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Structural characterization of the solid residue produced by hydrothermal treatment of sunflower stalks and subsequent enzymatic hydrolysis

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ABSTRACT

This study was to structurally characterize solid residues obtained from sunflower stalks hydrothermally treated at 180 and 200 °C for 30 min, followed by enzymatic hydrolysis. Recovered solid residue were 25.3% and 24.1% of fresh biomass, respectively. Each ethanol soluble fraction could be obtained up to 30% of the solid residue. The fraction from the solid residue at 200 °C was composed of smaller-sized lignin macromolecules with higher phenolic hydroxyl group, but lower aliphatic hydroxyl due to enhanced cleavage reactions related to side chain and aryl ether linkages of lignin, compared to that of the solid residue at 180 °C.

Introduction

Recently, due to rising concern over the depletion of fossil fuels and accelerating global warming, the demand for lignocellulosic biomass has increased dramatically worldwide. Lignocellulosic biomass has been considered a sustainable and renewable primary energy resource that can be converted to alternative transportation fuels, including bioethanol or biodiesel, bio-chemicals, and bio-polymers [1]. Among the promising lignocellulosic biomass, sunflower is being spotlighted as a huge energy source. Sunflower is commonly cultivated due to its high oil content in the seeds, representing up to 80% of its economic value, and its oilseed yield accounts for 8% of the total global production of oilseeds (404 million tons in 2008/2009), corresponding to the fourth largest production in the world [2]. In addition, sunflower can be cultivated throughout the world due to their relatively short cultivation period with a high yield up to 3 ton/ha. Top sunflower producing countries are in the order of Russia (5.7 billion tons per year), Ukraine (4.2 billion tons per year), and Argentina (3.5 billion tons per year) [2]. Even though it is also useful to produce pectins and essential oil from sunflower heads, sunflower stalks have been studied as an attractive lignocellulosic biomass [3].

In contrast to 1st generation feedstocks, such as starch or sugar-based crops, lignocellulosic biomass, a promising 2nd generation renewable material, is composed of three major biopolymers – cellulose, hemicelluloses and lignin, the amount of each which varies depending on the lignocellulosic biomass species. Generally, cellulose accounts for around 35–50%, hemicellulose around 15–35%, and lignin around 15–25% based on the dry weight biomass, and these components construct the plant cell wall structure in which lignin and polysaccharides are linked together. Therefore, to use cellulose and hemicelluloses as carbohydrate sources, pretreatment is an essential processing step in biochemical conversion of the lignocellulosic biomass to biofuels and biochemicals. The purpose of pretreatment is to break down the plant cell wall structure, due to the recalcitrance nature of lignocellulosic biomass, and then make cellulose more accessible to hydrolytic enzymes [4].

Among the various existing pretreatment methods, hydrothermal treatment, also called liquid hot water or autohydrolysis, has been evaluated as being a suitable process for producing fermentable sugars. Hydrothermal treatment is favorable for lower capital and operating investment, due to its use of only water as a reaction medium, lack of any requirement for acid or alkaline catalyst, and lower inhibitor generation such as furfural, 5-hydroxymethyl-2-furaldehyde (HMF) and phenolics that may adversely affect microbial fermentation [1]. To date, various studies have evaluated hydrothermal treatment as an effective

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pretreatment for converting cellulose from various herbaceous biomasses such as switchgrass, sunflower stalks and wheat straw to fermentable sugars for subsequent fermentation [3,5–8]. These studies reported that complicated conditioning for subsequent enzymatic hydrolysis was not needed, due to the absence of any mineral acid and alkali catalyst. Moreover, these biomasses can be effectively converted to fermentable sugars with low yields of inhibitory products interfering with downstream operations, such as microbial fermentation.

During fermentable sugar production, non-hydrolyzable solid residue that is mainly composed of lignin can be recovered but, unlike fermentable sugars for subsequent microbial fermentation, it is utilized as a low-grade boiler fuel. Lignin is a polyphenolic polymer derived from three phenylpropanoid units as precursors for lignin biosynthesis: *p*-coumaryl, coniferyl and sinapyl alcohol. The relative portions of each monolignol in lignocellulosic biomass differ depending on the biomass species. The lignin macromolecule is linked by carbon-carbon and carbon–oxygen bonds via radical coupling polymerization of three monolignols. The aryl ether bond (β-O-4) is major interunit linkage, and other linkages, such as biphenyl, β-5, and α-O-4 can also be detected [9]. Hydrothermal treatment has less affect on delignification, thus most of the lignin in the starting material remains in the pretreated biomass. Lignin has been regarded as the most recalcitrant component to enzymatic and microbial attack. Therefore, with the steady acceleration in industrial biofuel and biochemical production from lignocellulosic biomass, large quantities of solid residue are also generated as a by-product [10]. Improved cost-effectiveness and optimized biomass usage will necessitate effective valorization of solid residue.

