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An advanced understanding of the specific effects of xylan and surface lignin contents on enzymatic hydrolysis of lignocellulosic biomass

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HIGHLIGHTS

- Demonstrated a new approach to investigation of biomass recalcitrance.
- Established an XPS method to quantify surface lignin on biomass substrates.
- Gained new insight into the effect of xylan on enhancing cellulose swelling.
- Provided new evidence to confirm non-productive binding between lignin and cellulase.

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ABSTRACT

In this study, chemical pulping techniques were applied to create a set of biomass substrates with intact lignocellulosic fibers and controlled morphological and chemical properties to allow the investigation of the individual effects of xylan and surface lignin content on enzymatic hydrolysis. A high resolution X-ray photoelectron spectroscopy technique was established for quantifying surface lignin content on lignocellulosic biomass substrates. The results from this study show that, apart from its hindrance effect, xylan can facilitate cellulose fibril swelling and thus create more accessible surface area, which improves enzyme and substrate interactions. Surface lignin has a direct impact on enzyme adsorption kinetics and hydrolysis rate. Advanced understanding of xylan and surface lignin effects provides critical information for developing more effective biomass conversion process.

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1. Introduction

There is little doubt that sugar will become a primary currency in the future bioeconomy, and lignocellulosic biomass has been regarded as the most promising and sustainable source for supplying non-food sugar (Himmel et al., 2007). However, the lack of highly efficient and cost effective cell-wall degrading enzymes is a major obstacle that prevents commercialization of a biologically-based lignocellulosic biomass conversion process. The effectiveness of enzymatic hydrolysis is inextricably linked to the structural and chemical characteristics of the biomass material. Cellulose crystallinity, degree of polymerization (DP), lignin and hemicellulose content, and their distributions, particle size, and accessible surface area are among the most frequently cited substrate parameters associated with this effectiveness (Gregg and Saddler, 1996; Wyman, 1999; Zhang and Lynd, 2004). From an anatomical viewpoint, lignocellulosic biomass fiber can be separated into different structural levels (i.e. fiber, fibril and molecular). The morphological properties of fiber cells such as length, width, cell wall thickness, and lumen diameters, *etc.* are revealed at the fiber level. An individual fiber typically consists of primary (P) and secondary (S) cell wall layers; each of these layers is composed of numerous fibrils which encompass macro, micro and elementary fibril (Chinga-Carrasco, 2011; Ding and Himmel, 2006). At the fibril level, the interactions between cellulose, hemicellulose and lignin (macrofibril level) can be revealed (Gibson, 2012), as well as the arrangement of amorphous and crystalline cellulose (microfibril/elementary fibril level) (Frey-Wyssling, 1954; Heyn, 1969). Lignin-carbohydrate linkages and hydrogen bond/Van de Waals network of crystalline cellulose are the molecular level lignocellulosic substrate characteristics. When the substrate parameters are examined across these structural levels, complexity arises.

A considerable amount of research effort has been directed towards delineating the relationship between specific biomass

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substrate properties and the efficacy of cellulase enzyme components (Himmel, 2008; Himmel et al., 2007; Wyman, 1999; Zhang and Lynd, 2004). These studies have significantly advanced the knowledge of major factors that limit enzymatic hydrolysis. However, it is recognized that pretreated biomass substrates have intricate morphological structure and heterogeneous chemical composition. These characteristics have significantly impeded a complete understanding of the specific effect of individual biomass components on enzyme hydrolysability. For example, while the removal of xylan during pretreatment enhances cellulose enzymatic digestion by reducing xylan coating and linkages to cellulose at the fibril level (Hendriks and Zeeman, 2009; Lee et al., 2010), the mechanism by which xylan impacts cellulose digestion is still unclear. Furthermore, xylan is inextricably embedded in the cell fiber matrix with lignin; thus, the individual xylan impact can hardly be separated and studied from other substrate parameters. On the other hand, substrate lignin content has also been considered as one of the most influential substrate factors that limit enzymatic hydrolysis (Berlin et al., 2006; Chang and Holtzapple, 2000; Palonen et al., 2004). Lignin can physically impede access of an enzyme to cellulose and/or reversibly or irreversibly adsorb cellulase enzymes, which impairs their activity (Berlin et al., 2006; Eriksson et al., 2002; Palonen et al., 2004). In previous studies, bulk lignin content is often the only parameter used to interpret the "lignin effect" on enzyme hydrolysability. However, there has been great difficulty in drawing a clear correlation between bulk lignin content and enzyme hydrolysability. Previous work has shown that different lignin contents in either grass or hardwood substrate did not significantly influence enzyme digestibility (Sathitsuksanoh et al., 2012).

During the pretreatment, lignin can migrate to the fiber surface and lead to a redistribution throughout the biomass substrate. Enzymatic hydrolysis of lignocellulosic biomass starts with the adsorption of enzymes onto the fiber surface. The topographic characteristics of the substrates have a dominant effect on the hydrolysis rate. An understanding of the specific effect of surface lignin on enzymatic hydrolysis is lacking.

