Industrial Crops and Products 97 (2017) 498–508

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Microstructure change in wood cell wall fracture from mechanical pretreatment and its influence on enzymatic hydrolysis

Jinxue Jiang[a], Jinwu Wang[b], Xiao Zhang[c], Michael Wolcott[a,*]

[a] Composite Materials and Engineering Center, Washington State University, PACCAR 250, PO BOX 645815, Pullman, WA, 99164-5815, USA
[b] Forest Products Laboratory, United States Department of Agriculture Forest Service, Orono, ME, 04469, USA
[c] Voiland School of Chemical Engineering and Bioengineering, Washington State University, Richland, WA, 99354, USA

ARTICLE INFO

Article history:
Received 2 September 2016
Received in revised form 9 December 2016
Accepted 2 January 2017

Keywords:
Mechanical pretreatment
Cell wall fracture
Morphology
Structure
Digestibility
Energy efficiency
Wood

ABSTRACT

Mechanical pretreatment is an effective process for chemical or biochemical conversion of woody biomass. The deconstruction features of the wood cell wall play an important role in its chemical or biochemical processing. In this work, we evaluated the wood cell wall fracture in the early stage of mechanical pretreatment process conducted with various initial moisture contents. Electronic microscopy (i.e., SEM and TEM) and confocal laser scanning microscopy (CLSM) were used to visualize the cellular structure changes due to cell wall fractures.

Results reveal that the enzymatic digestibility of micronized wood produced from different initial moisture contents was improved by 2–6 folder than that of the raw material. The types of cell wall fractures after mechanical pretreatment were distinguished by the initial moisture contents of wood. In wood samples with lower moisture content, interwall fracture occurred predominantly at the middle lamella region, while intrawall fracture occurred primarily at inner cell wall layers, with sever breakage in wood fibers for high moisture content samples. Differences in the distribution of surface chemical composition also resulted from different cell wall fractures. Lignin preferentially covered the fracture surface of low-moisture content samples, while carbohydrates were more predominate in high-moisture content samples. These morphological and structural alternation contributed to improving enzymatic digestibility of micronized wood.

Findings from this study demonstrate how mechanical pretreatment modifies the fracture features of wood cell wall for further chemical/biochemical reactions.

1. Introduction

Currently, there is great interest in utilizing lignocellulosic biomass as a global energy source to reduce reliance of modern societies on fossil resources and mitigates greenhouse gas emissions. However, complex macromolecular interaction networks among biopolymer components in the plant cell wall matrix create natural recalcitrance. This, in turn, technically and economically limits the cost-effective release of fermentable sugars for subsequent liquid biofuels production (Zhao et al., 2012). The effectiveness of enzymatic saccharification of biomass is intricately related to their inherent properties, such as structural and chemical characteristics. Significant particle size reduction after pretreatment has been found to improve enzyme accessibility and mass/heat transfer efficiency (Zhao et al., 2012). The distribution of chemical composition is also integral to subsequent digestibility or a post pretreatment if required. Zhu et al. (2009) found that wood fiber with surface exposure of cellulose after chemimechanical pretreatment was more effective than wood fiber with lignin covering the surface in terms of subsequent enzymatic hydrolysis. Ju et al. (2013) found that, despite a similar bulk lignin content in wood fibers, the variation of surface lignin after chemical pulping pretreatments directly affected enzyme adsorption kinetics and hydrolysis rate.

From an anatomical viewpoint, the wood cell wall is composed of a hierarchical ultrastructure assemble ranging from the molecular level to micrometers cell wall level (Chundawat et al., 2011). Adjacent cells are separated by the middle lamella, while the individual cell wall is typically composed of three layers (i.e., the

* Corresponding author. Tel.: +1 509 335 6392.
E-mail address: wolcott@wsu.edu (M. Wolcott).

http://dx.doi.org/10.1016/j.indcrop.2017.01.001
0926-6690/

middle lamella, primary cell wall, and secondary cell wall). The secondary cell wall can be further divided into sublayers (i.e., S1 outer, S2 middle, and S3 inner layer), with different self-assembly and hierarchical organization (Chundawat et al., 2011). In the layered cell wall, semi-crystalline cellulose microfibrils are assembled as the reinforcement structure and coated with amorphous hemicellulose-lignin matrix through hydrogen bonds or covalent bonds. In addition, the distribution of chemical composition is heterogeneous throughout the cell wall. For example, the highest concentration of lignin can be found in the middle lamella area (especially cell wall corner), while S2 layer has the highest concentration of cellulose (Panshin and DeZeeuw, 1970). Thus, it is conceivable that mechanical action on wood cell wall deconstruction would result in substrates with distinguished morphological and physicochemical characteristics.

