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Towards understanding structural changes of photoperiod-sensitive sorghum biomass during sulfuric acid pretreatment

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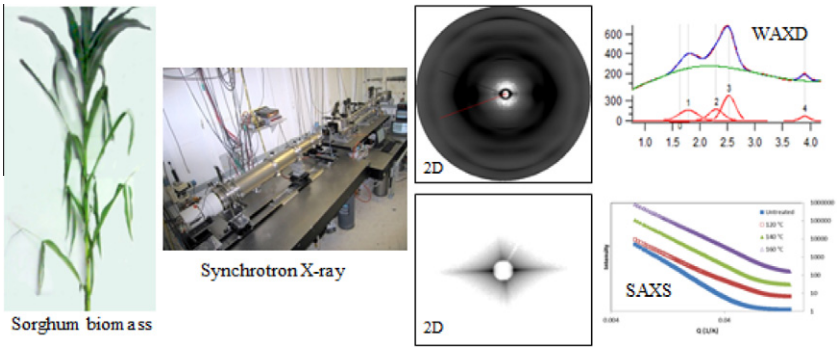
HIGHLIGHTS

- Wide-angle and small-angle X-ray scattering were used to study biomass structure.
- Both cellulose crystallinity and crystal size increased as the temperature increased.
- Simultaneous hydrolysis and crystallization of cellulose was found at 160 °C.
- The increase of microvoids volume contributes to the increased total surface area.

GRAPHICAL ABSTRACT



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ABSTRACT

Wide-angle X-ray diffraction (WAXD) and small-angle X-ray scattering (SAXS) were employed to investigate the structural changes of photoperiod-sensitive sorghum biomass in both crystalline and amorphous domains after sulfuric acid pretreatment. WAXD results suggested that the crystalline cellulose melted at 120 °C without significant polymer degradation and cellulose went through a simultaneous hydrolysis and crystallization at 160 °C. Both the cellulose crystallinity and the crystal size increased as the temperature increased, which might lower cellulose digestibility. The efficiency of enzymatic hydrolysis (EEH) increased because the cellulose protective structure was compromised and most hemicellulose was removed, resulting in an increase in total surface area as suggested by SAXS results. The radius of gyration of the polymer structure decreased and the lamellar structure was destroyed after pretreatment. Both the EEH and cellulose degradation increased as the temperature increased. The total glucose yield increased to 79.7% as the temperature increased to 160 °C.

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1. Introduction

Lignocellulosic biomass for biofuel production has attracted much attention because of its abundance and renewability. Photoperiod-sensitive (PS) sorghum, due to its advantages of drought tolerance, relatively low lignin content, and high soluble sugar content, is a promising biomass source for bioethanol production (Xu et al., 2011a). Pretreatment is a crucial step in biomass-ethanol conversion; its goals are to break the lignin/hemicellulose seal, disrupt the crystalline structure of cellulose, and increase the total surface area of cellulose to make the polysaccharides more susceptible to enzyme hydrolysis (Mosier et al., 2005; Whitfield et al., 2012). Numerous pretreatment methods have been employed for biomass processing, such as steam explosion, dilute acid, alkali, ammonia fiber explosion, and supercritical CO_2 (Wu et al., 2011). Among these methods, diluted sulfuric acid pretreatment has been used frequently in biomass-ethanol processing (Saha et al., 2005). An important advantage of acid pretreatment is that it is able to remove most hemicellulose from biomass and increase the

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total surface area of cellulose for enzymatic hydrolysis (Zeng et al., 2007). The removed hemicellulose could be degraded to monosaccharides, which could be used for ethanol production or be further degraded to furfural for chemical use. Another effect of acid hydrolysis on cellulose is the decrease of degree of polymerization, leading to the creation of reducing ends of cellulose (Knappert et al., 1980).

A review of the literature reveals that the structural changes of biomass during pretreatment are complex and not completely understood. There are arguments about the changes of crystalline structure of biomass during pretreatment. The question whether the crystallinity of biomass substrate affects enzymatic hydrolysis is in debate (Mansfield et al., 1999). Biomass crystallinity, which represents the mass percentage of crystalline component in whole mass rather than in cellulose, has been used frequently as a parameter of structural changes (Park et al., 2010). However, biomass crystallinity could be affected by the compositional changes of non-cellulose amorphous components (e.g., hemicellulose) and even extractives, providing limited information about crystalline structure. Thus, cellulose crystallinity, a percentage of crystalline part in cellulose, is suggested and was used in this study.

Both the compositional and structural changes of non-cellulose components (e.g., hemicellulose) by acid pretreatment could affect the digestibility of cellulose in biomass, but it is critical to understand the structural changes of cellulose after pretreatment. Wide-angle X-ray diffraction (WAXD) is effective for studying the crystalline structure of polymers (Park et al., 2010), and small-angle X-ray scattering (SAXS) is able to probe structure from approximately 1 nm to hundreds of nanometers (Chu and Hsiao, 2001). Recent studies using small-angle neutron scattering have revealed biomass structural changes during various pretreatments (Li et al., 2011; Pingali et al., 2010), but questions about the detailed changes of cellulose remained unanswered. Our previous study using both WAXD and SAXS has provided the structural information of different botanic parts of biomass and has shown that biomass goes through structure change in both crystalline and amorphous domains during acid and alkali pretreatments (Xu et al., 2012). Although the results are very promising, a study with multiple acid pretreatment conditions (e.g., severity) is suggested to provide detailed information on the structure changes of biomass, allowing us to understand the mechanism of pretreatment. In addition, further study is necessary to reveal the relationship between structural changes and hydrolysis efficiency.

