Biomass and Bioenergy 94 (2016) 12–20

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: http://www.elsevier.com/locate/biombioe

Research paper

The utilization of soybean straw III: Isolation and characterization of lignin from soybean straw

Zhulan Liu, Lingkun Meng, Junqing Chen, Yunfeng Cao*, Zhiguo Wang**, Hao Ren

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

ARTICLE INFO

Article history:
Received 17 January 2015
Received in revised form
10 July 2016
Accepted 14 July 2016

Keywords:
Soybean stem
LiCl/DMSO
Lignin isolation
Dissolution
Regeneration

ABSTRACT

To improve the yield of cellulolytic enzyme lignin (CEL) and to minimize the structural changes induced by isolation, the dissolution-regeneration of ball-milled soybean stem in a lithium chloride (LiCl)/dimethyl sulfoxide (DMSO) solvent was applied prior to enzymatic hydrolysis. The regenerated stem was enzymatically hydrolyzed for 72 h to remove the carbohydrates and then extracted with 1, 4-dioxane to obtain the cellulolytic enzyme lignin (named as DR-CEL). As expected, the yield of DR-CEL was much higher than that of CEL. With 2 h of ball-milling, the yield of DR-CEL was 40.1% (based on the total lignin in the raw stem). As the ball-milling time increased to 4 h, the yield of DR-CEL improved up to 82.4%, which was much higher than that of CEL with a yield of 26.0%. All the isolated lignin preparations were characterized using FT-IR, ^{1}H NMR and ^{1}H-^{13}C HSQC NMR. The results showed that the main structural characteristics of these two kinds of lignin (CEL & DR-CEL) were similar, except for a slightly higher amount of carbohydrates in DR-CEL. As a result, the combination of the dissolution–regeneration and enzyme hydrolysis of ball-milled sample has a great potential in isolating lignin with higher yield and minimal structural changes.

1. Introduction

Lignocellulosic biomass from agricultural residues, forestry waste, waste paper, and energy crops are natural composed of three major biopolymers: cellulose, hemicellulose, and lignin with complex structures [1]. Efficient utilization of these resources is becoming an important topic in upcycling of biomass to bioenergy and other bioproducts, including bioplastic and feedstock for biochemical industries [2]. In an attempt to select better biomass substrates or to optimize biomass conversion processes, it is essential to assay the composition and detailed chemical structure of plant cell-wall materials [3,4]. Among these three main components, lignin acts as glue that binds cellulose and hemicellulose, imparting rigidity and microbial resistance to the cell wall. Because lignin is synthesized after the polysaccharide matrix has been laid down, isolation of pure lignin is problematic [5].

The structure and biosynthetic pathway of lignin have been studied for more than a century. However, they have not been completely elucidated. A primary problem in clarifying the structure of native lignin is that it cannot be isolated in a chemically unaltered form [5]. In spite of many efforts, the isolation of truly representative and completely unaltered lignin is still a major challenge [6,7]. The first major advancement was made by Björkman [8], who extracted lignin from extensive ball-milled wood by neutral solvents at room temperature. However, concerns exist over the similarity between milled wood lignin (MWL) and native lignin based on low yields (25–50% of protolignin) and structural alterations due to extensive ball-milling, which can reduce the degree of polymerization, creating new free-phenolic hydroxyl groups [9,10].

A further important contribution to the isolation of lignin was made by Pew and Weyna [11]. They treated ball-milled wood with cellulolytic enzymes and obtained an insoluble residue containing almost all of the lignin present in spruce and aspen woods. However, the residue had as much as 12% carbohydrates, and no further characterization was attempted. Chang et al. also treated milled wood with enzymes but using an enzymatic preparation with greater cellulolytic and hemicellulolytic activities than the enzyme used by Pew. They extracted the insoluble residue obtained after the enzymatic hydrolysis successively with 96% and 50% aqueous dioxane. The combination of these two fractions

* Corresponding author.
** Corresponding author.
E-mail addresses: yunfcao@163.com (Y. Cao), zhiguojj@126.com (Z. Wang).

http://dx.doi.org/10.1016/j.biombioe.2016.07.021
0961-9534/

offered higher yields of lignin than MWL, and this kind of lignin was named as cellulolytic enzyme lignin which also abbreviated as CEL [5]. Clearly, cellulose hydrolysis is the key step for preparation of CEL because the efficiency of cellulose hydrolysis affects the following CEL extraction by aqueous dioxane. It is generally accepted that the accessibility of cellulases to the limited adsorption sites on crystalline cellulose structures plays an important role in determining the hydrolysis rate [12–14]. Cellulase could readily hydrolyze the more accessible amorphous portion of cellulose but is not as effective in degrading the less accessible crystalline portions [15,16]. Thus, cellulose with high crystallinity will be more resistant to enzymatic hydrolysis. As a result, it is essential to decrease the crystallinity of cellulose for more efficient cellulase hydrolysis of lignocellulosic materials to obtain higher yields of CEL [17].

