Industrial Crops and Products 50 (2013) 510–516

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Response of chemical profile and enzymatic digestibility to size reduction of woody biomass

Zhaojiang Wang*, Menghua Qin, Yingjuan Fu, Minggang Yuan, Yangyang Chen, Mingyu Tian

Key Laboratory of Paper Science and Technology of Ministry of Education, Qilu University of Technology, China

ARTICLE INFO

Article history:
Received 23 June 2013
Received in revised form 3 August 2013
Accepted 8 August 2013

Keywords:
Digestibility
Enzymatic hydrolysis
Pretreatment
Size reduction
Woody biomass

ABSTRACT

Size reduction is a prerequisite step for enzymatic saccharification of woody biomass. The existing controversies on its effectiveness reflect the complexity thereof. The present study focused on the dependence of enzymatic digestibility on substrate size among wood species. The results showed that the size reduction not only resulted in the augment of surface area, but also lignin enrichment and cellulose decrystallization. However, the changes in surface area, chemical profile, and crystallinity were different among wood species, which confirmed the diversity of woody biomass in bioconversion, and could be used to interpret the various responses of glucan conversion to size reduction.

1. Introduction

Woody biomass is an attractive feedstock that can be sustainably obtained from nature though photosynthesis for bioethanol production (Arato et al., 2005; Zhu et al., 2010b). Considering the massive renewable energy supply chain, purpose-grown short rotation woody crops are immensely cultivated for woody biomass production in most regions of the world (Vance et al., 2010). Among woody crops, intensively grown hybrid Populus (hereafter referred to as poplars) has gained substantial attention in the midwestern United States. Its productivity can be up to 9 Mg ha−1 yr−1, eight times greater than native aspen (*Populus tremuloides* Michx., *Populus grandidentata* L.) as demonstrated by Zalesny et al. (2009) and Netzer et al. (2002). Besides hybrid poplars in well-managed plantations, native lodgepole pine represents a major wood species from forest thinnings of the unmanaged forests that is available in large volumes and requires value-added utilizations to mitigate expensive thinning cost for sustainable healthy forest and ecosystem management in the United States. Thus the utilization of lodgepole pine for bioethanol provides an important part of the feedstock supply mix for the future biobased economy.

For bioconversion, besides the availability and productivity, plant recalcitrance is also an important factor for processing economics evaluation (Zhu et al., 2010a, 2011). Woody biomass has tough and strong physical structure and high lignin content than other feedstocks such as agriculture residues and grasses, which makes it very recalcitrant to enzymatic destruction (Sassner et al., 2008). This suggests research efforts on woody biomass should focus on upstream processing (e.g., pretreatment and size reduction) to overcome the inherent recalcitrance and enhance the subsequent enzymatic saccharification of polysaccharides. The chemical pretreatments are capable of improving the enzymatic digestibility of biomass by means of removing noncellulosic constituents (Chen et al., 2009; Rawat et al., 2013; Shi et al., 2009), increasing pore size (Grethlein, 1985) and breaking down fiber crystallization (Hall et al., 2010; Kamireddy et al., 2013; Öztürk et al., 2010). In addition to chemical approaches, size reduction is also recognized as a useful strategy to enhance the enzymatic digestibility because it can increase the accessible surface area of substrate, which caters for the interfacial heterogeneous reaction of enzymatic hydrolysis (Liu et al., 2013). Study of Zhu et al. (2010b) showed the glucan conversion of pine from hot-water, sulfuric acid, and sulfite pretreatments was greatly enhanced by decreasing the disk-plate gap of disk milling. Such enhancement was ascribed to the production of fiber bundles during size reduction (Zhu et al., 2009b). The effects of substrate size on enzymatic hydrolysis rate and hydromechanics properties of sawdust slurries was investigated by Dasari and Eric (2007). The results showed that up to 50% more glucose was produced from the smaller substrate ($33\ \mu m < x < 5\ \mu m$) than the larger ones ($590\ \mu m < x < 850\ \mu m$) after 72 h enzymatic hydrolysis. A similar study by Yeh et al. (2010)

* Corresponding author at: Qilu University of Technology, University Avenue 3501, Changing, Jinan 250353, China. Tel.: +86 13805407303.
E-mail addresses: wzj@spu.edu.cn, wzj820415@gmail.com (Z. Wang).

0926-6690/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.indcrop.2013.08.025

showed the production rate of cellobiose of microcrystalline cotton cellulose was increased at least 5-folds due to the size reduction. The advantages of size reduction on enzymatic hydrolysis were also reported for bioethanol production from corn stalk by Chundawat et al. (2007). However, opposite views were reported by Del Rio et al. (2011b), that fiber size did not appear to influence the ease of enzymatic hydrolysis of organosolve-pretreated pine. Similarly, research performed by Michael and Ely (2011) showed that the substrate size had a negligible influence on glucan conversion for various cellulosic materials, such as bagasse, switchgrass and rice straw.

