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Organic amine catalytic organosolv pretreatment of corn stover for enzymatic saccharification and high-quality lignin

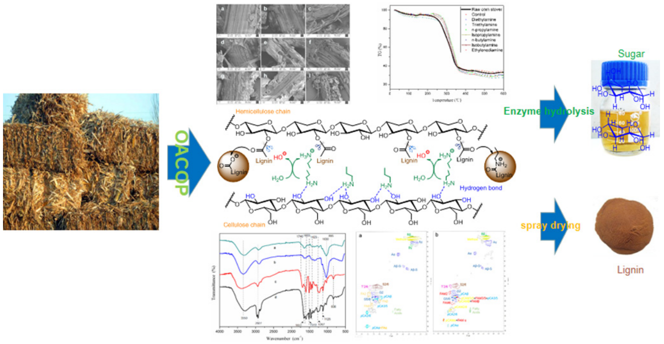
Chenglun Tang [a,b], Junqiang Shan [a,b], Yanjun Chen [a,b], Lingxia Zhong [a,b], Tao Shen [a,b], Chenjie Zhu [a,b,c,*], Hanjie Ying [a,b,c]

[a] College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing 210009, China
[b] National Engineering Technique Research Center for Biotechnology, Nanjing, China
[c] Jiangsu National Synergetic Innovation Center for Advanced Materials, Nanjing, China

HIGHLIGHTS

- Organic amine catalytic organosolv pretreatment was developed.
- High yield of monosaccharide was obtained after pretreatment and enzymatic hydrolysis.
- High-quality and salt-free lignin was produced from pretreatment.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel and efficient organic amine and organosolv synergetic pretreatment method was developed to overcome the recalcitrance of lignocellulose to produce fermentable sugars and high-quality salt-free lignin. After optimization of the process parameters, a delignification of 81.7% and total sugar yield of 83.2% (87.1% glucose, 75.4% xylose) could be obtained using *n*-propylamine (10 mmol/g, biomass) as a catalyst and aqueous ethanol (60%, v/v) as a solvent. The susceptibility of the substrates to enzymatic digestibility was explained by their physical and chemical characteristics. The physical structure of extracted lignin showed higher β-aryl ether bonds content and functionalities, offering the potential for further downstream upgrading. The role of organic amine catalyst and a synergistic mechanism is proposed for the present system.

1. Introduction

Due to the depleting fossil fuel reserves and increasing greenhouse gas emission, the exploration of feasible pathways for the utilization of abundant and renewable biomass is highly desirable. In this sense, lignocellulosic biomass is a promising candidate because it is an abundant and carbon-neutral energy resource (Somerville et al., 2010). Sugars derived from lignocellulose are the key platform chemicals, which can be further converted to biofuels and some other high value-added chemicals via biological and/or chemical routes (Wettstein et al., 2012; Sheldon, 2014). Enzymatic hydrolysis is considered as a promising method to obtain fermentable sugars from lignocellulose due to its mild processing condition and high selectivity (Chaturvedi and Verma,

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* Corresponding author at: College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, No. 30, Puzhu South Road, Nanjing 210009, China.
E-mail address: zhucj@njtech.edu.cn (C. Zhu).

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2013). However, the recalcitrant structure of lignocellulose resists the enzymatic hydrolysis process (Himmel et al., 2007). Therefore, an efficient and economical pretreatment technology is a prerequisite to enhance the bio-digestibility of enzymes.

Pretreatment techniques can overcome the recalcitrant structure by disrupting cell wall physical barriers as well as cellulose crystallinity and removing the lignin so that hydrolytic enzymes can access the biomass macrostructure. A large number of pretreatment approaches such as alkaline pretreatment, dilute acid pretreatment, hydrothermal pretreatment, steam explosion, and organosolv pretreatment have been investigated on a wide variety of feedstock types and there are several recent review articles which provide a general overview of this field (Ravindran and Jaiswal, 2016; Zhang et al., 2016). Among these methods, organosolv pretreatment provides an efficient fractionation of the lignocellulose into its main constituents, thus allowing the valorization of all fractions. Furthermore, the presence of organic solvent reduces the viscosity of the pretreatment medium, improves penetration into the lignocellulosic matrix, and facilitates a more efficient removal of lignin (Oliet et al., 2002). Various organic solvents especially for the low boiling point polar solvents such as ethanol, methanol, tetrahydrofuran, or acetone were used for organosolv pretreatment due to their easy recovery and low cost (Zhang et al., 2016).

