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Specific effects of fiber size and fiber swelling on biomass substrate surface area and enzymatic digestibility

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HIGHLIGHTS

- External surface area changes had insignificant effect on substrate digestibility.
- Fiber swelling can improve internal surface area and substrate reactivity.
- Confirmed that xylan can improve cellulose swelling while lignin restricted swelling.

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ABSTRACT

To clarify the specific effect of biomass substrate surface area on its enzymatic digestibility, factors of fiber size reduction and swelling changes were investigated by using poplar substrates with controlled morphological and chemical properties after modified chemical pulping. Results showed that fiber size changes had insignificant influence on enzymatic hydrolysis, although the external surface area increased up to 41% with the reduction of fiber size. Swelling changes caused by increased biomass fiber porosities after PFI refining showed a significant influence on the efficiency of enzymatic hydrolysis. It is also found that chemical properties such as xylan and lignin content can influence the swelling effect. Xylan is confirmed to facilitate substrate hydrolysability by swelling, while lignin restricts swelling effect and thus minimizes the enzyme accessibility to substrates.

1. Introduction

Discovering and identifying highly efficient and cost effective plant cell wall degrading enzymes is one of the most important research topics in developing an economically viable lignocellulosic biomass to fuel and chemical conversion process. Plant cell walls are constructed by nature to resist chemical, physical and microbial degradation. Understanding the relationship between lignocellulosic biomass substrate characteristics and their impact on enzymatic digestibility has attracted a significant amount of research activities. Among various factors, the amount of substrate surface area (available or accessible surface area) has been cited as a key substrate characteristic closely related to enzymatic hydrolysability of the substrate (Gregg and Saddler, 1996; Wyman, 1999; Zhang and Lynd, 2004). Lignocellulosic biomass is assembled by fibrous elements constructed in a matrix mainly consists of cellulose, hemicellulose and lignin (Fig. 1). The amount of total substrate surface area is intricately linked to morphological characteristics across biomass fiber structural levels. Surface area of pulp fibers can be divided into external surface area affected mainly by fiber length and width, or internal surface area, which is governed by the size of the lumen and the volume of pores and other void within fiber cell wall. The varying fiber lengths and widths produced during pulping can be viewed in a similar manner as the array of particle sizes produced during the pretreatment of lignocellulosic substrates for bioconversion (Chandra et al., 2007). Changing the fiber length, width, or lumen diameter of fiber cells in biomass will collectively affect substrate particle size, resulting in changes of the substrate external surface area. Disruption of physical and chemical interactions among cellulose, hemicellulose and lignin can influence layer and porous structures within fiber cell walls, leading to changes in substrate internal surface area. A precise measurement of external, internal, or even total surface area of biomass substrate associated with cellulase accessibility is proven to be difficult to attain. The generally used external surface measurement for biomass substrate by Brunauer–Emmett–Teller (BET) method is proven to be difficult to represent the cellulase accessibility to substrate (Zhang and Lynd, 2004). Measurement of the pore volume or porosity has been frequently used as an alternative to represent the amount of accessible substrate surface area to cellulase enzymes (Chandra et al., 2008;

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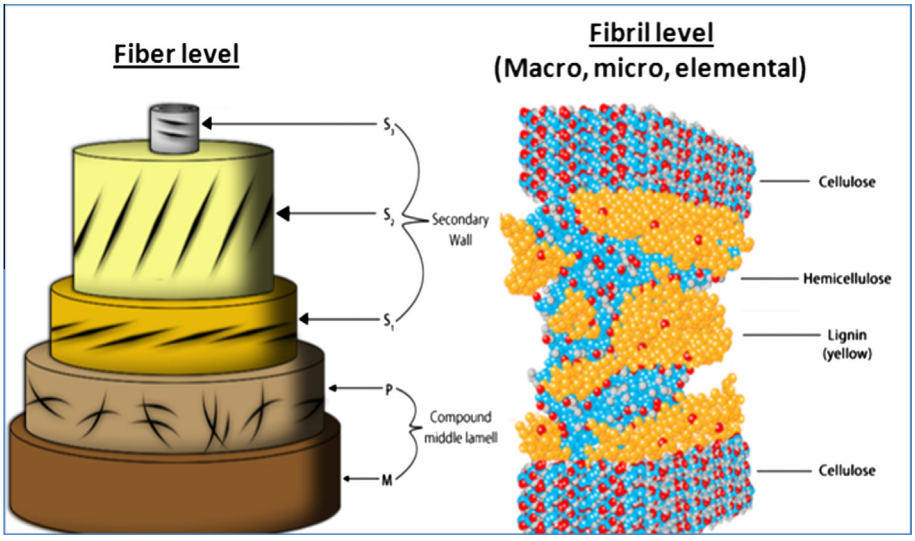

Fig. 1. Illustration of different fiber structural levels of plant cell wall.