In this study, modified chemical pulping technique was applied to create a set of biomass substrates with controlled fiber properties (length, width, cell wall thickness, lumen diameter) and selectively altered substrate parameters (xylan, surface and bulk lignin contents) at the fibril level. Chemical pulping specifically targets the lignin-enriched compounded middle-lamella layer and thus separates adjacent fiber cells. Maintaining fiber integrity is one of the most important objectives of chemical pulping. Pulped substrates can serve as a better model to mimic the structural complexity of the plant cell wall with controlled chemical composition and surface properties. The objective of this study is to gain an advanced understanding of the specific effects of xylan and surface lignin on enzyme hydrolysability with these substrates.

2. Methods

2.1. Substrate preparation and characterization

Poplar was used to produce substrates in this study. Two kraft pulping conditions (Table 1) were chosen to produce substrates with kappa numbers at 13 (KP13) and 40 (KP40), respectively. Kappa number is a measurement of the bulk lignin content of substrates that have relatively low lignin content. An empirical conversion factor of kappa number multiplied by 0.147 can be used to estimate Klason lignin. Pulping to a higher kappa number was also attempted by lowering the pulping severity (H factor); however, this condition was insufficient for attaining a fiber separation point, which resulted in a pulp sample with a significant amount of unseparated fiber (rejects). Pulping poplar at a higher H factor led to severe damage to the cellulose fiber. To obtain a lignin free substrate KP0, KP13 was treated by acid chlorite solution at room temperature for 24 h following a previously described procedure (Browning, 1967). Two sulfite pulping conditions were used to produce substrates SP13 and SP40 to attain similar lignin contents as KP13 and KP40, respectively (Table 1). After pulping, the substrates were screened through a laboratory flat screen plate (0.25 mm wide slots) to remove shives and uncooked material. The chemical compositions of all substrates were determined by standard Tappi procedures (T236, T204, T222, T211, and T249). Fiber length and coarseness were measured by fiber quality analysis (FQA). The available surface areas of the substrates were evaluated by Simons' staining (SS) procedure (Chandra et al., 2008). The crystallinity of substrates was measured by the general purpose X-ray diffractometer with Philips X'Pert MPD system and a vertical Θ–Θ goniometer (190 mm radius). The X-ray source was ceramic X-ray tube with Cu anode. Operating power was 40 kV, 50 mA (2.0 kW). X-ray diffraction pattern of samples obtained after freeze-drying were recorded at room temperature from 10° to 75°. The scan was carried out with a step size of 0.05°. Crystallinity index (CrI) was calculated from the height ratio between the intensity of the crystalline peak (I_{002}–I_{AM}) and total intensity (I_{002}) after subtraction of the background signal measured without substrate, based on the "Segal method".

2.2. X-ray photoelectron spectroscopy (XPS) measure of surface lignin coverage on lignocellulosic biomass substrate

The amount of surface lignin on the substrates was determined using XPS. As XPS can be used to determine surface lignin content on cellulose substrates by either O/C ratio or high resolution C1s spectra, both methods were employed in this study to determine the differences in surface lignin content in all substrates. A procedure described by Johansson (Johansson et al., 1999) was used to measure surface lignin contents of KP0, KP13, KP40, SP0, SP13, and SP40. The O/C ratio was used to estimate surface lignin content via a linear relationship between the O/C ratio of 0.74 for pure cellulose (Whatman filter paper) and an O/C ratio of 0.33 for lignin. Prior to XPS analysis, the samples were extracted with acetone to remove lipophilic extractives and then air dried. No plastic containers were used to avoid possible hydrocarbon contamination. Samples were pumped inside the chamber overnight to eliminate any residual moisture content. The XPS analysis was carried out with Kratos Axis Ultra spectrometer, using a monochromatic Al K (alpha) source (10 mA, 15 kV). XPS has capability to probe the surface of the sample to a depth of 5–7 nm, and has detection limits that range from 0.1 to 0.5 atomic percent depending on the element. The Kratos charge neutralizer system was used on all specimens. Survey scan analysis was carried out with an analysis area of 300 × 700 μm and pass energy of 160 eV. High resolution scan was performed with the same

Table 1
Chemical pulping conditions for reference substrate preparation.

Substrates	Pulping chemicals (% on wood)	Temperature (°C)	Total time/time at temperature (min/min)	Liquid: wood ratio
KP13	16% Na_2O (active alkaline) and 25% Na_2S (sulfidity)	170	160/70	4:1
KP40	16% Na_2O (active alkaline) and 25% Na_2S (sulfidity)	170	107/37	4:1
SP13	5.46% Total SO_2 (Mg_2SO_3), pH: 2.88	165	218/92	6:1
SP40	5.46% Total SO_2 (Mg_2SO_3), pH: 3.8	165	160/40	6:1