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Availability of four energy crops assessing by the enzymatic hydrolysis and structural features of lignin before and after hydrothermal treatment

Han-Yin Li, Bing Wang, Jia-Long Wen, Xue-Fei Cao, Shao-Ni Sun*, Run-Cang Sun*

Beijing Key Laboratory of Lignocellulosic Chemistry, Beijing Forestry University, Beijing 100083, China

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ABSTRACT

The anatomical characteristics, chemical composition, enzymatic hydrolysis efficiency and lignin characteristics of four energy crops (switchgrass, *Miscanthus* × *giganteus*, Hybrid *Pennisetum*, and *Triarrhena lutarioriparia*) before and after hydrothermal treatment (180 °C, 1 h) were investigated comprehensively to evaluate their potential for the bioenergy production. The hydrothermal treatment resulted in the deconstruction of the biomass cell walls and redistribution of the main components, accompanying with the removal of most hemicelluloses and partial lignin as well as favorable exposure of cellulose. These alterations significantly increased the efficiency of enzymatic hydrolysis, and the different recalcitrance of the four feedstocks was evenly erased by hydrothermal treatment. Among the four crops, Hybrid *Pennisetum* with high cellulose and low lignin contents had a better performance in the enzymatic hydrolysis process both before and after treatment. Meanwhile, the maximum concentration of low degree of polymerization xylo-oligosaccharides (2-6) and the lowest amount of degraded products were obtained from Hybrid *Pennisetum* during the treatment process. Moreover, the structural features of the residual lignin in the treated substrates were elaborately analyzed for deeply understanding the fundamental chemistry of lignin during the treatment. The present study is beneficial to the fully effective utilization of biomass components and extension of the energy sources.

1. Introduction

With the depletion of fossil fuels, the issues of energy demand and global warming have become growingly prominent, which force the sustainable and green resources attracting more attention to generate the energy and valuable chemical products. Lignocellulosic biomass as a sustainable, renewable and environmentally friendly energy source, has been emphasized increasingly to undergo enzymatic bioconversion into monomeric sugars for bioethanol production to reduce the dependence on fossil fuels [1]. Among biomass feedstocks, energy crops contain many valuable advantages, such as perennial growth, abundant sources, high biomass yields, low nutrient and water requirements, and low production costs, which make them draw considerable attention and are viewed as interesting raw materials for industrial bioconversion processes [2,3]. In order to dig new feedstock, the current study was to evaluate the application prospect of four energy crops, namely, switchgrass (SG), *Miscanthus* × *giganteus* (MG), Hybrid *Pennisetum* (HP), and *Triarrhena lutarioriparia* (TL). The former two crops, especially switchgrass, are the representative energy crops which have been attracted widespread interest and are considered to be the major lignocellulosic biomass for biofuel production [2,4–6]. However, study of the latter two is rather scarce up to date.

The bioconversion strategies are limited by the intrinsic recalcitrance of biomass which is resulted from the structural complexity and heterogeneous distribution of components (mainly cellulose, lignin and hemicelluloses) in cell walls. In the plant cell walls, cellulose fibrils show crystalline-like order, which is interacted with intermolecular hydrogen bonds forming cross-linking and sealed by a polymeric matrix of hemicelluloses and lignin [7,8]. The formed rigid and compact cell wall structure restricts the availability of the main components for conversion into biofuels or platform chemicals [9]. Therefore, to make the biomass suitable for efficient bioconversion, pretreatment is deemed as a prerequisite in industry to overcome the natural biomass recalcitrance, thus enhancing the accessibility of cellulose.

In recent decades, numerous pretreatment technologies have been adopted for improving the accessibility of enzymes to biomass substrates during hydrolysis step by altering the compositions and structures of cell walls [10–12]. Hydrothermal pretreatment has been considered as a leading and promising pretreatment technology, since it does not require rapid decompression, and water is the only reaction medium, resulting in the low-cost reactor construction with no need for corrosion resistant. Hemicelluloses, one of the major factors restricting

* Corresponding authors.
E-mail addresses: sunshaoni@126.com (S.-N. Sun), rcsun@bjfu.edu.cn (R.-C. Sun).

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enzymatic hydrolysis of biomass, can be largely dissolved and hydrolyzed into soluble mono- and oligosaccharides which can be further converted into value-added chemicals [13]. Simultaneously, partial depolymerization and dissolution of lignin would be occurred during the hydrothermal pretreatment process to further reduce the biomass recalcitrance. Therefore, this technology could make the biomass more accessible accompanying with less inhibitors [14–16]. The process of enzymatic hydrolysis was devoted to transform cellulose into glucose for the bioethanol production. The residual lignin remaining in the hydrothermally treated samples can be viewed as feedstocks for generating high value-added products. The hydrothermal pretreatment is in accord with the concept of biorefinery that almost all kinds of biomass components can be converted into different types of biofuels and chemicals [12]. Therefore, the hydrothermal pretreatment as a promising pretreatment technology in industry was used to assess the potential of the four feedstocks in the bioconversion industry.

The residual lignin in the bioconversion process with a relatively higher heating value is usually burned for generating steam or supplying the process heat requirements [17]. However, the low-value applications of lignin result in the waste of resource, since lignin is the most abundant renewable aromatic polymer and has the potential to be converted into higher value products. Prior to the valorization of lignin, the structural features of lignin remaining in the residues should be clarified, which also could elucidate the deconstruction mechanism of the given hydrothermal treatment on the biomass. In the present study, a residual lignin which isolated from biomass by double ball-milling and enzymatic hydrolysis (Double enzymatic lignin, DEL) was used to stand for the native lignin, owing to its high yield and structural integrity as compared with cellulolytic enzyme lignin (CEL) [18].