In recent years, complete dissolution of lignocellulose using an appropriate solvent gives a broad insight for lignin separation. A variety of cellulose-dissolution solvents might be considered for dissolving the lignocellulose to disrupt cellulose crystallinity, even though many of these are not suitable for isolation of relatively intact lignin from lignocellulose. Lu and Ralph reported that dimethyl sulfoxide-tetrabutyl ammonium fluoride (DMSO/TBAF) and dimethyl sulfoxide-imidazole binary (DMSO/NMI) solvent systems could dissolve wood after extensive ball-milling [18]. However, both of these two systems require a rather long ball-milling time, which causes lignin structural alternation and cellulose degradation. Kilpeläinen et al. reported that wood can be dissolved in various imidazolium-based ionic liquids (ILs) under moderate conditions [19]. However, the elevated temperature required for this dissolution is simply too destructive to be used for isolating lignin preparation useful for structural studies [17,20,21]. Recently, a dimethyl sulfoxide and lithium chloride (DMSO/LiCl) dissolution system, which is a much stronger wood solvent, has been developed by Wang et al. [22]. It was proposed that wood samples that only need 2 h of ball-milling can be completely dissolved in this system with the concentration reaching as high as 10%. Furthermore, the lignin structure did not change significantly due to the short duration of ball-milling.

In our previous work [23], both the soybean stem and pod after only 4 h of ball-milling could be completely dissolved in this solvent system without remarkable structural change in lignin. In addition, few efforts have been made to reveal the characterization or utilization of soybean straw. All these facts encourage us to continue working on the isolation and structural characterization of soybean straw components, as well as on converting this abundant agricultural waste to valuable chemicals and novel, functional, composite biomaterials. The aim of the present study is to introduce a novel moderate approach for high yield and minimum structural change in the lignin isolation procedure, which consists of dissolution-regeneration in LiCl/DMSO, followed by mild enzymatic hydrolysis of ball-milled lignocellulose.

The aim of the present study is to introduce a novel moderate approach for isolating the lignin with high yield and minimum structural change. In this procedure, the ball-milled lignocellulose was first dissolved in LiCl/DMSO and regenerated by distilled water, followed by mild cellulolytic enzymatic hydrolysis and dioxane extraction which are similar to the isolation of CEL. Because of the existence of dissolving and regenerating, this isolated lignin sample was named as DR-CEL. Then some of the lignin samples isolated via different methods were subjected to a series of instrumental analyses, such as components analysis, FT-IR, ^{1}H NMR and ^{1}H-^{13}C HSQC NMR, which focus on elucidating the structural characteristics of the lignin fractions.

2. Materials and methods

2.1. Materials

Soybean (*Glycine max*) straw was collected from the north area of Jiangsu, China. Air-dried soybean stem was fractionated from soybean straw then ground to pass through 40- to 80-mesh screens using a Genetic Electric Wiley mill. Then, the ground sample was extracted with benzene-ethanol (2:1, v/v) for 8 h to remove the extractives. The extractive-free sample was vacuum-dried and stored in sealed plastic bags as a starting material.

2.2. Methods

2.2.1. Chemical composition characterization

The lignin and sugar contents of the extractives-free samples were analyzed using the NREL/TP-510-42618 protocol [24]. The Klason lignin content was taken as the ash free residue after acid hydrolysis. The hydrolysate was collected for the determination of the acid-soluble lignin and the structural sugars. The acid-soluble lignin was measured by absorbance at 205 nm in a UV–vis spectrometer (TU-1810, Puxi, Beijing, China). The ash content was determined by combustion at 575 °C. The monomeric sugars were quantitatively measured with a high performance liquid chromatography (HPLC, Agilent 1200 Series, Santa Clara, CA) equipped with the refractive index detector (RID). The HPLC analysis was carried out using a Bio Rad Aminex HPX-87H 20n exclusion column (300 × 7.8 mm, Bio-Rad Laboratories, Hercules, CA) with a Cation-H Refill Cartridge guard column (30 × 4.6 mm, Bio-Rad Laboratories, Hercules, CA). The column temperature was 55 °C. A 5 mmol/L H_2SO_4 solution prepared with degassed super-purified deionized water was used as eluent at a flow rate of 0.6 ml/min. Aliquots (10 μl) were injected after passing through a 0.22 μm nylon syringe filter. Monomeric sugars were quantified with reference to standards using the same analytical procedure. The concentration of monosaccharide was corrected by calibration curve of standard sugars. Data of glucose, xylose, rhamnose, galactose, arabanase, and mannase contents were corrected to anhydro units, i.e., glucan, xylan, rhamnosan, galactan, arabinan, and mannan.

2.2.2. Planetary ball-milling

The sample (approximately 8 g) was milled for 0.5, 1, 2, and 4 h, respectively in a planetary ball mill (Fritsch GMBH, Pulverisette 7 premium line, Idar-Oberstein, Germany). Two zirconium dioxide bowls (80 ml) containing 25 zirconium dioxide balls (1 cm diameter) were used. The milling frequency was 600 rpm. The ball-milling was conducted at room temperature and paused for 10 min between every 5 min of milling to prevent overheating. The ball-milled samples were then vacuum-dried at 40 °C for 24 h.

2.2.3. Dissolution and regeneration of ball-milled sample

The dissolution and regeneration scheme is shown in Fig. 1. The samples ball-milled with different milling time were suspended in 8% LiCl/DMSO and stirred continuously at room temperature for 24 h [22]. The dissolved samples were regenerated in 5 vol of distilled water, then centrifuged and washed with distilled water thoroughly, until no Cl^- was detected in the supernatant, to get the dissolved-regenerated sample (DR).

2.2.4. Isolation of cellulolytic enzyme lignin

CEL and DR-CEL were isolated according to the scheme shown in Fig. 1. The ball-milled sample and the dissolved-regenerated sample (DR) were suspended in sodium acetate buffer (pH 4.8) with 5% (w/w) consistency. A commercial enzyme cocktail Cellic CTec2, VCNI0002 (Novozymes Inc. Bagsvaerd, Denmark; filter paper