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Structural features and antioxidant activities of degraded lignins from steam exploded bamboo stem

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ABSTRACT

A two-step process based on steam explosion pretreatment and alkali/alkaline ethanol post-treatments of bamboo stems was developed to produce bioethanol efficiently. In this study, alkali/alkaline ethanol post-treatments were applied to isolate lignins from steam exploded (1.8 and 2.0 MPa for 5 min, and 2.0 MPa for 8 min) bamboo stems, which resulted in the dissolution of 28.9, 53.3 and 60.1% of acid-insoluble lignins, respectively. The structural features of the isolated lignins were comparatively and comprehensively investigated by FT-IR and NMR techniques. The lignins showed low molecular weights (M_w, 860–1190 g/mol), narrow polydispersities (M_w/M_n, 1.46–1.92), and high content of phenolic hydroxyl groups (0.53–1.79 mmol/g). Antioxidant activities investigation showed that the radical scavenging index (RSI) of the extracted lignins (2.02–5.56) was higher than BHT (0.19) but lower than that of BHA (11.11). The relatively high antioxidant activity of the lignins suggested that they may be a potential antioxidant in food and other industries.

1. Introduction

The main objective of biomass fractionation according to the biorefinery was to achieve the separation of cellulose, hemicelluloses and lignin from lignocelluloses through an effective way, in which the fractions obtained can be converted into a wide variety of high-valued products such as biofuels (Alonso et al., 2010; Parajó et al., 1993), xylitol (Walther et al., 2001), and food additives (Dizhbite et al., 2004). Noteworthily, pretreatment method is the Gordian technique in the biorefinery process. Various pretreatment methods have been developed, including steam explosion, dilute acid treatment, and hot water treatment to overcome the recalcitrance of lignocelluloses (Mosier et al., 2005). Among them, steam explosion is a commonly used process for the separation of biomass constituents, which can disrupt the lignocellulosic structure and increase its chemical reactivity and enzyme accessibility. Moreover, the advantages of steam explosion also include significantly less environmental pollution, smaller capital investment, and less hazardous process chemicals (Wang et al., 2010).

Lignin can physically impede the access of an enzyme to cellulose and/or reversibly or irreversibly adsorb cellulase enzymes in the process of enzymatic hydrolysis, and thus impairs their activities (Eriksson et al., 2002; Ju et al., 2013). Therefore, it is necessary to remove lignin before enzymatic hydrolysis. Steam explosion pretreatment is a very important and effective method. When there were no other chemicals adding to the process, the main reaction is autohydrolysis of the ether bonds between polysaccharides and lignin. The high-temperature steam leads to the release of acetic acid from biomass, which catalyzes the hydrolytic reactions of constituent polymers. These reactions result in a loss of hemicelluloses and amorphous cellulose and a decrease in the amount of β-*O*-4′ structure of lignin (Martin-Sampedro et al., 2011). The important process which influences steam explosion is the pre-soaking treatment before steam explosion. The main purpose of pre-soaking treatment is to realize the fiber softening, which makes fiber separation escape mechanical damage. In addition, the fiber can be swelled, which can increase water vapor infiltration intensity and hydration, thereby, improving the effect of the treatment. The common reagents of the treatment are alkaline liquor, water and dilute acid solution. Among them, the alkaline liquor is generally considered to have a strong penetrating ability to make fiber swelled.

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Moreover, it can prevent cellulose and hemicelluloses from acidic hydrolysis by adding NaOH, $NaHCO_3$ or $MgCO_3$, etc., so cellulose and hemicelluloses can be reserved as much as they can (Ben et al., 1993; Wang et al., 2006). In the post-treatment process, the removal of lignin can increase the efficiency of enzymatic hydrolysis of the raw materials, and at the same time, the lignin obtained can also be recycled for further use (Sukumaran et al., 2009).

Alkali treatment of lignocellulosic biomass is a very effective method to remove and recycle lignin. It can disrupt the cell wall by partially dissolving hemicelluloses, and lignin, or by hydrolyzing uronic, acetic, and hydroxycinnamic acid esters. The increase of the degradability of the cell walls is also due to the cleavage of the bonds between lignin and hemicelluloses or between lignin and hydroxycinnamic acids (Jackson, 1997; Sun et al., 1999). Compared with cellulose and hemicelluloses, lignin is a complex polymer composed of heterogenous units and this heterogeneity leads to the limitation of lignin usage in many aspects. Besides, as lignin could generally be degraded and dissolved in solutions and the extraction is also difficult, the awareness of lignin is not high (Mosier et al., 2005; Gosselink et al., 2010). Therefore, it is necessary to obtain a better understanding of the structural features of the lignin, which determined the final utilization of lignin. For example, lignins can be used as a substitution for phenol in producing phenol–formaldehyde (Mansouri and Salvadó, 2006). Moreover, lignins have phenolic hydroxyl group that can act as a potential antioxidant in the food industry, preventing the loss of food flavor, color, and active vitamin content (Dizhbite et al., 2004; Pan et al., 2006).