After enzymatic hydrolysis of the pretreated biomass, the remaining solid residue, with lignin as its main component, is an attractive aromatic resource for producing aromatic chemicals and materials. To date, a number of researchers have attempted to develop novel valorization of lignin, including fractionation methods of lignin, and synthesis of lignin-based polymers [11–13]. However, significant challenges remain in the application of lignin due to its structural complexity. Moreover, the physico-chemical properties of lignin differ depending on the biomass and recovery processes. Therefore, it is important to evaluate the potential use of solid residue after hydrothermal treatment, and subsequent enzymatic hydrolysis.

The majority of the lignin research, to date, has focused on organosolv lignin recovered from organosolv-pretreated supernatants, and organosolv lignin has been comprehensively characterized and evaluated for further use [6,10,14–17]. On the other hand, little research has investigated the valorization of solid residue as a by-product obtained from hydrothermally treated lignocellulosic biomass, and subsequent enzymatic hydrolysis for producing fermentable sugars. During hydrothermal treatment without adding any chemicals, lignin is rarely thermally decomposed and hydrolyzed, whereas it retains its native lignin properties [18]. As the lignin in the solid residue could be also as heterogeneous and insoluble as natural lignin, it might not be suitable for chemical purpose. If the solid residue is fractionated into more suitable materials for value added applications, this solid residue could potentially serve as an attractive source for phenolic-based chemical industries [19,20].

The objectives of this study were to structurally characterize the solid residue recovered from the hydrothermally treated sunflower stalks at 180 and 200 °C, followed by enzymatic hydrolysis. In addition, lignin-enriched fractions were collected and characterized by compositional analysis, elemental analysis, thermogravimetric analysis (TGA), gel permeation chromatography (GPC), and ^{31}P nuclear magnetic resonance (NMR) spectroscopy in order to elucidate the structural information on a starting material for bio-based polymers and fine/bulk chemicals.

Materials and methods

Raw material

Sunflower stalks (*Helianthus annuus* L.) were supplied by Chungbuk Agricultural Research and Extension Services (Ochang, Chungcheongbuk-Do, Korea) and air-dried in a greenhouse. The stalks were ground, using a knife mill equipped with a 20-mesh aperture screen, and the ground stalks containing 2.3% moisture were stored at −20 °C in a refrigerator in sealed plastic bags until use. Cellic® CTec2 as a cellulase, Cellic® HTec2 as a xylanase, and Novozyme 188 as a beta-glucosidase were purchased from Novozymes Korea (Seoul, Korea). All organic solvents at the highest purity grades were purchased from Sigma–Aldrich.

Hydrothermal treatment followed by enzymatic hydrolysis

Hydrothermal treatment was carried out using a 2 L-scale Parr reactor (Parr Instruments, Moline, IL). The pretreatment method has been specifically described in a previous study [5]. The powdered sunflower stalks equivalent to 120 g dry weight were added to reactor vessels, and then filled with de-ionized water, to a total weight of 1500 g. The reactor vessels were sealed, kept at room temperature through the night, and pretreated at 180 and 200 °C for 30 min, respectively. All the pretreated biomasses were transferred to 7 L-scaled batch-type fermenters (BioTron, Seoul, Korea), and a mixture of Cellic® CTec2, HTec2, and β-glucosidase (volumetric ratio 18:2:1) was loaded at a volume equivalent to 0.1 ml (ca. 8.7 FPU) per 1 g dry biomass. Enzymatic hydrolysis was carried out at 50 ± 1 °C with a rotating speed of 200 rpm for 72 h. The hydrolyzates were separated into solid and liquid fractions by centrifugation at 3727 × *g* for an hour, with a swing-type centrifuge (Model Combi-514, Hanil Scientific Co., Seoul, Korea). The solid residue was washed out with de-ionized water, followed by centrifugation. This washing/centrifugation cycle was repeated until the initial supernatants of the hydrolyzates remained less than 1%. The residue was then dried at 45 °C until the moisture content dropped below 10%. The solid residues obtained from sunflower stalks hydrothermally treated at 180 and 200 °C, followed by enzymatic hydrolysis, are herein referred to as 180 °C-solid and 200 °C-solid, respectively.

Sequential extraction of solid residue

To prepare homogeneous lignin-enriched fractions, the solid residues were sequentially extracted with water and organic solvents using a Soxhlet apparatus. Around 5 g of the solid residues were extracted with 150 ml de-ionized water, and then oven-dried at 45 °C, until the moisture content dropped below 10%. The water extractives (WE)-free solid residues were sequentially extracted with 150 ml of organic solvents, including ethanol, methanol, acetone, and isopropyl alcohol. The dissolved fractions in water and organic solvents were evaporated and freeze-dried.

Compositional and elemental analysis

The compositional analysis of the solid residues and extractives with water or organic solvents was performed according to National Renewable Energy Laboratory (NREL) standard procedures [21,22]. The method is briefly described as follows. The carbohydrates and lignin in the sample were measured by the two-step acid hydrolysis. First, concentrated acid hydrolysis was conducted with 72% (w/w) H_2SO_4 at 30 °C for 60 min. In the second step, the above hydrolyzates were diluted to 4% (w/w) H_2SO_4 with de-ionized water, and then diluted acid hydrolysis was performed at 121 °C for 60 min in an autoclave. The hydrolyzates