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Comparative analysis of lignin chemical structures of sugarcane bagasse pretreated by alkaline, hydrothermal, and dilute sulfuric acid methods

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ABSTRACT

To understand the lignocellulose properties of sugarcane (*Saccharum* sp.) bagasse with a view to its application to produce fermentable sugars, we comparatively analyzed lignocellulose properties, especially lignin chemical structures, of bagasse before and after alkaline, hydrothermal, and dilute sulfuric acid pretreatments. Wet chemical analyses and two-dimensional nuclear magnetic resonance spectroscopy determined that lignocellulose from untreated bagasse contained similar levels of guaiacyl (G) and syringyl (S) lignins with lower levels of *p*-hydroxyphenyl (H) and tricin (T) lignins. Alkaline pretreatment preferentially removed S and T lignins from bagasse, whereas the hydrothermal and dilute sulfuric acid pretreatments had the effect of enriching lignins (except T lignin) by reducing xylans and arabinans. Our data indicated that the alkaline and dilute sulfuric acid pretreatments relatively enriched β–5 linkages, while reducing β–*O*–4 and β–β substructures, in the residual lignin polymers. In contrast, hydrothermal pretreatment reduced all three major linkage types.

1. Introduction

Lignocellulosic biomass, which is derived from plant cell walls, is the most abundant of terrestrial feedstocks (Yoda, 1982) available for both producing materials and harvesting energy. It represents a renewable and carbon-neutral resource, and is important not only for sustainability of natural resources but also for reduction of greenhouse gas emissions (Yu and Chen, 2008). Annual production of lignocellulosic biomass is estimated to be approximately 1.5–2.0 × 10^{11} tons (Kamm and Kamm, 2004; Mohanty et al., 2000; Pauly and Keegstra, 2008; Reddy and Yang, 2005; Zhang, 2008); however, the uses of lignocellulosic biomass are currently limited to only 2.0–3.5% of the produced lignocelluloses (Kamm and Kamm, 2004; Pauly and Keegstra, 2008; Zhang, 2008). To facilitate the application of lignocelluloses to various biomass refineries, technological advances based on a deeper understanding of lignocellulose characteristics are essential.

Sugarcane (*Saccharum* spp.), belonging to the Gramineae (Poaceae) family, is the most important crop for sugar production, and is a convenient source of biomass. World sugarcane production has steadily increased to more than 1.88 Gt at 2014, with an average productivity of 69.5 t ha^{-1} (FAOSTAT, 2017). The process of juice extraction from sugarcane produces bagasse as a fibrous by-product. During 2010/2011, 625 million tons of sugarcane was processed and 208 million tons of bagasse was generated in Brazil (Rocha et al., 2012), where sugarcane has the highest level of production in the world. Sugarcane bagasse is usually consumed as a solid fuel to generate energy required by the sugar production process (Rocha et al., 2012). However, as innovations in sugar processing continue to emerge, the energy requirements for sugar manufacture are declining (Rocha et al., 2011). This means that an increasing surplus of sugarcane bagasse is available for use as a lignocellulosic feedstock.

Enzymatic conversion of cell wall polysaccharides (cellulose and hemicelluloses) into fermentable sugars is one of the promising biorefinery applications of sugarcane bagasse. Lignocellulosic biomass, however, generally displays a recalcitrance to enzymatic hydrolysis. To reduce such recalcitrance, chemical and thermochemical pretreatments, which affect the composition and structure of lignocellulose, are often applied. In particular, alkaline, hydrothermal, and dilute acid methods are the most popular pretreatments used to improve enzymatic saccharification of lignocellulosic biomass. Earlier studies showed that these three pretreatment methods boost enzymatic saccharification by differentially altering the composition and structure of various grass lignocelluloses (Asgher et al., 2013; Canilha et al., 2011; Carvalho de

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et al., 2016; Elander et al., 2009; Garlock et al., 2011; Kumari and Das, 2015; Rocha et al., 2011; Silva et al., 2011; Yu et al., 2013). In addition, the three pretreatments also differentially affect the crystallinity and ultrastructure of lignocellulose (Yu et al., 2013). However, the three pretreatment methods have different advantages and disadvantages regarding energy requirements and pretreatment process management (Yu et al., 2013). Accordingly, process optimization and development of combined pretreatments have been actively pursued for improving sugar production from lignocellulosic biomass (Binod et al., 2012; Rezende et al., 2011; Silva et al., 2011; Yu et al., 2013; Zhao et al., 2009; Zeng et al., 2014).

Lignins are complex phenylpropanoid polymers that fill the interspaces of cell wall polysaccharides (Umezawa, 2018). They are considered to be one of the main inhibition factors in lignocellulosic polysaccharide utilization, including fermentable sugar production (Hsu et al., 2010; Li et al., 2016; Mooney et al., 1998; Nakagame et al., 2011). Sugarcane bagasse typically contains 18–20% lignins (Canilha et al., 2011; Kumari and Das, 2015; del Río et al., 2015; Sun et al., 2003). As typical lignins found in grasses, sugarcane lignins are composed mainly of guaiacyl (G) and syringyl (G) units with a small amount of *p*-hydroxyphenyl (H) units (del Río et al., 2015; Sun et al., 2003). Sugarcane lignins are highly acylated (mostly by *p*-coumaroylation) at their side chains and contain tricin flavonoid units (del Río et al., 2015). In addition, ferulate residues, participating in cross-coupling reactions between arabinoxylans and between lignin and arabinoxylan polymer chains (Ralph, 2010), are an important component of sugarcane lignocellulose (Kumari and Das, 2015; del Río et al., 2015).

