Journal of Cleaner Production 181 (2018) 217–223

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Comparison of various pretreatment strategies and their effect on chemistry and structure of sugar beet pulp

Guanhua Li, Yanxin Sun, Wenjing Guo, Lin Yuan*

School of Life Sciences, Inner Mongolia University, Hohhot 010021, Inner Mongolia, China

ARTICLE INFO

Article history:
Available online 3 February 2018

Keywords:
Biomass
Pretreatment
Sugar beet pulp
Enzymatic digestibility

ABSTRACT

Pretreatment is deemed as a key step to destroy biomass recalcitrance and improve enzyme accessibility to cellulose during the second generation bioethanol production. A series of pretreatments were applied to treat sugar beet pulp (SBP) to uncover their impact on chemical and structural changes, and enzymatic digestibility. The results showed that all the four pretreatments had effects on the physical structure and chemical compositions of SBP. When being subjected to aqueous ammonia pretreatment, SBP exhibited high cellulose content due to the degradation of neutral detergent soluble fraction and other amorphous components. FTIR analysis showed that aqueous ammonia pretreatment could cleave ester linkages between carbohydrates and lignin, and modify the phenolic hydroxyl in lignin, yet without obvious delignification. In addition, the aqueous ammonia pretreatment led to cell wall dislocation and lignin redistribution, and enhanced enzymatic hydrolysis. The results obtained here indicated that biomass recalcitrance was destroyed by morphological and chemical alternation. The reducing sugar yield of SBP pretreated by AA could reach 487.8 mg/g, which was 2.73 times higher than that of the control. Consequently, AA might be more suitable to pretreatment for SBP, compared to the other pretreatments.

1. Introduction

Sugar beet is a potential underutilized feedstock for ethanol and other bio-based chemicals production (Vargas-Ramirez et al., 2017). In 2005, the first demonstration project of ethanol production using saccharose from sugar beet was established in the Netherlands (Langeveld et al., 2008). At present, sugar beet ethanol pathway has been deemed as an “Advanced Biofuel” (Alexiade et al., 2018). Sugar beet pulp (SBP) is the by-product from sugar beet industry. Almost 20% of the starting sugar beet ends up as SBP by the end of the extracting process. The large output and high content of cellulose enable SBP act as a potential feedstock for ethanol production. Furthermore, SBP has always been centralized stacking, which facilitates collection and utilization process.

Cellulose conversion by enzymatic hydrolysis is a vital step in ethanol production process. The compositions of SBP are structurally heterogeneous and complicated in nature. The effective utilization of SBP requires techniques to destroy the naturally ordered and rigid structure. Pretreatments of lignocellulosic biomass are essential strategies that can reduce the particle size of substrate, facilitate the exposure of carbohydrate components from biomass, and increase accessibility of cellulose (Li and Chen, 2014). Biomass pretreatment are broadly classified into biological, physical, and chemical process. Additionally, different technologies are suitable for various conversion of biomass (Rabemanolontsoa and Saka, 2016). Several researches have been performed on pretreatments of SBP (Zheng et al., 2013; Li et al., 2017a). However, there was seldom report on comprehensive insight into the effect of various pretreatment on SBP obtained from uniform source, to avoid any variations or discrepancies in observations. Cell wall structures of biomass are highly dynamic and vary obviously among different biomass. Additionally, the highly cross-linked and complex cell wall structure might be the nature of biomass recalcitrance. Therefore, the impact of pretreatment on SBP at cellular level was systematically analyzed. In this study, four pretreatments including hydrochloric acid (HA), aqueous ammonia (AA), ammonium oxalate (HO) and pectinase (P) as catalyst were applied to treat SBP. The mechanism of the various pretreatment on SBP was investigated. Due to the special physical and chemical structure, the enzymatic hydrolysis of SBP might be enhanced by pretreatment under mild condition, which could improve the development of ethanol production.

* Corresponding author.
E-mail address: yuan0079@163.com (L. Yuan).

https://doi.org/10.1016/j.jclepro.2018.01.259
0959-6526/

2. Materials and methods

2.1. Materials

The SBP with moisture content of 80% was collected from Sugar Company around Huhhot in 2016. The SBP was washed using tap water to remove residual sugars and other impurities. After being air-dried at room temperature (routinely stirred with hand, around 25 °C), the SBP was ground by high-speed rotary cutting mill (BJ-300A, Baijie, China). The powder was sieved using a 20 mesh aperture standard screen to provide particle sizes arranged from 0.84 to 0.42 mm. Cellulase produced from *Trichoderma vride* G was purchased from Shanghai Ye Yuan Co., Ltd. The cellulase, xylanase and β-glucanase activity was 160 filter paper unit (FPU)/g, 4.63 U/g and 5.91 U/g, respectively, according to the standard method (Ghose, 1987; König et al., 2002). All other analytic grade reagents were purchased from Zhaocheng Chemical Reagents Company (Huhhot, China) unless otherwise noted.

