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Quantitative characterization of enzyme adsorption and hydrolytic performance for ultrafine grinding pretreated corn stover

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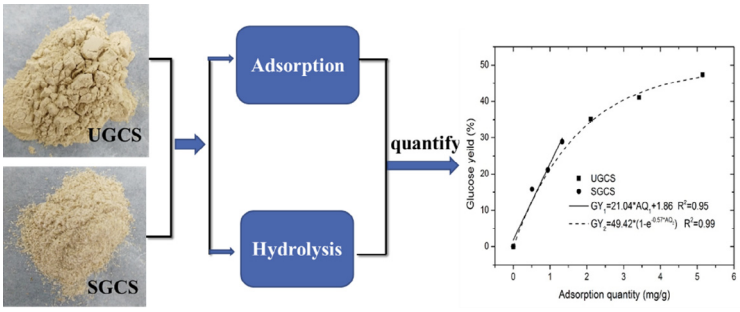
HIGHLIGHTS

- Ultrafine grinding is efficient in changing the microstructure properties.
- The quantitative analysis of enzyme adsorption and hydrolysis were presented.
- The enzyme adsorption was exponential to hydrolytic production for UGCS.
- The binding enzyme proportion was inversely proportional to enzyme consumption.

GRAPHICAL ABSTRACT



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ABSTRACT

Quantitative analysis of enzyme adsorption and hydrolysis were performed for sieve-based grinding corn stover (SGCS) and ultrafine grinding corn stover (UGCS)[1] with different enzyme consumptions. The UGCS presented significantly higher enzyme adsorption quantity (5.15 mg/g for UGCS, 1.33 mg/g for SGCS), higher glucose yield (49.75% for UGCS, 28.75% for SGCS) under 20 FPU/g and higher binding enzyme proportion (41.32% for UGCS, 10.64% for SGCS under 5 FPU/g) which can be attributed to the more accessible microstructure properties. The relationship between enzyme adsorption and hydrolytic production was directly proportional for SGCS ($GY_1 = 21.04 \times AQ_1 + 1.86$ ($R^2 = 0.95$)) while was exponential for UGCS ($GY_2 = 49.42 \times (1 - e_2^{-0.57 \times AQ})$ ($R^2 = 0.99$)),[2] indicating that overmuch enzyme consumption was not advisable for UGCS at economical aspect.

1. Introduction

Lignocellulosic biomass utilization has attracted tremendous attention all over the world due to its potential as an alternative to fossil fuels. Corn stover is the typical lignocellulose resource because of its large-scale output across the world and its feasibility of conversion to biofuels (Kim et al., 2016b). Enzyme hydrolysis is among the popular methods that have been vigorously studied in the conversion of lignocellulose (Mesa et al., 2016; Pihlajaniemi et al., 2015). However, the high cost make it a serious challenge in extensive practical application (Zhou et al., 2012). To make the conversion of lignocellulose commercially successful, efficient pretreatment methods still need to be optimized to destroy the natural recalcitrant structures, and the inner mechanism of hydrolysis need to be further understood (Sun et al., 2016). Several pretreatment methods have been applied for the purpose of changing the components or structures of the substrate to increase hydrolysis efficiency, such as dilute sulfuric acid, ammonia fiber expansion,

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[1] SGCS: Sieve-based grinding corn stover; UGCS: ultrafine ground corn stover.

[2] GY1 and GY2: glucose yield of SGCS and UGCS; AQ1 and AQ2: adsorption quantities of SGCS and UGCS.

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hot water, and mechanical milling, among others (Fougere et al., 2014; Jiang et al., 2016; Kim et al., 2016a). Among these methods, mechanical pulverization is efficient in destroying the cross-linked fibers and is an environmentally friendly method that saves the trouble of disposing waste water produced by chemical pretreatment (Lin et al., 2010; Mesa et al., 2016). And this method is receiving increasing attention around the world. For example, Fougere et al. studied the effects of mechanical downsizing on the enzymatic digestibility of hardwood (Fougere et al., 2014) and Chen et al. investigated the enzymatic digestibility of corn stover pretreated by different mechanical refining technologies (Chen et al., 2013). These studies mainly used traditional mechanical methods such as knife-milling, disc-milling and screw extrusion, which can reduce fiber length, produce shorten chips, but fibrillated fiber bundles can still exist (Chen et al., 2013; Fougere et al., 2014). These mechanical methods can-not produce efficient hydrolysis yields and are usually applied along with other chemical pretreatment methods (Chen et al., 2015; Kim et al., 2016b).