In the mechanical pulping process, wood fiber fibrillation has been found to affect the characteristics of pulps, such as fiber length, aspect ratio and surface composition. These characteristics drastically impacted subsequent post treatment and final performance of paper (Fernando, 2007; Zhu, 2011). Mechanical pretreatment aimed at overcoming the recalcitrant structure to facilitate enzymatic saccharification differs from traditional mechanical pulping processes because maintaining fiber integrity is not necessary for sugar production. Considerable research has focused on maximizing fermentable sugar release by disrupting biomass cell wall structure after mechanical pretreatment (Inoue et al., 2008; Takahashi et al., 2013; Zakaria et al., 2014). In fact, the fracture of fiber bundles and the fragmentation of individual fibers at specific positions during the early stage of mechanical pretreatment may significantly affect substrate properties and/or post-treatment requirements for improving enzymatic hydrolysis (Ju et al., 2013; Zhu et al., 2009). However, there is still a research gap in the literature addressing the fundamental characteristics of cell wall fracture and the corresponding influence on surface chemical composition and enzymatic hydrolysis.

In addition, mechanical pretreatment is generally considered to be energy intensive, which elicits particular attention for scaling bioconversion systems (Zhu, 2011). Studies show that energy consumption in mechanical wood pulping depends significantly on the mechanism of the wood fractured (Gorski, 2010; Walter, 2009). The energy input for the mechanical refining process also affects final morphological and structural properties of wood fibers, e.g., fiber length/width and fineness (Fernando et al., 2011; Gorski, 2010). However, additional research is needed to elucidate the fundamentals of wood cell fracture and its corresponding energy consumption during mechanical pretreatment.

This study is aimed at obtaining a better understanding of the structural and morphological characteristics of cell wall fractures of wood in the early stage of mechanical pretreatment and the influence of these characteristics on enzymatic hydrolysis. The influence of moisture content on the type of cell wall fracture in micronized wood is examined with a series of characterization techniques. These included electronic microscopy to delineate the structural changes in wood cell wall and, as it turned out, to reveal surface morphology and ultrastructural features of fractured cell walls. Fluorescence microscopy was applied as a rapid, effective way to identify and classify the fracture surface chemical composition distribution of micronized wood after mechanical pretreatment. We also evaluated the enzymatic hydrolysis of micronized wood and energy consumption of the mechanical pretreatment process in an effort to assess the change in the recalcitrance corresponding to wood cell wall fracture. Together, these data were integrated to provide insight into overcoming woody biomass recalcitrance for producing digestible substrate with mechanical pretreatment, or a combination of a second chemical treatment.

2. Materials and methods

2.1. Materials

Douglas-fir (*Pseudotsuga menziesii*) wood chip was obtained locally (Vaagen Brothers Lumber Inc., Colville, WA). Prior to pretreatment, the received chips were passed through a vibrating screen with 25.4-mm aperture and then hammer-milled to pass a 3.18-mm screen. The pre-processed feedstock was subsequently conditioned to different equilibrium moisture content (EMC) values (i.e., 5–30%, dry weight). Before conducting fine milling pretreatment, all conditioned material was stored in sealed plastic bags and the moisture content was validated using gravimetric methods according to standard protocol (Sluiter et al., 2008a).

2.2. Mechanical pretreatment process

Mechanical milling pretreatment of woody feedstock was performed using a high-energy vibratory Standard Ring and Puck mill with motor power of 1.1-kw (Rocklab Pty Ltd, New Zealand). The milling chamber had an inner diameter of 128-mm and height of 43-mm. The grinding media were a ring (inner diameter 78-mm, outside diameter, 100-mm, height 41-mm) and a puck (diameter 52-mm and height 41-mm). Both milling chamber and grinding media were made of tungsten carbide. The samples (10-g, oven-dry base) with different moisture content were loaded to the milling chamber and milled for 2 min. The milling time was chosen based on preliminary test showing that the particle size of the milled substrate for 2-min milling was in the micrometer range with a discernable cell wall structure. Thus, the milled samples were also noted as micronized wood (or micronized particles) in this study.

2.3. Measurement of specific energy consumption

The specific energy consumed during mechanical milling process was measured using a Fluke 1735 power logger (Fluke, USA). The active power, active energy, power factor, frequency, and time were acquired by a computer. The specific energy consumption was calculated according to the following equation:

$$E_p = \int_0^t (P_t - P_0)d_t/m = \int_0^t \Delta P_t d_t/m$$

where: $E_p$is the specific net energy consumption (kJ/kg); P_t is the power consumed at time t; $P_0$is the average power consumption under idle condition measured from an empty mill; and m is the mass charge in kg of wood to be pulverized. All measurements were performed in duplicate.

2.4. Composition analysis of the wood sample

The chemical composition of wood material was assessed according to the two-step acid hydrolysis procedure from the NREL standard protocol (Sluiter et al., 2008b). Briefly, a 300-mg sample and 3-mL of 72% H_2SO_4 was added to a 100-mL pressure tube and incubated at 30 °C for 1 h and stirred every 15 min. The sample was then diluted with 84 mL deionized water and autoclaved for 1 h. Sugars were detected using a high-performance anion exchange chromatography (HPAEC) (Dionex, ICS-3000) as described below.

2.5. Enzymatic hydrolysis

Enzymatic hydrolysis was performed with 15 FPU/OD g of substrate Cellic CTec2 cellulase and cellic HTec2 hemicellulase (1/9 of the cellulase amount). Digestion was carried out in 125-mL flasks