Esteghlalian et al., 2001; Grethlein, 1985; Ogiwara and Arai, 1968; Stone et al., 1969).

A number of studies have demonstrated that reduction in substrate size can help improve the enzymatic digestibility of biomass. For example, both Dasari et al. and Yeh et al., have reported that reducing the size of sawdust or microcrystalline cellulose by ball milling improved sugar and reduced the total hydrolysis time for enzymatic hydrolysis (Dasari and Berson, 2007; Yeh et al., 2010). However, ball milling has been shown to reduce cellulose DP and crystallinity in addition to particle size (Yeh et al., 2010). In contrast to these reports of the beneficial effect of particle size on hydrolysis efficiency, Ballesteros has shown that particle size reduction does not necessarily lead to improvement in enzymatic digestibility since larger steam-exploded chips (8–12 mm) exhibited higher enzymatic digestibility (Ballesteros et al., 2000). It is inevitable that, along with the biomass substrate size changes, other substrate characteristics were also modified which likely affected substrate hydrolysability. Mansfield has shown that with the reduction in particle size of the pretreated biomass substrate, there is also an increase in the amount of surface lignin coverage which would hinder enzymatic hydrolysis (Mansfield et al., 1999). Besides these two contradictive observations, Sinitsyn et al. have also reached to a conclusion that "particle average size" does not noticeably influence the efficiency of enzymatic hydrolysis since the mixture of geometrically large and small particles may distort the size measurement (Sinitsyn et al., 1991). In all the previous studies, biomass substrates pretreated by physical or chemical methods were used. Selectively removing hemicellulose and/or lignin as well as drying the biomass during pretreatment has been shown to alter the substrate porosity. It is conceivable that the particle size reduction would primarily change the external surface area of the biomass substrate, while fiber internal structural changes will have direct impact on the porosity. In general, an increase in substrate porosity would likely lead to improved enzymatic digestibility. However, the specific effect of substrate porosity changes without the interference of other substrate factors is difficult to determine. Due to the complex structural properties and heterogeneous surface topology of biomass substrate, especially after chemical pretreatments, it is a challenge to draw a quantitative relationship between substrate surface area (or available/accessible surface area) and its enzymatic digestibility.

Based on reviewing of existing information, it becomes apparent that a further understanding and clarification is needed for the effects that changes in substrate surface area, either through changes in fiber size or porosity, have on the biomass substrate hydrolysability. This information will provide critical guidance to the development of biomass construction methods to produce "ideal" substrates for efficient enzymatic hydrolysis. In a previous study, a methodology to prepare biomass "reference substrates" with controlled parameters was developed to allow the investigation of individual substrate factors and their effect on enzymatic hydrolysis (Ju et al., 2013). The main objective of this study was to attain a clear understanding of the effect of fiber size and porosity on the enzyme accessibility and hydrolysis efficiency. In this study, representative reference substrates with different fiber size and porosities were prepared by fiber fractionation and PFI refining. All the other substrates characteristics were maintained at a similar level. These substrates allowed us to investigate the individual effects of fiber size and swelling on substrate surface area and their enzymatic digestibility.

2. Methods

2.1. Substrate preparation

Reference substrates prepared by modified kraft pulping of poplar, KP0 and KP13, as well as modified sulfite pulping of poplar SP0 and SP13 (Ju et al., 2013), were used to produce substrates with different fiber size and swelling fibers. KP0 is a lignin-free substrate with 82.20% glucan and 16.78% xylan, while KP13 consists of 79.91% glucan, 16.05% xylan and 2% lignin (Kappa number is 13). SP0 is a lignin-free substrate with 87.41% glucan and 9.65% xylan, while the SP13 consists of 85.65% glucan, 9.52% xylan and 2% lignin (Kappa number is 13).

Fractionation of KP0 and SP0 to different fiber lengths (long, medium and short) was carried out in a Bauer–McNett fiber classifier fitted with 14-, 28-, 48-, 100-, and 200-mesh screens following Technical Association of Pulp and Paper Industry (TAPPI) standard method T222. Laboratory beating of substrates KP0, SP0, KP13 and SP13 was conducted on a PFI mill following TAPPI standard method T248. Two different revolution counts were applied for the PFI refining in this study (5000 and 10,000 revolutions).

2.2. Chemical analysis

Moisture content of all substrates was determined by the mass loss after drying to constant weight at 105 °C in a convection oven.