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Short Communication

Comparison of organosolv and hydrotropic pretreatments of eucalyptus for enhancing enzymatic saccharification

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HIGHLIGHTS

- Hydrotropic method could dramatically remove lignin from fiber surface and fiber cell wall.
- Effect of organosolv pretreatment on reducing cellulose crystallinity was notable.
- Water treatment without addition chemicals was able to displace the lignin on fiber surface.
- Cellulase adsorption capacity of hydrotropic pretreated substrates was better.
- Hydrotropic lignin contains more phenolic group and syringyl unit than organosolv lignin.

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ABSTRACT

The objective of this study was to investigate the effects of organosolv and hydrotropic pretreatments on improving enzymatic hydrolysis of eucalyptus. The chemical composition of the fiber surface was analyzed using X-ray photoelectron spectroscopy (XPS) to determine the surface characteristics of pretreated eucalyptus. Other than the significant decrease of surface coverage by lignin, hydrotropic pretreatment was more effective in removing the lignin and xylose from fiber cell walls than organosolv pretreatment. The restriction of acetyl and phenolic groups in pretreated substrates was typically eliminated by hydrotropic pretreatments. Moreover, fiber structure and morphology after pretreatments were more suitable for enzymatic hydrolysis. Cellulase adsorption capacity was notably improved by hydrotropic pretreatment, which indicating the better enzyme accessibility of cellulose in pretreated substrates. Eventually, higher glucose yield was obtained with hydrotropic pretreatment. In addition, the precipitated lignin as an important by-product of pretreatments was characterized by Fourier transforms infrared spectroscopy (FTIR) also.

1. Introduction

Conversion of lignocellulosic biomass into glucose by enzymatic hydrolysis is a crucial step for production of biofuels and biochemicals (Himmel et al., 2007). The pretreatment prior to enzymatic hydrolysis is a mandatory process in order to disrupt the recalcitrant structure of lignocellulosic biomass, so that the accessibility of enzymes to cellulose could be improved. Up to now, several pretreatment technologies have been investigated including biological, physical (e.g., milling and grinding), physic-chemical (e.g., hydrothermal and steam explosion), and chemical (e.g., acid, alkali and organosolv) methods (Li et al., 2012). Of these technologies, organosolv pretreatment is one of the most promising for biofuel production due to its advantage in delignification and increasing enzymatic digestibility. Ethanol is the most commonly used organic solvent because it is easy to recycle for reducing the cost (Zhang et al., 2016). Using ethanol for pretreatment of biomass, the cellulose-to-glucose yield was over 80%, meanwhile, a considerable amount of lignin was able to be obtaining from pretreatment process (Wildschut et al., 2013). Hydrotropic pretreatment has similar merits as organosolv pretreatment such as effective delignification, simple lignin recovery and the hydrotrope agent recyclable (Mou et al., 2016). While, energy consumption for recovery of hydrotrope agent and ethanol by distillation need cut off in the real biorefinery production. Sodium xylenesulphonate (SXS) was the best hydrotrope agent for fractionation lignin and cellulose from biomass (Mou et al., 2013a). In our previous studies on the production of fermentable sugars from lignocellulosic materials, hydrotropic treatment could remarkably remove the lignin while increasing the cellulose-to-glucose conversion yield from

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enzymatic hydrolysis (Mou et al., 2013b). By comparison with room-temperature ionic liquid method, diluted alkaline and hydrothermal pretreatment, hydrotropic pretreatment can particularly remove the lignin distribution on fiber surface, which leading to the improvement of the enzymatic accessibility of cellulose (Mou et al., 2013b, 2014a). Combination with acid treatment or addition little acid during hydrotropic treatment could further reinforce the delignification and hemicelluloses solublization, while cellulose retained well in the pretreated solid fraction (Mou et al., 2014a,b). With the purpose of extraction lignin from biomass, hydrotropic treatment was carried out using 36% SXS at 170 °C for 2 h, the precipitated lignin yield was about 70% (Gabov et al., 2013). However, high concentration of hydrotrope agent brings challenge for the utilization of hydrotropic technology in industry. Either organosolv or hydrotropic pretreatment can be used to fractionate lignocellulosic biomass into cellulose, lignin and hemicelluloses or hemicellulosic-degradation products. Nevertheless, the distinct mechanism of organosolov and hydrotropic pretreatments on disrupting the recalcitrant structure of biomass have not been extensively explained yet.