To reconcile these conflicting results in the literatures, an enlightening classification of size reduction was proposed recently by Leu and Zhu (2013). For Class I size reduction, fibers are separated, cut, fragmented, and mildly externally fibrillated by shear forces to produce hairs or fibrils from the fiber surface which increase fiber external surface area, but with very minor cell wall deconstruction and decrystallization. For Class II size reduction, biomass size is reduced to the level beyond fibers toward micro or even nanofibrils with significant breakup of microfibril cross-links and crystallization. Such classification provides a better understanding of size-related factor affecting enzymatic saccharification. From another perspective of view, these controversial views mentioned above indicate that the effect of size on enzymatic digestibility not only relates to pretreatment methods, but also traits of feedstocks. The study of Kamireddy et al. (2013) on two types of forage sorghum showed the difference of enzymatic digestibility caused by genotypes. Generally, size reduction is more critical to bioconversion of woody biomass than herbaceous biomass because of the stronger physical structure and higher lignin content. To our knowledge, the diversity of wood species in terms of size-induced digestibility was little concerned. Furthermore, contrary to the conventional belief that the size reduction does not change the chemical composition of biomass, our experimental results showed different chemical profile of substrate fractions from size classification. Actually, the size-induced change of chemical profile had been reported by Zhu et al. (2009b). Hence, its important to pay more attention to the chemical profiles of substrates, especially the non-cellulosic constituents, because they are closely associated with the enzymatic digestibility of biomass.

In the present study, hybrid poplars (*Populus deltoides Bartr. ex Marsh* × *Populus nigra* L. 'NE2′; *P. nigra* × *Populus maximowiczii A. Henry* 'NM6′) from well managed plantation, and native lodgepole pine (syn. *P. contorta var. latifolia*. 'LP3′) from unexploited forest were investigate to examine the diversity of woody biomass in terms of enzymatic digestibility. We focus on the response of chemical profile and enzymatic saccharification to size reduction in the scale of Class I with the hope that the results of our study can provide objective and practical information to researchers, landowners, and policy makers for the production of wood as a bioenergy crop.

2. Materials and methods

2.1. Materials

Fourteen-year-old trees of poplar clones (*P. deltoides* × *P. nigra* 'NE2′; *P. nigra* × *P. maximowiczii* 'NM6′) were harvested from the US Forest Service, Hugo Sauer Nursery in Rhinelander, WI. The genotypes were selected because they belong to the most common genomic groups utilized in the northern Lake States region, and because they have exhibited broad variation in yield potential, growth phenologies, and recalcitrance levels as we previously reported (Wang et al., 2012). Logs of lodgepole pine trees (*syn. P. contorta var. latifolia* 'LP3′) were harvested from the Pringle Falls Experimental Forest, Deschutes National Forest, Oregon. The trees were about 100 years old with a typical diameter of 12–20 cm at breast height. These trees were grown in suppressed conditions in most of their life because of the lack of forest management. All logs were hand debarked and then chipped at the US Forest Service, Forest Products Laboratory (Madison, WI) using a laboratory chipper. The wood chips were then screened to remove all particles greater than 38 mm in length and less than 6 mm in length. The thickness of the accepted chips ranged from 1 to 5 mm. The chips were kept frozen at a temperature of −16 °C until used.

Commercial enzymes Celluclast 1.5 L (cellulase) and Novozyme 188 (β-glucosidase) were generously provided by Novozymes North America (Franklinton, NC, USA). A monocomponent endoglucanase (EGV) Novozym 476 (Novozymes A/S, Denmark) was used as received. Bio-Rad (Bradford) protein assay kit (Hercules, CA, USA) and bovine serum albumin (Food grade, SeraCare, Milford, MA) were used to calibrate protein concentration of Novozym 476. Sodium acetate and sulfuric acid were acquired from Sigma–Aldrich (St. Louis, MO), while all other chemicals, including culture media ingredients, were received from Fisher Scientific (Hanover Park, IL). All chemicals were of analytical quality.

2.2. Dilute acid pretreatment

Pretreatment was conducted as described in our previous study (Wang et al., 2012). Wood chips of 150 g and sulfuric acid solution were added to vessels in a digester which was heated via steam externally while rotating at the speed of 2 rpm. Pretreatment was conducted at temperature 170 °C, liquor to wood ratio of 3:1, 1.1% (w/w) sulfuric acid on oven-dry wood, and 25 min of pretreatment duration. Following pretreatment, the residual solid of pretreated wood chips was separated from the hydrolysate using a simple screen. The pretreated wood chips were stored at a temperature of −4 °C until used.

2.3. Size reduction and classification

Substrate with relatively uniform size was obtained by screening. First, the pretreated wood chips were hammer-milled (Montgomery-Ward ModelWB9A, 5 HP, 3600 rpm). The milled particles were then subjected to successive classification using 9 sieves with different opening size of mesh from 0.053 to 2.81 mm (Fischer Scientific Co. Ltd.) for substrate fractions production. Chemical analysis of substrate fractions was conducted, and the results were listed in Table 1.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out at 2% substrate solids (w/v) in 100 mL of sodium acetate buffer (pH 4.8, concentration 100 mM) on a shaker/incubator (Thermo Fisher Scientific, Waltham, MA) at 50 °C and 120 rpm. Cellulase loading was Celluclast 1.5 L at 5 FPU/g glucan and β-glucosidase at 7.5 IU/g glucan. This relatively low enzyme dosage was chosen for detecting differences among woody species Hydrolysate was sampled periodically and concentrations of glucose were measured for glucan conversion. Each data point was the average from two replicates.

2.5. Cellulase adsorption measurement

Cellulase adsorption was measured by an in situ UV–vis spectrophotometry method according to the literature of Liu et al. (2010). An endoglucanase (EGV) Novozym 476 was used as model cellulase to evaluate the adsorption capacity of substrate fractions. Cellulase adsorption was conducted in 50 mM pH 4.8 acetic buffer at a solid consistency of 1% (1 g substrate and 99 g cellulase