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NOTE

Characterizations of poplar catkin fibers and their potential for enzymatic hydrolysis

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Abstract
This study focused on poplar (*Populus tomentosa*) catkin fiber as a new resource for bioethanol production via enzymatic hydrolysis. The poplar catkin fiber was found to have advantages of relatively high α-cellulose content (44.5%), and low lignin content (2.9%), which indicated the potential for facile enzymatic hydrolysis. The results indicated that the pretreatment improved the cellulose-to-glucose conversion yield (CGCY), the high concentration alkaline pretreatment resulted in highest CGCY (83.3%), followed by dewaxing (31.5%), dilute alkaline (29.8%), and dilute acid pretreatment (24.4%) after enzymatic hydrolysis with cellulase of 15 filter paper units per gram glucan, while the poplar catkin fiber only achieved 18.7% of CGCY by enzymatic hydrolysis after 48 h at 50 °C without any pretreatment.

Keywords Poplar catkin fiber · Enzymatic hydrolysis · Dilute acid pretreatment · Alkali pretreatment · Dewaxing pretreatment

Introduction

Poplars, as short-rotation woody crop, are well known woody plants throughout the northern hemisphere for bioenergy utilization [1]. Poplar catkin fibers are cotton-like substance that surrounds the seeds on the branches of poplar tree [2]. The flying white catkin fibers floating in air cause a lot of trouble for people, such as respiratory ailments or skin anaphylaxis. Traditionally, they are considered as a waste and often just burned or buried, which cause severe environmental pollution. Recently, researchers begun to utilize poplar catkin fibers as precursor to prepare carbon microtubes (CMTs) based on its micro-tubular structure [3, 4]. However, there was little known literature related to the characteristics of poplar catkins fibers.

Bioethanol production converted from lignocellulosic biomass has offered great promise to replace fossil fuels [5]. It is important to note that poplar catkin fibers are lignocellulosic wastes, and may be available as feedstock for the production of bioethanol, instead of being disposable wastes. Poplar catkin fiber, similar like kapok fiber or cotton, consisting of single cell fiber, contains up to more than 90% carbohydrates, so it is a potential substrate for enzymatic hydrolysis and further for bioethanol production. The complete conversion of cotton and kapok fiber into bioethanol has been approached by employing simple pretreatments prior to enzymatic hydrolysis [6, 7]. Therefore, it is possible to utilize poplar catkin fibers as raw material to produce value-added bioethanol and thereafter products. Moreover, poplar catkin fibers with a natural fine fiber size have benefit that can be used to produce ethanol directly without size reduction process. Herein, the aim of this research is to investigate the characteristics of poplar catkin fiber for the further bioethanol production and value-added products.

Previous studies about the characterization and utilization of poplar catkin fiber are rare. Few studies stated the conversion of poplar catkin fiber for the bioethanol production via enzymatic hydrolysis. In this study, to evaluate the feasibility of bioethanol production from poplar catkin fiber, the chemical composition and structure of poplar catkin fiber were investigated. The response and behavior of three different pretreatments, namely dilute acid (DA), alkaline, and dewaxing pretreatments, were investigated and compared prior to the enzymatic hydrolysis. The enzymatic digestibility of the

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pretreated substrates was evaluated for exploring the potential of poplar catkin fiber for further bioethanol production.

Experiments

Materials

The poplar (*Populus tomentosa*) catkins were collected from the poplar tree (Fig. 1a) in Beijing, China in April 2016. The poplar catkin fiber was manually isolated from the seed after air-dried process, and then stored in plastic bags until further process (Fig. 1b, c). Commercial enzymes, Celluclast 1.5 L (cellulase) and Novozyme 188 (β-glucosidase) were purchased from Novozmes A/S Denmark (Franklinton, NC, USA). All the chemical reagents were supplied from Sigma-Aldrich.

Fig. 1 Photos of poplar catkins that are still on the poplar tree (**a**) and collected (**b**), catkin fibers were isolated from the seed by hand (**c**), SEM images of poplar catkin fiber: **d** cross section, and **e** longitudinal

Pretreatments

Pretreatments were carried out in the pressure bottles with screw caps (hydrothermal synthesis reactor). DA, alkaline, and dewaxing pretreatments were carried out in different pretreatment conditions as shown in Table 1. Each pretreatment was carried out in duplicate. The conditions for pretreatments were determined according to our previous studies [8]. After pretreatments, the pretreated substrates were separated by filtration and washed with deionizer (DI) water until the pH was neutral, and then stored at condition chamber at 4 °C for enzymatic hydrolysis.

Enzymatic hydrolysis

The solid substrate with 2% solid loading recovered before and after pretreatments was hydrolyzed by two different enzymes, cellulase and β-glucosidase, at 50 °C and 220 rpm for 48 h in a shaking incubator (KYC-100C, Shanghai Fuma Laboratory Instrument Co., Ltd., China). Sodium acetate buffer (0.05 M) was used to adjust the pH at 4.8, while 1.5 mg of tetracycline chloride was added to control the growth of microorganisms and prevent consumption of liberated sugars.

