Bioresource Technology 181 (2015) 7–12

Contents lists available at ScienceDirect

Bioresource Technology

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

Influence of lignin addition on the enzymatic digestibility of pretreated lignocellulosic biomasses

Wangxia Wang, Yangsu Zhu, Jing Du, Yiqin Yang, Yongcan Jin *

Jiangsu Provincial Key Lab of Pulp and Paper Science and Technology, Nanjing Forestry University, Nanjing 210037, China

HIGHLIGHTS

- Effects of hydrophilic and hydrophobic lignin on enzymatic hydrolysis were compared.
- Sulfonated lignin enhanced enzymatic hydrolysis of AS and GL pretreated samples.
- SED of GL pretreated masson pine increased from 42% to 75% with SL addition.
- SL on enhancing hydrolysis may associate with hydrophilic groups in residual lignin.
- Hydrophobic interaction between lignin and enzyme may drive the enzyme adsorption.

ARTICLE INFO

Article history:
Received 6 December 2014
Received in revised form 6 January 2015
Accepted 8 January 2015
Available online 17 January 2015

Keywords:
Sulfonated lignin
Kraft lignin
Enzymatic digestibility
Pretreatment
Lignocellulosic biomass

ABSTRACT

The presence of lignin in lignocellulosic biomass is correlated with its enzymatic digestibility. Their correlation and mechanism have been investigated widely but have not been elucidated clearly. In this study, hydrophilic sulfonated lignin and hydrophobic kraft lignin were introduced into the enzymatic hydrolysis process to investigate their effects on the enzymatic digestibility of different pretreated lignocellulose. The influence of lignin addition on the enzymatic digestibility varied with both introduced lignin type and the pretreatment methods of substrates. Slight enhancement of enzymatic hydrolysis was observed for all substrates by adding kraft lignin. The addition of sulfonated lignin could effectively improve the enzymatic digestibility of green liquor and acidic bisulfite pretreated materials, but had little effect on sulfite–formaldehyde pretreated samples. The enzymatic digestibility of green liquor pretreated masson pine increased from 42% without lignin addition to 75% with 0.3 g/g-substrate sulfonated lignin addition.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Lignocellulosic biomass as an abundant renewable resource has attracted much attention for bioethanol production (Demirbas, 2008). Cellulose, hemicellulose and lignin account for approximately 90% of the entire lignocelluloses. Both of cellulose and hemicellulose can be liberated by enzymatic hydrolysis and subsequently fermented to ethanol (Novy et al., 2013). Lignin forms a solid seal around cellulose micro-fibrils and exhibits limited covalent association with hemicellulose (Kim, 2012), which is generally accepted as an obstacle restricting cellulose accessibility to cellulase for saccharification, thereby limiting the bioconversion of lignocellulosic biomass into liquid fuels (Rahikainen et al., 2011).

The influences of lignin on the enzymatic digestibility of lignocellulose have been widely investigated. Many studies showed that the enzymatic digestibility is related to lignin content and its cross linking to other components (Studer et al., 2011; Yu et al., 2011). Chemical pretreatment of lignocelluloses may reduce lignin content, simultaneously disrupt its cross linkage and increase the surface area of pretreated lignocelluloses and to improve its enzymatic digestibility. The enzymatic digestibility of lignocellulosic materials can be increased several times with a certain degree of delignification (Chang and Holtzapple, 2000; Ohgren et al., 2007). Recent studies suggest that not only the amount of lignin in lignocellulose, but also the chemical structure and distribution of lignin after chemical pretreatment affect the enzymatic digestibility (Nakagame et al., 2011b). Nakagame et al. (2010) found that lignin isolated from softwood with guaiacyl unit has more obvious inhibitory effect on the enzymatic hydrolysis than herbaceous materials with guaiacyl, syringyl and *p*-hydroxyphenyl units in lignin. Lignin enriched in carboxylic acid content can decrease

* Corresponding author at: Laboratory of Wood Chemistry, Department of Paper Science and Technology, Nanjing Forestry University, 159 Longpan Rd., Nanjing 210037, China. Tel.: +86 (25) 8542 8163; fax: +86 (25) 8542 8689.
E-mail address: jinyongcan@njfu.edu.cn (Y. Jin).

http://dx.doi.org/10.1016/j.biortech.2015.01.026
0960-8524/© 2015 Elsevier Ltd. All rights reserved.

the non-productive binding of cellulase and consequently increase the enzymatic hydrolysis (Nakagame et al., 2011a). The enzymatic degradability of middle lamella and primary wall is often less than that of secondary wall, because the middle lamella and primary wall contain more branched lignin and highly branched lignin is more inhibitory to cell wall degradability than liner lignin (Jung and Deetz, 1993).

Conflicting conclusions have been reported about the influence of lignin on the enzymatic saccharification of lignocellulosic biomass, and the mechanisms are still unclear. Physical blockage and unproductive enzyme binding were recognized as two mechanisms that affect the enzymatic digestibility (Kumar et al., 2012). Hydrophobic interaction between cellulase and lignin seems to be the primary driving force that governs cellulase unproductive binding. As the hydrophobicity of cellulase or substrate increase, there is a great tendency for adsorption/binding to occur (Schmaier et al., 1984). This suggests that the enzyme adsorption could be different with different hydrophobicity of substrate or lignin. Lignocellulose is a very complex matrix, it is difficult to evaluate the influence of one lignin characteristic on the enzymatic digestibility. Any change will correlate to concurrent change in other lignin properties. Using simple model system such as adding lignin in untreated/pretreated lignocellulose can be an effective approach to understand the influence of lignin on cell wall digestibility (Nakagame et al., 2011b). In this work, two commercial lignins, Indulin AT (KL, purified softwood kraft lignin) and Reax 85A (SL, sulfonated softwood kraft lignin) were used as hydrophobic and hydrophilic lignin in the enzymatic hydrolysis of lignocellulosic substrates with different chemical pretreatment to figure out the influence of lignin hydrophobicity on the enzymatic digestibility.