To the best of our knowledge, the structural changes of biomass during acid pretreatment, especially the detailed changes of crystalline cellulose, have not been fully understood. In this study, both WAXD and SAXS with synchrotron radiation helped us understand the structural changes of biomass in a large length scale and their effects on enzymatic hydrolysis. Both the biomass rind and powder were used for the investigation of structural change. Biomass rind is the out layer of sheath and crystal structure of cellulose in rind has orientation (Xu et al., 2012). A study using biomass rind is helpful for understanding the nanostructure related to orientation; a study using biomass powder is more beneficial for understanding the direct effects of pretreatment on subsequent processing such as enzymatic hydrolysis. Furthermore, a better understanding of biomass structural change will enable development of a cost-efficient strategy for biomass processing.

2. Methods

2.1. Materials

The PS sorghum was harvested at physiological maturity from Riley County, Kansas, in 2008. Three rinds were prepared by knife-cutting PS sorghum and air-drying in an oven at 50 °C for 12 h. Detail information about the biomass preparation could be found in our previous report (Xu et al., 2012). Powder samples (<1 mm particle size) were prepared using a cutting mill (SM 2000, Retsch Inc., Newtown, PA). The compositional analysis of biomass was conducted by following the procedures from National Renewable Energy Laboratory (Sluiter et al., 2004). Accellerase® 1500, an enzyme complex including cellulase and β-glucosidase (Endoglucanase activity: 2200–2900 CMC U/g (one CMC U unit of activity liberates 1 μmol of reducing sugars (expressed as glucose equivalents) in 1 min under specific assay conditions of 50 °C and pH 4.8)), and Accellerase® XY, a hemicellulose enzyme complex (activity: 20,000–30,000 ABXU/g), were generously provided by Genencor (Rochester, NY). All the enzyme activity data were from the data sheets coming with the enzymes. All chemicals used in this research were of analytical grade and purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA).

2.2. Acid pretreatment

Both the PS sorghum powder and the rind were used for acid pretreatment. For biomass powder pretreatment, a prewashed sample in which the water-soluble extractive had been washed out as described (Xu et al., 2011a) was used as an untreated sample. For biomass rind pretreatment, a piece of PS sorghum rind (0.5 × 2 cm) was submerged in sulfuric acid solution. A reactor (Swagelok, Kansas City Valve & Fitting Co., Shawnee Mission, KS) made from 316L stainless steel with a measured internal volume of 75 mL (outside diameter of 38.1 mm, length of 125 mm, and wall thickness of 2.4 mm) was used for this study. A working volume of 50 mL was used to allow space for expanding liquid water at a high temperature during pretreatment. The loading of the prewashed PS sorghum was 6.1% (w/v, 3.07 g dry mass in 50 ml 1.27% sulfuric acid solution). A sandbath (Techne Inc., Princeton, NJ) with a temperature controller was used. After the heating medium attained a certain temperature, the reactor was submerged in the medium for 40 min. Then the reactor was immediately transferred and cooled by room-temperature water to decrease the internal temperature to below 50 °C in 2 min. The slurry removed from the reactor was washed, and the solid fraction was separated by filtration. Part of the solid mass from filtration was used for enzymatic hydrolysis, part of the air-dried sample was used for WAXD and SAXS measurement, and the remaining part and the liquid were used for composition analysis. Two replicates were obtained. Cellulose recovery yield (Y_{REC}) was defined as shown in Eq. (1).

$$Y_{REC} = \frac{M_{pret} \times C_{pret}}{M_{OR} \times C_{OR}} \times 100\% \quad (1)$$

where M_{pret} is the dry mass weight after pretreatment, M_{OR} is the original dry mass weight, C_{pret} is the cellulose percentage of the solid part after pretreatment, and C_{OR} is the percentage of the cellulose in original dry mass.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was conducted with the pretreated powder at 2% solids concentration (grams dry weight per 100 mL) in 50 mM sodium acetate buffer (pH 5.00) with the addition of 40 μg/mL of tetracycline and 30 μg/mL of cycloheximide. The enzymes, Accellerase® 1500 and Accellerase® XY, were used at the recommended dosages of 0.5 mL per gram cellulose and 0.1 mL per gram cellulose, respectively. Total monosaccharide was analyzed at the end of hydrolysis (72 h) on supernatants using high-performance liquid chromatography (HPLC) equipped with reflective index detector (RID 10A, Shimadzu, MD, USA) and a Rezex RPM-monosaccharide column (Phenomenex, CA, USA) operated at 80 °C. Two replicates were obtained. The efficiency of