The chemical structure of lignins in raw sugarcane straw and bagasse, without any pretreatment, has been investigated in detail (del Río et al., 2015). In contrast, however, studies on lignin chemical structures of pretreated sugarcane bagasse are still limited to several reports (Chen et al., 2011; Liu et al., 2007; Rezende et al., 2011; Zeng et al., 2014). Therefore, comparative analysis of the details of lignin chemical structures, such as aromatic unit composition and intermonomeric linkage distribution, of sugarcane bagasse pretreated by alkaline, hydrothermal, and dilute acid methods would be informative for the applications of these pretreatment methods in a sugarcane biorefinery. Hence, in this study, we comparatively investigated the impacts of alkaline, hydrothermal, and dilute sulfuric acid pretreatment methods on the lignocellulose characteristics of sugarcane bagasse, especially focusing on their lignin chemical structures.

2. Materials and methods

2.1. Sample preparation

The sugarcane bagasse sample used in this study was provided by a local sugar factory in Okinawa, Japan. The raw bagasse collected from the sugar mill was stored in a refrigerator for 2 weeks and then dried in sunlight. The dried bagasse samples were ground to pass a 1.0-mm mesh. The bagasse sample was treated with 1% (w/v) NaOH solution at 120 °C for 20 min (alkaline pretreatment), with distilled water at 230 °C for 10 min (hydrothermal pretreatment), or with 1% (v/v) H_2SO_4 at 170 °C for 5 min (dilute sulfuric acid pretreatment). The untreated sugarcane bagasse and three pretreated bagasse samples were pulverized and extracted with solvents as described previously (Yamamura et al., 2013).

2.2. Lignin content measurement

Klason lignin and ash contents were measured in a similar manner to that described previously (Sluiter, 2012). Lignin content was calculated as follows:

$$\text{AIL}\ (\%) = (DW_{aif} - DW_a)/DW_s \times 100, \quad (1)$$

where AIL is acid-insoluble lignin content, and DW_{aif}, DW_a, and DW_s are the dry weights (mg) of the acid-insoluble fraction (pellets remaining after the acid hydrolysis), ash, and the sample before the acid hydrolysis, respectively.

2.3. Determination of polysaccharide contents

Polysaccharide content was determined as described previously (Miyamoto et al., 2018). Samples were de-starched (Hattori et al., 2012) and hydrolyzed with 2 M trifluoroacetic acid (TFA). The resulting monomeric sugars were acetylated (Hayashi, 1989) and quantified by gas chromatography–mass spectrometry (GC–MS) (GCMS-QP 2010 Plus, Shimadzu, Kyoto, Japan). Myo-inositol (Nacalai Tesque, Kyoto, Japan) was used as an internal standard for quantification. In parallel, a crystalline cellulose fraction that remained after TFA hydrolysis was purified using the Updegraff method (Updegraff, 1969) and completely hydrolyzed with H_2SO_4 (Hattori et al., 2012). The released glucose was quantified using a Glucose CII test kit (Wako Pure Chemicals Industries, Osaka, Japan). The amount of polysaccharides from neutral sugar monomers were calculated as follows (Sluiter, 2012):

$$\text{PS}\ (\%) = M_{mns} \times C_{corr}/DW_s \times 100, \quad (2)$$

where PS is polysaccharide content, and M_{mns}, C_{corr}, and DW_s are the mass (mg) of monomeric neutral sugars, correction constant: 0.90 for hexoses (glucose, mannose, and galactose); 0.88 for pentoses (xylose and arabinose), and dry weight (mg) of sample used for the analysis, respectively.

2.4. Enzymatic saccharification

Enzymatic saccharification was performed as described previously (Hattori et al., 2012). The enzymatic saccharification efficiency (ESE) was determined using the following equation:

$$\text{ESE}\ (\%) = Glu_E/Glu_T \times 100, \quad (3)$$

where Glu_E and Glu_T are amounts (mg) of glucose obtained by enzymatic saccharification of samples and total glucose in the samples (Sect. 2.3), respectively.

2.5. Thioacidolysis

Analytical thioacidolysis was performed and released lignin β–*O*–4-derived monomers were quantified by GC–MS (GCMS-QP 2010 Ultra, Shimadzu) according to an established method (Yamamura et al., 2012). For quantification of the lignin β–*O*–4-derived monomers, 4,4′-ethylenebisphenol was used as an internal standard with the response factors obtained from the literature (Yue et al., 2012).

2.6. Quantification of cell wall-bound hydroxycinnamates

Cell wall-bound hydroxycinnamates were hydrolyzed with a mild alkaline solution and quantified by GC–MS (Shimadzu QP-5050A GC–MS System, Shimadzu) as described previously (Yamamura et al., 2011). The released monomers were derivatized with *N*,*O*-bis(trimethylsilyl)acetamide.

2.7. 2D NMR

Whole cell wall and lignin-enriched cell wall samples for analysis by nuclear magnetic resonance (NMR) spectroscopy were prepared as described previously (Koshiba et al., 2017; Takeda et al., 2017). NMR spectra were acquired on an Avance III system (800 MHz, Bruker Biospin, Billerica, MA, USA) equipped with a cryogenically cooled 5-mm TCI gradient probe. Adiabatic two-dimensional (2D) ^{1}H–^{13}C heteronuclear single-quantum coherence (HSQC) NMR experiments were