The cellulase activity was assayed [9] and expressed as filter paper units (FPUs) based on the Filter Paper Unit Assay. β-glucosidase activity was determined using *p*-nitrophenyl-β-D-glucoside as the substrate and expressed as international units (IUs) [10]. The enzyme loadings were 15 FPU cellulase and 30 IU β-glucosidase per gram substrate. The hydrolysis was conducted in duplicates for each substrate, and the average is reported here.

Table 1 Pretreatment conditions of poplar catkin fiber

Pretreatment type	Liquid/solid ratio	Temperature (°C)	Time (min)	Chemical load[a] (%)	Solid yield[b] (%)
DA[c]	20:1	180	30	2	41.6
NaOH	20:1	120	60	2	77.1
NaOH	20:1	120	30	20	43.8
$CHCl_3$	20:1	60	10	100	–

[a]All the chemical loadings were based on oven-dry biomass

[b]Solid yield percentage of substrate obtained after pretreatment to untreated raw biomass, both in dry matter

[c]DA-dilute acid pretreatment with 2% H_2SO_4 on dry biomass

Analytical methods

The poplar catkin fibers were dried using oven for 24 h at 80 °C. Catkin fiber samples were extracted by alcohol-benzene (1:2) using a Soxhlet extractor (6 h), and then extract-free samples were analyzed for Klason lignin, holocellulose and α-cellulose content according to the method of TAPPI T222om-98 [11], and Chinese National Standards (GB/T2677.10-1995 and GB/T 744-1989) described by previous literature [12]. Hemicellulose was isolated from poplar catkins to conduct sugar analysis. Initially, the extract-free sample was treated with acidified sodium chlorite solution at 75 °C for 2 h to remove lignin. Hemicellulose was extracted by an alkaline treatment (5 wt % potassium hydroxide solution) at 60 °C for 4 h. The suspension was subsequently filtered and rinsed with DI water, and finally freeze-dried to yield the hemicellulose product. The sugar composition in hemicellulose was determined by high performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAE-PAD, ICS-3000, Dionex, USA). The hemicellulose was hydrolysised with 72% sulfuric acid before the analysis. The chromatographic separation was performed on a CarboPac PA20 column (4 × 250 mm, Dionex, USA) using 5 mmol/g sodium hydroxide (NaOH) and 0–75 mmol/g sodium acetate (NaAc) gradient solution at 0.5 mL/min flow rate.

The structure of poplar catkins were characterized by field emission scanning electron microscopy (FE-SEM, XL30, FEI, USA). The sample was coated with platinum using an ion sputter coater (Leica EM SCD 005, Germany).

The cellulose content of substrates was analyzed according to National Energy Laboratory (NREL) analytical procedure: Determination of Structural Carbohydrates and Lignin in Biomass [13]. The glucose content of the enzymatic hydrolysate was measured by a bio-sensor analyzer (SBA-40E, Biology Institute of Shandong Academy of Sciences, China). The results in this study were reported using average means calculated by three replicates. The cellulose-to-glucose conversion yield (CGCY) was calculated by Eq. (1):

$$\mathrm{CGCY}\ (\%) = \frac{M_g \times 0.9}{M_c} \times 100, \tag{1}$$

where M_g is the weight of glucose yield after enzymatic hydrolysis (g), M_c is the weight of cellulose in pretreated substrate (g), and 0.9 is the conversion factor of glucose to equivalent glucan.

Results and discussion

Characterization of poplar catkin fiber

The chemical composition of untreated poplar catkin fiber is listed in Table 2. In this study, holocellulose content was up to 96.91% while 44.5% cellulose content was determined by α-cellulose. The monomer of hemicelluloses in polar catkin fiber was up to 91.1% and dominated by xylose and glucose. The high proportion of carbohydrates of poplar catkin indicated that the polar catkin fiber is a good potential feedstock for bioethanol production. Notably, the results showed that Klason lignin content was only 2.9%. The literature stated that lignin is the major deterrent to enzyme accessibility to cellulose [14]. Therefore, the extremely low lignin content, exhibited great potential to lower the cost of pretreatment processing step prior to bioethanol production compared to other lignocellulosic biomass such as woody biomass [15], rice straw [16], wheat straw [17].

Appearance and morphological structure of poplar catkin fibers are shown in Fig. 1. Abundant white catkin appears on poplar branches in every spring, as shown in Fig. 1a-c. Poplar catkin fibers showed cylindrical hollow structure (Fig. 1d) with smooth surface (Fig. 1e). Similar hollow structure was also observed with other natural fibers such as kapok and milkweed fibers [18, 19]. The average outer diameter of poplar catkin fibers was 8 μm which a range from 4 to 14 μm, more than a half size less compared with kapok fiber at 20 μm [19]. Because of its fine fiber structure feature, poplar catkin fiber can be used directly without high economic and energetic costs of grinding process compared with lignocellulosic materials (such as wood), which accounting for 33% of the power requirement of the entire process [20]. In addition, its hollow structure provided a large and effective surface area, for enzyme adsorption and catalytic reactions during enzymatic hydrolysis.

