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Short Communication

Investigating lignin and hemicellulose in white rot fungus-pretreated wood that affect enzymatic hydrolysis

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HIGHLIGHTS

- Selective delignification and hemicellulose removal were performed on bio-treated residues.
- Lignin in fungi-pretreated residues played a dominant role in hindering enzymatic hydrolysis.
- Fungal pretreatment prefer to integrate with alkaline pretreatment to maximize the synergy.

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ABSTRACT

Selective delignification and hemicellulose removal were performed on white rot fungus-pretreated residues to investigate the effects of lignin and hemicellulose removal on enzymatic hydrolysis. 43.66 – 77% of lignin with small part of hemicellulose were degraded by chlorite treatment, while 79.97–95.09% of hemicellulose with little lignin were degraded by dilute acid treatment, indicating that cross effect between lignin and hemicellulose was minimized. In subsequent enzymatic digestion, regardless of the cellulase loading, residues from series-grade delignification released more glucose and xylose than that from hemicellulose removal, suggesting that lignin rather than hemicellulose in fungi-pretreated residues played a dominant role in hindering enzymatic hydrolysis. Based on the fundamental mechanisms of acidic/alkaline pretreatments in literature, it is proposed that fungal pretreatment prefers to integrate with alkaline pretreatment rather than acidic pretreatment to maximize the synergy. This indication would be helpful to optimize and renovate the integrated pretreatment.

1. Introduction

Bioethanol, a carbon neutral, clean burning, sustainable and renewable fuel, can be produced from wood, grass and agriculture residues as a promising alternative to fossil fuel (Demirbas, 2008). However, native biomass has limited its accessibility to enzymes or microorganisms due to its compact and rigid structure known as biomass recalcitrance. Therefore, pretreatment is an essential prerequisite to make biomass accessible by breaking the lignin seal, removing hemicellulose, or disrupting the crystalline structure of cellulose. Thermochemical pretreatment (steam-explosion, acid and alkaline pretreatment etc.) usually need a high consumption of energy and chemicals, and a series of toxic compounds are generated during the process of pretreatment (Sun and Cheng, 2002).

Recently, fungal pretreatment using white rot fungi has attracted extensive attention for biorefinery as the remarkable abilities of delignification and certain advantages of low-cost, environmentally friendly and no emission of inhibitors to fermentation (Alvira et al., 2010). However, the drawback of fungal pretreatment is the low efficiency of enzymatic hydrolysis, indicating that quantities of recalcitrant barriers still exist in the fungi-pretreated residues. Fungal pretreatment followed by physical or chemical pretreatment can potentially overcome the recalcitrance, since the combined pretreatment process could initiate a synergistic effect, improving the yields of end products (Wang et al., 2012). However, different physical or chemical pretreatments own different fundamental mechanisms (Zhao et al., 2012). To maximize the synergy of combination of fungal pretreatment and physical or chemical pretreatment, the second-step pretreatment should be compatible with the first-step fungal pretreatment, which necessitate the understanding of what is the dominant recalcitrant factor in fungi-pretreated residues.

During white-rot-fungi pretreatment, part of lignin and hemicelluloses are degraded, with an associated increase of cellulose accessibility to enzymes (Wan and Li, 2012). Lignin and hemicellulose are most possible to be the principal barrier to enzymatic

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hydrolysis. Moreover, they can be directly manipulated by pretreatment process. Consequently, the two chemical components, lignin and hemicelluloses, were selected to study as the independent variables. In the present work, white rot fungi-pretreated residues were either delignified using sodium chlorite or removed hemicellulose using dilute acid, to create three spectrums of lignin contents and hemicellulose contents. Cross effects were minimized. Finally, these residues with different contents of lignin or hemicelluloses were subjected to enzymatic hydrolysis. Through this design, components in fungi-pretreated residues as recalcitrant barrier that affect enzymatic hydrolysis were investigated. This might be helpful to further understand the mechanisms of fungal pretreatment and optimize the combined pretreatment processes, as well as develop novel pretreatment methods.

2. Methods

2.1. Microorganisms and inoculum preparation

White rot fungus, *Trametes velutina* D10149, a common fungus on angiosperm wood (Dai, 2012), was isolated from Jilin Province in North China. The organism was preserved on 2% (w/v) malt-extract agar (MEA) plates at 4 °C in the Institute of Microbiology, Beijing Forestry University. The fungus was activated in 100 mL basic medium (g/L: glucose 20, yeast extract 5, KH_2PO_4 1, $MgSO_4$ 0.5, VB_1 0.01) (Wang et al., 2012), and cultured on a rotary shaker at 28 °C with a speed of 150 rpm. Mycelial pellets were harvested after 5 days, added 100 mL distilled water and then mixed with a laboratory blender for 30 s at 5000 rpm. This suspension would act as inoculum.