The purpose of this work was to investigate the structural features and properties of the lignin fractionations released from steam-exploded bamboo stems by a successive extraction with 0.5% NaOH aqueous solution and 60% aqueous ethanol containing 1.5% NaOH post-treatments. The influences of steaming pressure/time on the subsequent isolation, chemical structures and properties of the lignin fractions were comprehensively studied to evaluate the possibility of lignin being an antioxidant. The lignin fractions were characterized by their yields, associated polysaccharides, and molecular weights. In addition, the information of lignin structure, including some main substructures (intercoupling bonds, β-O-4′, C—C, etc.) and PCA/FA as well as S/G ratios, were qualitatively and quantitatively obtained by 2D-HSQC NMR spectroscopy. The functional groups (phenolic hydroxyl, aliphatic hydroxyl and carboxyl groups) in the lignin fractions were quantitatively determined by ^{31}P NMR and the antioxidant ability of the lignins was also detected by *in vitro* DPPH radical scavenging test.

2. Methods

2.1. Materials

Bamboo (*Phyllostachys pubescen*) stems were obtained from Jiangxi province, China. The chemical composition (%, w/w) was determined to be cellulose 36.7%, hemicelluloses 23.3% and Klason lignin 25.1%, according to National Renewable Energy Laboratory's (NREL) standard analytical procedure (Sluiter et al., 2008). The bamboo stems were dried in an oven at 50 °C, followed by cutting into an average size of around 100 mm × 15 mm × 15 mm. The chips were soaked using 1% KOH aqueous solution for 24 h at 25 °C followed by a steam explosion treatment.

2.2. Steam explosion pretreatment and successive alkali/alkaline ethanol solution post-treatments

The steam explosion pretreatment was performed in a flash hydrolysis laboratory pilot unit (7.5 L reactor) designed especially for the processing of lignocellulosic materials. All the experiments were carried out in the same amount of bamboo stem chips (500 g). The reactor was heated with saturated steam, at the end of the steaming, the pressure was instantaneously released to stop the reaction, and the sample was blown in the receiver, then the exploded material was collected and oven-dried at 50 °C for 24 h. In this study, steam explosion was conducted at 1.8 and 2.0 MPa for 5 min (samples 1 and 2), and 2.0 MPa for 8 min (sample 3), respectively.

The scheme for a successive extraction procedure (alkali/alkaline ethanol extraction post-treatments) of lignin fractions from the three steam-exploded samples is illustrated in Fig. 1. The three steam-exploded samples were extracted with 0.5% NaOH aqueous solution and 60% aqueous ethanol containing 1.5% NaOH at 80 °C for 3 h with a solid to liquor ratio of 1:25 (g/mL), respectively. The six alkali-extracted filtrates were neutralized to pH 5.5 with 6 M HCl and evaporated to about 30 mL at reduced pressure. Subsequently, each concentrated solution was poured into 3 volumes of 95% ethanol with vigorously stirring, and the hemicellulosic pellet was recovered by filtering. The pellet was washed with 70% aqueous ethanol and freeze-dried. Then each filtrate was concentrated to about 30 mL and poured in 5 volumes of acidic water (pH 2, HCl). The degraded acid-insoluble lignin fractions were obtained by centrifugation and freeze-drying. All lignin samples were stored in a desiccator for further characterization. L_{1a}, L_{2a}, L_{3a} represented the degraded acid-insoluble lignin fractions isolated by extraction with 0.5% NaOH and L_{1b}, L_{2b}, L_{3b} represented the degraded acid-insoluble lignin fractions isolated by extraction with 60% ethanol containing 1.5% NaOH from the corresponding steam-exploded samples 1, 2, and 3, respectively. Moreover, L_{1a}, L_{2a}, L_{3a} (alkali extraction) were named as L_a, whereas L_{1b}, L_{2b}, L_{3b} (alkaline ethanol extraction) were labeled as L_b, respectively.

2.3. Characterization of the isolated acid-insoluble lignin

The associated sugars (neutral sugars and uronic acids) in the lignins were calculated by using high-performance anion exchange chromatography (HPAEC) according to the literature (Sun et al., 2013a,b). Molecular weights of the lignin fractions were determined by gel permeation chromatography (GPC). The GPC system was calibrated with polystyrene standards. Fourier-transform infrared (FT-IR) spectroscopic measurements were performed on a Nicolet iN10 spectrophotometer with an MCT detector. The samples were scanned between 4000 and 800 cm^{-1} at 4 cm^{-1} resolution with 128 scans. The solution-state NMR spectra of the samples were acquired on a Bruker AVIII 400 MHz spectrometer at 25 °C. For HSQC spectroscopic experiments, the data were acquired in HSQC experiment mode using 20 mg lignin in 1 mL DMSO-d_6. The parameters for data acquisition were set according to a previous literature (Sun et al., 2013a,b).

2.4. Functional groups and antioxidant activities against DPPH radical

Functional groups (phenolic hydroxyl, aliphatic hydroxyl and carboxyl groups) of lignin samples were determined by ^{31}P NMR spectra according to previous literatures (Faix et al., 1994; Wen et al., 2013a). Each dried lignin sample (20 mg) was dissolved in 500 μL anhydrous pyridine and deuterated chloroform (1.6:1, v/v) under stirring. This was followed by the addition of 100 μL cyclohexanol (10.85 mg/mL) as an internal standard (IS), and 100 μL chromium (III) acetylacetonate solutions (mg/mL in anhydrous pyridine and deuterated chloroform 1.6:1, v/v) as a relaxation reagent. Finally, the mixture was treated with 100 μL