2.2. Pretreatment

The SBP was pretreated in 100 mL screw-capped bottles with AA, HA, AO and P as catalyst, respectively. The pretreatments with the solid-liquid ratio of 1:20 were performed at the temperature of the set value ± 1 °C that controlled by water bath. The specific conditions of the various pretreatments were stated in Table 1. Upon completion of various pretreatment, the samples were cooled down to room temperature, collected by filtration using a vacuum pump and washed by distilled water to pH 6.5–7.0. Then samples were oven dried at 61 °C, weighted and kept for the following analysis. The raw SBP was used as control.

2.3. Enzymatic hydrolysis

Two grams of the pretreated SBP were put into 250 mL erlenmeyer flasks containing 40 mL citrate buffer (0.05 mol/L, pH 4.8) and 40 FPU cellulase (20 FPU/g dry SBP). Enzymatic hydrolysis was conducted in water-bath incubator with gentle agitation at 50 °C for 48 h. The enzymatic hydrolysate was collected, deactivated at 90 °C for 10 min, and centrifuged at 5000 rpm for 10 min. The supernate was used for determination of reducing sugars. The reducing sugar content (measured as glucose) was assessed by dinitrosalicylic acid method that was described by Miller (1959). The results were expressed as reducing sugar yield (RSY) and calculated according to the following equation.

$$RSY\ (\%) = \rho V \times 100/m \quad (1)$$

Where RSY represents reducing sugar yield (mg/g), ρ represents the concentration (mg/mL) of generated reducing sugars during the enzymatic hydrolysis, V represents the total volume (mL) of enzymatic hydrolysate, m is the initial weight of various SBP.

2.4. Chemical composition determination

The chemical compositions of SBP before and after pretreatment were determined by the Van Soest method with minor modifications (Van Soest, 1967; Li et al., 2017a). The extraction process was conducted by soxhlet apparatus using neutral detergent, 2 mol/L hydrochloric acid, 7.34 mol/L sulfuric acid, and 0.41 mol/L sulfuric acid in sequence. After each step, the filtration residue was washed with water and acetone, and then oven-dried to constant weight. The neutral detergent fiber (NDF) fraction, acid detergent fiber (ADF) fraction, and acid detergent lignin (ADL) fraction were calculated from the difference of weight between the initial material and the treated residue. The neutral detergent soluble (NDS) content was obtained from the difference between initial weight and NDF, hemicellulose content was measured from the difference between NDF and ADF, cellulose content was calculated according to the difference between ADF and ADL. The ash content was determined by heating the ADL at 550 °C for 180 min, and lignin content was the difference between ADL and ash.

Table 1
The conditions of various pretreatment.

	Conditions
HA pretreatment	1 wt%, 80 °C, 6 h
AA pretreatment	10 wt%, 80 °C, 6 h
AO pretreatment	5 wt%, 80 °C, 6 h
P pretreatment	30 U/g, 50 °C, 6 h

2.5. Fourier transform infrared spectroscopy analysis

Fourier transform infrared (FTIR) spectroscopy of SBP was analyzed by FTIR spectrophotometer (IR-Affinity-1S, Shimadzu, Japan). Exactly 1 mg of dried finely ground sample was mixed with 99 mg KBr (spectroscopic grade), and compressed at around 1.5 ton pressure for 15 min to form a KBr disk. The spectra of KBr disk without SBP were used as control to subtract background spectra from each sample (Karimi and Taherzadeh, 2016).

2.6. Specific surface area analysis

To analyze the Brunauer, Emmett and Teller (BET) surface area, the oven dried sample of 0.5 g was degassed with high purity nitrogen (99.999%) at 200 °C and tested with a surface area analyzer (Micromeritics ASAP2020 apparatus, Micromeritics, USA) at −196 °C.

2.7. Crystallinity measurement

The crystallinity of sample was determined by employing powder X-ray diffractometry technique using X-ray diffractometry system (X'Pert PRO, PANalytical, Netherlands). Scans were recorded at 40 kV and 40 mA with a step size of 0.02° from 5° to 40° 2θ. The crystallinity of SBP was expressed by crystallinity index (CrI), determined from X-ray diffractometry (XRD) data and calculated with the following equation (Mahmood et al., 2017).

$$CrI = (I_{cr} - I_{amor}) \times 100/\ I_{cr} \quad (2)$$

Where CrI represents the crystallinity index, I_{cr} represents the peak intensity of crystalline regions in cellulose at 2θ between 22° and 23°, and I_{amor} represents the minimum intensity of amorphous regions in cellulose at 2θ between 18° and 19°.

2.8. Microscope imaging analysis

The cell wall structure of SBP was analyzed by fluorescent microscope (FM). For better images, SBP were embedded in paraffin, frozen at −20 °C and sectioned by cryostat (Leica, RM2015) (González-Melendi et al., 2008). Sections (8 μm) containing SBP were maintained onto adhesive glass slide, observed by fluorescence microscope (Olympus, Japan) using 40× objectives with ultraviolet sources (280 nm). Photographs were captured with color camera. The fluorescence intensity and fluorescence intensity variance were analyzed based on the method described by Banerjee with some modifications using Image J software (Banerjee et al., 2009). The scanning electron microscopy (SEM) images of SBP