2. Methods

2.1. Materials

Masson pine (*Pinus massoniana*) was provided by a paper mill in Fujian, China. Poplar (*Populus nigra*) and silvergrass (*Triarrhena lutarioriparia*) were respectively collected from Jiangsu and Hunan, China. Air dried masson pine (~3 cm × 1.5 cm × 0.2 cm), poplar (~3 cm × 1.5 cm × 0.2 cm) and silvergrass (~3 cm in length) were stored in a refrigerator at 4 °C before use. Part of these three materials were ground by a Wiley mill and a fraction between 40 and 80 mesh of each untreated milled particles was collected for the analysis of main components and enzymatic hydrolysis. Two kinds of commercial lignin, Reax 85A and Indulin AT kindly provided by Meadwestvaco (Charleston, SC, US), were used in the enzymatic hydrolysis to evaluate their effects on the digestibility of different pretreatment substrates. Reax 85A is the sodium salt of a chemically modified medium molecular weight kraft lignin polymer (with a weight average molecular weight of 10,000). As sulfonate group was introduced onto the molecular after modification, Reax 85A exhibits good solubility in water. Indulin AT is a purified form of kraft pine lignin completely free of all hemicellulosic materials, with a poor solubility in neutral and acidic aqueous media. Cellic® CTec2 (filter paper activity 250.2 FPU/mL) generously provided by Novozymes A/S (Bagsværd, Denmark) was used for the enzymatic hydrolysis of lignocellulosic materials. All the chemicals were analytical grade and purchased from Nanjing Chemical Reagent Co., Ltd. of China and used as received without further purification.

2.2. Pretreatment of raw materials

Three pretreatments under alkaline (green liquor, GL), neutral (sulfite–formaldehyde, SF) and acidic conditions (acidic bisulfite, AS) were used to produce different pretreated substrates for enzymatic hydrolysis (Jin et al., 2010, 2013; Wang et al., 2013a). All pretreatments in this work were carried out in a rotary lab-scale cooking system with an electrically heated oil bath (YRG2–10 × 1.25, Nanjing Jiezheng, China). Ten 1.25 L-stainless steel bomb reactors with screw cap were contained in the cooking system. Three raw materials, masson pine, poplar and silvergrass, were subjected to GL pretreatment. The detail conditions of the pretreatment, including total titratable alkali (TTA) charge as Na_2O on the basis of oven dry (o.d.) material, sulfidity of the pretreatment liquor, the ratio of liquor to biomass (L:B, mL/g), final temperature and the time at temperature were listed in Table 1. Only poplar was used for SF and AS pretreatments under the conditions in Table 2. All chemicals charged in the pretreatments were on the basis of o.d. materials. The molar ratio of sodium sulfite to formaldehyde in SF pretreatment was 1:1 (Jin et al., 2013). The pretreatment conditions of AS and SF were optimized from series experiments to obtain the similar lignin content with GL pretreated poplar (~22%).

The materials were first impregnated with the pretreatment liquor at 80 °C for 30 min. Then the temperature was raised with the rate of 2 °C/min to the pretreatment temperature and maintained for 1 h. The pretreatment was immediately terminated while the designed time at temperature was reached. The bombs were cooled in cold water to room temperature and the samples were filtered through cheese cloth. The original filtrates were collected for pH measurement. The remaining solids were washed with deionized water to remove residual chemicals and dissolved compounds from raw materials. The pretreated solids were defiberized by a laboratory disk refiner (KRK, Φ 300 mm, Jilin, China) to produce substrates for enzymatic hydrolysis.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of different substrates was carried out in sodium acetate buffer (pH 4.8) at 5% (w/v) consistency and 50 °C using a shaking incubator (DHZ-2102, Shanghai Jinghong, China) at 180 rpm for 6–96 h. The activity loading of Cellic® CTec2 was based on a cellulase charge of 20 and 40 FPU/g-cellulose. Varied amount of Reax 85A (SL) and Indulin AT (KL) were added into the enzymatic hydrolysis system prior to enzyme addition. Enzymatic hydrolysis without lignin addition was used as control. Sodium azide was charged at 3 mg/mL of buffer as an antibiotic to inhibit the microbial infection. Enzymatic hydrolysis residue and hydrolysate was separated by centrifugation (5000 rpm, 20 min). Hydrolysate was sampled for monomeric sugar (glucose, xylose, arabinose and mannose) analysis.

2.4. Analytical methods

Cellulase activity of Cellic® CTec2, in terms of "filter paper unit" (FPU) was measured by the filter paper method using Whatmann No. 1 filter paper as a standard substrate (Ghose, 1987). The content of sulfonate and carboxylic acid groups in the substrates was determined using the conductometric titration technique described by Katz et al. (1984).

Monomeric sugars in enzymatic hydrolysate were determined using a high performance liquid chromatography (HPLC, Agilent Technology 1100 series, Palo Alto, CA) with refractive index detector (RID). A SP0810 column (8.0 mm × 300 mm) and a SP-G Pb^{2+} column (6.0 mm × 50 mm) (Shodex, Showa Denko, Japan) were used as analytical column and guard column. The column temperature was 70 °C. Degassed super-purified deionized water was used as eluent at a flow rate of 0.5 mL/min. Aliquots (10 μL) were injected after passing through a 0.22 μm nylon syringe filter. The concentration of monosaccharide was corrected by calibration

Download English Version:

https://daneshyari.com/en/article/680087

Download Persian Version:

https://daneshyari.com/article/680087

Daneshyari.com