Recently, ultrafine grinding has begun to come into notice by the researchers around the world, which can achieve a median particle size of approximately 25 μm and can get a high conversion yield without any other pretreatments. Ultrafine grinding technology is extensively used in the food processing industry but has been sporadically studied as a lignocellulose pretreatment method (Zhu et al., 2010). According to the few existing studies, ultrafine grinding had been confirmed as a high-efficiency method, which can deeply destroy the fiber structure and generate low cellulose crystallinity, a large surface area and loose fibers with more pore volume, thus exhibiting a significantly high enzymatic digestibility (da Silva et al., 2010; Ji et al., 2016). Silva et al. reported it can improve the release of carbohydrates 10-fold compared with coarse milled substrates (Silva et al., 2012). However, the existing reports mainly focused on the morphological changes of the substrates and on the final increased sugar yields induced by ultrafine grinding. It is also important to determine if enzymatic digestion features can be related to other characteristics, such as enzyme consumption and enzyme adsorption. Since enzyme cost is always an expensive input in the field of industrial enzyme hydrolysis, obtaining a high hydrolysis yield with higher enzyme utilization at a low enzyme consumption is an economically viable option. However, the hydrolysis performance behind different enzyme consumptions, especially low enzyme consumptions, has not been elucidated. Furthermore, enzyme adsorption is the precondition of hydrolysis, and the study of cellulase adsorption kinetics on ultrafine grinding substrate would be helpful in understanding the mechanism of ultrafine grinding and for optimizing the hydrolysis reaction. In a recent article, the effects of ultrafine grinding on enzyme adsorption using both experimental and theoretical evidence was thoroughly discussed (Zhang et al., 2016). Nevertheless, the characteristics of adsorption at different enzyme consumptions have not been reported. There was a study that indicated that bound enzymes play a leading role in enzymatic hydrolysis, while the contribution of free enzymes was insignificant after a certain time, and the production of recycled free enzymes was too low to recycle (Yu et al., 2013). However, some other scholars showed conflicting observations that enzyme carbohydrate-binding modules might be not relevant to hydrolysis rate and hydrolytic performance (Pakarinen et al., 2014; Varnai et al., 2013). Gao et al. found that a reduction of enzyme binding to substrate followed an increased cellulose decrystallization rate, thus concluding that hydrolysis efficiency matters little with enzyme binding (Dahai Gao et al., 2013). Considering these contradictions, it is meaningful to further investigate and accurately quantify the relation between adsorption and hydrolysis, especially for binding enzyme functions and for optimizing cellulase effectiveness against enzyme consumption. Reports of the quantitative relationship between enzyme adsorption and hydrolysis performance are rare, and lack a viewpoint on cellulase effectiveness against enzyme consumption.

In accordance with these objectives, in this study, the relation between enzyme adsorption and hydrolysis was quantified for the SGCS and UGCS. The binding enzyme proportion and the cellulase effectiveness with different enzyme consumptions were also analyzed to investigate the efficiency of enzyme utilization. In addition to enzyme adsorption and sugar yield data, the changes in composition and structure of the native material and solids in the hydrolysis process were also analyzed to elucidate the mechanism of the efficiency of ultrafine grinding.

2. Materials and methods

2.1. Sample and enzyme preparation

All corn stover used was collected in October 2015 from the Shangzhuang agronomy farm of the China Agricultural University, located in Beijing, China. First, the whole-plant corn stover was dried naturally and coarsely milled to approximately 1–2 cm. Next, it was milled using a RT-34 hammer mill (Rong Tsong Precision Technology Co., Taiwan). The SGCS samples were obtained by sieving the milled material entirely through a 40-mesh screen JH-300A sieve shaker (Jiahe Machinery Co., Henan province, China). The UGCS samples were obtained by further milling the SGCS samples using ZrO_2 balls (6–10 mm diameter) in a 1:2 vol ratio using a CJM-SY-B ultrafine vibration grind mill (Taiji Ring Nano Products Co., Hebei, China) for 0.5 h. Before analysis, all samples were sealed at room temperature. The carbohydrates and lignin content were determined according to the analytical procedures recommended by the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008). 300 g samples were fully mixed with 3 mL sulfuric acid (72%, w/v) in pressure tubes and incubated for 60 min in a water bath at 30 °C. Add 84 mL deionized water to the tubes to dilute the acid concentration to 4% and autoclaved at 121 °C for 60 min. After incubation, the supernatants were filtered and took 20 mL to determine the sugar concentrations with a HPLC system (Hitachi L-7200 with refractive index detector L-2490, Hitachi Ltd., Tokyo, Japan) according to the laboratory analytical procedures proposed by the NREL, USA (NREL/TP-510-42618). Took another 20 mL to determine the concentration of acid-soluble lignin. The acid-insoluble lignin was calculated by subtracting the ash content after drying the hydrolysis residues.

Celluclast 1.5 L (cellulase) was used in the adsorption and hydrolytic experiments and was purchased from Sigma-Aldrich (St. Louis, MO, USA); the protein content was 47.95 mg/mL. Furthermore, Novozyme 188 (β-glucosidase) was also used in enzymatic hydrolysis to promote catalysis. The cellulase filter paper unit (FPU) activity was measured based on the NREL Analytical Procedure and it was 67.27 FPU/mL (Baker, 2008). The β-glucosidase cellobiase unit (CBU) activity was determined according to the methodology reported by Kim et al. (Kim et al., 2013) and it was 376.0 CBU/mL.

2.2. Particle size determination of raw materials

A laser diffraction particle size analyzer Mastersizer3000 (Malvern Instruments Ltd., United Kingdom) was used to measure the particle size distribution, which can measure the particle range from 0.01 μm to 3500 μm. The samples were dispersed with distilled water and then were poured into the measurement instrument with ultrasonic intensity of 10% and stirred at the rate of 2000 rpm to form a uniform liquid suspension.