2.2. Raw materials

Fresh poplar wood (*Populus tomentosa*) from Shandong Province was chopped into small pieces and air-dried. The samples were ground, and the particles between 20 and 80 mesh were prepared for the subsequent pretreatment with white rot fungi.

2.3. Fungal pretreatment of poplar wood

The Fungal pretreatment was carried out in a 500 mL Erlenmeyer flask with 15 g of air-dried poplar wood and 37.5 mL of distilled water. The samples were sterilized in the autoclave for 20 min at 121 °C and inoculated with 15 mL inoculum. The cultures were incubated statically at 28 °C for 8 weeks. The non-inoculated samples were served as the control. All experiments were performed in triplicate.

2.4. Selective delignification and hemicelluloses removal

Chlorite treatment was used to extract lignin from the fungi-pretreated poplar wood. 2 g substrate was treated with an aqueous solution containing 80 mL of distilled water, 2.5 g $NaClO_3$ and with 2 mL acetic acid at 80 °C for 30, 60 and 90 min (abbreviated to L1, L2, L3), respectively. After being dried at 35 °C for 24 h, the samples with different lignin content were obtained.

Hemicelluloses removal was conducted in batch tube reactors. The reactor was filled with 2 g bio-pretreated poplar wood and 20 mL 1% sulfuric acid to achieve a 10% w/v of dry matter mixture. An IKA (C-MAG HS 7) equipped with oil bath was employed to heat the tube reactor. When the oil reached 140 °C, the tube reactor was placed into the oil bath with a vigorous magnetic stirring (300 rpm) and then the residence time began to record. After 30, 60 and 120 min (abbreviated to H1, H2, H3), respectively, the reaction was ended by quenching the tube in room-temperature water. Wet material was vacuum filtered to obtain water-insoluble residues. The residues after filtration were washed to neutralize with distilled water, and then dried at 35 °C for 24 h for further analysis.

2.5. Enzymatic hydrolysis

Commercial cellulase preparation (Celluclast 1.5 L) and β-glucosidase were purchased from Sigma–Aldrich. Enzymatic hydrolysis was conducted in 10 mL of 50 mM sodium acetate buffer (pH 4.8) supplemented with 40 μL antibiotics tetracycline and 20 μL cycloheximide. The substrate was loaded at 2% (w/v). Cellulase loading was 14 and 35 FPU/g substrate, respectively (with 15 and 37.5 CBU/g substrate of β-glucosidase, respectively). The mixture was incubated at 50 °C in a rotary shaker at 150 rpm. Samples were taken from the reaction mixture periodically, centrifuged for 10 min at 10000 rpm, and stored at −20 °C for sugar assay.

2.6. Analytical methods

The chemical composition of the raw material and pretreated residues was determined according to NREL LAP (Sluiter et al., 2008) using HPAEC. The monosaccharides in the supernatant after enzymatic hydrolysis were also analyzed by HPAEC. The cellulose and hemicellulose conversion were calculated as follows:

$$\text{Cellulose conversation}\ (\%) = \frac{\text{amount of glucose in enzyme hydrolysate} \times 0.9 \times 100}{\text{amount of cellulose}}$$

$$\text{Hemicellulose coversion}\ (\%) = \frac{\text{amount of xylose in enzyme hydrolysate} \times 0.88 \times 100}{\text{amount of hemicellulose}}$$

3. Results and discussion

3.1. Selectively decay of poplar wood

After 8-week solid-state fermentation with white rot fungus, *T. velutina*, the bio-pretreated residues were further subjected to chlorite and dilute acid treatment, respectively. The changes of chemical components in residues after various treatments were shown in Table 1.

With respect to fungi-pretreated sample, as expected, part of lignin and hemicellulose were degraded, which was consistent with previous reports about fungal degradation (Yu et al., 2009). In the subsequent chlorite pretreatment, lignin decreased gradually (43.66–77%) with residence time but only a small portion of hemicellulose (18.44–22.25%) was removed in the residues. Besides, the delignification was mainly attributed to the removal of acid insoluble lignin (AIL) since large quantities of AIL were decayed at each treatment level, whereas only a little change happened to the soluble lignin (ASL). Contrary to chlorite pretreatment, dilute acid pretreatment caused large amounts of hemicellulose removal, from 79.97% to 95.09%, while lignin increased slightly with the prolonged residence time because of the lignin coalescence and re-location during acid pretreatment (Selig et al., 2007; Donohoe et al., 2008). As a result, it can be concluded that chlorite pretreatment and dilute acid pretreatment employed in this work are fairly selective and cross effects between lignin and hemicellulose during these two pretreatments are minimized. In this case, major attention can be focused on the effect of one variable on enzymatic hydrolysis regardless of another variable.