Steam pretreatment of agricultural residues facilitates hemicellulose recovery while enhancing enzyme accessibility to cellulose

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HIGHLIGHTS

- Steam pretreatment showed similar results with 7 agricultural residues.
- Hemicellulose solubilized correlated ($r^2 = 0.98$) with enzymatic hydrolysis yields.
- Simons staining correlated ($r^2 = 0.83$) with hemicellulose removal/hydrolysis.

ARTICLE INFO

ABSTRACT

The origins of lignocellulosic biomass and the pretreatment used to enhance enzyme accessibility to the cellulosic component are known to be strongly influenced by various substrate characteristics. To assess the impact that fibre properties might have on enzymatic hydrolysis, seven agricultural residues were characterised before and after steam pretreatment using a single pretreatment condition (190 °C, 5 min, 3% SO$_2$) previously shown to enhance fractionation and hydrolysis of the cellulosic component of corn stover. When the fibre length, width and coarseness, viscosity, water retention value and cellulose crystallinity were monitored, no clear correlation was observed between any single substrate characteristic and the substrate's ease of enzymatic hydrolysis. However, the amount of hemicellulose that was solubilised during pretreatment correlated ($r^2 = 0.98$) with the effectiveness of enzyme hydrolysis of each pretreated substrate. Simons's staining, to measure the cellulose accessibility, showed good correlation ($r^2 = 0.83$) with hemicellulose removal and the extent of enzymatic hydrolysis.

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1. Introduction

Steam pretreatment has been shown to be an effective, rapid, low-cost front end for enzyme based cellulose based biorefineries (Gnansounou, 2010; Jørgensen et al., 2007). Earlier work has shown that the prior impregnation of lignocellulosic substrates with sulfur dioxide can facilitate the solubilization of hemicellulose into the water soluble stream, improving overall sugar recovery while enhancing subsequent enzymatic hydrolysis of the water insoluble cellulosic component (Bura et al., 2009; Carrasco et al., 2010). Unlike alkali based pretreatments such as ammonia fiber explosion (AFEX) or lime pretreatment, the solubilization of the hemicellulose component into the water soluble (WSF) stream during SO$_2$ catalyzed steam pretreatment reduces the need for additional hemicellulolytic enzymes to provide complete hydrolysis of the cellulosic component (Kumar and Wyman, 2009). Previous work has indicated that the hemicellulose appears to have the most influence on the ease of subsequent enzymatic hydrolysis of pretreated agricultural biomass (Bura et al., 2009). However, many of these previous studies utilized a single biomass sample and varied the steam pretreatment conditions (Bura et al., 2009; Jeoh et al., 2007) rather than assessing the effect of a single steam pretreatment condition on range of biomass substrates. Rather than looking at the collective biomass substrate, the ability to isolate intact fiber cells from agricultural residues (Jin et al., 1986) and the availability of instruments such as High Resolution Fiber Quality analyzers (FQA) have now made it possible to evaluate the effects of fiber heterogeneity on the effectiveness of pretreatment and subsequent hydrolysis.

In the work reported below, seven Canadian agricultural biomass substrates were harvested using a "stripper" harvesting technique which had previously been shown to result in up to 38% more residual straw being withdrawn for a particular area as compared to conventional combine and rotary harvesting systems

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(Stumborg et al., 2008). The substrates were pretreated using a pretreatment condition of 190 °C, 5 min and 3% SO_2 that had previously been used to recover about two-thirds of the hemicellulose and result in enzymatic hydrolysis of more than 90% of the cellulose using reasonable enzyme loadings (Bura et al., 2009). A range of substrate characterization techniques were used to try and elucidate the influence that different substrate characteristics might have on the effectiveness of steam pretreatment and subsequent enzymatic hydrolysis of the agricultural residues. As is described in more detail below, cellulose accessibility was shown to be the substrate characteristic which most influenced the effectiveness of cellulose hydrolysis, with hemicellulose solubilization during pretreatment contributing to the observed increase in cellulose accessibility.

2. Methods

2.1. Biomass substrates and chemicals used

Triticale, Canadian prairie spring wheat (SW), durum wheat, feed barley, malt barley, oat and flax straws were provided by the Agricultural Biomass Innovation Program (ABIP), Saskatoon, Saskatchewan. These samples were obtained via the "stripper" harvesting technique (Stumborg et al., 2008). Each biomass sample was ground to pass a 2 cm screen on a large Wiley mill.

Direct Orange 15 (Pylam products Inc.) was processed prior to use for determining cellulose accessibility, as described below. Cupriethylene diamine (Fisher Scientific), sulfur dioxide (Praxair), glacial acetic acid (Fisher Scientific), sodium chlorite (Sigma–Aldrich) were used as described.