In this work, hydrotropic and organosolv technologies were subjected to eucalyptus for increasing enzymatic digestibility. Effects of pretreatments on chemical and surface chemical compositions, structural features and enzymatic hydrolysis were comprehensively evaluated. In order to deeply explain the mechanism of pretreatments for enhancing enzymatic accessibility, the cellulase adsorption capacity of pretreated substrates was studied. And the chemical structure of lignins recovered from pretreatment process was investigated also.

2. Materials and methods

2.1. Raw materials composition

Eucalyptus chips collected from Chen Ming pulp mill (Zhanjiang, China) with a size of 4–6 mm were air dried for pretreatments. The chemical composition of eucalyptus before and after pretreatments was analyzed according to National Renewable Energy Laboratory (NREL) analytical method. Chemicals were purchased from commercial sources and were used without further purification. In this study, all experiments were carried out in duplicates and the results were averaged.

2.2. Pretreatment methods

2.2.1. Hydrotropic pretreatment

Eucalyptus chips were treated with 30% (w/w) sodium xylenesulphonate (purity 90%, Sigma-Aldrich) and 2.0% (w/w) formic acid at 160 °C for 90 min, and liquor to solid ratio (L/S) is 8:1. After treatment, the solid fractions were disintegrated and washed as published before (Mou et al., 2013a). The pretreated solids fractions were stored at 4 °C for enzymatic hydrolysis. Eucalyptus treatment with water at same condition was carried out as comparison.

2.2.2. Organosolv pretreatment

A mixture of ethanol and water (60:40 (w/w)) was used for organosolv pretreatment, the L/S ratio is 6:1. The organosolv treatment was run at 160 °C for 90 min. After pretreatment, the solid fraction substrates were washed with ethanol before washing with water. Afterwards, the sample was centrifuged and stored at 4 °C for enzymatic hydrolysis. Hydrotropic and organosolv lignins were recovered from the spent liquor for further analysis.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of pretreated substrates has been published elsewhere (Mou et al., 2014a). The hydrolyzates were sampled at 6, 8, 12, 24, 48, 72 h for determination of the glucose concentration. The glucose monomer was detected by ion chromatography (ICS-900, USA). And the glucose yield was calculated on the basis of the glucose content in the initial raw material.

2.4. Enzyme adsorption

The adsorption of cellulase onto pretreated eucalyptus was performed in serum bottles using 0.5 mL cellulase in 4 ml of sodium citrate buffer (pH 4.8) at room temperature for 90 min. Prior to addition cellulase, bovine serum albumin (BSA, 0.2 mg.mL^{-1}) was conducted to avoid the adsorption of enzyme on residual lignin in pretreated samples for 60 min. The supernatant was separated by centrifuge for protein analysis.

2.5. Chemical analysis methods

2.5.1. FTIR analysis

The pretreated substrates and isolated lignins were determined by FTIR (Nicolet IS50, Thermo Fisher Scientific, USA). Prior to analysis, same amount of sample was prepared through KBr pellet, the weight ratio of KBr to sample is 100:1. Spectra were collected at a resolution of 4 cm^{-1} in the range of 500–4000 cm^{-1}.

2.5.2. XRD analysis

Crystallinity of untreated and pretreated eucalyptus was analyzed by XRD (D8 Avance, Bruker, Germany). The crystallinity index (CrI) calculation method was illustrated before by Segal et al. (1959).

2.5.3. SEM analysis

The morphology of eucalyptus before and after treatment was measured by SEM (Hitachi S-4800, Japan). Before analysis, the freeze-dried samples were pasted on the specimen stub and coated with platinum.

2.5.4. XPS measurement

The surface chemical components of eucalyptus before and after treatment were investigated by a Axis Ultra DLD XPS instrument. Analyzed area was 700 × 300 μm and at least three different spots were measured on each sample. The surface coverage by lignin (S_{lig}), carbohydrates (S_{car}) and extractives (S_{ext}) calculation method was published elsewhere (Ström and Carlsson, 1992; Mou et al., 2013a,b).

2.5.5. Protein analysis

The amount of free protein in solution was determined according to the method reported by Bradford (1976). Samples lacking enzyme were used as reference. And the cellulase adsorption capacity was calculated based on the following equation:

$$\text{Cellulase adsorption capacity } \% = \frac{(\text{Total protein} - \text{Free protein})}{\text{Total protein}} \times 100 \quad (1)$$

3. Results and discussion

3.1. Chemical compositions of eucalyptus after pretreatments

The major chemical components of eucalyptus after organosolv and hydrotropic pretreatments at specific conditions are presented