Table 2 Chemical composition of untreated poplar catkin fiber

%	Holocellulose	α-cellulose	Klason lignin	Carbohydrates in hemicellulose				
				Glucose	Xylose	Arabinose	Galactose	Glycuronic acida
Raw mateiral	96.9	44.5	2.9	2.2	88.9	6.2	0.4	2.4

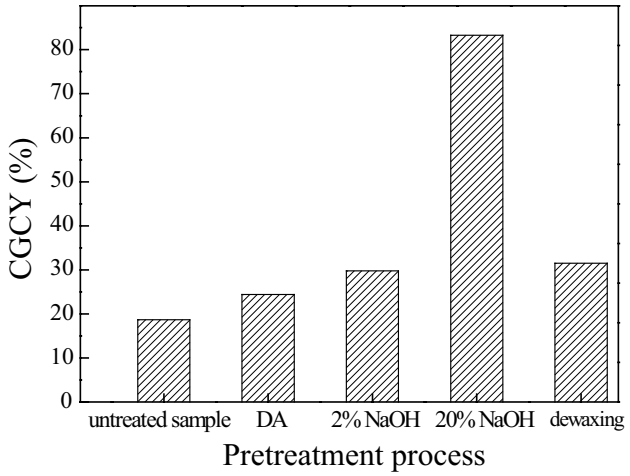

Fig. 2 Comparison of CGCY of treated poplar cakin fiber with different pretreatments (*CGCY* cellulose-to-glucose conversion yield)

The effect of pretreatments on enzymatic digestibility

The enzymatic digestibility of untreated raw and pretreated poplar catkin fiber of different pretreatments is presented in Fig. 2. It was expected that untreated raw poplar catkin fiber should have performed well in enzymatic hydrolysability in terms of its low lignin content (2.9%, Table 2). However, without any pretreatment, the CGCY of poplar catkin fiber after a 48-h hydrolysis were only 18.7%, and the digestibility slightly increased to 19.1% (data not included in Fig. 2) even with high cellulase loading (30 FPU/g substrate). This low enzymatic digestibility is primarily limited by its natural physical properties, since the main function of poplar catkin fiber is served as seedcoat enabling seeds to be transported long distance, which require biomass recalcitrance to resist assault through evolved complex structural and chemical mechanisms, which may limit it for enzymatic hydrolysis [21, 22].

The pretreated poplar catkin fiber showed different improvements on its enzymatic digestibility, as expected, in Fig. 2. The DA pretreatment did not enhance the sugar conversion significantly, which the glucose conversion only increased from 18.7 to 24.4%. The sample solid yield after DA pretreatment was 41.6% (Table 1). It is suggested that most of the hemicellulose was removed, since DA pretreatment has performed well and been used widely for a wide range of feedstocks to effectively remove hemicellulose prior to enzymatic hydrolysis [23]. The alkaline pretreatment was conducted at two level of NaOH concentration. The 2% NaOH concentration gave moderate enhancement of glucose yield from 18.7 to 29.8%. It is well known that the alkaline pretreatment have ability to eliminate the lignin and hemicellulose, which may allow for enhancement of enzymatic saccharification [24]. It is noted that the low NaOH concentration pretreatment was not sufficient to endow the fiber with complete enzymatic accessibility and hydrolysability as the retained solid yield was rather high (77.1%), similar to the solid yield of kapok fiber (79%, pretreated with 2% NaOH, at 100 °C for 60 min) [25]. Increasing the NaOH concentration can further remove lignin and hemicellulose while the solid yield decreased to 43.8%, thus the enzymatic digestibility significantly enhanced to 83.3% when 20% NaOH was applied. Considering the waxy surface of poplar catkin fiber, dewaxing pretreatment using $CHCl_3$ was also studied, and approximately 31.5% glucose conversion rate was obtained, which verified that the waxy surface is also a hindrance to enzyme attack. Among the four pretreatments, high concentration alkaline pretreatment was the most effective and followed by dewaxing, dilute alkaline, and DA pretreatment. The results indicated that the poplar catkin fiber has the potential for bioethanol production, and the further study will be aimed to overcome biomass recalcitrance by optimizing pretreatment conditions prior enzymatic hydrolysis for sugar production, better understanding of poplar catkin fiber cell wall structure at the nanometer scale will also be studied to overcome biomass recalcitrance.

Conclusion

In this study, poplar catkin fiber was creatively introduced as a new resource for enzymatic hydrolysis to produce bioethanol. Poplar catkin fiber has high α-cellulose content (44.5%), extreme low lignin content (2.9%), therefore showing a considerable potential feedstock for bioethanol production via enzymatic hydrolysis. The CGCY of poplar catkin fiber can only achieve 18.7% without any pretreatment. By employing simple pretreatments, namely DA, dilute NaOH, and dewaxing pretreatments, the CGCY of the biomass was increased to 24.4, 29.8 and 31.5%, respectively. A relative high value of CGCY (83.3%) can be achieved using high concentration (20%) NaOH pretreatment followed by enzymatic hydrolysis. It indicates the potential of preparing high value-added products from biomass waste for bioenergy application.

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