Compared to the pure organosolv pretreatment technology, catalytic organosolv pretreatment (COP), which usually use acid or alkali as a catalyst combined with organic solvent have proven to be an efficient method for the lignocellulosic biomass pretreatment due to its positive effect to enhance the delignification process and enzymatic digestibilities of cellulose (Zhang et al., 2016). In view of the issues derived from acid catalytic organosolv pretreatment (ACOP) such as generating inhibitors, equipment corrosion problems, base catalytic organosolv pretreatment (BCOP) have recently drawn considerable attention (Kim et al., 2016). BCOP could suppress the dissolution of cellulose and hemicellulose, which are often observed in the acid or hydrothermal pretreatment processes (Alvira et al., 2010). Furthermore, it usually causes the swelling of the biomass, thus increasing the accessibility of enzyme to the cellulose and improve the saccharification. Numerous inorganic base catalysts such as NaOH (Li et al., 2013), KOH (Sun et al., 2011) have been used for the BCOP process. However, these processes usually generate significant waste water streams that must be neutralized, which leads to additional disposal cost. Using solid base catalyst such as CaO, MgO still suffers from the separation problem between the catalyst and pulp. From an economic and environmental perspective, the development of a new BCOP method which can simplify the process, promote clean manufacturing, and decreased the disposal cost is highly desirable.

In continuation of our efforts to develop new BCOP method for the lignocellulosic biomass pretreatment (Tang et al., 2017), herein, a novel and efficient organic amine catalytic organosolv pretreatment system (OACOP) was reported. In this study, the catalytic effects of organic amine on the pretreatment results were investigated, and analysis techniques were employed to characterize the structure and property changes of stock before and after pretreatment to evaluate the effect of organic amine catalyst. A mechanism of OACOP was proposed. Furthermore, the recyclability of organic amine along with organic solvent was also studied.

2. Materials and methods

2.1. Materials and reagents

Corn stover, provided by local farmer in Lianyungang, Jiangsu, China, was knife milled and screened to 30–50 mesh and dried to constant weight. The chemical composition of the raw corn stover (on a dry weight basis) was 38.7% cellulose, 20.0% hemicellulose, 18.1% lignin, 3.9% ash and 19.3% unknown components. Organic amine (99.5%) and ethanol (99.5%) were purchased from Aladdin (China).

Cellulase (245 FPU/mL), was obtained from Tianguan Co. (Nanyang, China).

2.2. Experimental section

2.2.1. Pretreatment of corn stover by OACOP

The OACOP reactions were carried out in high-pressure reactors (Yanzheng Co. in China) and were performed using corn stover (4.0 g on a dry basis) with aqueous ethanol (60%, 40 mL), organic amine as a catalyst (10 mmol/g dry biomass), anthraquinone (AQ) (0.2% w/w dry biomass), and air pressure (1 bar). The pretreatment was conducted at 140 °C for 40 min. After pretreatment, the reactor was cooled down to room temperature by cooling water, and the obtained solid and liquid fractions were separated by filtration. The solids were washed with water (2 × 20 mL) and dried at 105 °C to constant weight. The amount of solid remaining was calculated and sampled for composition analysis. Ethanol and organic amine were recovered under vacuum to obtain lignin. Finally, the pretreated corn stover was used for enzymatic hydrolysis.

2.2.2. Composition analysis of corn stover

Composition of raw corn stover and pretreated solid was analyzed by two-stage acid hydrolysis, according to National Renewable Energy Laboratory (NREL) procedures (Sluiter et al., 2008). A high performance liquid chromatography (HPLC) (Agilent 1200 series; Hewlett-Packard, Palo Alto, CA, USA) with a refractive index detector, using an Aminex HPX-87H ion exclusion column (300 × 7.8 mm; Bio-Rad Laboratories, Hercules, CA, USA) was used to analyse sugars in the acid hydrolysate and enzymatic hydrolysate. The mobile phase was 5.0 mM H_2SO_4 at a flow rate of 0.6 mL min^{-1}, the temperature of column was 55 °C. The acid-insoluble lignin and acid-soluble lignin were determined by gravimetric analysis (calcined acid insoluble residue at 575 °C for 24 h) and UV spectroscopy, respectively (Tang et al., 2017).

2.2.3. Enzymatic hydrolysis of biomass

Enzymatic hydrolysis of raw corn stover and pretreated solids was carried out at 50 °C with an agitation speed of 150 rpm on an insulation shaker. All experiments were performed in 20 mL of 50 mM sodium citrate buffer (pH 4.8) at solid consistency of 5%. Cellulase was added at loading of 15 FPU per gram of substrate. Periodically, aliquot samples of the hydrolysate were taken for monosaccharide analysis by HPLC.

2.3. Characterization of corn stover and extracted lignin

2.3.1. Microstructure analysis

The pretreated solid samples were dehydrated using a freeze dryer (LABCONCO, 2.5 Free Zone, USA), since lyophilization preserves morphology. The different surface morphology of corn stover before and after pretreatment were characterized by scanning electron microscope (SEM) (FEI Quanta 200FEG SEM at 2 kV at 1000× magnification).

The porosity and specific surface area of raw and pretreated corn stover samples were determined by a Brunauer-Emmet-Teller (BET) surface area analyzer (Micromeritics, ASAP2020, USA). Before the measurement, each sample was dried using freeze dryer for 24 h and degassed under vacuum for 1 h.

The crystallinity of corn stover was determined by an X-ray diffractometer (Rigku ultima IV, Janpan). The samples of particle