In this study, to assess the application prospect of the four energy crops in the bioconversion industry, chemical compositions and enzymatic hydrolysis efficiency of the four samples before and after hydrothermal treatment were employed. In addition, how the microstructures of substrates respond to the hydrothermal treatment were analyzed by the morphological and topochemical changes for deeply understanding the treatment mechanisms of enzymatic hydrolysis enhancement. Moreover, the structural characteristics of DELs prepared from the untreated and hydrothermally treated samples were thoroughly characterized.

2. Materials and methods

2.1. Materials

Switchgrass (SG), *Miscanthus* × *giganteus* (MG), Hybrid *Pennisetum* (HP), and *Triarrhena lutarioriparia* (TL) were manually collected from National Experiment Station for Precision Agriculture, Xiaotangshan, Beijing, China. The stems were air dried after removal of leaves, and then ground. The particles were extracted with toluene-ethanol (2:1, v/v) in a Soxhlet apparatus for 6–8 h, and subsequently dried. For microscopic measurements, the block samples prepared from the internode of the four raw materials stem were also subjected to the same extraction process with particles.

2.2. Hydrothermal treatment

The hydrothermal treatments were carried out at a solid to liquid ratio of 1:10 (g/mL) in a 100 mL batch reactor (Sen Long Instruments Company, Beijing, China) with a magnetic stirrer for 1 h at 180 °C, which was the optimum reaction temperature in view of the recovery of sugars and reduction of energy consumption [19]. At the end of the desired time, the reactor was cooled down by flowing water. The solid residues were separated from the liquor by filtration with Buchner funnel, washed repeatedly with distilled water, and then dried. The hydrothermally treated residues of switchgrass (SG), *Miscanthus* × *giganteus* (MG), Hybrid *Pennisetum* (HP), and *Triarrhena lutarioriparia* (TL) were labeled as RSG, RMG, RHP, and RTL, respectively.

2.3. Preparation of samples for microscopic measurements

The untreated and hydrothermally treated block samples were embedded with water-soluble polyethylene glycol (PEG 1500). Cross sections of 10 μm thickness were cut from the embedded blocks by a sliding microtome. All sections were moved to clean glass slides immediately and washed with distilled water repeatedly to remove the water-soluble PEG, and then mounted in water covered with 0.17 mm thickness coverslips for further microscopic measurements.

2.4. Preparation of DELs

The raw materials and the hydrothermal treated residues (2 g) were milled in a planetary ball mill (Fritsch GMBH, Idar-Oberstein, Germany) with the milling frequency of 450 rpm for 5 h, respectively. The ball-milled samples were enzymatically hydrolyzed in a 50 mM sodium acetate buffer (pH = 4.8) with 2% of substrate (w/v) in a double-layer shaking incubators (ZWYR-2102C) (Shanghai, China) (150 rpm) at 50 °C for 72 h. A commercial cellulase (Novozyme, Beijing, China) was used for enzymatic hydrolysis at enzyme activity of 50 FPU/g ball-milled samples. Subsequently, the residual lignin was collected by centrifugation, washed repeatedly with sodium acetate buffer (pH = 4.8) and water, and then freeze-dried. After that, the recovered residual lignin relived the processes of ball-milling (2 h) and enzymatic hydrolysis again as mentioned above to obtain DEL. The prepared DELs were named as DEL-SG, DEL-MG, DEL-HP, DEL-TL, DEL-RSG, DEL-RMG, DEL-RHP, and DEL-RTL, respectively, according to the corresponding samples of SG, MG, HP, TL, RSG, RMG, RHP, and RTL, respectively.

2.5. Analysis procedures

The fluorescence microscopy (FM) images of the untreated and treated cell walls were obtained on a Leica DM 2000 fluorescence microscope illuminating by an ultrapressure mercury lamp. The excitation wavelength was 435–480 nm and the emission wavelength at 495–600 nm was used for lignin autofluorescence imaging.

The Raman images of the four energy crop cross sections before and after the hydrothermal treatment were acquired using a LabRam Xplora confocal Raman microscope (CRM) (Horiba Jobin–Yvon, Longjumeau, France) [20]. A linear-polarized laser (λ = 532 nm) focused with a diffraction-limited spot size (0.61 λ/NA) and an MPlan 100 × oil immersion microscope objective (Olympus, NA = 1.40) were used to achieve a high spatial resolution. The laser power on the samples was about 8 mW. Confocal aperture was set at 100 μm and slit width at 100 μm. An integration time of 2 s per spot and 0.6 μm steps were chosen for mapping, and every pixel corresponded to one scan. The Labspec 5 software was used for image processing.

The chemical compositions of the four energy crops before and after treatments were detected according to the National Renewable Energy Laboratory (NREL) standard analytical procedure [21]. The sugars were determined by high-performance anion-exchange chromatography (HPAEC) system (Dionex ICS 3000, USA) on a Carbopac^{TM} PA-20 analytical column (3 mm × 150 mm, Dionex, Sunnyvale, USA).

The liquors obtained from the hydrothermal treatment were stored to detect monosaccharides, xylo-oligosaccharides (XOS), and inhibitory products. 3 mL liquor sample was post-hydrolyzed with 4% H_2SO_4 for 1 h at 121 °C to determine the total concentration of XOS which was calculated by the increased concentration of xylose after post-hydrolysis. Based on this procedure, saccharides with two or higher degree of polymerization (DP) were considered as XOS. All the liquor fractions were filtered by 0.22 μm filters for the subsequent analysis by HPAEC system. The inhibitors in the liquid fractions were quantitatively