2.2. Chemical composition of biomass and pretreated water insoluble and water soluble fractions

The chemical composition including the extractive components of the raw biomass samples was determined according to the National Renewable Energy Laboratory (NREL) procedures (Sluiter et al., 2005, 2008). The carbohydrates, acid insoluble and acid soluble lignin and ash content of the substrates prior to extraction were determined. In brief, water extraction was performed for 24 h using a conventional Soxhlet apparatus with a reflux rate of five draining cycles per hour with a subsequent extraction of the material using ethanol for 7 h at approximately ten draining cycles per hour. The extractives were subsequently weighed and the chemical composition was determined using the extractive-free material. Monosaccharides were measured on a Dionex (Sunnyvale, CA) HPLC (ICS-2500) equipped with an AS50 autosampler, ED50 electrochemical detector, GP50 gradient pump, and anion exchange column (Dionex, CarboPac PA1). Post-hydrolysis analyses of the liquid samples allowed quantification of the oligomeric sugars (Bura et al., 2009).

2.3. Characterization of raw biomass and pretreated substrates

The various substrates water retention values (WRV) were determined and calculated according to TAPPI Useful Method-256. The carboxylic acid group content of the pretreated substrates and biomass were measured using the conductometric method according to Katz et al. (1984). Simons' staining was performed according to the modified method of Chandra et al. (2008) with the exception that only the >100 k Direct Orange fraction was used. In order to conserve the isolated 100 k Direct Orange fraction, the steam pretreated biomass was measured in 2.0 ml screw-top vials in a total volume of 1 mL. The maximum amount of Direct Orange dye absorbed to the substrate (mg dye/g substrate) is calculated

using the Langmuir adsorption isotherm to calculate the maximum adsorption value (A_{max}) as detailed in Chandra et al. (2008).

The viscosity of both the raw and pretreated materials was determined according to TAPPI method T-230. Prior to the viscosity measurement, the samples were delignified using an acetic acid sodium chlorite solution according to PAPTAC method G10.U, with subsequent extraction of the hemicellulose component according to TAPPI T203cm-99.

Fiber quality analysis (FQA), which measures the length, width and coarseness of pulp fibers, was performed using an Optest Hi-Resolution benchtop fiber quality analyzer. The fiber analysis of the raw biomass was performed on the delignified material that was prepared for the cellulose viscosity measurement (as described above but prior to the extraction of hemicellulose). Prior to measurement on the FQA, the delignified raw biomass was extracted with 0.5% NaOH at 100 °C for 15 min to remove pectic substances and to facilitate fiber separation according to the procedure outlined by Jin et al. (1986). The suspension was filtered subsequently using a Britt Dynamic Drainage Jar (DDJ), stirred by an overhead stirrer at 200 rpm (based on Tappi T261cm-00) to collect fibers retained on the 200-mesh screen (105 μm) and to remove small non-fiber elements. The crystallinity of the substrates was estimated using a Spectrum One Fourier-transform infrared spectrometer (FT-IR) with a PIKE MIRacle™ single bounce attenuated total reflectance (ATR) accessory (Perkin Elmer, Wellesley, MA). The non-destructive nature of the ATR-FTIR facilitated the testing of the cellulose crystallinity on both the raw biomass and the pretreated substrates. The ratio of the absorbance peaks at 1425–898 cm^{-1} enables the Lateral Order Index (LOI) to be calculated based on CH_2 scissoring and the vibrational mode of C1 and the four atoms attached to it and is characteristic of amorphous β-glycosidic linkages (Jeihanipour et al., 2010). Briefly, approximately 0.1 g (oven dry basis) of never dried substrate was re-suspended in 50 ml of deionized water and shaken vigorously to disperse the material. The re-suspended sample was filtered in a Buchner funnel fitted with a Whatman No. 1 filter paper to create a pulp pad and subsequently air-dried overnight. The samples were measured against the diamond probe surface, and mid-IR spectra were obtained by averaging 64 scans from 4000 to 600 cm^{-1} at 4 cm^{-1} resolution. The spectra were normalized using the Spectrum One software supplied with the equipment.

2.4. Enzymatic hydrolysis

Cellulases (Celluclast 1.5 L, Novozymes, Bagsvaerd, Denmark) were added at 12 FPU/g cellulose and β-glucosidase (Novozymes 188, Novozymes, Bagsvaerd, Denmark) added at 40 CBU/g cellulose. Hydrolysis of pretreated agricultural biomass was carried out at 2% solids (w/v) in sodium acetate buffer 50 mmol/L pH 4.8, supplemented with 0.02% w/v tetracycline and 0.015% w/v cyclohexamide, to prevent microbial contamination. Glucose concentration was determined using HPLC high performance liquid chromatography as described above. Hydrolysis yields (%) of the pretreated substrates were calculated from the cellulose content as a percentage of the theoretically available cellulose in the pretreated substrate.

3. Results and discussion

As mentioned earlier, seven Canadian agricultural biomass substrates were harvested using a "stripper" harvesting technique which had previously been shown to result in up to 38% more residual straw being withdrawn for a particular area as compared to conventional combine and rotary harvesting systems (Stumborg