution number, but different operation conditions (*e.g.* beating gap) at a given revolution number for the same biomass may lead to different results of enzymatic conversion (Chen et al., 2012; Koo et al., 2011). Also, the revolution number cannot be applied to disc refiner for the evaluation of refining degree, especially for large-scale production.

In pulp and paper industry, the refining/beating degree is commonly assessed using Schopper-Riegler (SR) number or Canadian Standard Freeness (CSF). Both of them can be simply tested and converted with each other. In particular, the online freeness testers are widely used to monitor and predict the properties of resulting pulp and paper in actual production (Li et al., 2011a). The CSF value decreases with the increase of refining and fines content, while the SR number increases. In this paper, SR number was used to evaluate the beating degree of the PFI refining of various NaOH-pretreated corn stover, aiming to find the relationship between the beating degree and the effect of enzymatic conversion of the refined substrate. Quantifying the impact of refining degree on enzymatic conversion by simply testing the SR or CSF of the refined substrate is of significant importance for monitoring the process for the production of fermentable sugars, particularly for the future large scale application with the utilization of online testers.

2. Methods

Corn stover was sourced in the fall in 2012, from Qingdao, China. After milled by a plant grinder, the corn stover was screened to get the particle size of 0.18–0.85 mm, and then collected in plastic ziplock bags at room temperature for equilibrium moisture and subsequent experiments. The chemical compositions of raw corn stover are given in Table 1.

Sodium hydroxide was bought from Sinopharm Chemical Reagent Co. Ltd. Congo Red was purchased from BASF China Co. Inc. β-glucosidase (Novozyme 188) and Cellulase (Celluclast 1.5 L) were supplied by the Sigma–Aldrich China Inc. All enzymes and chemicals were used without further purification. The activities of β-glucosidase and cellulase were 741 IU/mL and 121 FPU/mL, respectively, as tested by IUPAC standards (Ghose, 1987).

2.1. Sodium hydroxide pretreatment of corn stover

The sodium hydroxide pretreatment of the screened corn stover was performed in a cooking reactor (Mode PL1-00, Xianyang TEST Equipment Co., Ltd., Xianyang, China). There were four cooking tubes with the size of 1 L for each in the reactor. Thus, four pretreatment tests could be conducted simultaneously. For each tube, 50 g (oven dried) corn stover was added with the desired alkaline dosage (varied from 7% to 10% based on the dried weight of corn stover) and the liquid to solid ratio of 6:1. The pretreatment was carried out at the desired temperature (varied from 120 to 160 °C) for 30 min. The period of heating up was 30 min for each set of tests and the rotational speed of the reactor was about 1 rpm. After pretreatment, the cooking tubes were cooled with tap water to room temperature. Then, the samples were moved to a Nylon bag (with the mesh of 300) and washed with tap water to neutral pH. At last, the washed samples were completely collected in pre-weighed ziplock bags and stored at 4 °C for further analyses.

2.2. PFI refining treatment

The sodium hydroxide-pretreated corn stover was treated by a PFI refiner (mode PL11-00, Xianyang TEST Equipment Co., Ltd., Xianyang, China). The PFI refining was operated at the refining consistency of 10% with the rotational speed of 1460 rpm and refining gap of 0.24 mm. The PFI refining revolution numbers used in this work were 100, 200, 400, 500, 1000, 1500, 2000, 4000, 6000, 8000, and 10,000, respectively. The beating degree was measured by a beating degree instrument (mode ZB-DJ100, Zhibang Automation Technology Co., Ltd., Hangzhou, China) following standard test method (ISO 5267-1, 1999).

2.3. Enzymatic hydrolysis

To evaluate the effectiveness of PFI refining, the enzymatic hydrolysis of PFI refined corn stover was performed at substrate consistency of 2% (w/v) with the addition of β-glucosidase (5 IU/g dry substrate), cellulase (20 FPU/g dry substrate), 0.02% sodium azide, and 0.05 M sodium citrate buffer (pH 4.8). The hydrolysis reaction run at 50 °C for 48 h in small bottles (25 mL) placed in a water bath shaker at 90 rpm. Upon completion, the hydrolyzate was filtered by a 0.22 μm membrane and frozen at −10 °C for further analysis.

2.4. Analysis methods

The chemical compositions of pretreated corn stover were tested by the National Renewable Energy Laboratory (NREL)